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TITLE OF THESIS... Hypotensive Hydroxamic Acids

UNIVERSITY... The University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED... Ph.D.

YEAR THIS DEGREE GRANTED... 1974

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DATED. April 18 1974

NL-91 (10-68)

THE UNIVERSITY OF ALBERTA

# HYPOTENSIVE HYDROXAMIC ACIDS

by

© FRANK DAVID SEMAKA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL CHEMISTRY

DEPARTMENT : FACULTY.OF.PHARMACY.AND.PHARMACEUTICAL.SCIENCES

EDMONTON, ALBERTA

SPRING, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled HYPOTENSIVE.HYDROXAMIC.ACIDS submitted by FRANK.DAVID.SEMAKA in partial fulfilment of the requirements for the degree of Doctor of Philosophy in PHARMACEUTICAL.CHEMISTRY.

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TO SHIRLEY

## ABSTRACT

An homologous series of methyl α-(and β)-methyl-β-($N^4$-substituted-$N^1$-piperazinyl) propionates was synthesized by reacting the appropriate amine with either an α, β-unsaturated ester or a suitably substituted bromoester. In a similar manner, a number of $N^4$-substituted-piperazinyl butyrates, acetates, formates and propionic acids as well as several 4-phenylpiperidino, 4-benzylpiperidino and 4-phenyl-1, 2, 5, 6-tetrahydropyridino esters were prepared. Many of the above aminoesters were converted to their corresponding hydroxamic acids by reacting the ester with hydroxylamine hydrochloride in methanol or ethanol.

In addition, three 1-hydroxyquinoxalin-2-one 4-oxides were prepared by reacting appropriate α-ketoaldehydes with o-quinone dioxime in water. These oxides were then catalytically reduced over palladium-charcoal to 1-hydroxyquinoxalin-2-ones. Several different strategies to synthesize the above quinoxaline cyclic hydroxamic acids by reductive cyclization of suitably substituted o-nitroesters with sodium borohydride catalyzed by palladium-charcoal were unsuccessful. The mass spectra of the 1-hydroxyquinoxalin-2-one 4-oxides and 1-hydroxyquinoxalin-2-ones have been recorded and interpreted. All gave abundant molecular ions, most of which fragmented to $[M-16]^+$; $[M-17]^+$ and $[M-45]^+$ ions by the loss of O, $\dot{O}H$, and $\dot{C}OOH$, respectively. These suggested fragmentation pathways were supported by the presence of

metastable peaks of appropriate mass. Further decompositions were largely influenced by the nature of the substituents on each molecule.

All of the compounds obtained were screened for hypotensive activity at dose levels of 1, 5 or 25 mg/kg by measuring the carotid arterial blood pressure of two rats anesthetized with urethane and comparing the blood pressure responses to acetylcholine chloride, noradrenaline bitartrate, 1,1-dimethyl-4-phenylpiperazine or nicotine and vagal stimulation before and after administration of the test drug intravenously.

The presence of an aromatic group in the 4-position on a cyclic 3-amino function provided optimal hypotensive activity in the methyl propionate and propionohydroxamic acid series. Whether an α- or β-methyl substituent was present or whether the ester was methyl or ethyl had no consistent advantage for hypotensive activity. Decreasing the length of the propionate function by one or two methylene groups resulted in diminished hypotensive activity but increasing the distance by one methylene enhanced activity. The hydroxamic acids generally had a similar magnitude of hypotensive activity to the corresponding esters, but some had a longer duration of action. Aminocarboxylic acids were substantially weaker hypotensive agents than either the corresponding aminoesters or aminohydroxamic acids, while the 1-hydroxyquinoxalin-2-one 4-oxides and 1-hydroxyquinoxalin-2-ones

were virtually devoid of hypotensive activity. Within the amino-ester and aminohydroxamic acid series, most of the active 4-alkyl substitued cyclic 3-amino compounds had a ganglionic blocking action, while the 4-ara-alkyl substituted derivatives often had a mixed ganglionic blocking and α-adrenoceptive blocking action; the 4-aryl substituted analogues usually possessed a purely α-adrenoceptive blocking action.

The rates of hydrolysis of methyl 2-methyl 3-[1-(4-phenyl-piperazinyl)] propionate hydrochloride and 2-methyl-3-[1-phenyl-piperazinyl)] propionohydroxamic acid hydrochloride in distilled water were compared by measuring at regular intervals of time the quantity of remaining ester or hydroxamate in a known concentration of initial solution by reacting aliquots with hydroxylamine in alkaline solution to give the hydroxamic acid (in the case of the ester) which then gave a quantifiable colored chelate complex with ferric iron, which was estimated colorimetrically. The ester was hydrolyzed much more rapidly in distilled water than the hydroxamate.

## ACKNOWLEDGEMENTS

My most sincere appreciation is extended to Dr. D. F. Biggs and Dr. R. T. Coutts for their able supervision, guidance and encouragement throughout the course of this project.

To Mr. W. Dylke, I extend my thanks for his patience in recording NMR spectra and doing microanalyses. Gratitude is also expressed to all members of the Faculty of Pharmacy and Pharmaceutical Sciences for their good-natured assistance. Finally, thanks are extended to Mrs. Harriet Stoker for typing this manuscript.

The studentship provided by the Medical Research Council was gratefully appreciated.

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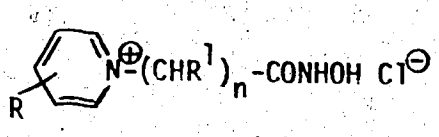
# INTRODUCTION

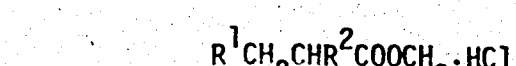
I. Hypotensive Aminoesters, Aminocarboxylic Acids and Aminohydroxamic Acids

Coe (1959) found that quaternary pyridinium hydroxamic acids of general structure (1), in which R was a hydrogen atom, an alkyl group or a substituted alkyl group and $R^1$ was a hydrogen atom (n = 1 or 2) or a methyl group (n = 1), were effective in the prophylaxis and treatment of organophosphate poisoning.



(1)

This publication led Coutts et al. (1969) to synthesize a series of methyl 3-aminopropionate hydrochlorides of general structure (2),



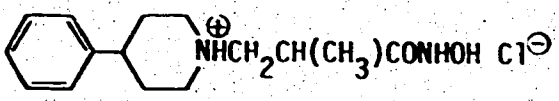
(2)

where $R^1$ was a substituted or unsubstituted piperidino, homopiperidino, morpholino, piperazino or benzylamino group and $R^2$ was a methyl group or a hydrogen atom. These esters were then converted to the corresponding

3-aminopropionohydroxamic acid hydrochlorides of general structure (3).

(3)

These hydroxamates were evisaged as potential protectives against organophosphate poisoning structurally resembling those reported by Coe (1959). However, Coutts and his co-workers noticed that intraperitoneal administration of large doses of the 3-aminohydroxamic acid hydrochlorides to rats produced disorientation, neurological effects (such as dyspnea) and CNS stimulation followed by depression. These observations led the authors to evaluate the effect of both the 3-aminopropionohydroxamic acids and the 3-aminopropionates on the carotid arterial blood pressure of anesthetized cats. All the hydroxamic acids and most of the esters caused a fall in blood pressure, with the hydroxamates exhibiting a longer duration of action. The most interesting of the compounds tested was 2-methyl-3-(4-phenyl-piperidino)propionohydroxamic acid hydrochloride (4) which produced a



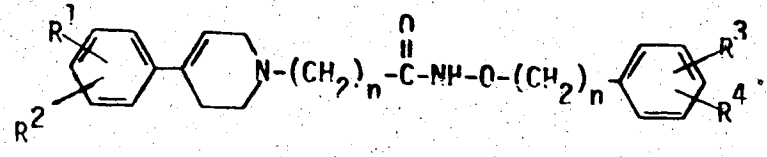
(4)

fall in arterial blood pressure. Subsequent detailed pharmacological investigation of the hypotensive action of this compound by Midha et al. (1970), suggested that its mechanism of hypotension may be sympathetic ganglion blockade. Midha et al. (1970) also found this derivative to be as effective a hypotensive agent as pentolinium tartrate on a molar basis.

Recognizing that the nature of the amino moiety $R^1$ in compounds of general structures (2) and (3) was important in determining the magnitude and duration of the fall in blood pressure observed, Coutts et al. (1971) extended their investigations to include esters and hydroxamates wherein $R^1$ was an alkyl or dialkylamino group, in addition to compounds wherein $R^1$ was a piperidino or morpholino group with $R^2$ remaining a methyl group or a hydrogen atom. Again these compounds were evaluated for their effect on the carotid blood pressure of anesthetized cats. However, none of the compounds were as active as 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid (4) in lowering the blood pressure.

Biel (1965) claimed that the aralkoxyamides of (4-phenyl-1,2,5,-6-tetrahydropyridino)alkanoic acids of general structures (5) and (6) could be used for the treatment of hypertension and peripheral vascular disease, where $R^1$, $R^2$, $R^3$ and $R^4$ were hydrogen, chlorine, bromine, iodine, or fluorine atoms or trifluoromethyl, amino, nitro, lower alkylamino, lower alkanoylamino, lower alkylthio, sulfamyl, lower

alkanoyl, lower alkylsulfonyl, or methylenedioxy groups or cycloalkoxy substituents and n was zero or an integer from 1 to 6. The claimed advantage of these compounds was the production of a potent and long lasting fall in blood pressure by a mechanism that did not involve the blockade of peripheral or autonomic ganglia.



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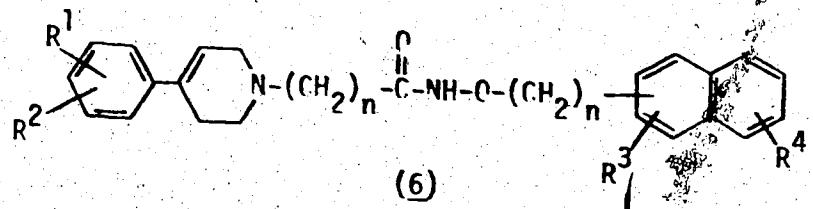
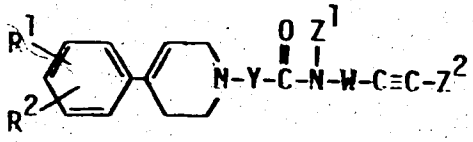


(6)

Concurrently, Biel and Hopps (1965) reported the analgesic activity of the closely related N-alkynyl and N-alkynyloxy derivatives (7), where W was a lower oxyalkyl or a lower oxyalkylene group, Y was a lower oxyalkylene group, $Z^1$ and $Z^2$ were hydrogen atoms, lower alkyl groups or functions of the formulae (8) or (9), and $R^1$, $R^2$, $R^3$, $R^4$ and n were the same as for general structures (5) and (6).



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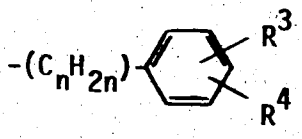

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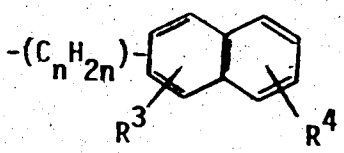
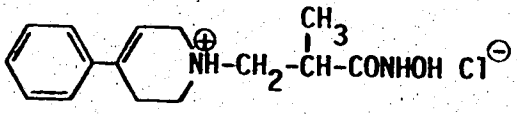


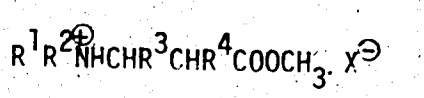
(9)

Stimulated by these findings, Biggs and co-workers (1972c) conducted a detailed pharmacological evaluation of the hypotensive action of the related compound, 2-methyl-3-(4-phenyl-1,2,5,6-tetrahydropyridino)propionohydroxamic acid hydrochloride (10), which they concluded produced a blood pressure fall via an α-adrenergic receptor blocking action.



(10)

Two investigations by Biggs et al. (1972 a; 1972 b) examined the hypotensive activity in anesthetized rats of compounds with structures (11) and (12), where $R^1$ was an alkyl, allyl or aralkyl group,



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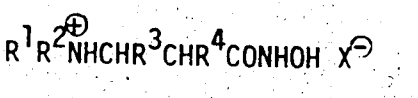
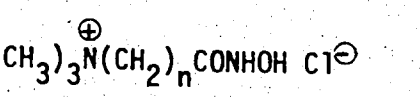


(12)

or a substituted or unsubstituted alicyclic ring and $R^2$, $R^3$ and $R^4$ were hydrogen atoms or methyl groups. They found many of the compounds [both esters (11) and hydroxamates (12)] possessed hypotensive properties of very short duration. The authors concluded that none of the compounds synthesized exhibited sufficient activity to warrant continued investigation. In addition, some of the hydroxamic acids (12) were screened for their ability to protect mice against a lethal dose of diisopropyl fluorophosphate (DFP), but none were active.

Gilbert et al. (1961) prepared a series of hydroxamic acids with general structure (13) [where n was 1, 2 or 3] in an investigation



(13)

of the relationship between structure and reactivating ability among hydroxamic acid reactivators of organophosphate-inhibited acetylcholinesterase. None of these derivatives were found to be an effective reactivator, thus indicating that the reactivation reaction probably does not require a molecular configuration similar to that of acetylcholine (14).

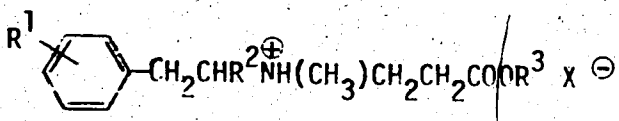
$$(CH_3)_3\overset{\oplus}{N}(CH_2)_2OCOCH_3\ Cl^{\ominus}$$

(14)

To summarize at this point, only a limited amount of research has been published concerning the synthesis and pharmacological evaluation of aminohydroxamic acids and aminoesters possessing interesting hypotensive properties. In addition to this property, aminoesters have a wide range of other biological activities. Some of these will now be discussed.

## II. Other Pharmacological Activities of 3-Aminoesters

In a series of papers, Raltzly et al. (1940 a; 1940 b; 1940 c) investigated the oxytocic activity of 3-aminoesters with structural formulas (15), (16) and (17), where $R^1$ was an alkoxy or phenoxy substituent, $R^2$ was a hydrogen atom or a methyl group, $R^3$ was a methyl or ethyl group, m and n were 1 or 2 and X was a chlorine or bromine atom. The maximum oxytocic activity found was only 5 - 10% that of ergonovine.



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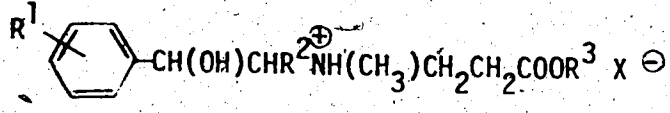

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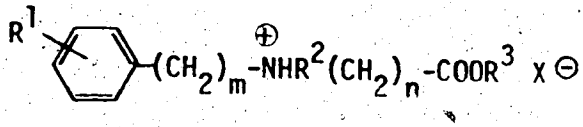
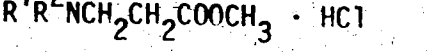


(17)

Phillips (1950) continued the search for oxytocic 3-aminopropionates by synthesizing compounds with general structure (18), where (a) $R^1$ and $R^2$ were simple aliphatic groups (or hydrogen), (b) $R^1$ was a



(18)

methyl, benzyl, 4-methoxyphenethyl or 3,4-dimethoxyphenethyl group and $R^2$ was 2-carbomethoxyethyl, or (c) $R^1R^2N$ was a saturated heterocyclic ring. The authors found none of these analogs to be active.

A compound belonging to the series with general structure (2), namely the reversed carboxyl analogue (19) of acetylcholine was synthesized by Bass et al. (1950) and found to be equipotent with acetylcholine in muscarinic activity when evaluated on guinea-pig ileum.

$$(CH_3)_3\overset{\oplus}{N}CH_2CH_2COOCH_3\ I^{\ominus}$$

(19)

Schueler and Keasling (1951) have reported that the reversed carboxyl analogs of acetyl-α-methylcholine and acetyl-β-methylcholine, (20) and (22) respectively, possessed less than one ten-thousandth of the muscarinic activity of their respective acetylcholine analogues (21) and (23).

$$(CH_3)_3\overset{\oplus}{N}CH(CH_3)CH_2COOCH_3\ I^{\ominus}$$

(20)

$$(CH_3)_3\overset{\oplus}{N}CH(CH_3)CH_2OOCCH_3\ I^{\ominus}$$

(21)

$(CH_3)_3\overset{\oplus}{N}CH_2CH(CH_3)COOCH_3\ I^{\ominus}$

(22)

$(CH_3)_3\overset{\oplus}{N}CH_2CH(CH_3)OOCCH_3\ I^{\ominus}$

(23)

A comparison of the differences in the reported relative activities of compounds (19), (20) and (22), prompted Biggs et al. (1971) to synthesize and re-examine the muscarinic and nicotinic activities of all three esters. They found that derivatives (20) and (22) exhibited significant muscarinic action, while compounds (19) and (20) possessed nicotinic action. Compounds (20) and (22) were noted to be more potent parasympathomimetics than was previously reported.

Adamson (1954) reported that compounds with general structure (24) have strong analgesic properties, when $R^1$ was a methyl group, $R^2$

$R^1R^2NCHR^3CH_2COOCH_3$

(24)

was a methyl or ethyl group and $R^3$ was a methyl or propyl group. In contrast, when Halverstadt et al. (1959) investigated the synthesis and hypotensive activity of a series of 2-ammonioalkyl-3-ammonioalkanoate

salts derived from general structure (25), where $R^1$, $R^2$, $R^3$, $R^4$, $R^5$ and

$$R^1R^2R^3\overset{\oplus}{N}CH_2CH_2COOCH_2CH_2\overset{\oplus}{N}R^4R^5R^6 \cdot 2X^{\ominus}$$

(25)

$R^6$ were various alkyl substituents or heterocycles, they found that a number of these diamino esters exhibited a marked hypotensive activity via ganglionic blockade.

Pacheo et al. (1962) screened a number of aminoesters with general formula (26) for antispasmodic activity, where $R^1$ was a piperidino, pyrrolidino or morpholino group, $R^2$ was a phenyl group and $R^3$ was a methyl or higher alkyl group. Many were active.

$$R^1CHR^2CH_2COOR^3 \cdot HCl$$

(26)

Horrii et al. (1962) evaluated different 3-aminoester derivatives of general structure (27) for oxytocic activity. In their compounds, $R^1$ was a variety of aliphatic or aromatic groups and $R^2$ and $R^3$ were methyl groups or hydrogen atoms. Several had significant activity.

$$R^1R^2\overset{\oplus}{N}HCH_2CHR^3COOCH_3\ Cl^{\ominus}$$

(27)

Matkovics et al. (1961; 1962) and Porszasz et al. (1961) synthesized and evaluated aminoesters with general formula (28), where $R^1$ was a piperidino, pyrrolidino or morpholino group, $R^2$ was a methyl, ethyl, butyl, phenyl or benzyl group and n was 1 or 2. The tertiary esters with lower aliphatic substituents exhibited nicotinic action, while those with higher molecular weight substituents or aromatic substituents reportedly produced ganglionic blockade. The authors found that the quaternary salts of all these esters were potent ganglionic blockers.

$$R^1(CH_2)_nCOOR^2$$

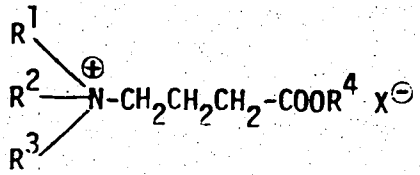
(28)

Barrass et al. (1968) also studied aminoesters of structural type (28), but in this case $R^1$ was a diethylamino or pyrrolidino group, $R^2$ was a methyl or ethyl group and n was 1 to 4. The authors found that these derivatives had predominately a nicotinic action and were more active when quaternized.

In a subsequent report Brimblecombe and Sutton (1968) investigated the effects of various quaternary aminoesters (such as methyl 3-dimethylaminopropionate methiodide and ethyl 3-dimethylaminopropionate methiodide) which exhibited both muscarinic and nicotinic activity on the superior cervical ganglion of the cat. They concluded that these compounds produced their effects by interaction with postsynaptic receptors in the ganglion.

Besides 3-aminopropionates, 4-aminobutyric acids and derivatives have been synthesized and screened for a variety of biological activities.

In series of publications, Takahashi et al. (1961 a; 1961 b; 1962 a; 1962 b) and Kumei (1961) studied the pharmacological actions of 4-aminobutyrates with general structure (29), where $R^1$ was a hydrogen atom, methyl, acetyl or phenyl group, $R^2$ and $R^3$ were hydrogen atoms or methyl groups, $R^4$ was a hydrogen atom, methyl, ethyl, n-butyl or benzyl group and X was either a chloride or bromide ion. Many of these derivatives produced vasodilation, lowered blood pressure and possessed antistimulant actions against acetylcholine, 5-hydroxytryptamine, nicotine and histamine.

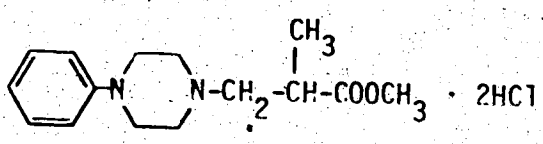


(29)

In addition to studying the blood pressure responses of anesthetized cats to various 3-aminoesters and 3-aminohydroxamic acids, Coutts et al. (1971) tested several 3-aminopropionic acids of general structure (30), where R was piperidino, 4-methylpiperidino, homopiperidino or ethylamino and $R^1$ was a hydrogen atom or a methyl group. These

(30)

carboxylic acids were found to have no effect upon the blood pressure, while the corresponding esters and hydroxamic acids possessed significant hypotensive properties (Coutts et al. 1969; 1971). Furthermore, Coutts et al. (1969) noted that although corresponding hydroxamates and esters had similar magnitudes of hypotensive activity, the duration of action of the hydroxamates was longer than that of esters. The above observations led the present author to suggest that the differences in duration of action between corresponding hydroxamates and esters could be attributed to differing rates of hydrolysis to give inactive carboxylic acids. To examine this hypothesis, a comparison of the rates of hydrolysis of methyl 2-methyl-3-[1-(4-phenyl-piperazinyl)]propionate dihydrochloride (31) and 2-methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic hydrochloride (32) in distilled water was undertaken. The results of this study are discussed later.



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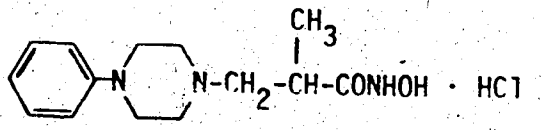
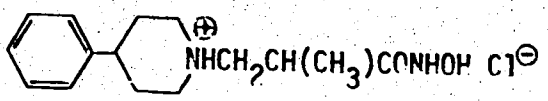


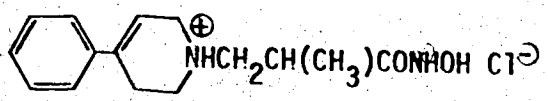
(32)

## III. Hypotensive Piperazine Derivatives

In view of the foregoing observations, it was decided that further investigation of the structural requirements for hypotensive activity of 3-aminoesters and 3-aminohydroxamates with general structures (2) and (3) would be worthwhile. The 3-position amino groups chosen for study were various substituted piperazines. The reason for this choice was twofold. First was the prominence in the scientific literature of potent hypotensive compounds incorporating a piperazine moiety in their structure. Second was the difference in pharmacological properties between compound (4), a potent ganglionic blocking agent (Midha et al. 1970) and compound (10), a potent α-adrenergic blocking agent (Biggs et al. 1972c). It should be noted that since the present study concerns itself only with hypotensive activity, an attempt to document the wide range of pharmacological properties displayed by compounds incorporating a piperazine nucleus was judged beyond the scope of the current undertaking. Consequently, the following discussion will review only the documentation of piperazine moieties occurring in potent hypotensive agents.

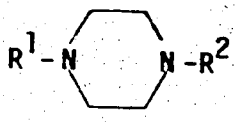



(4)

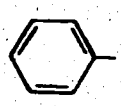
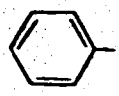
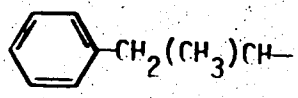
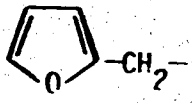
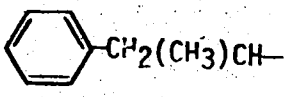
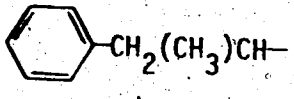
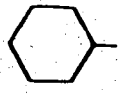
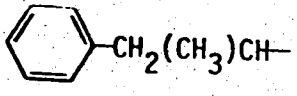



(10)

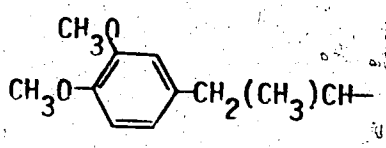
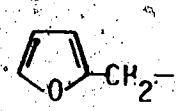
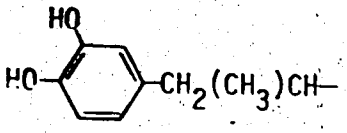
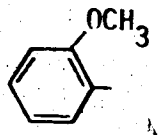
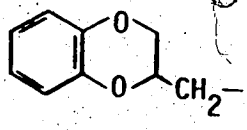
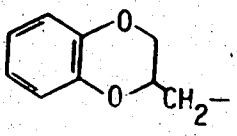
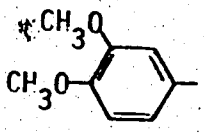
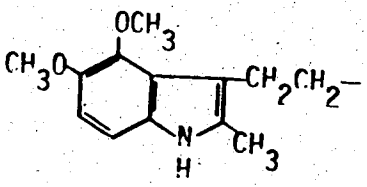
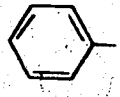
The adrenolytic, sympatholytic and antihistaminic properties of piperazine derivatives were first mentioned by Bovet and Bovet-Nitti (1948), who studied 1-phenylpiperazine (33a) and 1-methyl-4-phenylpiperazine (33b). Several years later, Olin (1953) reported that 1-(2-methylpropyl)-4-(1-methyl-2-phenylethyl)piperazine (33c) and 1-furfuryl-4-(1-methyl-2-phenylethyl)piperazine (33d) possessed hypotensive activity while 1-isoamyl-4-(1-methyl-2-phenylethyl)-piperazine (33e) and 1-cyclohexyl-4-(1-methyl-2-phenylethyl)piperazine (33f) were inactive.



(33)

| | $R^1$ | $R^2$ |
|---|---|---|
| (a) | H- |  |
| (b) | $CH_3$- |  |
| (c) | $CH_3(CH_3)CHCH_2$- |  |
| (d) |  |  |
| (e) | $(CH_3)_2CHCH_2CH_2$- |  |
| (f) |  |  |

(33)

| | $R^1$ | $R^2$ |
|---|---|---|
| (g) |  |  |
| (h) | $(CH_3)_2CHCH_2-$ |  |
| (i) |  | H– |
| (j) |  |  |
| (k) |  | H– |
| (l) | $(C_6H_5)_2CHNH-$ | $CH_3-$ |
| (m) | $(p\text{-}ClC_6H_5)_2CHNH-$ | $CH_3-$ |
| (n) |  |  |

Moffit and Lim (1965) found that 1-[2-(3,4-dimethoxyphenyl)-1-methylethyl]-4-furfurylpiperazine (33g) and 1-isobutyl-4-[2-(3,4-dihydroxyphenyl)-1-methylethyl]piperazine (33h) produced sustained hypotension in unanesthetized hypertensive (sinoaortic occlusion) dogs when given either intravenously or orally. Further investigation led the authors to conclude that the hypotension produced by these compounds was partly due to blockade of the vasomotor centre in the medulla and partly to blockade of adrenergic terminations at the periphery, with no involvement of ganglionic blockade and no interference with other cholinergic receptors.

O'Leary (1953) demonstrated that 1,4-bis-(1,4-benzodioxan-2-yl-methyl)piperazine (33j) was a new adrenergic blocking agent which lowered the blood pressure, antagonized blood pressure responses to epinephrine and inhibited the effects of directly (splanchic stimulation) or reflexly (carotid occulsion) stimulating the sympathetic nervous system in chloralose or pentobarbital anesthetized dogs. Page et al. (1959) found 1-(2-methoxyphenyl)piperazine (33i) produced a lowering of blood pressure and sedation in hypertensive patients, while 1,4-bis-(1,4-benzodioxan-2-yl-methyl)piperazine (33j) was less effective. This finding led Quesuel et al. (1960) to examine the action of 1-(3,4-dimethoxyphenyl)piperazine (33k) in dogs and cats. The authors concluded this compound produced its hypotensive effect by acting upon β-adrenergic receptors.

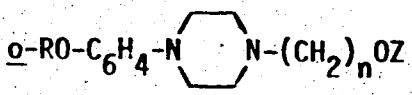
In an American patent, Rudner (1961) synthesized N-methyl-N$^1$-(p-chlorobenzhydryl)piperazine (33m), then claimed that

pharmacological studies showed the compounds to be antihypertensive and antihistamic agents.

Da Costa and Spector (1963) reported that 1-[4,5-dimethoxy-2-methyl-(3-indolyl)ethyl]-4-phenylpiperazine (33n) had both adrenolytic and catecholamine depleting actions in anesthetized dogs as well as anti-arrhythmic activity in petroleum-ether sensitized and in coronary artery ligated dogs.

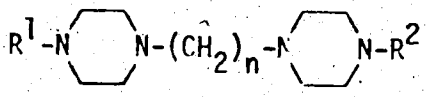
Roth (1954) also studied the pharmacological properties of a series of compounds of general structure (33), in this case where $R_1$ was a hydrogen atom or a phenyl, 2-pyridyl, benzyl, benzhydryl, p-bromobenzyl, 9-phenanthrylmethyl, p-chlorobenzhydryl or a p,p'-dichlorobenzhydryl group and $R^2$ was a hydrogen atom or a methyl, ethyl or n-propyl group. The author observed that 1-phenyl-4-methylpiperazine (33b) had the most potent adrenergic action, although the properties of the congenors did not reveal any consistent or definitive structure-activity relationships.

Parke, Davis and Company (1958) obtained a British patent for compounds with general structure (34), where R was a methyl, ethyl, n-propyl, n-butyl or allyl group; Z was a hydrogen atom or an acetyl group and n was 3,4,5 or 6. These compounds were claimed to be useful in the treatment of hypertension and anxiety states as well as in the prevention of nausea and vomiting.



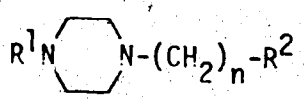
(34)

Bach et al. (1958) synthesized several bis-piperazine derivatives of general structure (35), in which $R^1$ was a 2-pyridyl or



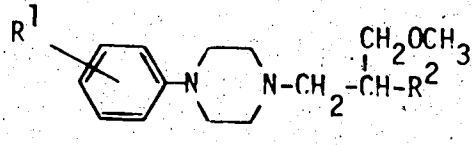
(35)

2-pyrimidyl group; $R^2$ was a carbethoxy, 2-pyridyl, 2-pyrimidyl or a 6-chloropyridazyl group and n was 2, 3, 6 or 10. In this patent, the authors claimed the above derivatives were effective hypotensive agents and vasodilators. The following year Bach et al. (1959) obtained a patent for chemically related hypotensive agents of general structure (36), where $R^1$ was a benzyl, phenyl, 2-pyridyl or carbethoxy group; $R^2$ was a 1-(1,2,3,4-tetrahydroquinoline) or 1-indoline group and n=2, 4 or 10.



(36)

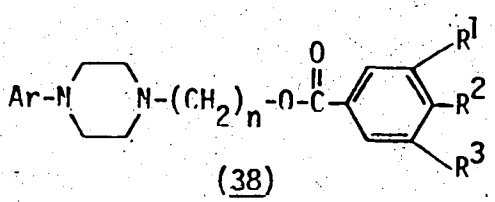
In the same year, Howell et al. (1963) prepared several piperazine analogs possessing the following general structure (37), where $R^1$ was a hydrogen atom or a 2-methoxy, 2-ethoxy, 4-ethoxy, 2-n-propylthio or a 2-ethylthio group and $R^2$ was a straight chained ester



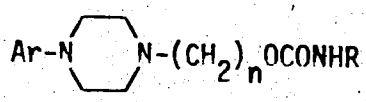
(37)

group (methyl to hexyl inclusive) or a methoxy, ethoxy, n-propoxy, n-butoxy, t-butoxy or benzyloxy group. The authors found several of these derivatives exhibited a moderate hypotensive activity at a dosage of 2 - 8 mg/kg in anesthetized rats. Their preliminary pharmacological testing indicated a slight to moderate adrenergic blockade for the active compounds.

Hayao et al. (1963) prepared several piperazine derivatives of general structure (38) and (39), where in the former Ar was a phenyl, 4-chlorophenyl or 4-methoxyphenyl group; $R^1$, $R^2$ and $R^3$ were a methoxy group or a hydrogen atom and n was 2, 3, 4 or 5 while in the latter, Ar was a phenyl or a 4-methylphenyl group; R was a 4-methoxyphenyl, 3,4-dimethoxyphenyl or 3,4,5-trimethoxyphenyl group and n was 2, 3, 4 or 5. Upon intravenous administration to pentobarbitalized dogs, derivatives of both general structures were found to produce sustained hypotension at least partially by α-adrenergic blockade. However, the authors noted that in normal unanesthetized dogs, the hypotensive effect of orally administered drug was uncertain and variable.

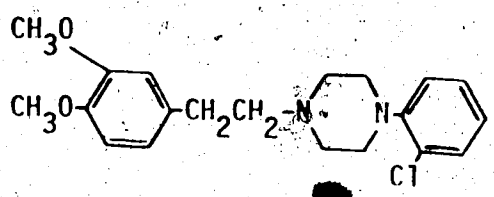


(38)



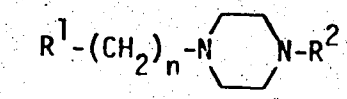
(39)

Boissier et al. (1961) reported that 1-(3,4-dimethoxyphenethyl)-4-(2-chlorophenyl)piperazine (40) possessed adrenolytic and central



(40)

sedative actions, and as a result of this observation, Boissier and co-workers (1963a; 1963b; 1963c; 1963d; 1963e; 1963f; 1963g) and Ratouis et al. (1965a; 1965b) synthesized and pharmacologically evaluated an extremely large number of related compounds of general formula (41), in which $R^1$ and $R^2$ were most often phenyl or substituted phenyl groups

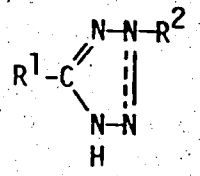


(41)

or heterocyclic ring systems and n was 1,2,3,4,6 or 10. Many of the compounds studied contained halogen or alkoxy substituents; other groups, such as acetyl, methyl and amino, were also incorporated. It proved difficult to rationalize pharmacological activity with substitution pattern. Some compounds, for example, were potent hypotensive agents with CNS depressant activity, while others of similar chemical structure were more active as antagonists of the α-adrenergic properties of epinephrine

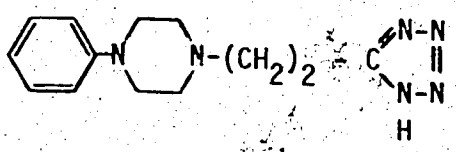
both in vivo in anesthetized dogs and in vitro on isolated guinea pig seminal vesicle. Others possessed weak histaminolytic properties.

A series of compounds was prepared by Rodriguez et al. (1965) with the following general structure (42), where $R^1$ and $R^2$ were usually



(42)

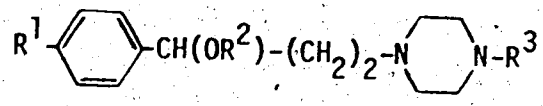
various aromatic groups and N-(substituted-aromatic)piperazine functions. The authors found these compounds to be potent α-adrenergic blocking agents with greater potency in vivo in anesthetized normotensive rats and dogs than in vitro on rabbit aortic strips. One of the more potent members, namely 4-phenyl-1-[2-(5-tetrazolyl)ethyl]piperazine (43), given



(43)

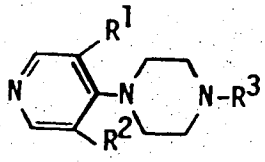
intravenously was shown to lower the blood pressure in the unanesthetized renal hypertensive dog and in the unanesthetized mecamylamine hypertensive dog. The compound was found to have a much longer duration of action than azapetine or phentolamine. Also, unlike phenoxybenzamine, it had an immediate onset and a competitive mechanism of action.

In two Swiss patents to De Stevens and Mull (1967a; 1967b), compounds of general structure (44), in which $R^1$ was a hydrogen atom or methyl group, $R^2$ was a methyl or ethyl group and $R^3$ was 2-chlorophenyl, 2-methylphenyl or a 2-methoxyphenyl group were claimed to possess adrenolytic, dilatatory, anti-hypertensive and diuretic properties.



(44)

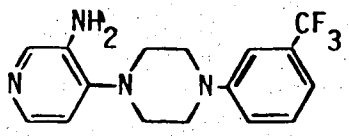
Several compounds which possessed marked anti-convulsant and anti-reserpine properties as well as hypotensive activity were synthesized by Jain et al. (1967). These derivatives had general structure (45), where $R^1$ was a nitro or amino group or a hydrogen atom; $R^2$ was a nitro or amino group or a bromine or a hydrogen atom; and $R^3$ was a methyl, benzyl, phenyl group or various monoalkoxy, polyalkoxy, chloro, alkyl, trifluoromethyl, fluoro, nitro or amino-substituted phenyl derivatives.



(45)

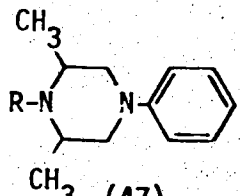
Ahmed (1967) found that 4-(m-trifluoromethylphenyl)-1-(3'-amino-4'-pyridyl)piperazine (46) caused a transient fall in the blood pressure of anesthetized cats, but did not alter blood pressure

responses to acetylcholine, adrenaline and histamine. It was also shown that this compound had no effect upon the cat sympathetic ganglion.



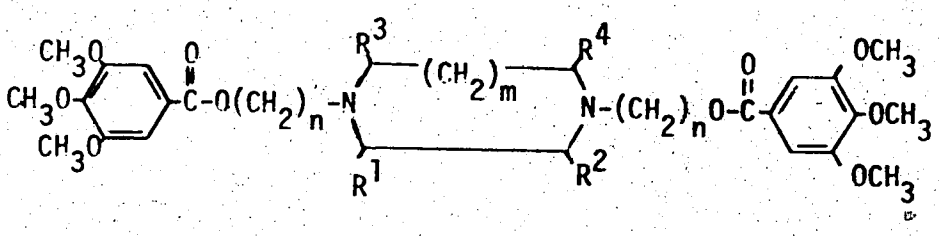
(46)

Lepetit (1967) prepared hypotensive piperazine derivatives with the following general structure (47), where R was a methyl, 4-pyridylethyl or a 3,4-dimethoxyphenylethyl group or a hydrogen atom.



(47)

Powerful coronary vasodilators with general structure (48) were reported by Arnold et al. (1968). In the compounds described, $R^1$, $R^2$, $R^3$ and $R^4$ were methyl groups or hydrogen atoms in various combinations, while n was 3 or 2 and m was 0 or 1.



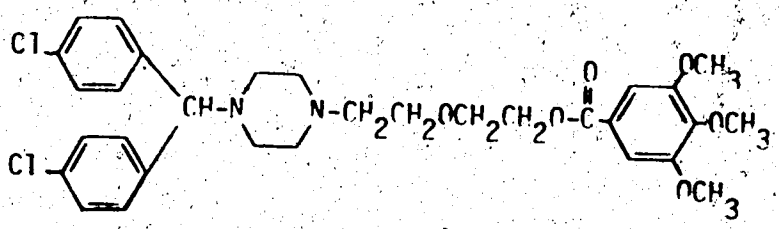
(48)

Coscia et al. (1968) synthesized several compounds of general structure (49), where $R^1$ was a 2,6-dimethylphenyl, 2,6-diisopropylphenyl, 2,6-dimethoxyphenyl, 2-methyl-6-chlorophenyl, or a 2,4,6-trimethylphenyl group; $R^2$ was a methyl group or a hydrogen atom and $R^3$ was a 4-methyl-1-piperazinyl group or a 1-piperidinyl group. Although the authors found several derivatives were more active than lidocaine as local anesthetics, all of the compounds caused a transitory hypotension in anesthetized cats.

$$R^1O_2CCH(R^2)R^3$$

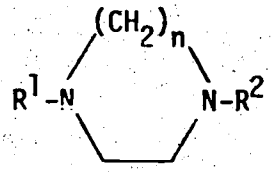
(49)

Hypotensive cardiovascular activity in cats and rabbits was found by De Marchi and Torrielli (1968) for 1-(p-chlorobenzhydryl)-4-[2-[2-(3,4,5-trimethoxybenzoyloxy)ethoxy]ethyl]piperazine (50).



(50)

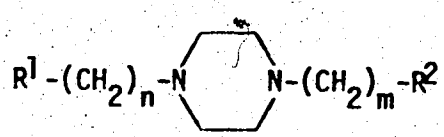
Prasad et al. (1968; 1969) prepared a series of unsymmetrically 1,4-disubstituted piperazines and other diazines with general structure (51), in which $R^1$ was usually a phenyl, methyl or o-methoxyphenyl group; $R^2$ was a hydrogen atom or variously substituted aromatic amines, ketones



(51)

thioketones or alkanes and n was 2 or 3. The authors found that only the 4-substituted derivatives of 1-phenyl- or 1-o-methoxyphenylpiperazine exhibited an appreciable sustained fall in blood pressure in anesthetized cats. None of the 1-methyl-4-substituted piperazines showed any significant hypotensive activity. In addition, they pointed out that no consistent difference in hypotensive activity existed between the amides and the amines.

Nikolova et al. (1969) prepared compounds of general structure (52) where $R^1$ was a substituted oxy-aromatic group; $R^2$ was a different substituted oxy-aromatic group; m was 1, 2 or 3 and n was also 1, 2 or 3. Tested at a dosage level of 1 - 100 mg/kg, the authors found all compounds had hypotensive effects lasting 5 - 15 minutes in anesthetized cats.



(52)

Several esters of general structure (53) were synthesized by Lespagnol et al. (1969), where $R^1$ was 1-(1,1-dimethyl)butyl, 1-(1-methyl-1-ethyl)pentyl or a tert-butyl group and $R^2$ was a diethylamino, morpholino, 1-pyrrolidinyl, 4-methyl-1-piperazinyl or piperidino group. In addition, the methyl iodide or butyl bromide quaternary derivatives of these amino esters were prepared. The authors observed that these esters were especially resistant to hydrolysis, exhibited antispasmodic activity on guinea pig ileum and possessed hypotensive effects in rabbits.

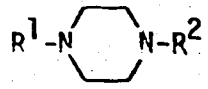
$$R^1CO_2(CH_2)_2R^2$$

(53)

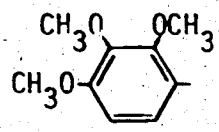
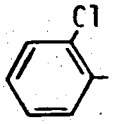
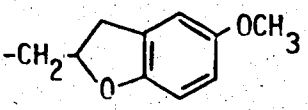
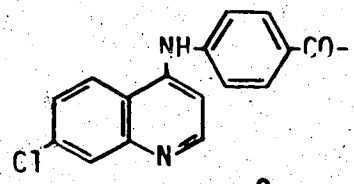
Nagata et al. (1969) demonstrated the anti-nicotinic action of 1-(2,3,4-trimethoxybenzyl)piperazine (54a) on guinea pig atria. A French patent to Ciba Limited (1970) claimed that 2-[1-(2-chlorophenyl)piperazinyl)methyl]-5-methoxy-2,3-dihydrobenzofuran (54b) was an androgenic blocking agent as well as a hypotensive agent.

Cambar and Aviado (1970) demonstrated that the hypotension caused by 1-methyl-4-[4-(7-chloroquinol-4-yl)benzoyl]piperazine (54c) in mice, rats and dogs was due to blockade of both α- and β-adrenergic receptors.

Lanyi et al. (1970) reported 1-carbethoxy-4-thiocarbamoylpiperazine (54d) exhibited hypotensive and hypothermic effects with little CNS activity.



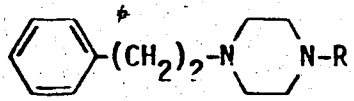
(54)

| | $R^1$ | $R^2$ |
|---|---|---|
| (a) |  | H- |
| (b) |  |  |
| (c) |  | $CH_3$- |
| (d) | $CH_3CH_2OC$- | -C-SH |

In their extensive review of the structure activity relationships of adrenergic-blocking compounds, Ghouri et al. (1969) concluded that the ring structure of piperazine was not essential for α-adrenergic blocking activity, but increased the activity over that found in open chain compounds. They also felt that the adrenolytic, but not the hypotensive property of piperazine derivatives was greatly susceptible

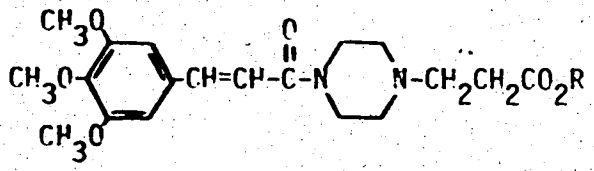
to structural changes.

In a German patent, Fauran et al. (1970) prepared many 1,4-disubstituted piperazines of general structure (55), where R was $-CH_2CO_2CH_2CH_3$, $-CH_2CN$, $-CO_2CH_2CH_3$, $-CH_2CCH_3(OH)$p-$CH_3OC_6H_4$ or $-CH_2CCH_3(OH)C_6H_4$. The authors claimed that the two hydroxy derivatives



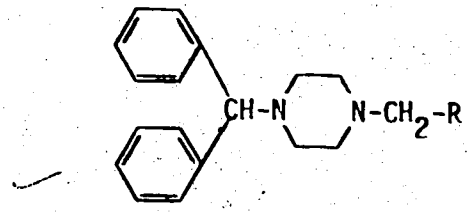
(55)

had hypotensive and vasodilatory effects in dogs, cats and rabbits as well as adrenolytic, spasmolytic and diuretic effects on rats. Subsequently, Fauran et al. (1971) obtained a patent on compounds with structure (56), where R was a methyl, ethyl, propyl, isopropyl, or butyl group. The authors described these compounds as useful hypotensive and β-adrenergic receptor inhibiting agents as well as coronary and vasodilators.



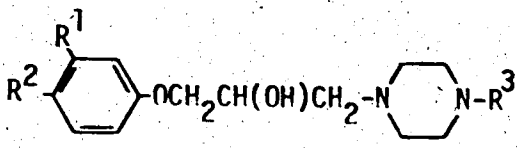
(56)

Turin et al. (1971) claimed compounds with general structure (57) were useful as antihistaminics, diuretics, hypotensives and analgesics if R was $-CONHCH(CH_3)_2$, $-CO_2CH_3$, $-CONH_2$, $-C(=O)-N$ (pyrrolidine), $-CONH$-3,4,5-$(CH_3O)_3C_6H_2$.



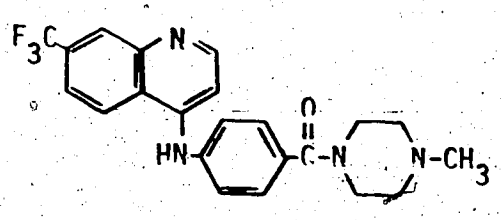
(57)

In their German patent Damilwicz and Kemp (1971) prepared compounds of general structure (58) which they stated were anti-hypertensives, when $R^2$ was a hydrogen atom or a nitro, carboxyamino or acetoxyamino group; $R^1$ was a hydrogen atom or an acetoxyamino group and $R^3$ was a phenyl, o-methoxyphenyl or 2,5-methoxymethylphenyl.



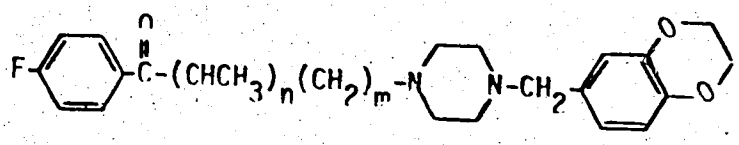
(58)

Coverdale (1971) reported that p-(7-trifluoromethyl-4-quinolylamino)-N,N-(3-methyl-3-azapentamethylene)benzamide (59) had hypotensive activity.

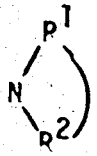


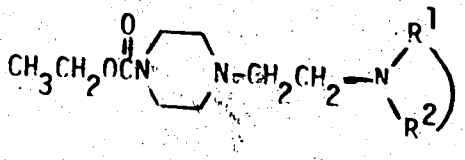
(59)

Beregi et al. (1971) found compounds with the following general structure (60), where n was 2 and m was 3 or n and m were both 1 had neuroleptic, vasodilative adrenolytic and anti-emetic properties.



(60)

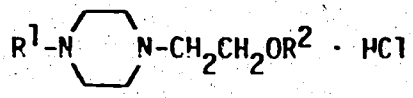
The hypotensive properties of a series of unsymmetrically substituted piperazines with general structure (61) was examined by Das et al. (1971), where  was a 1-piperidino, 1-morpholino, 1-pyrrolidino, N-1,2,3,4-tetrahydroisoquinolino, 4-phenylpiperazino, 4-benzyl-1-piperazino, diisopropylamino, 4-p-chlorophenyl-1-piperazino or a 4-m-chlorophenyl-1-piperazino group. The authors found the N-1,2,3,4-tetrahydroisoquinolino derivative exhibited maximal hypotensive activity in pentobarbital anesthetized cats.



(61)

Rushig et al. (1971) synthesized compounds of general structure (62), where $R^1$ was a phenyl, 2-pyridyl, o, m or p-methylphenyl group and $R^2$ was a 4,6-dimethyl-2-pyrimidinyl, 2-methyl-3-pyrazinyl, 2-quinolyl

2-benzothiazolyl or 2-methyl-3-quinoxalinyl group. Rushig et al. (1971) found these derivatives had α-sympatholytic, hypotensive and sedative effects.



(62)

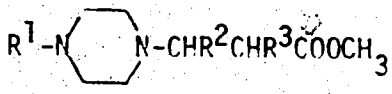
The occurrence of a quinoxaline moiety within an active hypotensive piperazine derivative in the above paper by Rushig et al. (1971) prompted an interest in the possible hypotensive properties of various quinoxaline derivatives alone, since quinoxaline could be viewed as a fused benzene plus piperazine ring-system.

No investigations of the blood pressure effects of quinoxaline derivatives were found upon searching the literature. However, the antibacterial properties of various quinoxaline derivatives were well documented (Padeiskaya et al. (1967); Nast et al. (1970); Galt and Pearce, (1971); Duerckheimer et al. (1971); Schweizer and Egli, (1971); and Seng et al. (1971)).

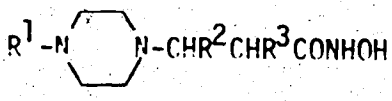
## IV. Purpose of the Present Study

Many N-substituted piperazines have been shown to possess hypotensive activity. The presence of a hydroxamate group, and to a lesser extent, an ester grouping in certain piperidine derivatives also confers hypotensive properties on these compounds. The purpose of the present study was to synthesize and screen for their hypotensive activity a series of N-substituted piperazinylpropionates (63),

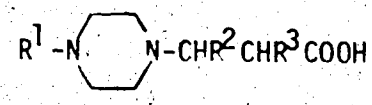
hydroxamates (64) and carboxylic acids (65) compounds which would combine both the structural features just described. In the envisaged compounds, $R^1$ was to be a hydrogen atom, methyl, ethyl, n-propyl, n-butyl, phenyl, benzyl or phenethyl group and $R^2$ and $R^3$ to be hydrogen atoms or methyl groups. In addition, a number of quinoxaline cyclic hydroxamic acids (66) in which R was a hydrogen atom, methyl or phenyl group were considered worth synthesizing for pharmacological evaluation.



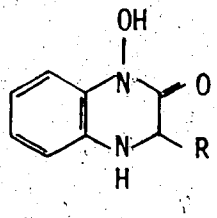
(63)



(64)



(65)



(66)

Also during the course of this investigation, the preparation and initial screening of a number of N-substituted piperazinyl butyrates, acetates and formates, as well as a number of 4-phenylpiperidino, 4-benzylpiperidino and 4-phenyl-1,2,5,6-tetrahydropyridino derivatives were successfully undertaken. These compounds were prepared to examine the effects of certain structural modifications upon the hypotensive activity of the parent structures (63) and (64).

All of the compounds synthesized in this study were evaluated at dosages of 1, 5 or 25 mg/Kg for their effect on the carotid arterial blood pressure of urethane anesthetized rats. At each dosage tested, every compound was evaluated at least twice - once in each of two different rats. The hydroxamates, esters and carboxylic acids were administered in 0.1 ml isotonic saline solutions via a cannula in the femoral vein, whereas the quinoxalines were given in 0.1 ml N/10 NaOH solutions by the same route.

# CHEMISTRY

# I. DISCUSSION

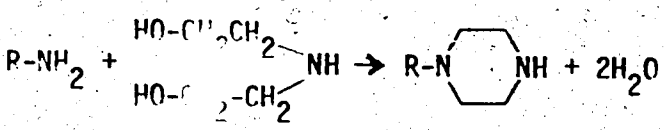
The basic synthetic plan of the present investigation was to prepare the desired 4-substituted piperazin-1-yl esters by reaction of the appropriate 1-monosubstituted piperazine with either methyl acrylate, methyl crotonate, methyl methacrylate, ethyl chloroformate, ethyl bromoacetate or ethyl 4-bromobutyrate. Subsequently, hydroxamic acids would be prepared by reaction of each ester with hydroxylamine.

The approach to the quinoxaline derivatives was to synthesize first the corresponding N-oxide by reacting o-quinone dioxime with an appropriate α-ketoaldehyde. The N-oxide would then be catalytically reduced to the desired quinoxaline analog.

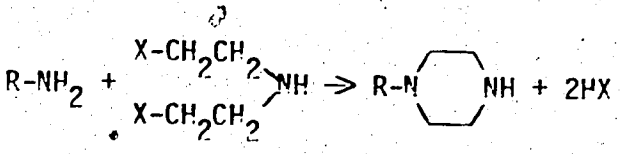
## Preparation of 1-Monosubstituted Piperazines

Five methods are commonly employed to obtain 1-monosubstituted piperazines.

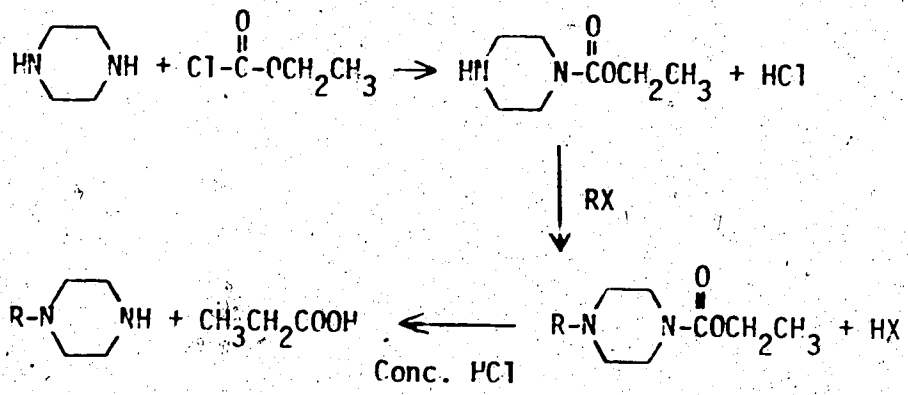
1) Condensation of primary amines with diethanolamine (Prelog et al. 1935; Pollard et al. 1934).



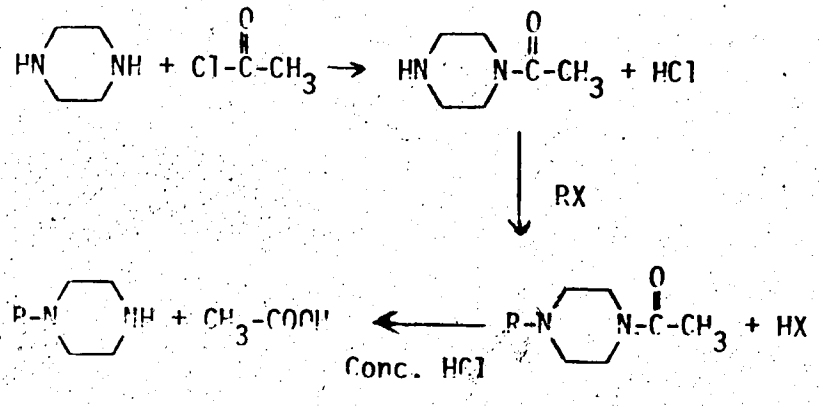
2) Condensation of primary amines with bis-(β-chloro or bromoethyl)-amine (Prelog et al. 1933; 1934).



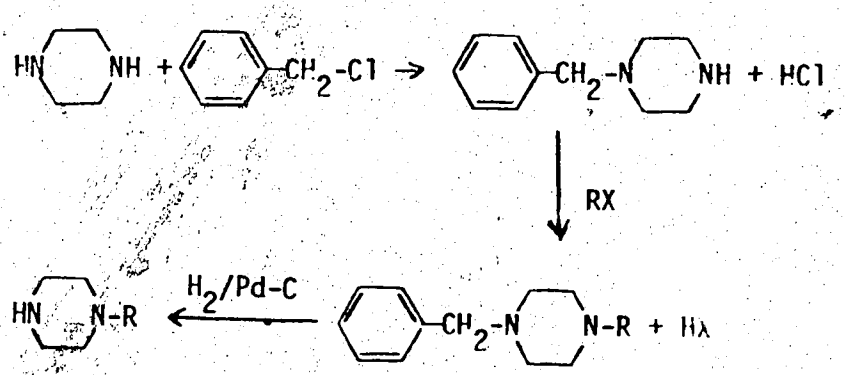
3)a) Reaction of piperazine with ethyl chloroformate to give a pure monosubstituted derivative followed by introduction of the desired substituent, then hydrolysis to remove the formate blocking group (Moore et al. 1929; Fourneau and Barrelet, 1929; Hamlin et al. 1949; Stewart et al. 1948).



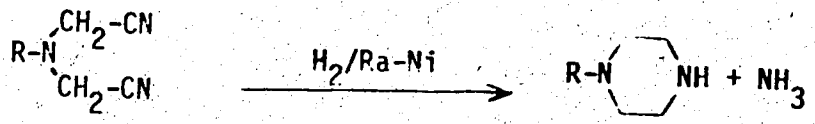
3)b) Reaction of piperazine with acetyl chloride to give the pure monosubstituted derivative followed by introduction of the desired substituent, then hydrolysis to remove the acetyl blocking group (Jacobi, 1933).



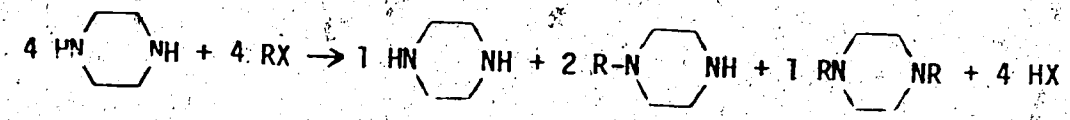
3)c) Reaction of piperazine with benzyl chloride to give the pure monosubstituted derivative followed by introduction of the desired substituent, and catalytic hydrogenation to remove the benzyl blocking group (Baltzly et al. 1944; U.S. patent, 1947; Morren et al. 1951).



4) Reduction of bis-(cyanomethyl)amine or its N-substituted derivative over W-2 Raney nickel (Mosher et al. 1953).



5) Reaction of piperazine with alkyl halides (Baltzly et al. 1944; Yung et al. 1968).

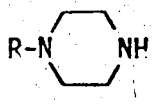


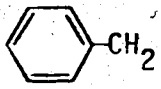
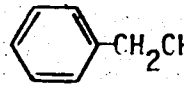
The last method, that of Yung et al. (1968) was chosen to prepare all the 1-monosubstituted piperazines required. This choice was based upon the ease of product purification by washing with 3N NaOH to remove most of the excess piperazine followed by fractional distillation under reduced pressure to give the pure 1-monosubstituted piperazine. Furthermore, this procedure had the great advantage of entailing only one synthetic reaction and purification step, thus requiring less time and effort than some of the other routes. In addition, the inexpensiveness and availability of sufficient quantities of piperazine and the various alkyl halides allowed preparative quantities of product to be synthesized. Utilizing the method of Yung et al. (1968), the following 1-monosubstituted piperazines were prepared (Table I).

All the 1-monosubstituted piperazines had an ammoniacal odor and were colorless or pale yellow mobile oils, except for the 1-benzyl- and 1-phenethyl-piperazine derivatives which crystallized to waxy white solids immediately after distillation. Their IR spectra all showed a single absorption band due to the stretching of the N-H bond at 3270 $cm^{-1}$. By comparison, the IR spectrum of

TABLE I

1-Monosubstituted Piperazines



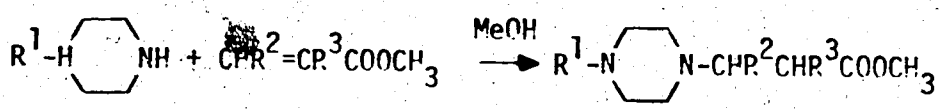
| Compound | R | % Yield |
|---|---|---|
| 67 | $CH_3CH_2$ | 56 |
| 68 | $CH_3CH_2CH_2$ | 43 |
| 69 | $CH_3CH_2CH_2CH_2$ | 61 |
| 70 |  | 57 |
| 71 |  | 75 |

piperazine showed N-H stretching as two much broader peaks at 3220 $cm^{-1}$ and 3360 $cm^{-1}$. Other than comparing the boiling points of these derivatives with those reported in the literature, no further characterization of the 1-monosubstituted piperazines was undertaken.

Preparation of Amino-esters

Most of the esters required for the present study were prepared by one of two general methods.

1) Michael addition of the appropriate amine to methyl acrylate, methyl crotonate, methyl methacrylate or ethyl acrylate.



Howton (1945) concluded that the esters of acrylic acid exhibited a greater tendency to combine with primary and secondary bases than do the corresponding esters of methacrylic acid. This was also shown in the present study, where condensation of the amines generally occurred much more readily with methyl or ethyl acrylate than with methyl crotonate or methyl methacrylate. Howton was able to condense aniline with methyl acrylate but not with methacrylate. Similarly, di-n-butylamine added to ethyl acrylate but not to ethyl methacrylate. Friedman and Wall (1966) made a more extensive study of the effect of introduction of methyl substituents near the reactive site of vinyl compounds and the subsequent reduction in their rate of reactivity with amino compounds. They concluded that this reduction might be due mainly to steric factors associated with the methyl groups. The lower reaction rate of methyl crotonate as compared to methyl acrylate must be due to the additional steric requirements of the methyl group as well as to its inductive effect which results in an altered electron density at the reaction site. It was suggested by these workers that the difference in rates of reactivity may also be due to a difference in the stability of the carbanion involved in the transition state of the reaction (Table II).

TABLE II

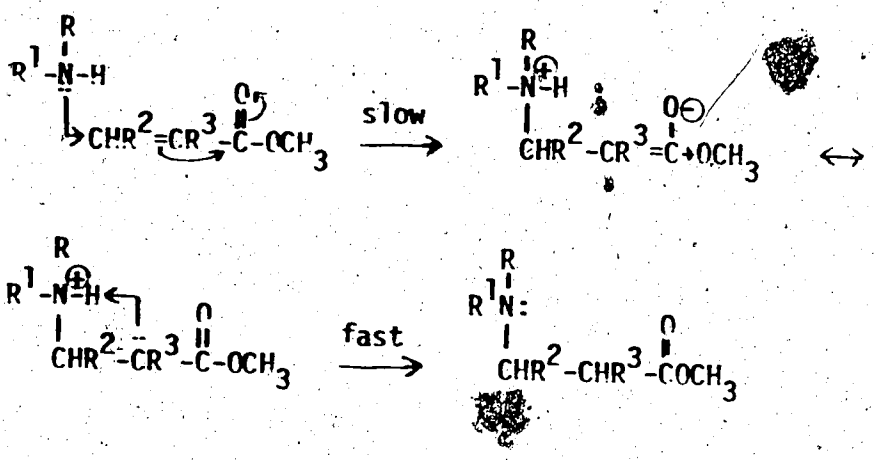
Transition State Involved in the Reaction of Acrylates with Secondary Amines

| Acrylate | Transition State | Type of Carbanion |
|---|---|---|
| Methyl acrylate | $RR^1\overset{\oplus}{N}H-CH_2-\overset{\ominus}{C}H-COOCH_3$ | Secondary |
| Methyl methacrylate | $RR^1\overset{\oplus}{N}H-CH_2-\overset{\ominus}{C}(CH_3)-COOCH_3$ | Tertiary |
| Methyl crotonate | $RR^1\overset{\oplus}{N}H-CH(CH_3)-\overset{\ominus}{C}H-COOCH_3$ | Secondary |

The transition state with methyl acrylate and methyl crotonate is a secondary carbanion whereas methyl methacrylate gives an intermediate tertiary carbanion which is less stable. The slower rate of reaction of methyl methacrylate with the amine is due primarily to electronic effects. The steric factors of methyl methacrylate appear less important than those of methyl crotonate. The authors also showed that the polar and steric factors associated with the vinyl and amino compounds make independent contributions to observed reactivities of the acrylates.

Given below is a mechanism for this nucleophilic addition suggested by Pfau (1967). The author indicated that the steric requirements of the amine are important in this reaction, since he was able to condense n-propylamine with ethyl acrylate, but the reaction with

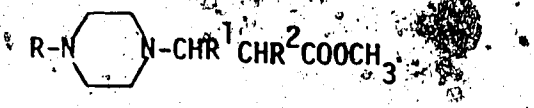
isopropylamine failed to proceed.



This difference between condensation with methyl methacrylate and methyl crotonate was not large in our experiments and seemed to slightly favor methyl crotonate rather than the methacrylate. This could be due to the fact that the amino nucleophile may have been large enough to experience approximately equal steric interaction in approaching and reacting with methyl methacrylate as with methyl crotonate.

The observation of very little variation in yield throughout the amino series reacting with methyl methacrylate or methyl crotonate was likely due to the fact that the changes in amino structure were occurring at a point quite remote from the nucleophilic portion of the amine. Thus the inductive, steric and mesomeric interactions of the nucleophile were largely unchanged throughout the series of amines.

The following esters with general formulas (72) and (73) were prepared in good yield by condensation of the appropriate amine with methyl acrylate, methyl crotonate, methyl methacrylate or ethyl acrylate (Table III, IV and V).



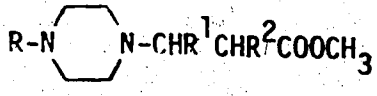
(72)

(73)

Methanol was used as the solvent for reactions involving methyl ester preparation, while ethanol was used as the solvent for reactions involving ethyl ester synthesis to prevent the possibility of transesterification of the respective esters from occurring. In order to minimize amide formation, the reaction mixtures were initially stirred at room temperature then heated under reflux for a minimum length of time. Bieber (1954) and Pfan (1967) showed that an increase in temperature increased the amount of amide formation and Coutts (1971) showed that prolonging the reaction time also increased amide formation.

TABLE III

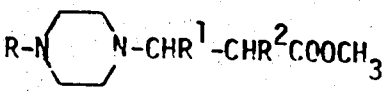
Methyl 3-[1-(4-Substituted piperazinyl)]propionates



| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 74 | H | H | $CH_3$ | 45 |

TABLE III cont'd

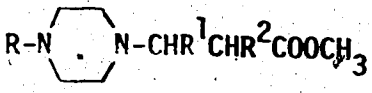
Methyl 3-[1-(4-Substituted-piperazinyl)]propionates



| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 75 | $CH_3$ | H | $CH_3$ | 30 |
| 76 | $CH_3CH_2$ | H | $CH_3$ | 32 |
| 77 | $CH_3CH_2CH_2$ | H | $CH_3$ | 51 |
| 78 | $CH_3CH_2CH_2CH_2$ | H | $CH_3$ | 46 |
| 79 | Ph | H | $CH_3$ | 51 |
| 80 | $PhCH_2$ | H | $CH_3$ | 47 |
| 81 | $PhCH_2CH_2$ | H | $CH_3$ | 57 |
| 82 | H | $CH_3$ | H | 41 |
| 83 | $CH_3$ | $CH_3$ | H | 40 |
| 84 | $CH_3CH_2$ | $CH_3$ | H | 57 |
| 85 | $CH_3CH_2CH_2$ | $CH_3$ | H | 46 |
| 86 | $CH_3CH_2CH_2CH_2$ | $CH_3$ | H | 63 |
| 87 | Ph | $CH_3$ | H | 59 |
| 88 | $PhCH_2$ | $CH_3$ | H | 72 |
| 89 | $PhCH_2CH_2$ | $CH_3$ | H | 63 |

TABLE III cont'd

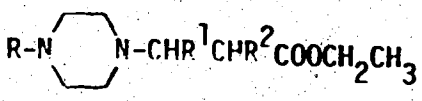
Methyl 3-[1-(4-Substituted-piperazinyl)]propionates



| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 90 | Ph | H | H | 92 |
| 91 | $PhCH_2$ | H | H | 78 |
| 92 | $PhCH_2CH_2$ | H | H | 97 |

TABLE IV

Ethyl 3-[1-(4-Substituted-piperazinyl)]propionates

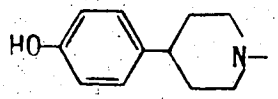
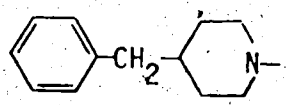
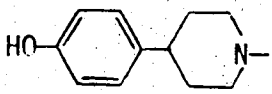
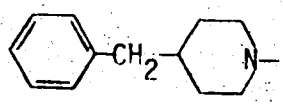
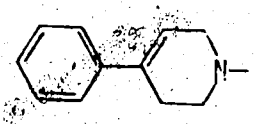


| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 93 | Ph | H | H | 73 |
| 94 | $PhCH_2$ | H | H | 59 |
| 95 | $PhCH_2CH_2$ | H | H | 80 |

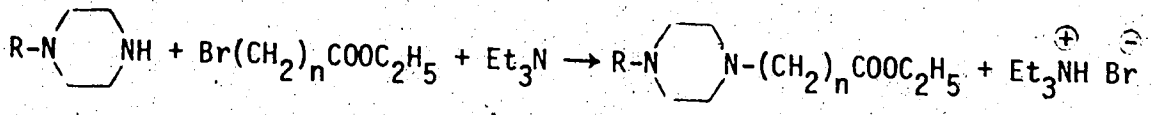
TABLE V

Methyl 3-Aminopropionates

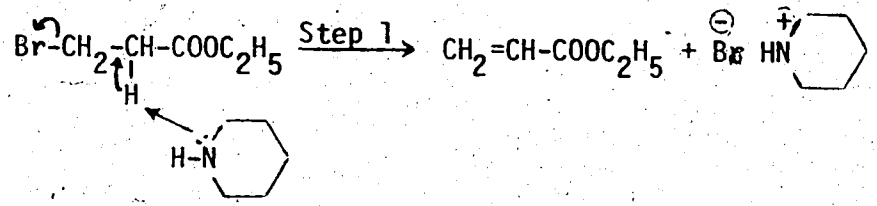
$RCHR^1CHR^2COOCH_3$

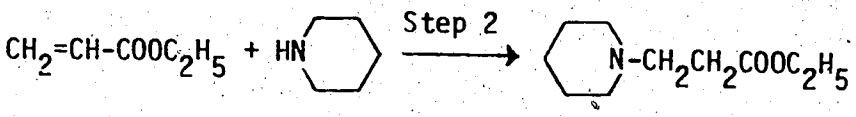
| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 96 |  | H | $CH_3$ | 68 |
| 97 |  | H | $CH_3$ | 91 |
| 98 |  | $CH_3$ | H | 83 |
| 99 |  | $CH_3$ | H | 51 |
| 100 |  | $CH_3$ | H | 47 |

2) Condensation of the appropriate amine with ethyl chloroformate, ethyl bromoacetate or ethyl 4-bromobutyrate in the presence of triethylamine.



In 1934, Drake and McElvain proposed that the reaction of organic amines with bromoesters occurred in two steps. These workers studied the reaction of piperidine with 3-bromoesters. They envisaged the first step as removal of a proton from the α-carbon of the ester by




the unshared electrons of the piperidine nitrogen followed by release of the bromide ion. This mechanism explains the formation of one mole of amine hydrobromide per mole of ester. The yield was found dependent upon the basic strength of the amine used and upon steric factors. Although diethylamine, pyrrolidine and piperidine have essentially the same basicity, the pyrrolidine derivative was obtained in greater yield due to its lesser steric requirements. The 1-monosubstituted piperazines whose preparation was previously discussed, were obtained in relatively limited quantities. Consequently, it was judged wasteful to discard one mole of 1-monosubstituted piperazine hydrobromide for every mole of ester produced. To overcome this disadvantage, an equimolar quantity of triethylamine was added to the reaction mixture to remove by-product hydrogen bromide in the form of triethylamine hydrobromide. A comparison of the pKa values of triethylamine and 1-methylpiperazine, a typical 1-monosubstituted piperazine, reveals that triethylamine is about 100 times as strong a

base. This means that the hydrogen bromide liberated in step 1 of the

| Compound | | pKa | Source |
|---|---|---|---|
| Triethylamine | | 11.01 | (Handbook of Chemistry and Physics, (1970) |
| 1-Methylpiperazine | First | 8.9 | (Erb-Debruyne, 1964) |
| | Second | 4.5 | (Erb-Debruyne, 1964) |

Drake and McElvain mechanism would predominately react with triethylamine giving triethylamine hydrobromide. This reaction would occur independently of which amine removed the α-carbon proton to initiate step 1 due to the occurrence of the following equilibria:

$$(CH_3CH_2)_3\overset{+}{N}H\ Br^- \rightleftharpoons (CH_3CH_2)_2N + HBr \qquad K = 1.2 \times 10^{-12}$$

$$CH_3\text{-}\overset{+}{N}H_2\ Br^- \rightleftharpoons CH_3\text{-}N\text{(ring)}NH + HBr \qquad K = 1.26 \times 10^{-9}$$

Any competition in step 2 between triethylamine (a 3° amine) and 1-monosubstituted piperazines (secondary amines) would greatly favor the much less sterically hindered 1-monosubstituted piperazines.

Using the Drake and McElvain procedure without the addition of triethylamine would allow a maximal yield of only 50% product based upon the amount of 1-monosubstituted piperazine used. With the addition of triethylamine, aminoesters were obtained in yields ranging from 46% to 98% based upon the amount 1-monosubstituted piperazine consumed (Table VI). Of the twelve esters prepared utilizing the addition of triethylamine, only one was obtained in slightly less than

50% yield and all others were prepared in greater than 50% yield (Table VI).

Analogously, Sonntag (1954) in an extensive review of the reactions of aliphatic acid chlorides, tabulated numerous examples where the bases pyridine, sodium hydroxide (Schotten - Baumann procedure) or sodium carbonate were used to remove or neutralize hydrogen chloride as it was formed in the reaction between the selected amine and acid chloride.

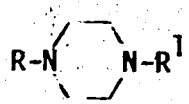
Guiliano and Stein (1956) used triethylamine to remove by-product hydrogen chloride as insoluble triethylamine hydrochloride in their preparation of 2-diethylaminoethyl carboxylic esters from 1-chloro-2-diethylaminoethane and the appropriate carboxylic acid.

In their examination of the partial reduction of tertiary amides by lithium aluminum hydride as a possible synthetic route to aldehydes, Brown and Tsukamoto (1961) found 1-acylaziridines exhibited unusually favorable characteristics for this synthesis producing the corresponding aldehydes in excellent yields. The authors synthesized the precursor 1-acylaziridines by adding the appropriate acid chloride to a stirred benzene solution of ethyl oximine and triethylamine cooled by an ice bath. The precipitated triethylamine hydrochloride was filtered off, then the 1-acylaziridine isolated from the filtrate.

Tabulated in Table VI are those esters prepared by reacting the appropriate amine with ethyl chloroformate, ethyl bromoactate or ethyl 4-bromobutyrate in the presence of triethylamine.

TABLE VI

1,4-Disubstituted Piperazines



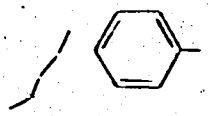
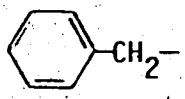
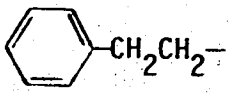
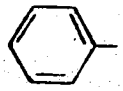
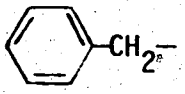
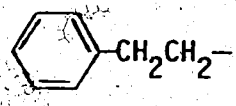
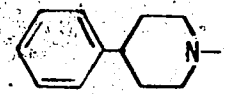
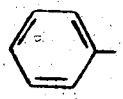
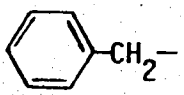
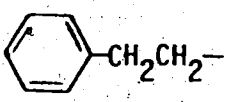
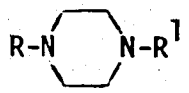
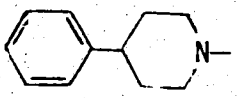
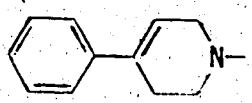
| Compound | R | $R^1$ | % Yield |
|---|---|---|---|
| 101 |  | $-COOC_2H_5$ | 57 |
| 102 |  | $-COOC_2H_5$ | 69 |
| 103 |  | $-COOC_2H_5$ | 64 |
| 104 |  | $-CH_2COOC_2H_5$ | 64 |
| 105 |  | $-CH_2COOC_2H_5$ | 98 |
| 106 |  | $-CH_2COOC_2H_5$ | 91 |
| 107 |  | $-CH_2COOC_2H_5$ | 80 |
| 108 |  | $-CH_2CH_2CH_2COOC_2H_5$ | 68 |
| 109 |  | $-CH_2CH_2CH_2COOC_2H_5$ | 90 |
| 110 |  | $-CH_2CH_2CH_2COOC_2H_5$ | 84 |

TABLE VI

1,4-Disubstituted Piperazines



| Compound | R | $R^1$ | % Yield |
|---|---|---|---|
| 111 |  | $-CH_2CH_2CH_2COOC_2H_5$ | 67 |
| 112 |  | $-CH_2CH_2CH_2COOC_2H_5$ | 46 |

The hydrochlorides of all amino esters were prepared by reacting the free amino bases with a methanolic solution of dry hydrogen chloride for methyl esters, or an ethanolic solution of dry hydrogen chloride for ethyl esters. All the salts were obtained in quantitative yield as colorless powders and were stable over an extended period of time.

The structures of all the aminoester derivatives were verified by microanalysis, IR and NMR spectra. Each compound had a band in the 1710-1750 $cm^{-1}$ region of its IR spectrum due to the ester (C=O). All NMR spectra were consistent with the proposed structure of that particular compound. The NMR spectrum in $D_2O$ of methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate dihydrochloride (75) (Figure 1a) represents a typical recording obtained for these derivatives. The signal occuring at $\delta$ 4.73 was assumed to represent $H_2O$ and was ignored. The three proton doublet at $\delta$ 1.28 was assigned to the

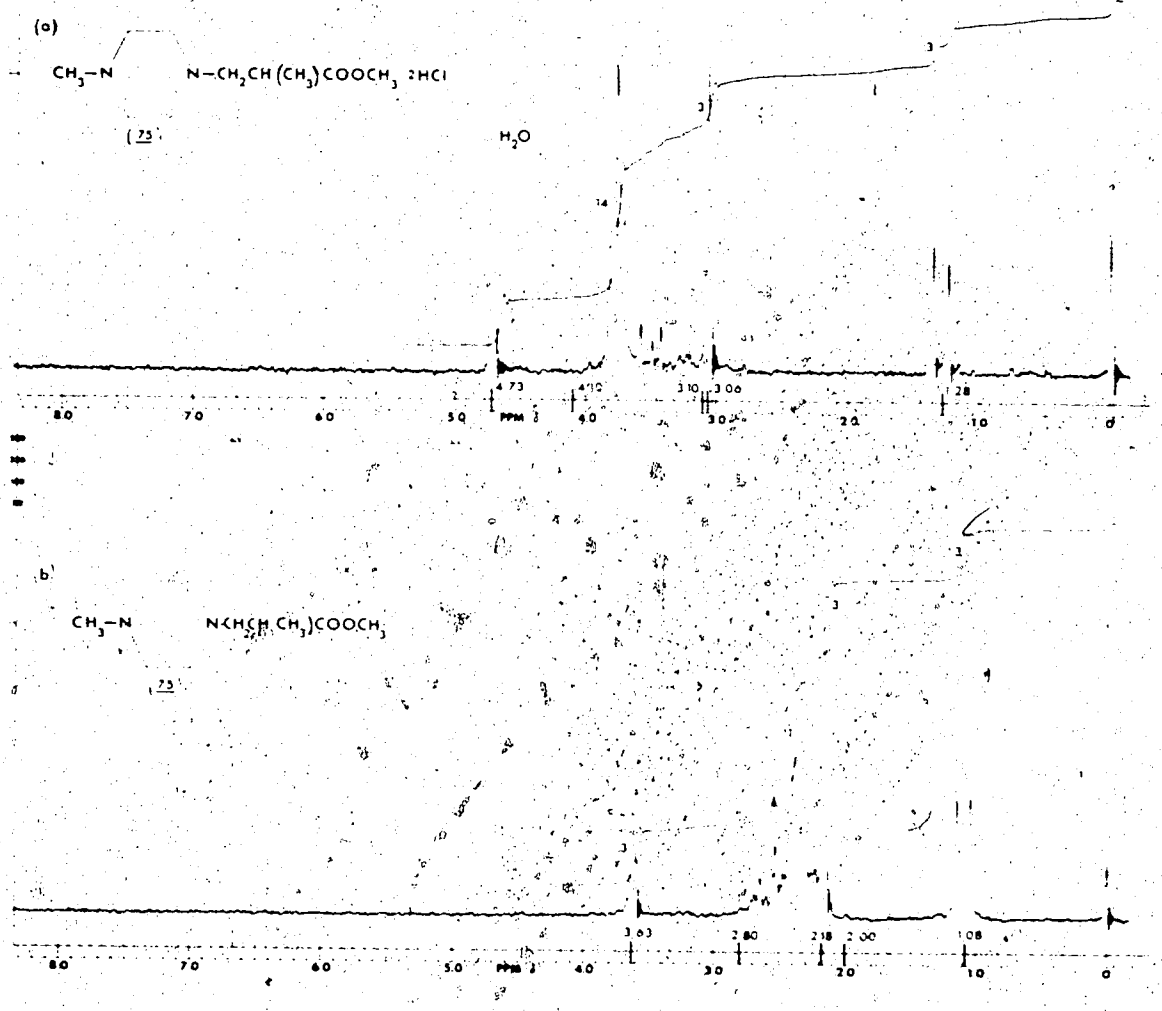



**Figure I: NMR spectra of (a) methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate dihydrochloride (75) in $D_2O$ and (b) the corresponding free base in $CCl_4$.**

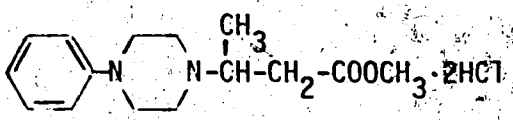
2-methyl substituent since coupling with the $C_2$ proton would be expected, while the three proton singlet at δ3.06 was assigned to the N-methyl group on the piperazine moiety. N-Methyl protons are known to come to resonance over the range δ2.1 - 3.0 (Dyer, 1965). The complex multiplet occurring over the region δ3.1 - 4.1 was assigned to the remaining protons, namely eight piperazine protons, three methoxy protons and three protons of the propionate chain. The complex nature of this multiplet was likely due to the fortuitous overlapping of chemical shifts for the various protons represented. Recording the NMR spectrum of the free base of the same compound in $CCl_4$ (Figure 1b) gave a 3-proton doublet at δ1.08, a 3-proton singlet at δ2.18, an 11-proton multiplet over δ2.0 - 2.8 and a 3-proton singlet at δ3.63 which were assigned to $CHC\underline{H}_3$, $NC\underline{H}_3$, $C_4\underline{H}_8C\underline{H}_2C\underline{H}$ and $OC\underline{H}_3$, respectively. A comparison of these two spectra of methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate revealed that the chemical shift of the 2-methyl substituent moved from δ1.28 for the dihydrochloride salt in $D_2O$ to δ1.08 for the free base in $CCl_4$. Similarly affected were the shifts of the N-methyl protons (δ3.06 to δ2.18) and the piperazine ring plus propionate protons (δ3.1 - 4.1 to δ2.0 - 2.8). This upfield shift occurring in the free base relative to the salt for all these protons is readily explained by the increased shielding occurring from the electron pairs on the two piperazine nitrogens which were present in the free base, but absent in the dihydrochloride salt. The isolated 3-proton singlet at δ3.63 in the NMR spectrum

of the free base was assigned to the methoxy protons. Since these protons are furthest removed from any shielding effects of the nitrogen atoms, they should exhibit the least upfield shift. For this reason, a single sharp peak present at δ3.65 within the multiplet occurring over the range δ3.1 - 4.1 in the NMR spectrum of the dihydrochloride in $D_2O$ could be assigned to the methoxy protons. Besides the knowledge that methoxy protons generally come to resonance over the range δ3.3 - 4.0 (Dyer, 1965), such an assignment would be supported by the fact that within this multiplet at δ3.1 - 4.1 is another taller, broader signal which may represent the piperazine protons and several much smaller signals which may represent the coupled propionate protons.

Because recording the NMR spectrum of the free base in $CCl_4$ as well as the dihydrochloride salt in $D_2O$ did not separate or illuminate the nature of the piperazine or propionate protons, usually only the NMR spectrum of the dihydrochloride salt in $D_2O$ was recorded for the purpose of confirming aminoester structures.

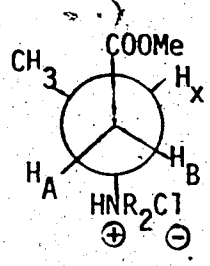
An interesting isolated 2-proton multiplet was observed at δ2.7 - 3.2 in the NMR spectra of all methyl 3-methyl-3-[1-(4-substituted piperazinyl)]propionate dihydrochlorides. This particular multiplet was not clearly present in the NMR spectra of methyl 2-methyl-3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides, methyl 3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides, ethyl 3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides or ethyl 4-[1-(4-substituted-piperazinyl)]butyrate dihydrochlorides.

The NMR spectrum of methyl 3-methyl-3-[1-(4-phenyl-piperazinyl)]propionate dihydrochloride (113a) in $D_2O$ (Figure IIa) was

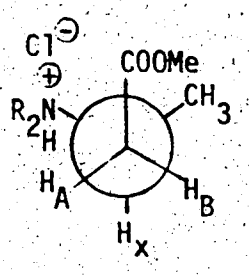


(113a)

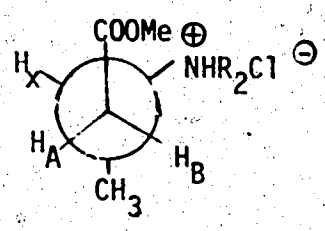
typical of those derivatives with a 2-proton multiplet at δ2.7 - 3.2. This multiplet was assigned to the $-CH_2-$ protons adjacent to the ester carbonyl group. Upon first inspection, one might expect this signal to be a doublet. However, examination of Newman projections along the $C_2$-$C_3$ bond of the molecule (114), (115) and (116) revealed that steric hindrance to rotation about this bond must exist and consequently,



(114)



(115)



(116)

unequal conformer populations must also exist. Jackman and Sternhell

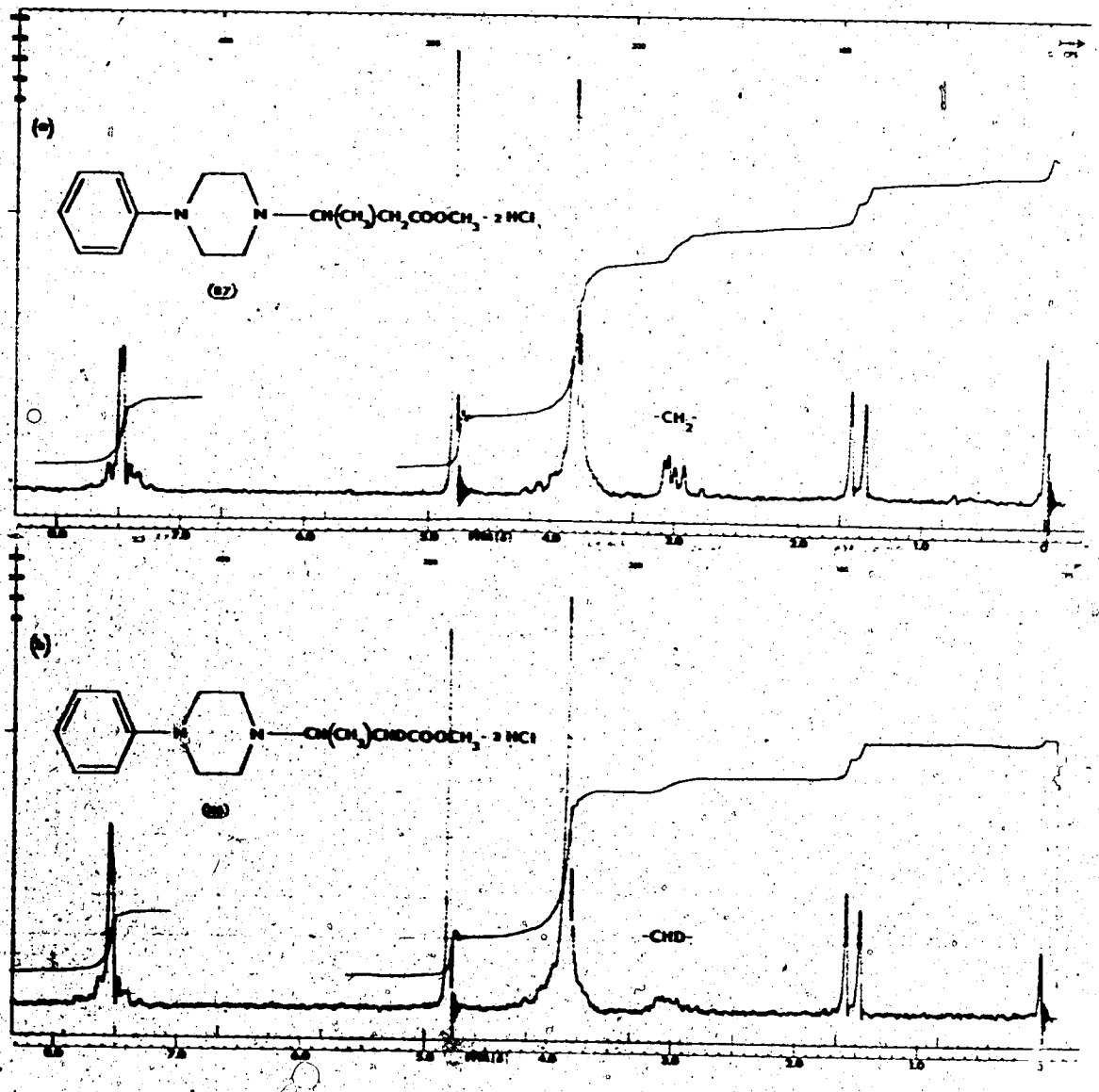
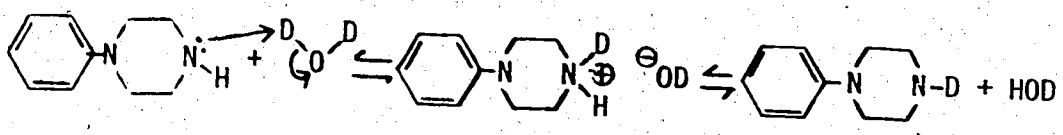



**Figure 11: NMR spectra in $D_2O$ of (a) methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate dihydrochloride (87) and (b) methyl 3-methyl-2-deutero-3-[1-(4-phenyl-piperazinyl)]propionate dihydrochloride (116).**

(1969) summarized the form of the NMR spectrum to be expected for each of the various possibilities of substituted ethanes. The system under examination in the $C_2$-$C_3$ bond of methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate dihydrochloride could be described as $CH_2X$-CHYZ. According to Jackman, this type of system could be expected to yield either a spectrum having the form of three ABC spectra in the slow rotation case (as would likely exist in this compound) or a single ABC spectrum in the fast rotation case. Jackman pointed out that the ABC system consists of up to 15 lines and has no characteristic appearance. Furthermore, no useful information can be obtained from ABC spectra by attempts at first-order analysis. In fact, computing and peak matching techniques must be applied to obtain precise information about chemical shifts and coupling constants. Such procedures are beyond the scope of the present investigation; consequently, no useful information could be obtained from the 2-proton multiplet in the NMR spectrum of methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate dihydrochloride (87) (Figure IIa). A similar analysis should apply to the NMR spectra of methyl 2-methyl-3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides, methyl 3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides, ethyl 3-[1-(4-substituted-piperazinyl)]propionate dihydrochlorides and ethyl 4-[1-(4-substituted-piperazinyl)]butyrate dihydrochlorides. However, in all these cases the complex multiplet that would arise from unequal distributions of conformers fortuitously overlaps other proton signals - effectively obscuring the nature of the multiplet.

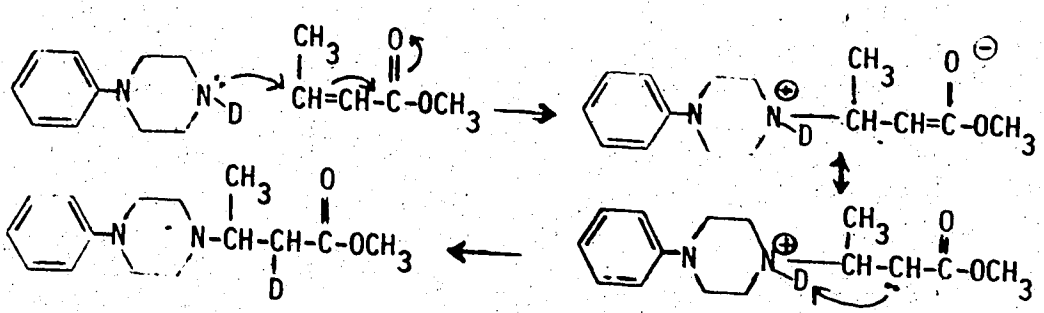
To confirm the assignment of this 2-proton multiplet to the two protons adjacent to the ester carbonyl, advantage was taken of the Michael addition mechanism. First, 1-deutero-4-phenylpiperazine was prepared by stirring a solution of 1-phenylpiperazine in anhydrous

dioxane with 15 ml of $D_2O$. Extreme caution was used to prevent any contact by the reaction mixture with traces of water from the atmosphere. The following reaction sequence summarizes this systhesis.



Since $D_2O$ was added in a 6-fold molar excess, these equilibrium reactions resulted in predominantly 1-deutero-4-phenylpiperaine as product. In the IR spectrum (film) of 1-deutero-4-phenylpiperazine, N-D stretch was present at 2440 $cm^{-1}$, while N-H stretch of 1-phenylpiperazine at 3280 $cm^{-1}$ was absent. The NMR spectrum of 1-deutero-4-phenylpiperazine in $CCl_4$ had a broad singlet at $\delta$1.24 integrating for only about one-quarter proton, instead of the full proton in 1-phenylpiperazine. This evidence would indicate that the deuterated product was contaminated with an amount of non-deuterated product. This was expected because pure $D_2O$, uncontaminated with water is practically impossible to attain.

The 1-deutero-4-phenylpiperazine was reacted with methyl crotonate in anhydrous dioxane to give methyl 3-methyl-2-deutero-3-[1-(4-phenylpiperazinyl)]propionoate (113b). This synthesis can be summarized by the following mechanism (Pfau, 1967):



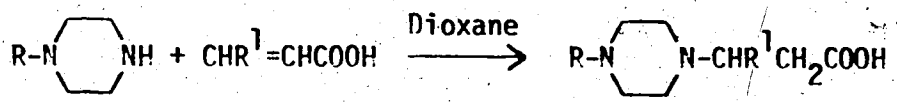
(113b)

Once again, great care was taken to ensure the reaction had no contact with moisture from the atmosphere. The IR spectrum (film) of the deuterated ester product possessed peaks at 2470 $cm^{-1}$ (C-D stetch) as well as at 1735 $cm^{-1}$ (C=O stretch). The NMR spectrum of the deuterated ester dihydrochloride in $D_2O$ (Figure IIb) possessed a broad collapsed multiplet at δ2.7 - 3.2 integrating for only one proton rather than the two protons of the undeuterated product. From a knowledge of the Michael addition mechanism, it can be safely assumed that a deuterium atom was introduced into a significant proportion of molecules of methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate at the 2-position. Consequently, one can conclude that assignment of the multiplet at δ2.7 - 3.2 in the NMR spectrum of methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate (87) in $D_2O$ (Figure IIa) to the protons adjacent to the ester carbonyl was correct.

Preparation of 3-Amino-carboxylic Acids

Two methods were used to prepare the carboxylic acids studied.

1) Michael addition of the appropriate amine to crotonic acid or acrylic acid in dioxane.

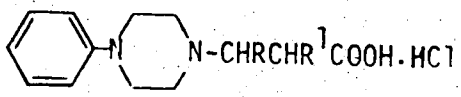



The mechanism and properties of this reaction have been previously discussed under the preparation of 3-aminoesters. Dioxane rather than methanol or ethanol was used as the solvent in this case

to prevent the possibility of ester formation. The carboxylic acids were not isolated by fractional distillation since the reaction residues were solids. Consequently, the residues were acidified with hydrogen chloride gas dissolved in acetone and the carboxylic acids isolated as hydrochloride salts. Using this procedure, the following carboxylic acids were synthesized (Table VII).

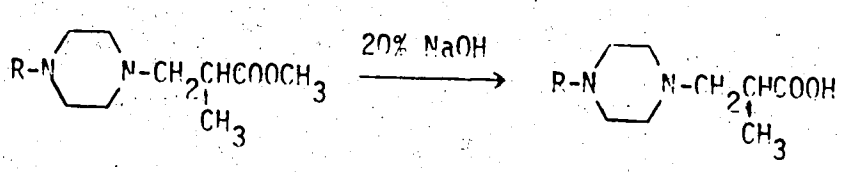
TABLE VII

3-[1-(4-Phenylpiperazinyl)]propionic Acids



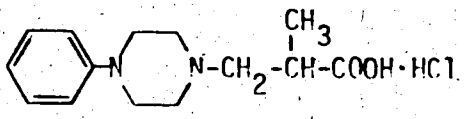
| Compound | R | $R^1$ | % Yield |
|---|---|---|---|
| 117 | H | H | 74 |
| 118 | $CH_3$ | H | 60 |

2) Aqueous alkaline hydrolysis of the appropriate 3-aminoester (Vogel, 1956).



Utilizing this base-catalyzed hydrolysis procedure of Vogel (1956), 2-methyl-3-[1-(4-phenylpiperazinyl)]propionic acid hydrochloride

(119) was prepared in 84% yield.



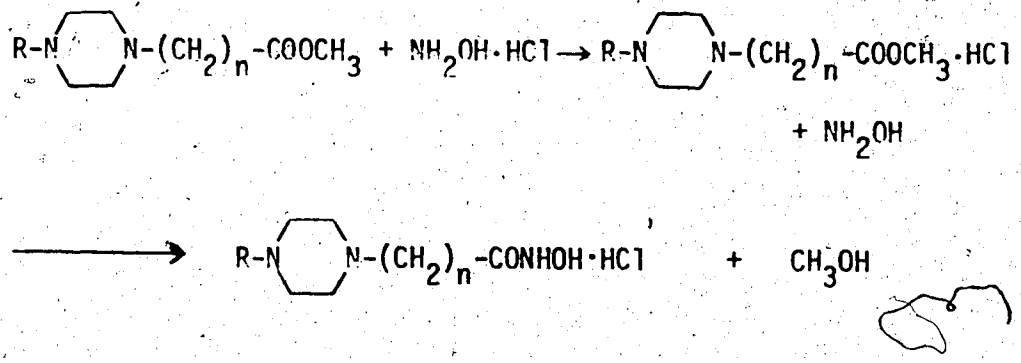
(119)

The three carboxylic acids prepared in the present investigation were all colorless compounds stable over a period of several months. They were characterized by microanalysis and their IR spectrum. All three infrared spectra showed a carbonyl stretching absorption band at either 1725 $cm^{-1}$ or 1715 $cm^{-1}$. None of these derivaties were sufficiently soluble in any of the commonly used solvents to permit useful NMR spectra to be recorded.

Preparation of Amino-hydroxamic Acids

Three methods were used for the synthesis of the aminohydroxamic acids examined in the present investigation.

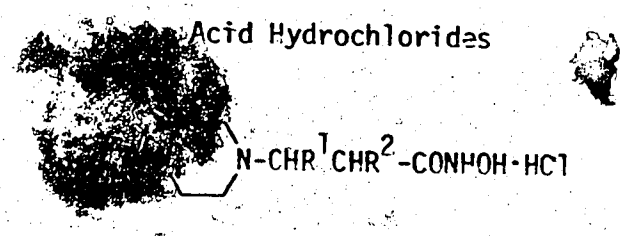
1) Reaction of the appropriate amino ester free base with hydroxylamine hydrochloride in methanol. (Coutts et al. 1969; 1971).



This procedure was employed to prepare the following amino-hydroxamic acids (Table VIII and Table IX).

TABLE VIII

3-[1-(4-Substituted-piperazinyl)]propionohydroxamic Acid Hydrochlorides



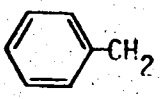
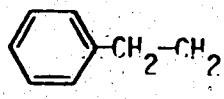
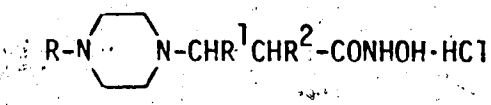

| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 120 | $CH_3$ | H | $CH_3$ | 38 |
| 121 | $CH_3CH_2$ | H | $CH_3$ | 50 |
| 122 | $CH_3CH_2CH_2$ | H | $CH_3$ | 51 |
| 123 | $CH_3CH_2CH_2CH_2$ | H | $CH_3$ | 32 |
| 124 |  | H | $CH_3$ | 30 |
| 125 |  | H | $CH_3$ | 51 |
| 126 |  | H | $CH_3$ | 37 |
| 127 | $CH_3$ | $CH_3$ | H | 38 |

TABLE VIII cont'd

3-[1-(4-Substituted-piperazinyl)]propionohydroxamic Acid Hydrochlorides



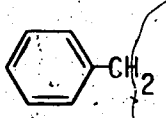
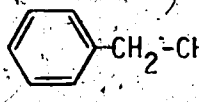
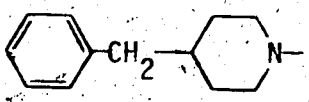
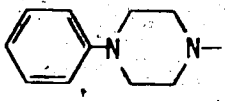
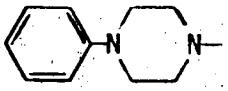
| Compound | R | $R^1$ | $R^2$ | % Yield |
|---|---|---|---|---|
| 128 | $CH_3CH_2$ | $CH_3$ | H | 45 |
| 129 | $CH_3CH_2CH_2$ | $CH_3$ | H | 42 |
| 130 | $CH_3CH_2CH_2CH_2$ | $CH_3$ | H | 48 |
| 131 |  | $CH_3$ | H | 45 |
| 132 |  | $CH_3$ | H | 33 |
| 133 |  | $CH_3$ | H | 44 |

TABLE IX

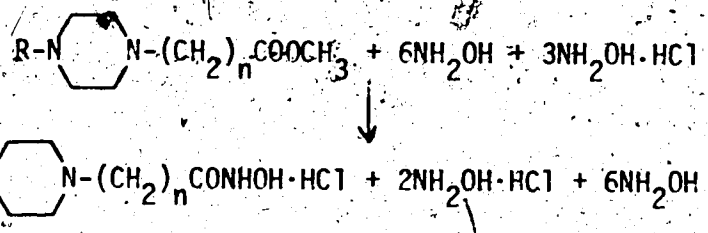
Aminopropionohydroxamic Acid Hydrochlorides

$R\text{-}(CH_2)_nCHR^1CONHOH\cdot HCl$

| Compound | R | $R^1$ | n | % Yield |
|---|---|---|---|---|
| 134 |  | $CH_3$ | 1 | 75 |
| 135 |  | H | 2 | 62 |
| 136 |  | H | 1 | 36 |

2) Reaction of the appropriate amino ester free base with hydroxylamine and hydroxylamine hydrochloride in methanol (Coutts et al. 1969; 1971).

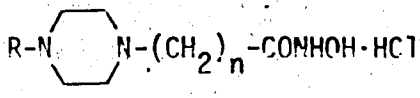
$$6NH_2OH\cdot HCl + 6KOH \longrightarrow 6NH_2OH + 6KCl + 3NH_2OH\cdot HCl$$



The following aminohydroxamic acids (Table X and Table XI) were prepared with this reaction.

TABLE X

1-(4-Substituted-piperazinyl)hydroxamic Acid Hydrochlorides



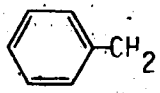
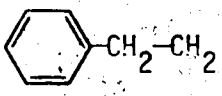
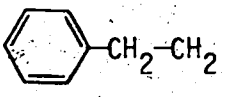
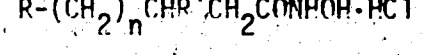
| Compound | R | n | % Yield |
|---|---|---|---|
| 137 |  | 3 | 40 |
| 138 |  | 3 | 42 |
| 139 |  | 1 | 83 |
| 140 |  | 1 | 67 |

TABLE XI

Aminohydroxamic Acid Hydrochlorides



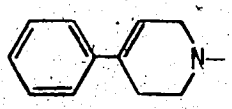
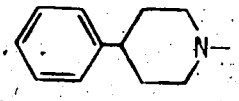
| Compound | R | $R^1$ | n | % Yield |
|---|---|---|---|---|
| 141 |  | H | 1 | 88 |

TABLE XI cont'd

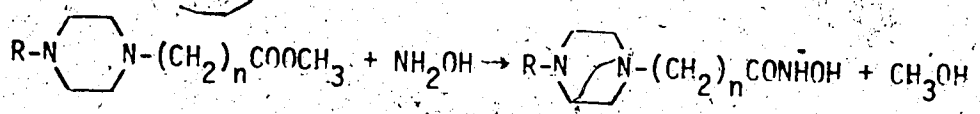
Aminohydroxamic Acid Hydrochlorides

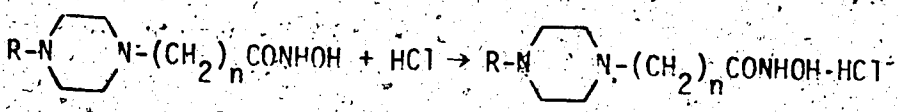
$R\text{-}(CH_2)_nCHR^1CH_2CONHOH\cdot HCl$

| Compound | R | $R^1$ | n | % Yield |
|---|---|---|---|---|
| 142 |  | $CH_3$ | 0 | 56 |

3) Reaction of the appropriate amino ester free base with hydroxylamine in aqueous methanol followed by acidification to pH 6 with hydrochloric acid (Fishbien et al. 1960).

$$NH_2OH\cdot HCl + 2\ NaOH \rightarrow NH_2OH + NaCl + NaOH$$

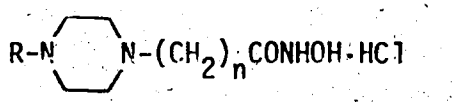


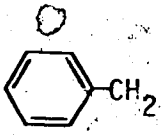
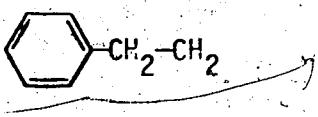


Using this technique, the following aminohydroxamic acid hydrochlorides (Table XII) were obtained.

TABLE XII

1-(4-Substituted-piperazinyl)hydroxamic Acid Hydrochlorides



| Compound | R | n | % Yield |
|---|---|---|---|
| 143 |  | 2 | 39 |
| 144 |  | 2 | 29 |

The aminohydroxamic acid hydrochlorides synthesized by all three methods were colorless solids which did not decompose when stored in a dessicator over a period of several months. The structure of all the hydroxamates were confirmed by microanalysis, IR and NMR spectra. In addition, each of the hydroxamic acids gave a violet color with alcoholic ferric chloride, consistent with the hydroxamate function (Coutts et al. 1971).

The infrared spectra of these compounds showed a carbonyl stretching absorption band between 1635 $cm^{-1}$ and 1695 $cm^{-1}$. These findings are in agreement with those reported by Coutts et al. (1971) and Biggs et al. (1972a and 1972b).

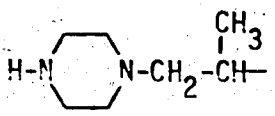
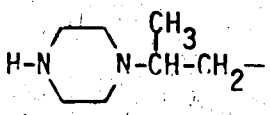
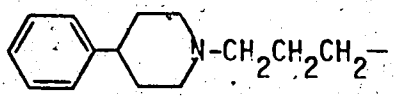
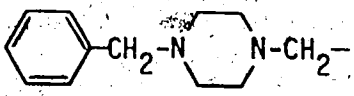
The NMR spectra of the aminohydroxamic acid hydrochlorides were run in DMSO-$d_6$. All spectra displayed a 3- or 4-proton broad signal in the $\delta$8.0 - 12.0 range which collapsed after addition of $D_2O$ to the sample. This signal was attributed to the two protons of the NHOH group and the proton of the $\overset{\oplus}{N}H$ or two $\overset{\oplus}{N}H$ groups. These observations parallel those reported by Coutts et al. (1971) who examined acyclic hydroxamic acids of general structure (3). These workers observed three 1-proton signals within the $\delta$8.33 - 11.66 range which disappeared after addition of $D_2O$ to the sample. The two protons of the NHOH group reportedly came to resonance in the ranges $\delta$8.68 - 9.00 and $\delta$10.75 - 11.05. A signal appearing at $\delta$10.75 - 11.05 was assigned to the $\overset{\oplus}{N}H$ proton, and was absent from the spectra of the corresponding bases. Comparable observations were reported by Selley (1971) and Towill (1971).

Four hydroxamates failed to recrystallize because of their extreme hygroscopicity. These aminohydroxamic acid hydrochlorides which were not characterized are listed in Table XIII.

TABLE XIII

Uncharacterized Aminohydroxamic Acid Hydrochlorides

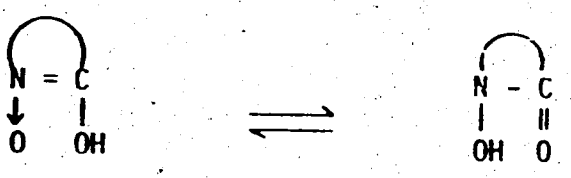
R-CONHOH·HCl

| Compound | R | Method of Attempted Synthesis |
|---|---|---|
| 145 |  | 1, 2 |
| 146 |  | 1, 2 |
| 147 |  | 2 |
| 148 |  | 2 |

Preparation of 1-Hydroxyquinoxalin-2-ones

Coutts (1967) and Bapat et al. (1969) have reviewed the synthetic procedures for obtaining a wide range of structures containing the cyclic hydroxamic acid function which can be generalized as the two tautomeric forms (149) ⇌ (150). Because of the great range of structures containing cyclic hydroxamic acid functions, it is difficult to give a concise summary of the available synthetic methods. Consequently, of the many general synthetic approaches available, only the two methods attempted in the present

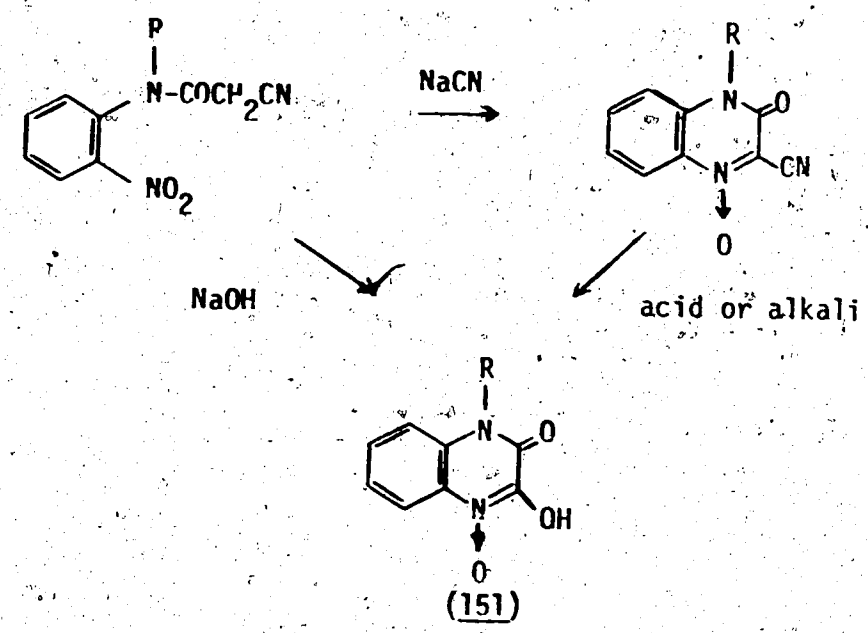
study will be discussed.



(149) (150)

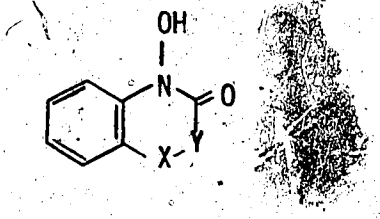
1) Synthesis by reductive cyclization

Tennant (1064) prepared quinoxaline hydroxamic acids (151) by nitro group side-chain interaction as shown below, where R was a hydrogen atom or a methyl group.



(151)

In addition to preparing certain quinolines, quinazolines, benzoxazines and benzothiazines containing the cyclic hydroxamic acid grouping, Coutts et al. (1964) synthesized three quinoxaline derivatives (152) by reductive cyclization of the appropriate o-nitroester with sodium borohydride catalyzed with palladium-charcoal.



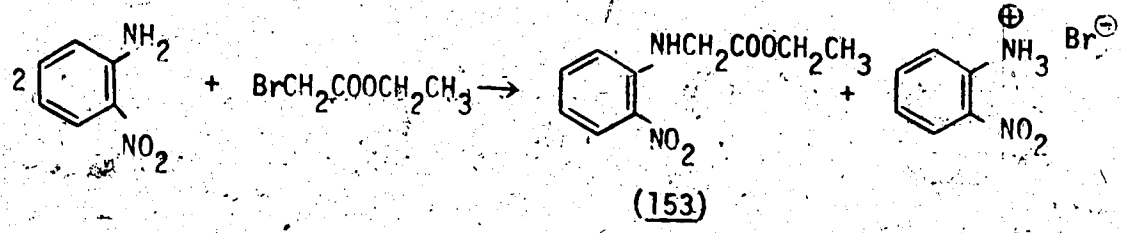
(152)

X-Y = $-NH-CH_2-$, $-N=CH_2-$ or $-NH-\underset{\displaystyle OH}{\underset{|}{CH}}-$.

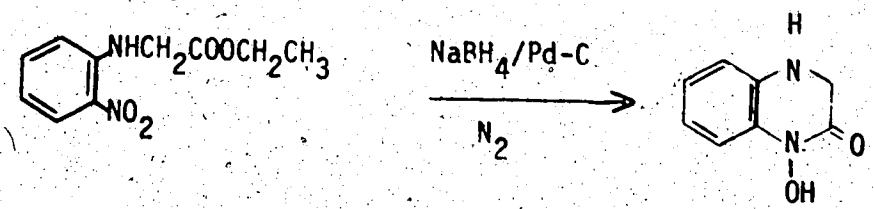
Coutts (1967) found that the sodium borohydride and palladium-charcoal reducing system was excellent for the preparation of a variety of cyclic N-oxy and N-hydroxy compounds from suitably substituted o-nitroesters. Consequently, this method was initially chosen in an attempt to prepare the quinoxaline hydroxamic acids of interest in the present investigation (Table XIV). This decision necessitated the synthesis of appropriately substituted o-nitroesters for subsequent reductive cyclization to the desired compounds.

King and Clark-Lewis (1951) prepared ethyl N-o-nitrophenylglycinate by the interaction of o-nitroaniline with 1 mole of ethyl bromoacetate followed by extracting with anhydrous ether. This

synthesis was duplicated in the present study and ethyl N-o-nitrophenylglycinate (153) was obtained in 20% yield. Subsequent reductive cyclization of this o-nitroester by the method of Coutts et al. (1964) as shown below, gave an impure product which produced a dark blue color with alcoholic ferric chloride. Extensive recrystallization from absolute methanol failed to improve the sample purity. Thin-layer chromatography of the product revealed two distinct spots (Rf = 0.43 and Rf = 0.36), indicating the presence of two different compounds. The spot with Rf = 0.36 corresponded to the spot obtained under the same conditions for pure 1-hydroxyquinoxalin-2-one prepared by another method. In contrast, Coutts et al. (1964) were able to obtain pure 3,4-dihydro-1-hydroxyquinoxalin-2-one (154) by the reductive cyclization of N-o-nitrophenylglycinate.




(153)




(154)

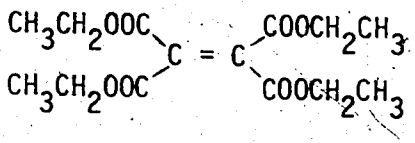
Attempts to broaden the scope of the King and Clark-Lewis (1951) reaction between o-nitroaniline and ethyl bromoacetate by reacting o-nitroaniline with a variety of ?-alkylsubstituted-2-bromoesters to give various 2-substituted-o-nitrophenylesters were unsuccessful. In each case, unreacted starting materials were recovered from the reactions of o-nitroaniline with ethyl 2-bromopropionate, ethyl 2-bromobutyrate and ethyl 2-bromohexanoate under the conditions used by King and Clark-Lewis (1951).

It was felt that these reactions may have failed because the electron-withdrawing nature of the o-nitro group might make o-nitroaniline too weak a base to attack the 2-bromoesters once the esters were substituted with electron donating alkyl substituents at the 2-position reaction site. In an effort to overcome this possible problem, o-nitroaniline was converted to its more nucleophilic conjugate base with sodium hydride by using the procedure which Zaugg et al. (1960) outlined. The conjugate base of o-nitroaniline was reacted in situ with ethyl 2-bromopropionate and ethyl 2-bromoisobutyrate. Although three different anhydrous solvents (xylene, dioxane and dimethyl sulfoxide) were tried, the crude reaction residue always displayed absorbances typical of a primary amine in the 3300-3500 $cm^{-1}$ region of the IR spectrum. Indeed, the two peaks attributed to a primary amine function had similar relative intensities and positions to the $-NH_2$ stretching absorbances in the IR spectrum of o-nitroaniline. It was concluded that these reactions had not succeeded since the desired product was a secondary amine which would display

only a single absorption peak in the 3300-3500 $cm^{-1}$ range.

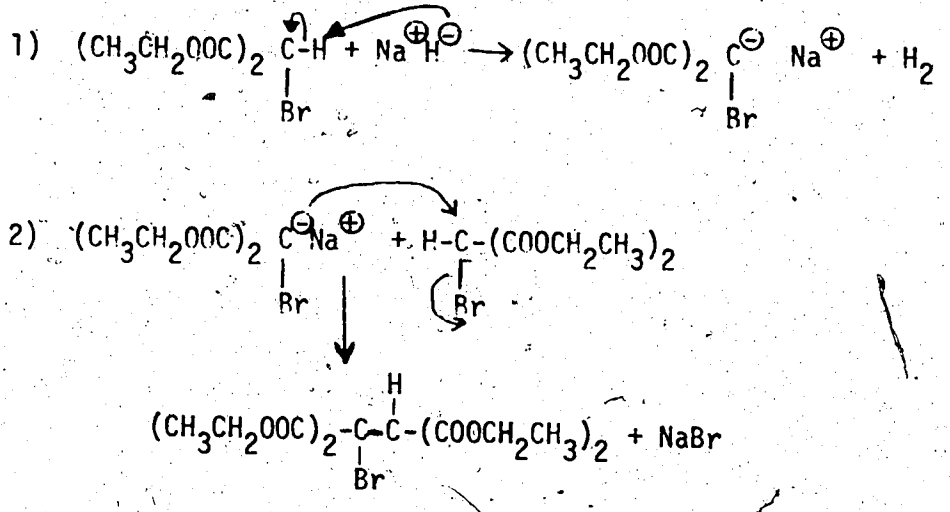
It was thought that these findings could be rationalized by concluding that the electron donating properties of the 2-alkyl-substituents on the 2-bromoesters were such that neither o-nitroaniline nor its conjugate base were strong enough nucleophiles to complete a successful reaction. To test this interpretation diethyl bromomalonate (a 2-bromoester substituted in the 2-position with an electron withdrawing ethyoxycarbonyl group) was synthesized then reacted with o-nitroaniline and its conjugate base. Once again, only unreacted starting materials were recovered when o-nitroaniline and diethyl bromomalonate were reacted under the conditions described by King and Clark-Lewis (1951). However, upon reacting the conjugate base of o-nitroaniline with diethylbromomalonate, pale yellow prisms physically unlike either starting material were obtained. After several recrystallizations, the yellow coloration (presumably contamination with o-nitroaniline) disappeared and shiny white prisms resulted. The IR spectrum of this compound had a peak at 1725 $cm^{-1}$ (C=O), but no peaks attributable to aromatic group or NH infrared absorbances. The NMR spectrum of the product possessed a triplet at $\delta$1.31 and a quartet at $\delta$4.26 which integrated in the ratio 3:2, respectively. The coupling constant for both signals was J=7.0 Hz, indicative of vicinal coupling. The above data as well as microanalysis was consistent with the structure of 1,1,2,2-tetraethoxycarbonylethylene (155) as the product. Confirmation of this structural assignment was obtained by repeating the above synthesis without

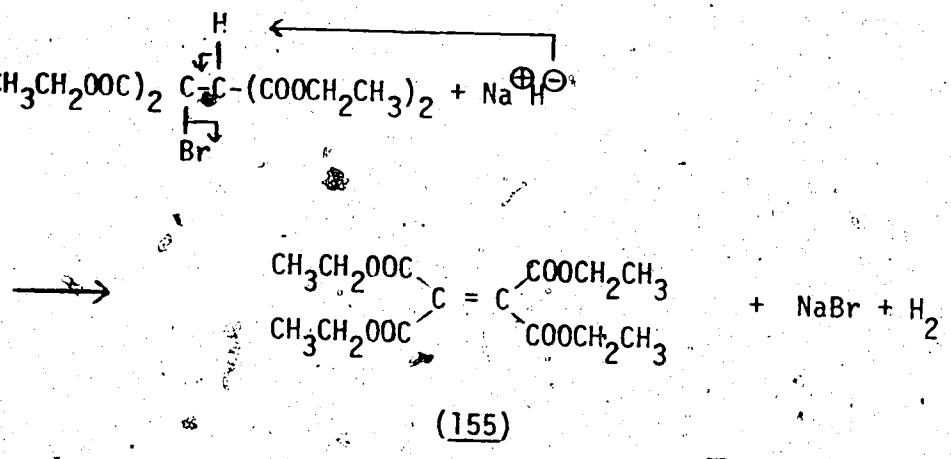
adding o-nitroaniline. In virtually all respects, an identical product was obtained.



(155)

1,1,2,2,-Tetraethoxycarbonylethylene (155) could be visualized as arising from the following reaction mechanism.





(155)

When o-nitroaniline was present, its conjugate base could take the place of the hydride ion in the above mechanism to give the same 1,1,2,2-tetraethoxycarbonylethylene product.

The above observations led to the conclusion that steric factors must be involved in hindering the approach and hence the reaction of o-nitroaniline to various 2-substituted-2-bromoesters. These synthetic difficulties resulted in the cessation of any further attempts to prepare o-nitroesters via the reaction of o-nitroaniline with appropriate 2-bromoesters.

Another approach to the synthesis of suitable o-nitroesters was suggested by the investigation of Ashton and Suschitzky (1957) in which o-nitrochlorobenzene and cyclohexylamine, benzylamine, phenethylamine, n-butylamine, allylamine, 2-aminopyridine, 4-chloro-2-aminopyridine or 4-methyl-2-aminopyridine were heated together to give the corresponding N-substituted-o-nitroanilines.

Foye and Feldmann (1957) were able to prepare several N-(nitrosubstituted)phenyl derivatives of D-glucamine in good yields

by using an analogous approach in reacting D-glucamine with p-nitrochlorobenzene, 2,4-dinitrochlorobenzene or 1,2-dichloro-4,5-dinitrobenzene using either pyridine or ethanol-sodium acetate as solvent.

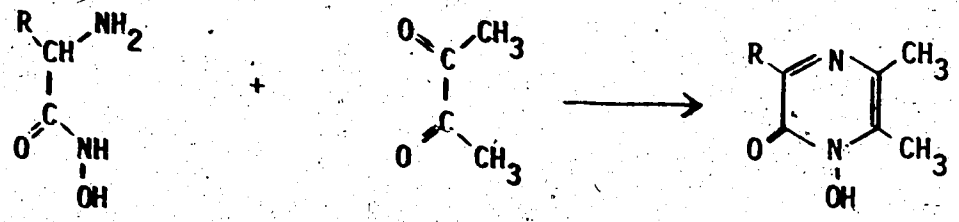
These syntheses prompted attempts to prepare suitably substituted o-nitroesters by reacting o-nitrochlorobenzene with various amino acid ethyl esters. However, ethyl glycinate failed to react with o-nitrochlorobenzene when refluxed for several days in absolute ethanol. Addition of bases such as triethylamine or sodium bicarbonate plus distilled water failed to shift any existing reaction equilibria to desired product formation. For each reaction, the IR spectrum of the crude residue possessed two absorption peaks in the 3300-3400 $cm^{-1}$ region characteristic of a primary amine and which had similar relative intensities and positions to the primary amine stretching absorptions of ethyl glycinate. If ethyl glycinate had reacted with o-nitrochlorobenzene, a secondary amine with only one peak in this region of the infrared spectrum would have been the product.

Other problems were encountered by Feldmann and Foye (1959) who tried to prepare N-2-nitrophenylglycines by reacting substituted o-nitrochlorobenzenes with glycine in the presence of pyridine. They found considerable decomposition of the reaction mixture after refluxing with no pure products isolatable.

In view of the synthetic difficulties involved in obtaining appropriately substituted o-nitroesters, the reductive cyclization approach to 1-hydroxyquinoxalin-2-ones was abandoned.

2) Preparations involving acyclic hydroxamic Acids

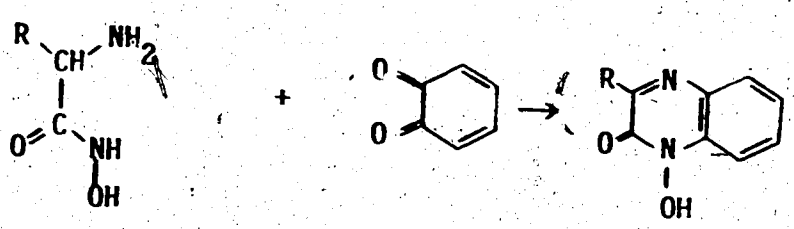
Safir and Williams (1952) synthesized cyclic hydroxamic acid derivatives of pyrazine (156) by reacting an α-aminohydroxamic acid with an α-diketone, where R was -H, -CONHOH, $-CH_2-CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$.



(156)

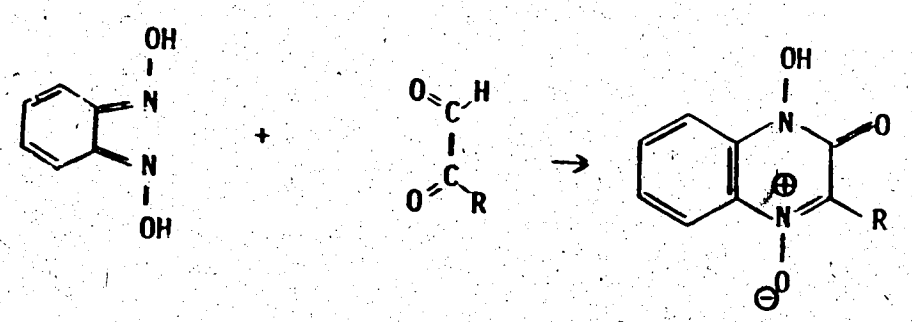
An effort was made to utilize this approach to prepare 1-hydroxyquinoxalin-2-ones (157) by reacting α-aminohydroxamic acids with the α-diketone, o-benzoquinone. Following the procedure outlined by Safir and Williams (1952) glycine hydroxamic acid was synthesized from glycine ethyl ester hydrochloride and hydroxylamine hydrochloride. At the same time, the relatively unstable o-benzoquinone was obtained in very low yield by oxidation of o-catechol with silver oxide according to the method described by Willstatter and Pfannenstiel (1904). A black solution, from which no pure products were isolated,

was obtained when glycine hydroxamic acid and o-benzoquinone were dissolved together in distilled water. It appeared that the reactants and/or products had decomposed.



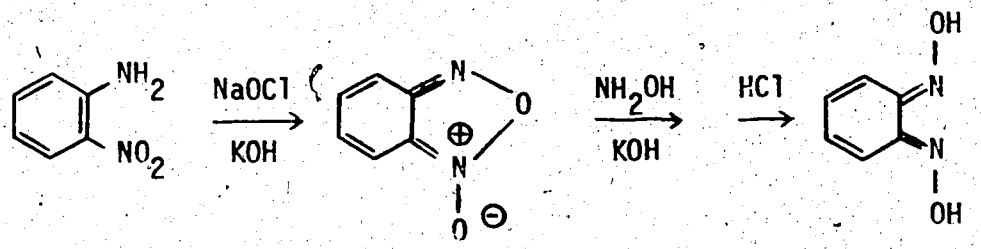
o-benzoquinone (157)

Abushanab (1970) prepared 1-hydroxy-quinoxalin-2-one 4-oxide (158a) and 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (158b) by reacting o-quinone dioxime with glyoxal and methyl glyoxal, respectively. Anticipating that these 4-oxides could be selectively deoxygenated to the corresponding 1-hydroxyquinoxalin-2-ones, the above syntheses of Abushanab were repeated.



(158a R=H); (158b R=$CH_3$)

The o-quinone dioxime needed for these reactions was obtained by reacting benzofuroxan with hydroxylamine hydrochloride in alkaline solution followed by acidification according to the method given by Ghosh and Whitehouse (1968). The procedure outlined by Mallory (1957) for the sodium hypochlorite oxidation of o-nitroaniline was employed to prepare the required benzofuroxan precursor. These syntheses are summarized in the following reaction sequence.



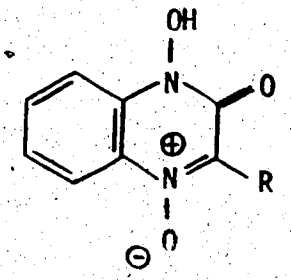

Of the α-ketoaldehydes reacted with o-quinone dioxime, glyoxal and methyl glyoxal were commerically available as 40% aqueous solutions, while phenyl glyoxal was prepared utilizing the procedure described by Vogel (1956) in which acetophenone was oxidized to phenyl glyoxal with selenium dioxide.

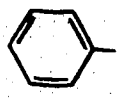
Using the procedure described by Abushanab (1970), the following 1-hydroxyquinoxalin-2-one 4-oxides were prepared from o-quinone dioxime and the appropriate glyoxal derivative (Table XIV). The structures of these 1-hydroxyquinoxalin-2-one 4-oxides were confirmed by microanalysis, IR, NMR and mass spectra. The IR spectra of these compounds possessed peaks in the 1225-1260 $cm^{-1}$ (N-O), 1610-1640 $cm^{-1}$ (C=O and C=N), and 2200-3200 $cm^{-1}$ (OH).

The NMR spectrum of each had a broad one-proton multiplet between δ9.5 - 11.8 (OH) that exchanged with $D_2O$. The mass spectrum of each compound was consistent with the proposed structure and are discussed in the mass spectrometry section of this thesis. In addition, each of these cyclic hydroxamic acids gave a violet color with alcoholic ferric chloride.

TABLE XIV

1-Hydroxyquinoxalin-2-one 4-Oxides



| Compound | R | % Yield |
|---|---|---|
| 159 | H- | 65 |
| 160 | $CH_3$- | 42 |
| 161 |  | 77 |

Although all three quinoxaline derivatives were recrystallized from absolute methanol, an unusual occurrence was noted with 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161) but not with 1-hydroxyquinoxalin-2-one 4-oxide (159) or 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (160). Upon vacuum drying 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161) in an acetone-heated pistol dessicator, the bright yellow air-dried compound turned to a pale yellow color. While the vacuum-dried compound melted at 196-198°, the air-dried compound changed from bright yellow to pale yellow at 85-90° without melting, then melted at 196-198°. The IR spectrum of the air-dried compound (Figure 3a) possessed significant absorbances at 1610 $cm^{-1}$ and 1655 $cm^{-1}$, while the IR spectrum of the vacuum-dried compound (Figure 3b) possessed only a single absorbance at 1610 $cm^{-1}$. Furthermore, the IR spectrum of the air-dried compound after being stored for about two months (Figure 3c) was virtually identical with the of the vacuum-dried compound. Additional differences occurred between the NMR spectrum of the air-dried compound (Figure 4a) which possessed a 3-proton singlet at $\delta$3.22 and a 9-proton multiplet at $\delta$7.3 - 8.6 and the NMR spectrum of the vacuum-dried compound (Figure 4b) which possessed a 9-proton multiplet at $\delta$7.3 - 8.7 and a broad collapsed 1-proton multiplet at $\delta$11.8 that exchanged with $D_2O$. The mass spectra of the air-dried and vacuum-dried 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide were virtually identical. However, in view of the ease with which heating converted one compound into the other, this similarity of mass spectra was expected since a temperature of 250° and a vacuum of $10^{-4}$ mm Hg exists within the ionizing chamber of a mass spectrometer under normal operating conditions.

The preceeding observations seemed to indicate that

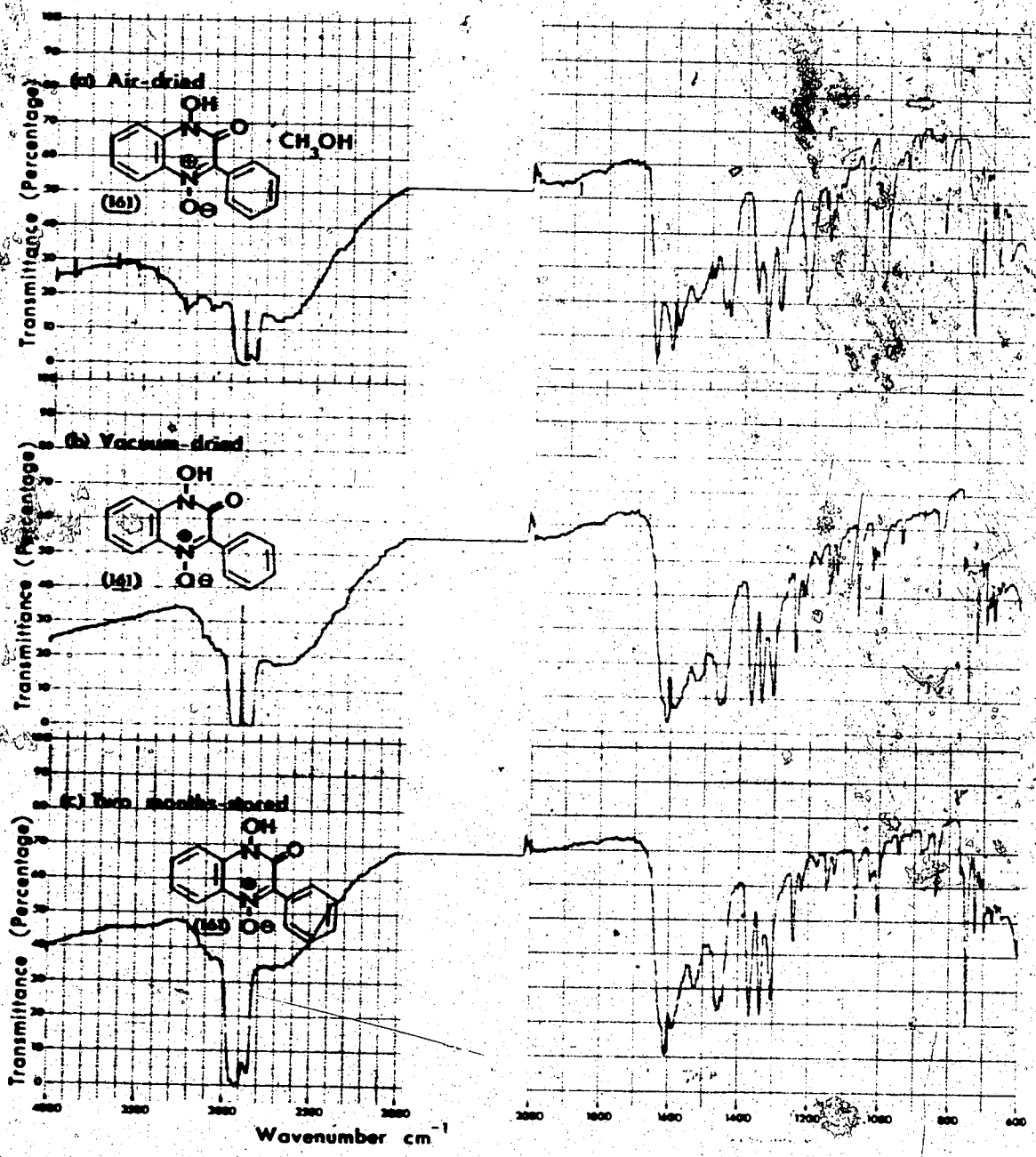



**Figure III : IR spectra (Nujol mulls) of (a) air-dried, (b) vacuum-dried and (c) two-months-stored 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161).**

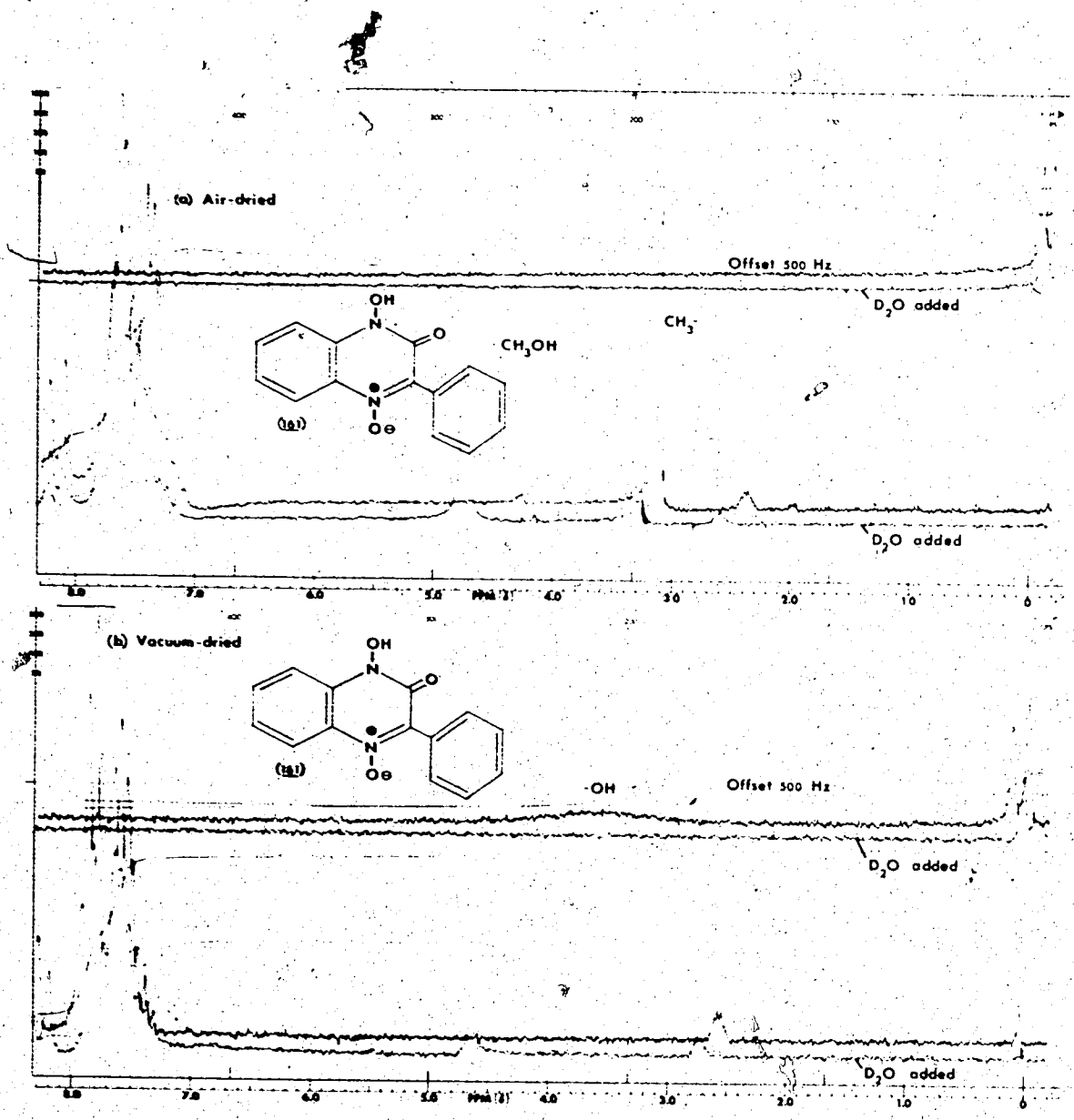
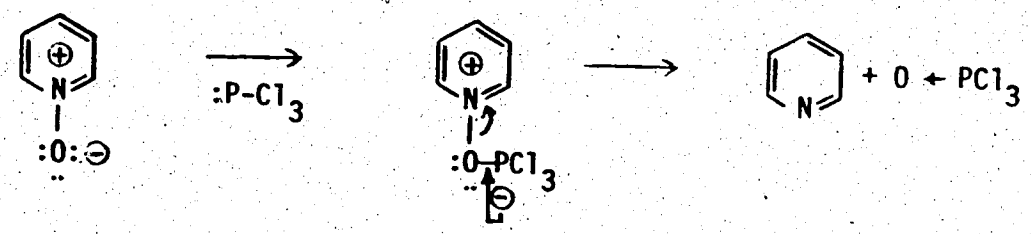



**Figure IV: NMR spectra in DMSO-$d_6$ of (a) air-dried and (b) vacuum-dried 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161).**

recrystallization of 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide from absolute methanol resulted in the isolation of a fairly stable 1:1 complex of 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide with methanol. Microanalysis of the air-dried complex agreed with the required analysis for a 1:1 complex ($C_{14}H_{10}N_2O_3 \cdot CH_3OH$), while microanalysis of the vacuum-dried compound was consistent with the expected CHN analysis for 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide ($C_{14}H_{10}N_2O_3$). Unfortunately, the data obtained does not allow an unequivocal assignment of a structure to the air-dried methanol complex.

Ochiai (1967) discussed the deoxygenation of aromatic N-oxides. This summary revealed that aromatic N-oxides had been reduced with hydrogen gas over palladium charcoal and Raney nickel catalysts as well as with a wide variety of reagents including phosphorus trichloride, iron powder and acetic acid, sodium borohydride and aluminum trichloride, and sulfur in liquid ammonia. He indicated that deoxygenation with phosphorus trichloride was a comparatively facile reaction giving good yields of pure reduced product under mild reaction conditions. Hamana (1955) assumed that following sequence represented the reaction mechanism for phosphorus trichloride deoxygenation.



The procedure for phosphorus trichloride deoxygenation which Ochiai (1967) described was initially chosen in an attempt to selectively reduce the N-oxide function of 1-hydroxyquinoxalin-2-one 4-oxides. In each case, the products isolated did not give any color with alcoholic ferric chloride, indicating that the hydroxamic acid functional group had been destroyed. The IR spectra of all three products had strong absorption peaks in the region 1655-1670 $cm^{-1}$, indicative of C=O and/or C=N stretching vibrations. Silica gel G thin-layer chromatography Rf values of each phosphorus trichloride reduced N-oxide did not match the Rf value obtained under the same conditions for the corresponding pure 1-hydroxyquinoxalin-2-one prepared, as described later, by reduction with another method ($H_2$/Pd-C). In the mass spectra of all three products, the ions of greatest mass were consistent with structures in which both the 4-oxide and the 1-hydroxy substituents were reduced and in which two chlorine atoms were substituted (Table XV). In addition, each mass spectrum also possessed peaks of significant relative abundance with mass to charge ratios consistent with either a molecular ion containing a single chlorine atom or a fragment ion resulting from the loss of a chlorine atom from the disubstituted molecular ion. The reason for making this statement is that although some of the phosphorus trichloride deoxygenation products could be purified to constant melting point by repeated recrystallizations, the microanalysis of the product was always much closer to that required for the monochloro substituted product than for the disubstituted one.

It was thus concluded that deoxygenation by phosphorus trichloride with Ochiai's conditions resulted in a mixture of monochloro and dichloro substituted products in which both the N-hydroxy and the N-oxide substituents were reduced. Consequently, the reduction of 1-hydroxyquinoxalin-2-one 4-oxides to 1-hydroxyquinoxaline-2-ones with phosphorus trichloride were judged unsuccessful.

TABLE XV

Possible Molecular Ions in the Mass Spectra of the $PCl_3$-Reduction Products of 1-Hydroxyquinoxalin-2-one 4-Oxides

| Compound Reduced | m/e Ratio of Product | Possible Formula of Ion | % Relative Abundance |
|---|---|---|---|
| 159 | 215 | $[C_8H_5Cl_2N_2O]^+$ | 0.18 |
| | 180 | $[C_8H_5ClN_2O]^+$ | 0.02 |
| 160 | 228 | $[C_9H_6Cl_2N_2O]^+$ | 7.2 |
| | 194 | $[C_9H_7ClN_2O]^+$ | 72.0 |
| 161 | 290 | $[C_{14}H_8Cl_2N_2O]^+$ | 0.51 |
| | 256 | $[C_{14}H_9ClN_2O]^+$ | 26.5 |

Ochiai (1967) noted that catalytic reduction of aromatic N-oxides over palladium-charcoal proceeded very gradually whereas

catalytic reduction over Raney nickel in a neutral solvent was a facile reaction which gave excellent yields. In their review of the syntheses and reactivity of cyclic hydroxamic acids, Bapat et al. (1969) pointed out that chemical methods have been preferred to catalytic hydrogenation for the reduction of cyclic hydroxamic acids, probably because the choice of catalyst for the latter is critical. For example, Hayashi et al. (1959) and Lott et al. (1949) found that 1-hydroxy-2-pyridone was inert to palladium catalysts, while Hayashi et al. (1959) showed that hydrogenolysis to 2-pyridone occurred readily over Raney nickel in methanol.

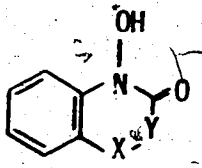
In view of these observations, it was decided to attempt the selective reduction of the N-oxide function by catalytic reduction over palladium-charcoal. It was envisaged that the hydroxamic acid group should be inert while the N-oxide should slowly undergo reduction.

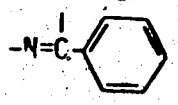
Catalytic reduction of 3,4-dihydro-, 3-methyl and 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxides (Table XIV) over palladium-charcoal in dioxane yielded 3,4-dihydro-1-hydroxyquinoxalin-2-one, 3-methyl-1-hydroxyquinoxalin-2-one, 3-phenyl-1-hydroxyquinoxalin-2-one, respectively (Table XVI). The structures of these compounds were confirmed by microanalysis, IR, NMR and mass spectra. The IR spectra of these compounds possessed absorbances in the 1620-1660 $cm^{-1}$ (C=O and C=N) and 2300-3200 $cm^{-1}$ (OH and/or NH) regions, while NMR spectra possessed a one-proton broad singlet between $\delta$10.4 - 11.2 (OH) which exchanged with $D_2O$. The mass spectra of these derivatives were

consistent with their respective assigned structure and are discussed in the mass spectrometry section of this thesis. In addition, each of the 1-hydroxyquinoxalin-2-ones gave a dark blue color with alcoholic ferric chloride consistent with the presence of an intact hydroxamic acid function.

TABLE XVI

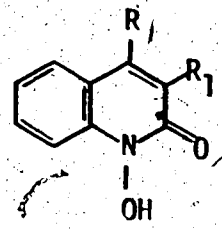
1-Hydroxyquinoxalin-2-ones



| Compound | -X-Y- | % Yield |
|---|---|---|
| 162 | $-NH-CH_2-$ | 80 |
| 163 | $-N=C(CH_3)-$ | 78 |
| 164 |  | 95 |

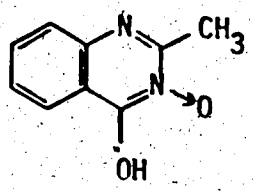
## II. MASS SPECTROMETRY

Coutts and Hindmarsh (1969) found that on electron impact, simple quinoline hydroxamic acids (165) lose an oxygen atom and an OH radical from the molecular ion with the resultant $[M-16]^{\ddagger}$ and $[M-17]^{+}$ ions decomposing further by the expulsion of CO or HCN molecules.



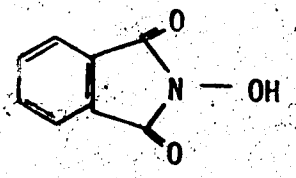

(165)

In contrast, these same authors observed that 4-hydroxy-2-methyl-quinazoline-3-oxide (166) expelled a nitric oxide radical from the molecular ion to give an abundant $[M-30]^{+}$ ion. N-Hydroxyphthalimide




(166)

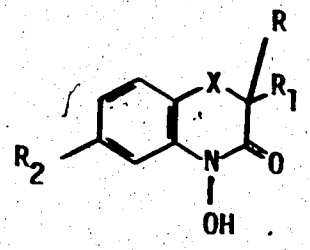
(167) was found by Bowie et al. (1969) to also expel a nitric oxide




(167)

radical. In these two instances, $[M-16]^{+\cdot}$ and $[M-17]^{+}$ ions were also observed, although the abundances of the latter ion were very low.

These findings led Coutts and Hindmarsh (1970) to extend their studies to cyclic hydroxamic acids of general structure (168),




(168)

in which X was O or S or $SO_2$. Although all three classes of compounds showed abundant molecular ions, each fragmented initially in distinctive ways. The 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazines (168, X = O) exhibited abundant $[M-45]^{+}$ ions and $[M-16]^{+\cdot}$ ions generally of low abundance; $[M-17]^{+}$ ions were not formed. The 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazines (168, X = S) showed strong $[M-16]^{+\cdot}$, $[M-17]^{+}$ and $[M-45]^{+}$ ions, whereas the molecular ions of the chemically related 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine-1,1-dioxides (168, X = $SO_2$) decomposed to give abundant $[M-16]^{+\cdot}$ and $[M-RR'C=C=O]^{+\cdot}$ ions, but neither $[M-17]^{+}$ nor $[M-45]^{+}$ ions were formed. Further decompositions were influenced by the nature of the substituents on each molecule. None of the compounds examined expelled nitric oxide from the molecular ion.

As a logical extension of these investigations, the mass spectra of the 1-hydroxyquinoxalin-2-one 4-oxides (Table XIV) and 1-hydroxyquinoxalin-2-ones (Table XVI) prepared in the present study were recorded and examined. The mass spectra of these compounds are included in Figures V and VI. Summarized in Table XVIIA are the relative abundances of the $[M]^{+\cdot}$, $[M-16]^{+\cdot}$, $[M-17]^{+}$, $[M-45]^{+}$ and $[M-28]^{+}$ ions for these compounds. As can be seen, all showed abundant molecular ions. Furthermore, each compound gave abundant $[M-16]^{+\cdot}$, $[M-17]^{+}$ and $[M-45]^{+}$ ions except 1-hydroxyquinoxalin-2-one 4-oxide (159) which had only an abundant $[M-16]^{+\cdot}$ ion. Only compounds (159), (163) and (164) gave abundant $[M-28]^{+\cdot}$ ions presumably by the loss of carbon monoxide. Thus the majority of these cyclic hydroxamic acids fragment initially in a manner analogous to the 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazines (168, X=S) studied by Coutts and Hindmarsh (1970).

TABLE XVIIA

Relative Abundance of $[M]^{+\cdot}$, $[M-16]^{+\cdot}$, $[M-17]^{+}$, $[M-45]^{+}$ and $[M-28]^{+\cdot}$ Ions and Location of Base Peaks in the Mass Spectra of 1-Hydroxyquinoxalin-2-one 4-oxides and 1-Hydroxyquinoxalin-2-ones

| Compound | Percentage Relative Abundance | | | | | |
|---|---|---|---|---|---|---|
| | $[M]^{+\cdot}$ | $[M-16]^{+\cdot}$ | $[M-17]^{+}$ | $[M-45]^{+}$ | $[M-28]^{+\cdot}$ | Base Peak |
| 159 | 100 | 11.3 | 0.2 | 1.6 | 63.2 | $M^{+\cdot}$ |
| 160 | 100 | 10.2 | 32.3 | 38.3 | 0.1 | $M^{+\cdot}$ |
| 161 | 100 | 21.5 | 62.0 | 31.6 | 2.5 | $M^{+\cdot}$ |
| 162 | 68.3 | 6.8 | 14.2 | 100 | 0.2 | $[M-45]^{+}$ |

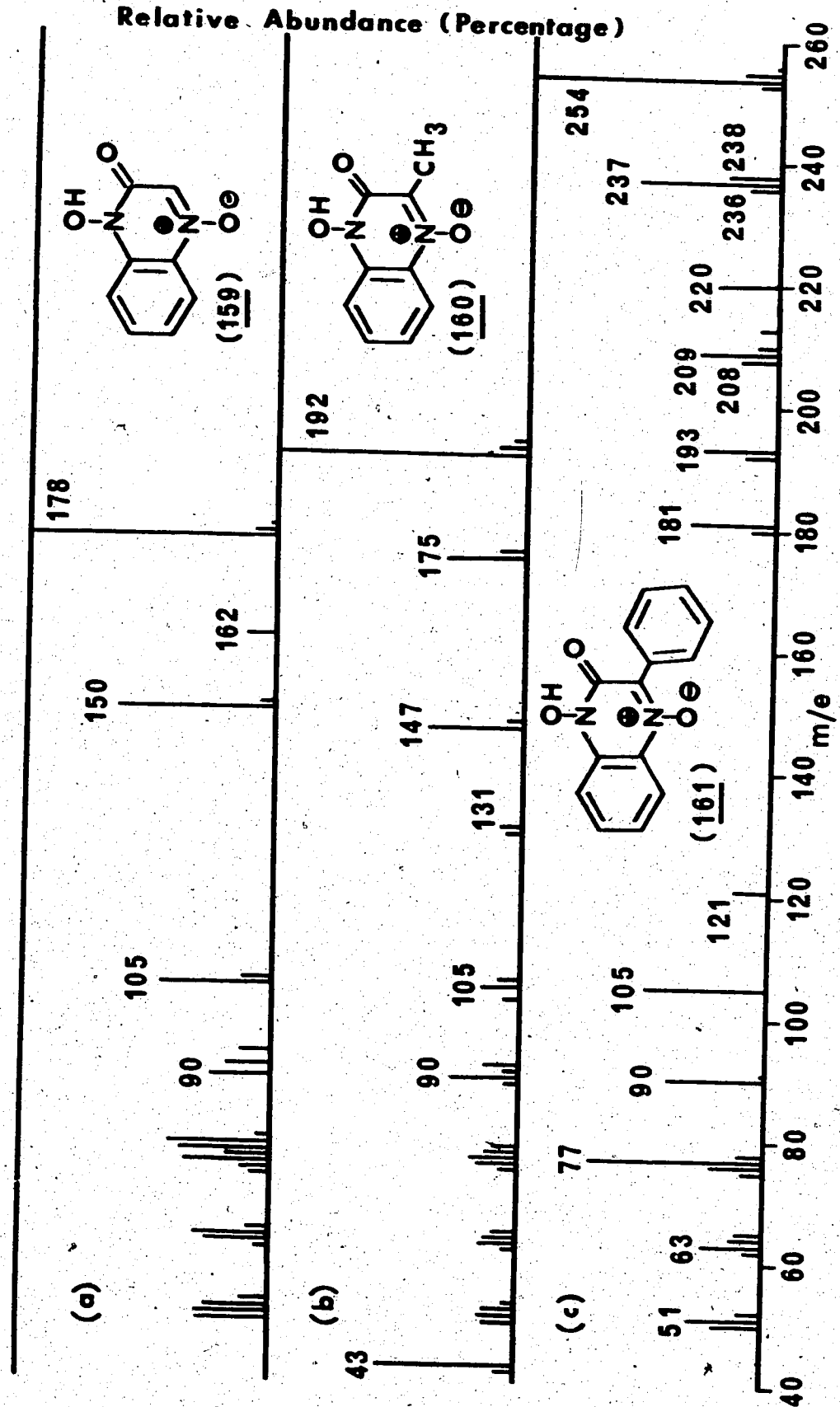



Figure V : Mass spectra of (a) 1-hydroxyquinoxalin-2-one 4-oxide (159), (b) 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (160) and (c) 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161).

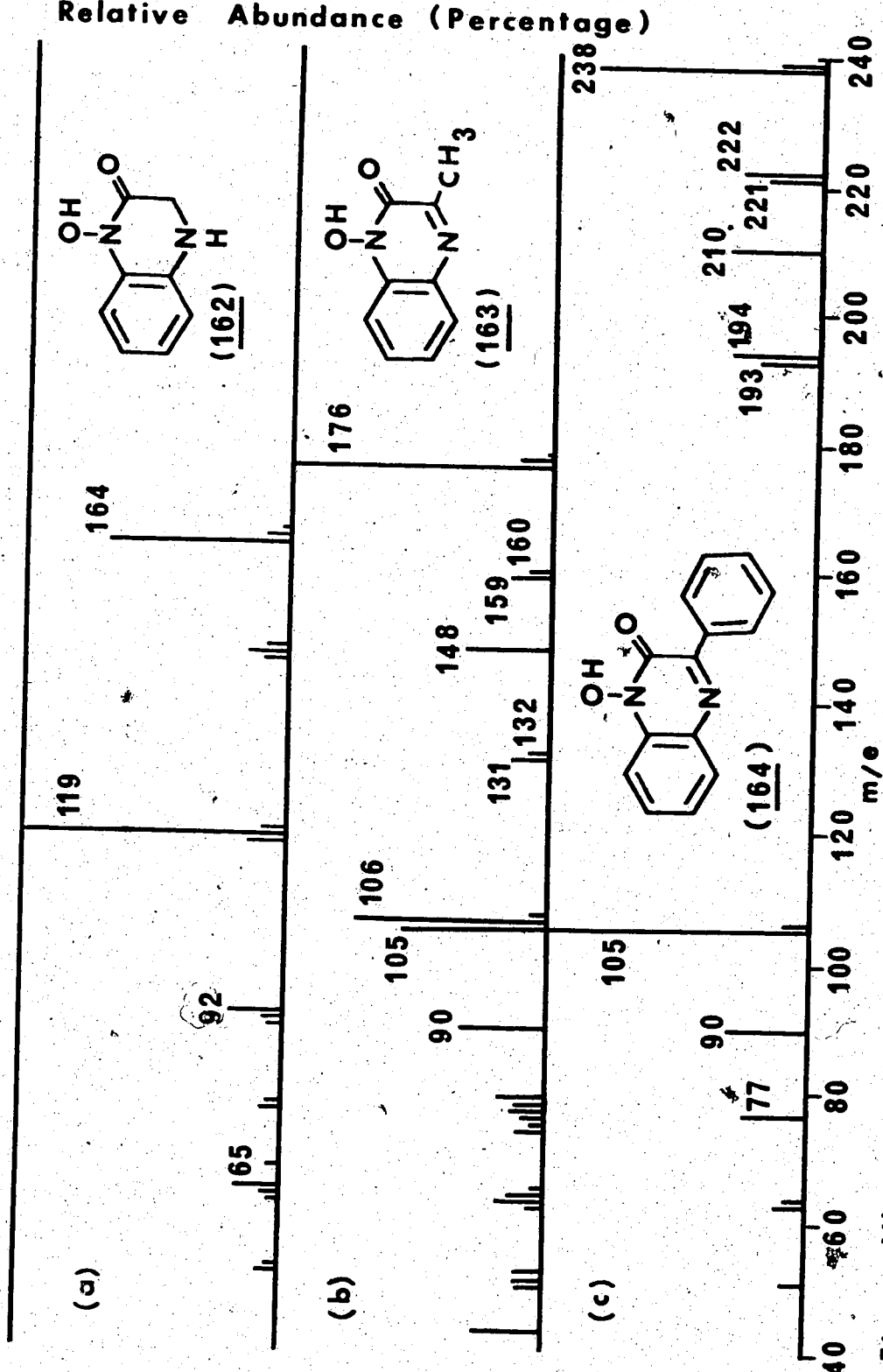
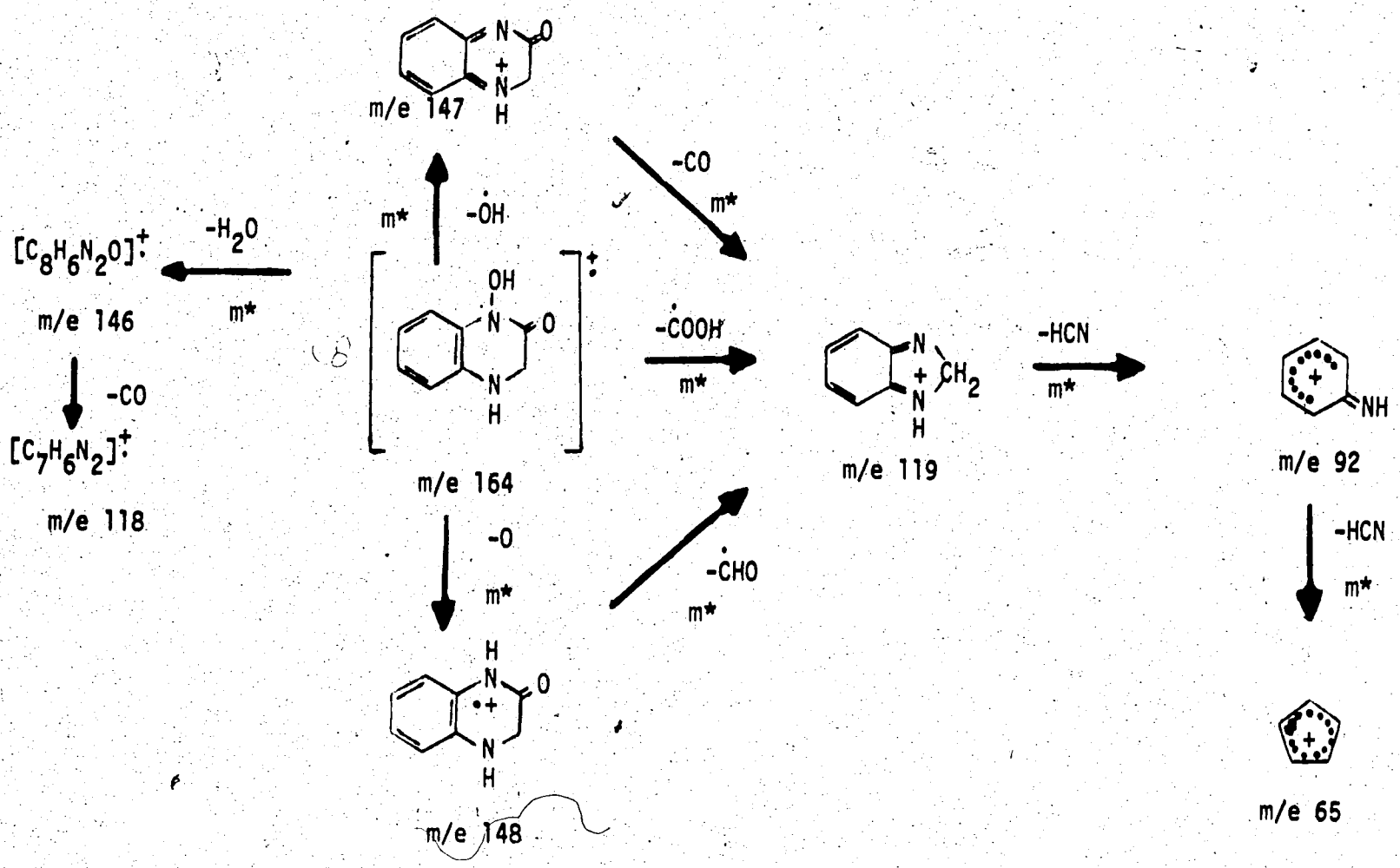



Figure VI : Mass Spectra of (a) 3,4-dihydro-1-hydroxyquinoxalin-2-one (162), (b) 3-methyl-1-hydroxyquinoxalin-2-one (163) and (c) 3-phenyl-1-hydroxyquinoxalin-2-one (164).

Relative Abundance of $[M]^{+\cdot}$, $[M-16]^{+\cdot}$, $[M-17]^{+}$, $[M-45]^{+}$ and $[M-28]^{+\cdot}$ Ions and Location of Base Peaks in the Mass Spectra of 1-Hydroxyquinoxalin-2-one 4-oxides and 1-Hydroxyquinoxalin-2-ones

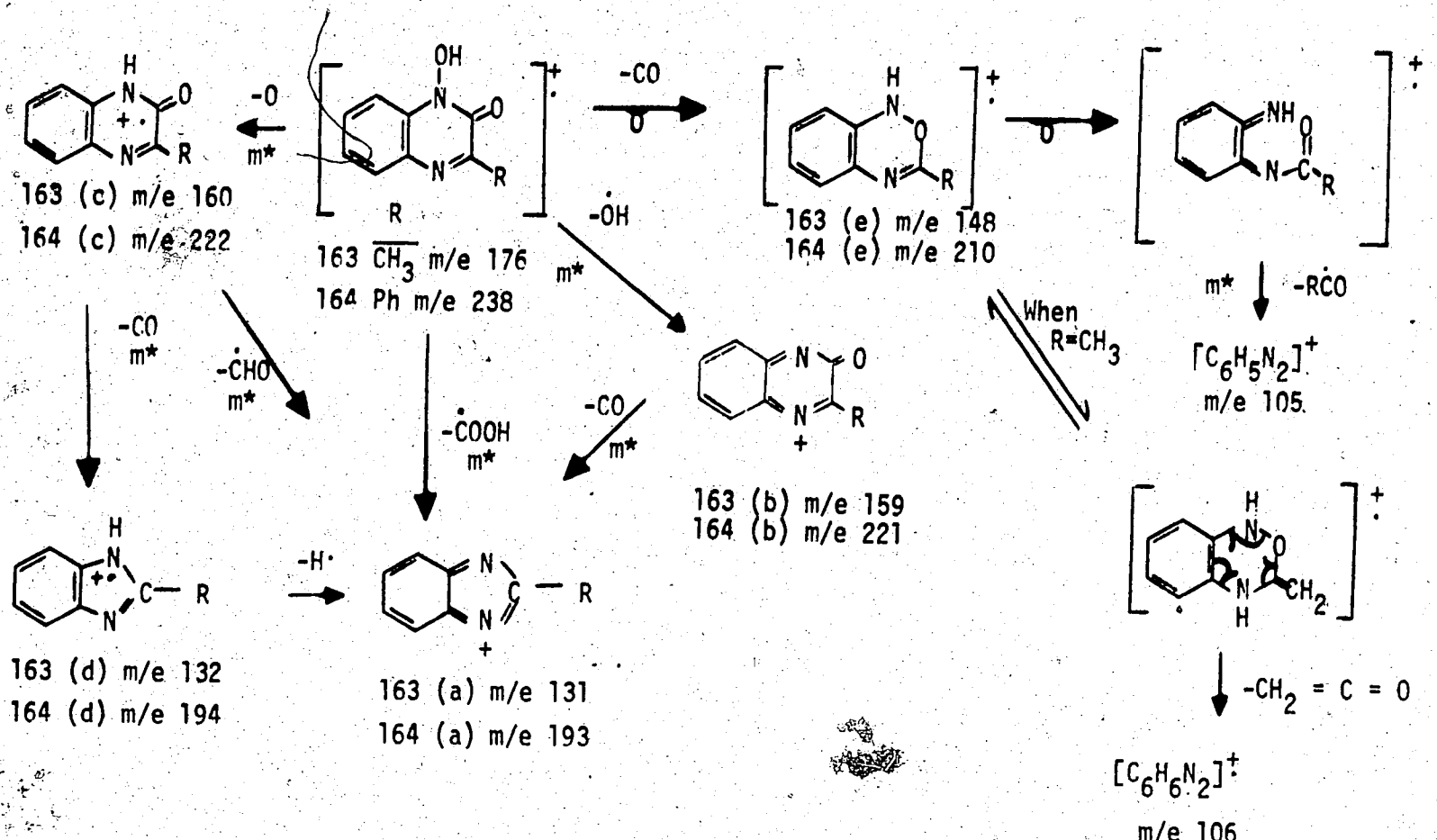
| Compound | Percentage Relative Abundance | | | | | |
|---|---|---|---|---|---|---|
| | $[M]^{+\cdot}$ | $[M-16]^{+\cdot}$ | $[M-17]^{+}$ | $[M-45]^{+}$ | $[M-28]^{+\cdot}$ | Base Peak |
| 163 | 100 | 7.5 | 14.7 | 14.1 | 31.3 | $M^{+\cdot}$ |
| 164 | 84.8 | 29.1 | 20.0 | 23.6 | 33.9 | $[M-133]^{+}$ |

Scheme I presents a possible fragmentation pathway for 3,4-dihydro-1-hydroxyquinoxalin-2-one (162). An abundant molecular ion of m/e 164 was formed which fragmented to ions of m/e 119, 146, 147 and 148 by the loss of COOH, $H_2O$, OH and O, respectively. Both ions m/e 147 and 148 then fragmented to give ion m/e 119 by the loss of CO and CHO, respectively. Subsequently, ion m/e 119 decomposed through the successive loss of two HCN molecules to give ions m/e 92 and 65. Although all the above described fragmentations are apparently supported by the presence of metastable peaks of appropriate mass, the assignments made for the structure of the various ions must remain very tentative until accurate mass measurements are determined, labelling experiments carried out and further directly related standard compounds investigated.



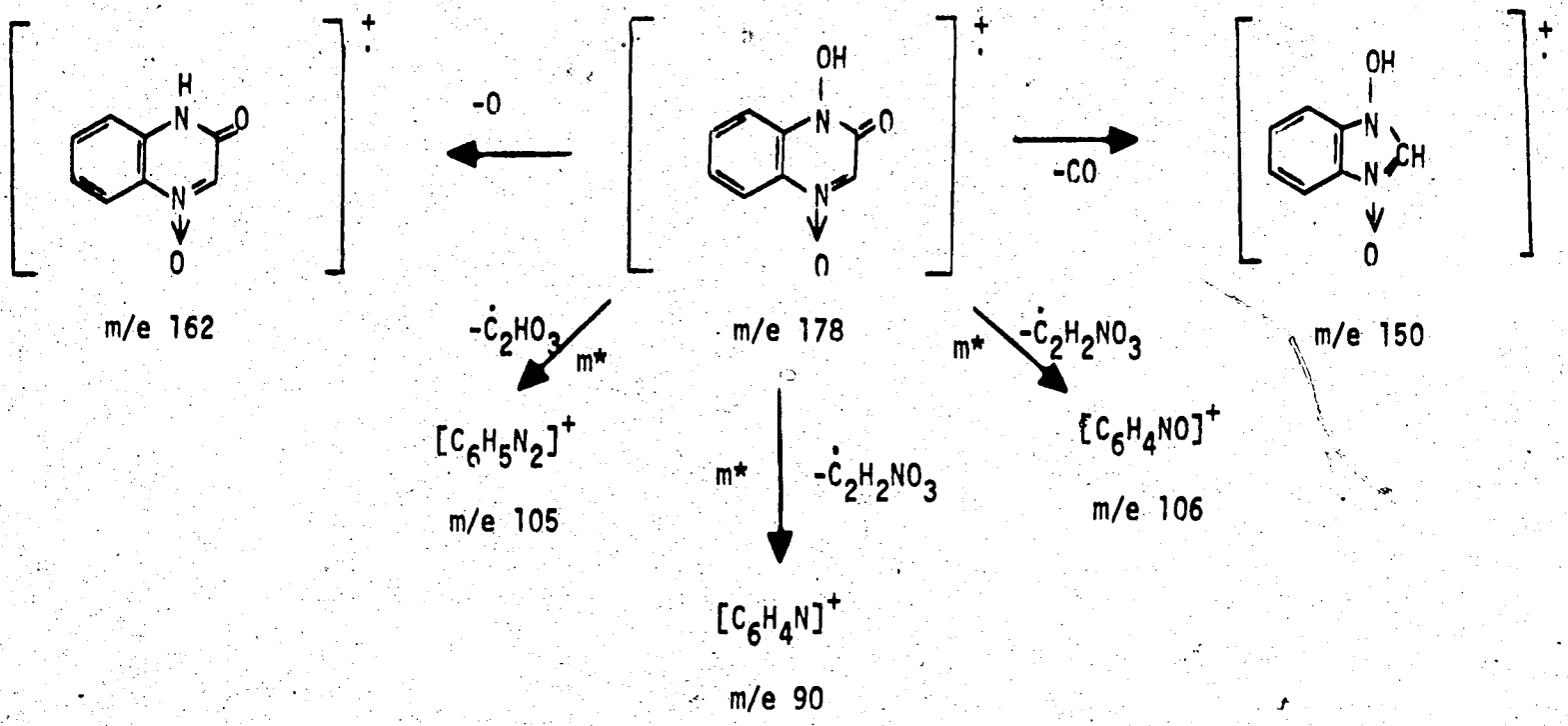

Scheme I: Fragmentation pathway for 3,4-dihydro-1-hydroxyquinoxalin-2-one (162).

Scheme II depicts a possible pathway for the fragmentation of 3-methyl-1-hydroxyquinoxalin-2-one (163) and 3-phenyl-1-hydroxy-quinoxalin-2-one (164). As can be seen, the molecular ion in both cases may fragment by the loss of O, $\dot{O}H$ and $\dot{C}OOH$ to give abundant $[M-16]^{+\cdot}$, $[M-17]^{+}$ and $[M-45]^{+}$ ions. The $[M-16]^{+\cdot}$ and $[M-17]^{+}$ ions may then fragment by the loss of $\dot{C}HO$ and CO, respectively, to give an $[M-45]^{+}$ ion. Next, the $[M-45]^{+}$ ion may decompose by the loss of $\dot{R}CN$, then HCN to give ions m/e 90 and 63, respectively. Once again, it must be pointed out that although each fragmentation described has an appropriate metastable peak supporting it, the structural designations made in Scheme II must remain merely speculative until further studies are done. One notable difference between the mass spectra of the two 3-substituted derivatives described in Scheme II was that the 3-methyl (163) compound had an abundant ion with m/e 106; whereas, the 3-phenyl (164) compound did not (Figures V and VI). A tentative suggestion to account for this difference is illustrated in Scheme II. Thus, although both compounds may rearrange upon losing carbon monoxide from the molecular ion to give an $[M-28]^{+\cdot}$ ion which is capable of yielding the ion m/e 105 by the loss of $\dot{R}CO$, only the 3-methyl (163) derivative may tautomerize as illustrated, then decompose through the loss of $CH_2 = C = O$ to give an m/e 106 ion.




Scheme II: Fragmentation pathway for 3-methyl-1-hydroxyquinoxalin-2-one (163) and 3-phenyl-1-hydroxyquinoxalin-2-one (164).

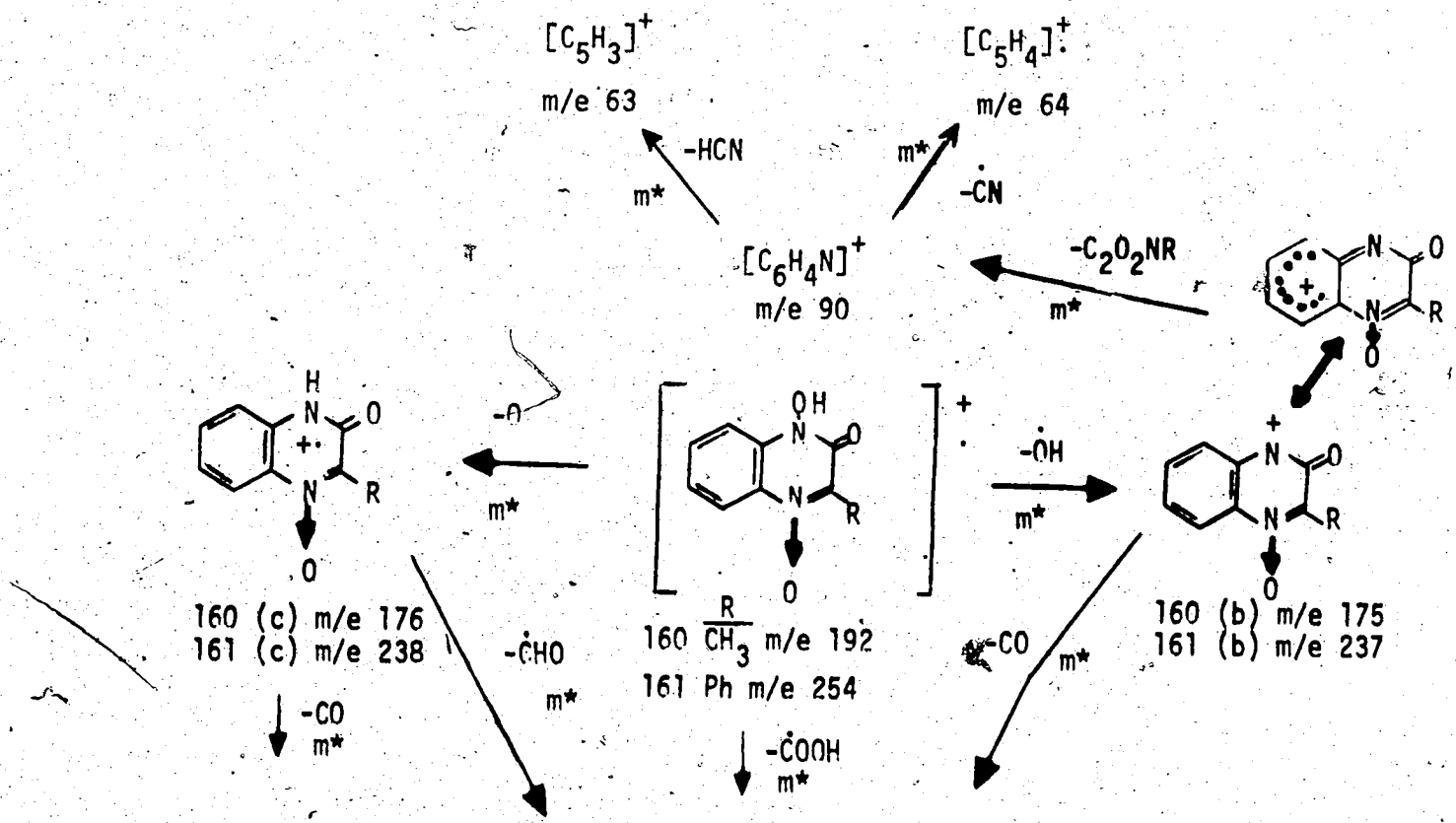
Table XVII A shows that unlike the three 1-hydroxyquinoxalin-2-ones just described, 1-hydroxyquinoxalin-2-one 4-oxide (159) did not give rise to abundant $[M-17]^+$ or $[M-45]^+$ ions. Apparently, the presence of the N-oxide function decreased the importance of fragmentation by the initial loss of an OH radical or COOH radical. However, abundant $[M]^{+\cdot}$, $[M-16]^{+\cdot}$ and $[M-28]^{+\cdot}$ ions were formed. Outlined in Scheme III the tentative fragmentation pathway postulated for 1-hydroxyquinoxalin-2-one 4-oxide (159). Although supportive metastable peaks were absent, the $[M-16]^{+\cdot}$ and $[M-28]^{+\cdot}$ ions were presumably formed in the same manner as for the 1-hydroxyquinoxalin-2-ones by the direct loss of oxygen and carbon monoxide from the molecular ion. Appropriate metastable peaks were present which apparently supported direct fragmentation of the molecular ion to ions of m/e 106, 105 and 90 through the loss of $C_2H_2NO_2$, $C_2HO_3$ and $C_2H_2NO_3$ radicals, respectively. Due to their complexity, the structures of both these radicals and the resultant ions can not be readily ascertained until accurate mass and labelling studies are carried out. However, in contrast, for all other derivatives, fragment ions of m/e 106, 105 and 90 always arose as the result of further fragmentation of $[M-16]^{+\cdot}$, $[M-17]^+$, $[M-45]^+$ or $[M-28]^{+\cdot}$ ions (Schemes I, II, IV).




Scheme III: Fragmentation pathway for 1-hydroxyquinoxalin-2-one 4-oxide (159).

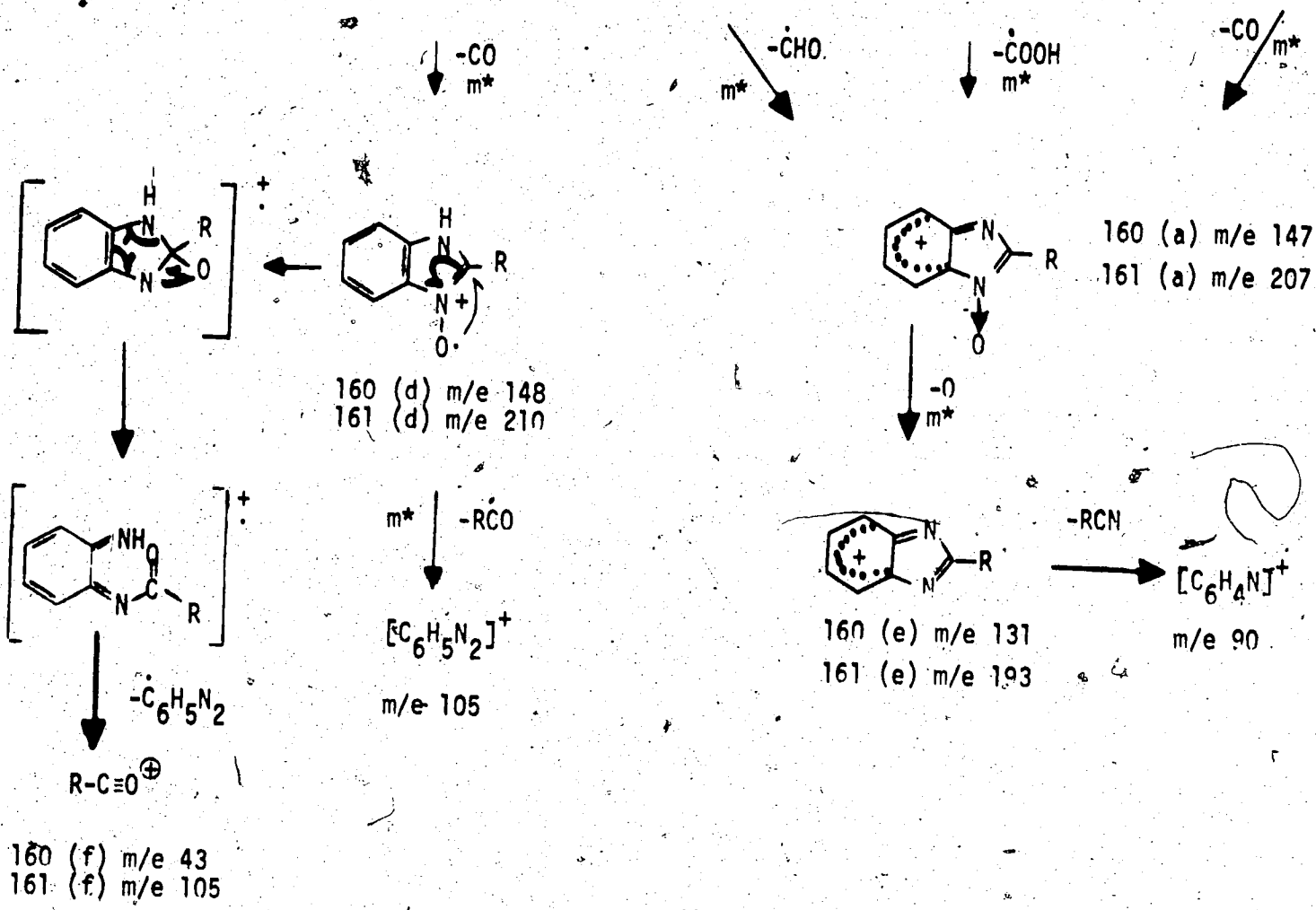
In contrast to 1-hydroxyquinolin-2-one 4-oxide (159), both 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (160) and 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161) did give abundant $[M]^{+\cdot}$, $[M-16]^{+\cdot}$, $[M-17]^{+}$ and $[M-45]^{+}$ ions (Table XVIIA). Occurrence of these fragments was parallel to the observations for the mass spectra of the 1-hydroxyquinoxalin-2-ones. Scheme IV is an attempt to explain some of the fragmentation pathway followed by 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (160) and 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161) though accurate mass measurements and labelling studies are obviously required to substantiate some of the claims. It is noticeable that the 3-substituted 1-hydroxyquinoxalin-2-one 4-oxides (Figure V ) did not give rise to abundant $[M-28]^{+\cdot}$ ions whereas the unsubstituted 4-oxide did (Figure V ). This behavior should be contrasted with that of the related compounds (163) and (164) which expel 28 mass units from the molecular ion. Unfortunately, the small number of compounds examined permit no useful conclusions to be drawn as to why these differences should exist.

In summary, the 1-hydroxyquinoxalin-2-one 4-oxides and 1-hydroxyquinoxalin-2-ones prepared in the present investigation fragmented initially in a manner analagous to the 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazines (168, X = S) investigated by Coutts and Hindmarsh (1970). That is, each compound, except 1-hydroxyquinoxalin-2-one 4-oxide (159) gave abundant $[M]^{+\cdot}$, $[M-16]^{+\cdot}$, $[M-17]^{+}$ and $[M-45]^{+}$ ions (Table XVII A). In certain cases, an additional initial $[M-28]^{+\cdot}$ fragment ion was also formed.




Scheme IV: Fragmentation pathway for 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide (160) and 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide (161).

Scheme 4: cont'd



## III. EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All melting points and boiling points are uncorrected. Most of the compounds synthesized were dried over Drierite under reduced pressure in an acetone reflux heated pistol dessicator. Infrared spectra were recorded on a Beckman Model 10 Infrared Spectrophotometer. NMR spectra were run on a Varian Model A-60D Spectrometer. Mass spectra were recorded on an A.E.I. MS 9 or MS 12 instrument equipped with a direct probe system; the electron beam energy was 70 eV. Elemental analyses were performed by the analytical laboratory of the Faculty of Pharmacy and Pharmaceutical Sciences. All chemicals obtained from commercial sources were used without further purification.

### Synthesis of 1-Monosubstituted Piperazines

### General Preparative Methods

Each of the N-monosubstituted piperazines prepared in this series was characterised by means of IR and NMR spectra, as well as by comparison of boiling points with literature values. Commercially available 1-monosubstituted piperazines included 1-methylpiperazine and 1-phenylpiperazine, which were purchased from Eastman Organic Chemicals.

Method Used - (Yung *et al.* 1968)

The appropriate alkyl halide (0.2 mole) was slowly added with stirring over 30 minutes to a solution of anhydrous piperazine (0.56 mole) in absolute ethanol (250 ml). The reaction mixture

was either let stand at room temperature overnight or refluxed overnight. The solution was then cooled and any precipitated piperazine hydrochloride filtered off. The bulk of the ethanol was removed *in vacuo*. The residue obtained was partitioned between excess 3N NaOH and diethyl ether (3 x 50 ml). The ether fractions were combined, dried over anhydrous sodium sulfate, filtered and the ether evaporated. The oil obtained was fractionally distilled under reduced pressure.

1-Ethylpiperazine

The title compound was synthesized in 56% yield by general method 1 for the synthesis of 1-monosubstituted piperazines using anhydrous piperazine and ethyl bromide. The reaction mixture was refluxed overnight. The product had b.p. 150-156° (760 mm). Reported (Yung *et al.* 1968), b.p. 57-60° (40 mm).

IR spectrum (film): 3270 $cm^{-1}$ (NH).

1-*n*-Propylpiperazine

The above compound was synthesized in 43% yield by general method 1 for the synthesis of 1-monosubstituted piperazines using anhydrous piperazine and 1-bromopropane. The reaction mixture was refluxed overnight. The product had b.p. 65-88° (24 mm). Reported (Yung *et al.* 1968), b.p. 75-77° (37 mm).

IR spectrum (film): 3270 $cm^{-1}$ (NH).

1-n-Butylpiperazine

General method 1 for synthesis of 1-monosubstituted piperazines gave the title compound in 61% yield from anhydrous piperazine and 1-bromobutane. The reaction mixture was refluxed overnight. The product had b.p. 95-100° (27 mm). Reported (Yung et al. 1968), 97-100° (40 mm).

IR spectrum (film): 3270 $cm^{-1}$ (NH).

1-Benzylpiperazine

Utilizing general method 1 for the synthesis of 1-monosubstituted piperazines, the above compound was obtained in 57% yield from anhydrous piperazine and benzyl chloride. The reaction mixture was let stand overnight at room temperature. The product had b.p. 160-170° (19 mm). Reported (Lutz and Shearer, 1947), b.p. 128-135° (6 mm).

IR spectrum (film): 3270 $cm^{-1}$ (NH).

NMR spectrum ($CCl_4$): δ 1.42 (s, 1, NH, absent after $D_2O$ exchange), 2.0-3.0(m, 8, $C_4H_8N_2$), 3.38 (s, 2, $CH_2N$), 7.20 (s, 5, $C_6H_5$).

1-Phenethylpiperazine

When general method 1 for the synthesis of 1-monosubstituted piperazines was used, the title compound was obtained in 75% yield from anhydrous piperazine and (2-chloroethyl)benzene. The reaction mixture was let stand overnight at room temperature. The product had b.p. 165-172° (16 mm). Reported (Baltzly et al. 1944),

b.p. 150-152°(8 mm).

IR spectrum (film): 3270 $cm^{-1}$ (NH).

NMR spectrum ($CCl_4$): δ 1.50 (s, 1, NH, absent after $D_2O$ exchange), 2.2-2.9 (m, 12, $CH_2CH_2C_4H_8N_2$), 7.12 (s, 5, $C_6H_5$).

## Synthesis of Amino-esters

### General Preparative Methods

Each of the esters prepared in this series was characterized by means of CHN elemental analysis, NMR and IR spectra. IR spectra were recorded as Nujol mulls or as thin films. NMR spectra were recorded at operating temperature employing a sample concentration of 100 mg/0.5 ml, with DSS used as the standard when $D_2O$ was the solvent. In all other solvents the reference was TMS. Chemical shifts are quoted in $\delta$ units. No attempt has been made to calculate the theoretical resonance positions of protons in complex second order spin systems. The quoted multiplet shift values indicate the center position of the multiplet. A chemical shift range is quoted for complex, unsymmetrical multiplets. Since most syntheses included a secondary amine as a starting material, absence of a band within the range of 3310 to 3500 $cm^{-1}$ (due to secondary amine NH strech) in the IR spectrum was considered a positive indication that the reaction had gone to completion. No attempts were made to separate optical or stereoisomers.

### Method 1

Methyl acrylate, methyl crotonate or methyl methacrylate

(0,1 mole) was added to a solution of an equimolar quantity of the appropriate amine in anhydrous methanol (25-75 ml). The reaction mixture was heated under reflux with stirring for 8 to 72 hours. The methanol was removed in vacuo, and the residual oil fractionally distilled under reduced pressure.

Method 2

Ethyl acrylate (0.1 mole) was added to a solution of an equimolar quantity of the appropriate amine in anhydrous ethanol (25-75 ml). The reaction mixture was heated under reflux with stirring for one to four days. The ethanol was removed in vacuo, and the residual oil fractionally distilled under vacuum.

Method 3

Ethyl bromoacetate, ethyl chloroformate or ethyl 4-bromobutyrate (0.1 mole) was added to a solution of triethylamine (0.1 mole) and the appropriate amine (0.1 mole) in anhydrous ethanol (100 ml). The reaction mixture was heated under reflux with stirring for one to six days. The reaction solution was cooled then poured into excess anhydrous ether (300 ml). The precipitated triethylamine halide salt was filtered off. The filtrate was evaporated in vacuo and the residual oil fractionally distilled. In those cases where the residue was a solid, the minimum amount of hot ethanol to effect dissolution was added and HCl gas bubbled through this solution. The colorless HCl salts obtained upon cooling were recrystallized from anhydrous ethanol.

Method 4

Methyl methacrylate or methyl crotonate (0.115 mole) was added to a solution of anhydrous piperazine (0.23 mole) in anhydrous methanol. The reaction mixture was refluxed for 12 to 24 hours. The methanol was removed in vacuo. The residue obtained was cooled and the excess piperazine filtered off. This filtrate was distilled under reduced pressure.

Preparation of Hydrochloride Salts of the Amino-Esters

The hydrochloride salts of the methyl esters were obtained by adding a solution of dry hydrogen chloride gas in anhydrous methanol to an anhydrous methanol solution of the appropriate ester. If upon cooling the salt did not crystallize, sufficient anhydrous ether was added to initiate crystallization. The hydrochlorides were recrystallized from methanol-ether.

The hydrochloride salts of the ethyl esters were obtained in the same manner, substituting anhydrous ethanol in place of anhydrous methanol.

All salts were obtained as colorless solids which were characterized by their infrared spectra and melting points. All showed strong $\overset{\oplus}{N}$-H stretching bands in the 2360-2790 $cm^{-1}$ region (Heacock and Marion, 1956).

Methyl 2-methyl-3-(1-piperazinyl)propionate

Utilizing general method 4 for the synthesis of aminoesters, the title compound was obtained in 45% yield from methyl methacrylate and piperazine. The reaction mixture was refluxed for

12 hours. The product had b.p. 65-74° (0.08 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O), 3330 $cm^{-1}$ (NH).

NMR spectrum ($CCl_4$): δ 1.06 (d, 3, J = 6.5 Hz, $CHC\underline{H}_3$), 1.24 (s, 1, N$\underline{H}$, absent after addition of $D_2O$), 2.0-2.9 (m, 11, $C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}$), 3.56 (s, 3, $OC\underline{H}_3$).

The <u>hydrochloride</u> of the above compound had m.p. 179-180°.

Analysis - Found: C, 41.42; H, 7.48; N, 10.73.

$C_9H_{20}Cl_2N_2O_2$ requires: C, 41.71; H, 7.77; N, 10.81.

<u>Methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate</u>

General method 1 for amino-ester synthesis gave the title compound in 30% yield from methyl methacrylate and 1-methylpiperazine when the reaction mixture was heated at reflux for eight hours. The product had b.p. 157-158° (70 mm). Reported (Barron <u>et al.</u> 1965), b.p. 60-62° (0.5 mm); (French Patent, 1958), b.p. 105-106° (11 mm); (Midha, 1969), b.p. 85-86° (3 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($D_2O$): δ 1.28 (d, 3, J = 7.0 Hz, $CHC\underline{H}_3$), 3.06 (s, 3, $NC\underline{H}_3$), 3.10-4.10 (m, 14, $C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}(CH_3)COOC\underline{H}_3$).

NMR spectrum ($CCl_4$): δ 1.08 (d, 3, J = 6.5 Hz, $CHC\underline{H}_3$), 2.18 (s, 3, $NC\underline{H}_3$), 2.00-2.80 (m, 11, $C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}$), 3.63 (s, 3, $OC\underline{H}_3$).

The <u>hydrochloride</u> of the title compound recrystallized from anhydrous methanol melted at 206-207°.

Analysis - Found: C, 44.16; H, 8.17; N, 10.46.

$C_{10}H_{22}Cl_2N_2O_2$ requires; C, 43.93: H, 8.11; N, 10.26.

Methyl 2-methyl-3-[1-(4-ethylpiperazinyl)]propionate

The title compound was synthesized in 32% yield by general method 1 for the synthesis of amino-esters using methyl methacrylate and 1-ethylpiperazine. The reaction mixture was refluxed for 16 hours. The product had b.p. 144-148° (24 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 207-209°.

NMR spectrum ($D_2O$): δ 1.30 (d, 3, J = 7.5 Hz, $CHC\underline{H}_3$), 1.36 (t, 3, J = 7.5 Hz, $CH_2C\underline{H}_3$), 3.2-3.9 (m, 16, $CH_3C\underline{H}_2C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}CO_2C\underline{H}_3$).

Analysis - Found: C, 45.92; H, 8.27; N, 10.05.

$C_{11}H_{24}Cl_2N_2O_2$ requires: C, 46.00; H, 8.42; N, 9.75.

Methyl 2-methyl-3-[1-(4-n-propylpiperazinyl)]propionate

When general method 1 for the synthesis of amino-esters was employed, the title compound was prepared in 51% yield from methyl methacrylate and 1-n-propylpiperazine. The reaction mixture was refluxed for 12 hours. The product had b.p. 146-156° (23 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 212.5-213.5°.

NMR spectrum ($D_2O$): δ 0.96 (t, 3, J = 6.5 Hz, $CH_2C\underline{H}_3$), 1.28 (d, 3, J = 7.0 Hz, $CHC\underline{H}_3$), 1.5-2.1 (m, 2, $CH_2C\underline{H}_2CH_3$), 3.1-4.0 (m, 16, $C\underline{H}_2C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}CO_2C\underline{H}_3$).

Analysis - Found: C, 47.70; H, 8.82; N, 9.32.

$C_{12}H_{26}Cl_2N_2O_2$ requires: C, 47.84; H, 8.70; N, 9.30.

Methyl 2-methyl-3-[1-(4-n-butylpiperazinyl)]propionate

The above compound was synthesized in 46% yield from methyl methacrylate and 1-n-butylpiperazine by general method 1 for the synthesis of amino-esters with refluxing for 12 hours. The product had b.p. 158-164° (24 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 215-215.5°.

NMR spectrum ($D_2O$): δ 0.7-2.1 (m, 10, $CH_3CH_2CH_2CH_2$ and $CHCH_3$), 2.8-4.1 (m, 16, $CH_2C_4H_8CH_2CHCO_2CH_3$).

Analysis - Found: C, 49.81; H, 8.69; N, 8.68.
$C_{13}H_{28}Cl_2N_2O_2$ requires: C, 49.53; H, 8.95; N, 8.88.

Methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate

Following the procedure of general method 1 for amino-ester synthesis, the title compound was obtained in 51% yield from methyl methacrylate and 1-phenylpiperazine. The reaction mixture was refluxed for 12 hours. The product had b.p. 140-145° (0.05 mm).

IR spectrum (film): 1738 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 210-211°.

NMR spectrum ($D_2O$): δ 1.40 (d, 3, J = 7.0 Hz, $CHCH_3$), 3.1-4.3 (m, 14, $C_4H_8N_2CH_2CHCH_3CO_2CH_3$), 7.66 (s, 5, $C_6H_5$).

Analysis - Found: C, 53.91; H, 7.11; N, 8.48.
$C_{15}H_{24}Cl_2N_2O_2$ requires: C, 53.74; H, 7.22; N, 8.35.

Methyl 2-methyl-3-[1-(4-benzylpiperazinyl)]propionate

Employing general method 1 for the synthesis of

amino-esters, the above compound was prepared in 47% yield from methyl methacrylate and 1-benzylpiperazine. The reaction mixture was refluxed for eight hours. The product had b.p. 140-144° (0.4 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 231.5-232.5°.

NMR spectrum ($D_2O$): δ 1.31 (d, 3, J = 7.0 Hz, $CHCH_3$), 2.9-4.0 (m, 14, $C_4H_8N_2CH_2CHCO_2CH_3$), 4.54 (s, 2, $C_6H_5CH_2$), 7.59 (s, 5, $C_6H_5$).

Analysis - Found: C, 55.05; H, 7.47. $C_{16}H_{26}Cl_2N_2O_2$ requires: C, 55.02; H, 7.50.

Methyl 2-methyl-3-[1-(4-phenethylpiperazinyl)]propionate

The title compound was prepared in 57% yield by general method 1 for amino-ester synthesis from methyl methacrylate and 1-phenethylpiperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 152-157° (0.4 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 225-225.5°.

NMR spectrum ($D_2O$): δ 1.34 (d, 3, J = 7.0 Hz, $CHCH_3$), 3.0-4.1 (m, 18, $CH_2CH_2C_4H_8N_2CH_2CHCO_2CH_3$), 7.42 (s, 5, $C_6H_5$).

Analysis - Found: C, 56.23; H, 7.65; N, 7.95. $C_{17}H_{28}Cl_2N_2O_2$ requires: C, 56.20; H, 7.76; N, 7.70.

Methyl 2-methyl-3-[1-(4-p-hydroxyphenylpiperidino)]propionate

General method 1 for amino-ester synthesis gave the title

compound in 68% yield from methyl methacrylate and 4-(p-hydroxyphenyl)-piperidine when the reaction mixture was heated at reflux for 72 hours. The product had b.p. 158-162°(0.5 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O), 3260 $cm^{-1}$ (OH).

The hydrochloride of the above compound had m.p. 176-178°.

NMR spectrum (DMSO-$d_6$): δ 1.30 (d, 3, J = 6.5 Hz, $CHCH_3$), 1.5-4.6 (m, 15, $C_5H_9NCH_2CHCH_3CO_2CH_3$), 5.58 (s, 1, $\overset{\oplus}{N}H$, absent after $D_2O$ exchange), 7.1-7.9 (m, 4, $C_6H_4$), 11.22 (s, 1, OH, absent after $D_2O$ exchange).

Analysis - Found: C, 61.01; H, 7.73; N, 4.52.
$C_{16}H_{24}ClNO_3$ requires: C, 61.24; H, 7.71; N, 4.46.

Methyl 2-methyl-3-[1-(4-benzylpiperidino)]propionate

The title compound was synthesized in 91% yield by general method 1 for the synthesis of amino-esters using methyl methacrylate and 4-benzylpiperidine. The reaction mixture was refluxed for 48 hours. The product had b.p. 152-155°.

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.04 (d, 3, J = 7.0 Hz, $CHCH_3$), 1.2-3.1 (m, 14, $CH_2C_5H_9NCH_2CH$), 3.56 (s, 3, $OCH_3$), 7.12 (s, 5, $C_6H_5$).

The hydrochloride of the above compound had m.p. 127-128°.

Analysis - Found: C, 65.43; H, 8.37; N, 4.19.
$C_{17}H_{26}ClNO_2$ requires: C, 65.47; H, 8.40; N, 4.49.

Methyl 3-methyl-3-(1-piperazinyl)propionate

Following the procedure of general method 4 for the synthesis of amino-esters, the title compound was prepared

in 41% yield from methyl crotonate and anhydrous piperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 80-84° (0.09 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O), 3270 $cm^{-1}$ (NH).

NMR spectrum ($CCl_4$): δ 1.01 (d, 3, J = g.5 Hz, $CHCH_3$), 1.81 (s, 1, NH, absent after $D_2O$ exchange), 2.0-3.2 (m, 11, $C_4H_8N_2CHCH_3CH_2$), 3.58 (s, 3, $OCH_3$).

The hydrochloride of the title compound had m.p. 190-191°.

Analysis - Found: C, 41.42; H, 7.78; N, 10.51.

$C_9H_{20}Cl_2N_2O_2$ requires: C, 41.71; H, 7.77; N, 10.81.

Methyl 3-methyl-3-[1-(4-methylpiperazinyl)]propionate

General method 1 for amino-ester synthesis gave the title compound in 40% yield from methyl crotonate and 1-methylpiperazine when the reaction mixture was heated at reflux for 10 hours. The product had b.p. 123-127° (13 mm).

IR spectrum (film): 1738 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 217-218°.

NMR spectrum ($D_2O$): δ 1.46 (d, 3, J = 7.0 Hz $CHCH_3$), 2.92 (d, 2, J = 4.0 Hz $CHCH_2$), 3.08 (s, 3, $CH_3N$), 3.5-4.3 (m, 12, $C_4H_8N_2CHCH_2CO_2CH_3$).

Analysis - Found: C, 43.61; H, 7.91; N, 10.22.

$C_{10}H_{22}Cl_2N_2O_2$ requires: C, 43.93; H, 8.11; N, 10.26.

Methyl 3-methyl-3-[1-(4-ethylpiperazinyl)]propionate

The title compound was synthesized in 57% yield by general

method 1 for the synthesis of amino-esters using methyl crotonate and 1-ethylpiperazine. The reaction mixture was refluxed for eight hours. The product had b.p. 147-153° (23 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 214-215°.

NMR spectrum ($D_2O$): δ 1.38 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 1.48 (d, 3, J = 7.0 Hz, $CH\underline{CH_3}$), 2.9-3.1 (m, 2, $CH\underline{CH_2}$), 3.41 (q, 2, J = 7.5 Hz $\underline{CH_2}CH_3$), 3.6-4.3 (m, 12, $C_4\underline{H_8}N_2\underline{CH}CH_2CO_2\underline{CH_3}$).

Analysis - Found: C, 46.24; H, 8.54. $C_{11}H_{24}Cl_2N_2O_2$ requires: C, 46.00; H, 8.42.

Methyl 3-methyl-3-[1-(4-n-propylpiperazinyl)]propionate

When general method 1 for the synthesis of amino-esters was employed, the title compound was prepared in 46% yield from methyl crotonate and 1-n-propylpiperazine. The reaction mixture was refluxed for nine hours. The product had b.p. 99-101° (0.5 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 230-231°.

NMR spectrum ($D_2O$): δ 0.98 (t, 3, J = 6.5 Hz, $CH_2\underline{CH_3}$), 1.46 (d, 3, J = 7.0 Hz, $CH\underline{CH_3}$), 1.6-2.1 (m, 2, $CH_2\underline{CH_2}CH_3$), 2.8-3.5 (m, 5, $\underline{CH_2}C_4H_8N_2\underline{CH}\underline{CH_2}$), 3.6-4.2 (m, 11, $C_4\underline{H_8}N_2CHCH_2CO_2\underline{CH_3}$).

Analysis - Found: C, 48.05; H, 8.81; N, 9.46. $C_{12}H_{26}Cl_2N_2O_2$ requires: C, 47.84; H, 8.70; N, 9.30.

Methyl 3-methyl-3-[1-(4-n-butylpiperazinyl)]propionate

Utilizing general method 1 for the synthesis of amino-esters, the above compound was obtained in 63% yield from methyl crotonate and 1-n-butylpiperazine. The reaction mixture was refluxed for 12 hours. The product had b.p. 150-162° (23 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 227-228°.

NMR spectrum ($D_2O$): δ 0.7-2.1 (m, 10, $CH_2\underline{CH_2}\underline{CH_2}\underline{CH_3}$ and $CH\underline{CH_3}$), 2.8-3.1 (m, 2, $CH\underline{CH_2}$), 3.1-3.5 (m, 2, $\underline{CH_2}N$), 3.6-4.2 (m, 12, $C_4\underline{H_8}N_2\underline{CH}\underline{CH_2}CO_2\underline{CH_3}$).

Analysis - Found: C, 49.25; H, 8.50; N, 8.92.

$C_{13}H_{28}Cl_2N_2O_2$ requires: C, 49.53; H, 8.95; N, 8.88.

Methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate

The above compound was synthesis in 59% yield from methyl crotonate and 1-phenylpiperazine by general method 1 for the synthesis of amino-esters with refluxing for 16 hours. The product had b.p. 158-166° (0.05 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

The hydrochloride of the title compound had m.p. 217.5-218°.

NMR spectrum ($D_2O$): δ 1.52 (d, 3, J = 7.0 Hz, $CH\underline{CH_3}$), 2.8-3.2 (m, 2, $CH\underline{CH_2}$), 3.5-4.3 (m, 12, $C_4\underline{H_8}N_2\underline{CH}\underline{CH_2}CO_2\underline{CH_3}$), 7.3-7.7 (m, 5, $C_6\underline{H_5}$).

Analysis - Found: C, 53.73; H, 7.35; N, 8.88.

$C_{15}H_{24}Cl_2N_2O_2$ requires: C, 53.74; H, 7.22; N, 8.35.

Methyl 3-methyl-3-[1-(4-benzylpiperazinyl)]propionate

Following the procedure of general method 1 for amino-ester synthesis, the title compound was obtained in 72% yield from methyl crotonate and 1-benzylpiperazine. The reaction mixture was refluxed for eight hours. The product had b.p. 147-149° (0.4 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

The hydrochloride of the above compound had m.p. 226-227°.

NMR spectrum ($D_2O$): δ 1.48 (d, 3, J = 7.0 Hz, $CHC\underline{H}_3$), 2.8-3.1 (m, 2, $CHC\underline{H}_2$), 3.6-4.3 (m, 12, $C_4\underline{H}_8N_2C\underline{H}C\underline{H}_2CO_2C\underline{H}_3$), 4.54 (s, 2, $C\underline{H}_2N$), 7.58 (s, 5, $C_6\underline{H}_5$).

Analysis - Found: C, 55.05; H, 7.46; N, 8.19.
$C_{16}H_{26}Cl_2N_2O_2$ requires: C, 55.02; H, 7.50; N, 8.02.

Methyl 3-methyl-3-[1-(4-phenethylpiperazinyl)]propionate

Employing general method 1 for the synthesis of amino-esters, the above compound was prepared in 63% yield from methyl crotonate and 1-phenethylpiperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 162-164° (0.5 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.01 (d, 3, J = 6.5 Hz, $CHC\underline{H}_3$), 2.1-3.7 (m, 15, $C\underline{H}_2C\underline{H}_2C_4\underline{H}_8N_2C\underline{H}C\underline{H}_2$), 3.60 (s, 3, $OC\underline{H}_3$), 7.14 (s, 5, $C_6\underline{H}_5$).

The hydrochloride of the title compound had m.p. 229-230°.

Analysis - Found: C, 56.50; H, 7.75; N, 7.88.
$C_{17}H_{28}Cl_2N_2O_2$ requires: C, 56.20; H, 7.76; N, 7.70.

Methyl 3-methyl-3-[1-(4-p-hydroxyphenylpiperidino)]propionate

The above compound was synthesized in 83% yield from methyl crotonate and 4-(p-hydroxyphenyl)piperidine by general method 1 for the synthesis of amino-esters with refluxing for six days. The product was not distilled, but isolated as the hydrochloride salt by acidifying the reaction mixture with HCl gas dissolved in absolute methanol. The hydrochloride of the title compound had m.p. 195.5-196.5°.

IR spectrum (Nujol mull): 1750 $cm^{-1}$ (C=O), 3400 $cm^{-1}$ (OH).

NMR spectrum (DMSO-$d_6$): δ 1.40 (d, 3, J = 6.5 Hz, $CHCH_3$), 1.6-4.2 (m, 15, $C_5H_9NCHCH_3CH_2CO_2CH_3$), 5.62 (s, 1, NH, absent after $D_2O$ exchange), 7.1-7.8 (m, 4, $C_6H_4$), 10.54 (s, 1, OH, absent after $D_2O$ exchange).

Analysis - Found: C, 61.48; H, 7.74; N, 4.22. $C_{16}H_{24}ClNO_3$ requires: C, 61.24; H, 7.71; N, 4.46.

Methyl 3-methyl-3-[1-(4-benzylpiperidino)]propionate

General method 1 for amino-ester synthesis gave the title compound in 51% yield from methyl crotonate and 4-benzylpiperidine when the reaction mixture was heated at reflux for eight days. The product had b.p. 130-138° (0.3 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 0.92 (d, 3, J = 6.5 Hz, $CHCH_3$), 1.1-3.3 (m, 14, $CH_2C_5H_9NCHCH_3CH_2$), 3.52 (s, 3, $OCH_3$), 7.08 (s, 5, $C_6H_5$).

The hydrochloride of the above compound had m.p. 145-147°.

Analysis - Found: C, 65.48; H, 8.27; N, 4.58.

$C_{17}H_{26}ClNO_2$ requires: C, 65.47; H, 8.40; N, 4.49.

Methyl 3-methyl-3-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]propionate

Following the procedure of general method 1 for amino-ester synthesis, the title compound was prepared in 47% yield from methyl crotonate and 4-phenyl-1,2,5,6-tetrahydropyridine. The reaction mixture was refluxed for eight days. The product had b.p. 160-162° (0.8 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): $\delta$ 1.02 (d, 3, J = 6.5 Hz, $CHCH_3$), 1.9-3.4 (m, 9, $C_3H_6NCHCH_3CH_2$), 3.54 (s, 3, $OCH_3$), 5.96 (m, 1, $C=CHCH_2$), 7.0-7.5 (m, 5, $C_6H_5$).

The hydrochloride of the above compound had m.p. 149-150°.

Analysis - Found: C, 64.53; H, 8.27; N, 4.47.

$C_{16}H_{22}ClNO_2$ requires: C, 64.97; H, 8.50; N, 4.74.

Methyl 3-[1-(4-phenylpiperazinyl)]propionate

General method 1 for amino-ester synthesis gave the title compound in 92% yield from methyl acrylate and 1-phenylpiperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 158-162° (0.7 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): $\delta$ 2.3-2.8 (m, 8, $C_2H_4NCH_2CH_2$), 3.0-3.3 (m, 4, $C_2H_4N$), 3.60 (s, 3, $OCH_3$), 6.6-7.3 (m, 5, $C_6H_5$).

The hydrochloride of the above compound had m.p. 211-212°.

Analysis - Found: C, 52.38; H, 6.87; N, 9.24.

$C_{14}H_{22}Cl_2N_2O_2$ requires: C, 52.34; H, 6.90; N, 8.72.

Methyl 3-[1-(4-benzylpiperazinyl)]propionate

The title compound was synthesized in 78% yield by general method 1 for the synthesis of amino-esters using methyl acrylate and 1-benzylpiperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 146-151° (0.6 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 2.2-2.7 (m, 12, $C_4\underline{H}_8N_2\underline{CH}_2\underline{CH}_2$), 3.42 (s, 2, $C\underline{H}_2N$), 3.60 (s, 3, $OC\underline{H}_3$), 7.20 (s, 5, $C_6\underline{H}_5$).

The hydrochloride of the above compound had m.p. 226-226.5°.

Analysis - Found: C, 53.99; H, 7.46; N, 8.31.

$C_{15}H_{24}Cl_2N_2O_2$ requires: C, 53.74; H, 7.22; N, 8.35.

Methyl 3-[1-(4-phenethylpiperazinyl)]propionate

When general method 1 for the synthesis of amino-esters was employed, the title compound was prepared in 97% yield from methyl acrylate and 1-phenethylpiperazine. The reaction mixture was refluxed for 24 hours. The product had b.p. 157-161° (0.5 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 2.2-2.9 (m, 16, $C\underline{H}_2C\underline{H}_2C_4\underline{H}_8N_2C\underline{H}_2C\underline{H}_2$), 3.58 (s, 3, $OC\underline{H}_3$), 7.12 (s, 5, $C_6\underline{H}_5$).

The hydrochloride of the above compound had m.p. 222.5-223.5°.

Analysis - Found: C, 55.04; H, 7.53; N, 8.01.

$C_{16}H_{26}Cl_2N_2O_2$ requires: C, 55.01; H, 7.50; N, 8.01.

Ethyl 3-[1-(4-phenylpiperazinyl)]propionate

General method 2 for amino-ester synthesis gave the title compound in 73% yield from ethyl acrylate and 1-phenylpiperazine when the reaction mixture was heated at reflux for two days. The product had b.p. 166-169° (0.8 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.18 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 2.2-3.5 (m, 12, $C_4\underline{H_8}N_2C\underline{H_2}C\underline{H_2}$), 4.06 (q, 2, J = 7.5 Hz, $C\underline{H_2}CH_3$), 6.5-7.5 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 207-209°.

Analysis - Found: C, 53.44; H, 7.51; N, 8.52.

$C_{15}H_{24}Cl_2N_2O_2$ requires: C, 53.74; H, 7.22; N, 8.35.

Ethyl 3-[1-(4-benzylpiperazinyl)]propionate

The title compound was synthesized in 59% yield by general method 2 for the synthesis of amino-esters using ethyl acrylate and 1-benzylpiperazine. The reaction mixture was refluxed for three days. The product had b.p. 157-159° (0.7 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.22 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 2.2-2.9 (m, 12, $C_4\underline{H_8}N_2C\underline{H_2}C\underline{H_2}$), 2.46 (s, 2, $C_6H_5C\underline{H_2}N$), 4.12 (q, 2, J = 7.5 Hz, $C\underline{H_2}CH_3$), 7.28 (s, 5, $C_6\underline{H_5}$).

Analysis - Found: C, 69.71; H, 8.81; N, 10.02.

$C_{16}H_{24}N_2O_2$ requires: C, 69.53; H, 8.75; N, 10.13.

The hydrochloride of the above compound had m.p. 231-233°.

Ethyl 3-[1-(4-phenethylpiperazinyl)]propionate

Utilizing general method 2 for the synthesis of amino-esters, the above compound was obtained in 80% yield from ethyl acrylate and 1-phenethylpiperazine. The reaction mixture was refluxed for four days. The product had b.p. 169-172°(0.7 mm).

IR spectrum (film): 1730 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.16 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 2.2-3.2 (m, 16, $\underline{CH_2}\underline{CH_2}C_4\underline{H_8}N_2\underline{CH_2}\underline{CH_2}$), 4.06 (q, 2, J = 7.5 Hz, $\underline{CH_2}CH_3$), 7.18 (s, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 234-236°.

Analysis - Found: C, 56.20; H, 7.89; N, 7.58.

$C_{17}H_{28}Cl_2N_2O_2$ requires: C, 56.20; H, 7.77; N, 7.71.

Ethyl 4-[1-(4-phenylpiperazinyl)]butyrate

When general method 3 for the synthesis of amino-esters was employed, the title compound was prepared in 68% yield from ethyl 4-bromobutyrate and 1-phenylpiperazine. The reaction mixture was refluxed for 16 hours. The product had b.p. 178-182° (1.0 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.20 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.5-3.2 (m, 14, $C_4\underline{H_8}N_2\underline{CH_2}\underline{CH_2}\underline{CH_2}$), 4.26 (q, 2, J = 7.0 Hz, $\underline{CH_2}CH_3$), 6.5-7.3 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 196-198°.

Analysis - Found: C, 55.46; H, 7.68; N, 7.95.

$C_{16}H_{26}Cl_2N_2O_2$ requires: C, 55.02; H, 7.50; H, 8.02.

Ethyl 4-[1-(4-benzylpiperazinyl)]butyrate

The above compound was synthesized in 90% yield from ethyl 4-bromobutyrate and 1-benzylpiperazine by general method 3 for the synthesis of amino-esters with refluxing for 48 hours. The product had b.p. 163-166° (0.6 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.20 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.5-2.6 (m, 14, $C_4\underline{H_8}N_2C\underline{H_2}C\underline{H_2}C\underline{H_2}$), 3.44 (s, 2, $C\underline{H_2}N$), 4.06 (q, 2, J = 7.0 Hz, $C\underline{H_2}CH_3$), 7.26 (s, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 229-230°.

Analysis - Found: C, 56.53; H, 7.59; N, 7.83.
$C_{17}H_{28}Cl_2N_2O_2$ requires: C, 56.20; H, 7.77; N, 7.71.

Ethyl 4-[1-(4-phenethylpiperazinyl)]butyrate

Employing general method 3 for the synthesis of amino-esters, the title compound was obtained in 84% yield from ethyl 4-bromobutyrate and 1-phenethylpiperazine. The reaction mixture was refluxed for 36 hours. The product had b.p. 180-182° (0.6 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.20 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.5-3.2 (m, 18, $C\underline{H_2}C\underline{H_2}C_4\underline{H_8}N_2C\underline{H_2}C\underline{H_2}C\underline{H_2}$), 4.08 (q, 2, J = 7.0 Hz, $C\underline{H_2}CH_3$), 7.18 (s, 5, $C_6\underline{H_5}$).

The hydrochloride of the above compound had m.p. 262-264°.

Analysis - Found: C, 57.07; H, 8.04; N, 7.75.
$C_{18}H_{30}Cl_2N_2O_2$ requires: C, 57.33; H, 8.01; H, 7.42.

Ethyl 4-(4-phenylpiperidino)butyrate

General method 3 for amino-ester synthesis gave the title compound in 67% yield from ethyl 4-bromobutyrate and 4-phenylpiperidine when the reaction mixture was heated at reflux for 24 hours. The product had b.p. 144-146° (0.8 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.18 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.5-3.2 (m, 15, $C_5\underline{H_9}NC\underline{H_2}C\underline{H_2}C\underline{H_2}$), 4.06 (q, 2, J = 7.0 Hz, $C\underline{H_2}CH_3$), 7.12 (s, 5, $C_6\underline{H_5}$).

The hydrochloride of the above compound had m.p. 61-63°.

Analysis - Found: C, 65.30; H, 8.60; N, 4.70.

$C_{17}H_{26}ClNO_2$ requires: C, 65.47; H, 8.40; N, 4.49.

Ethyl 4-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]butyrate

When general method 3 for the synthesis of amino-esters was employed, the title compound was prepared in 46% yield from ethyl 4-bromobutyrate and 4-phenyl-1,2,5,6-tetrahydropyridine. The reaction mixture was refluxed for 72 hours. The product had b.p. 160-166° (0.5 mm).

IR spectrum (film): 1735 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.16 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.5-3.2 (m, 12, $C_3\underline{H_6}NC\underline{H_2}C\underline{H_2}C\underline{H_2}$), 4.04 (q, 2, J = 7.0 Hz, $C\underline{H_2}CH_3$), 5.96 (m, 1, C = $C\underline{H}CH_2$), 7.0-7.5 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 161-164°.

Analysis - Found: C, 66.00; H, 8.02; N, 4.67.

$C_{17}H_{24}ClNO_2$ requires: C, 65.90; H, 7.81; N, 4.52.

Ethyl 2-[1-(4-phenylpiperazinyl)]acetate

Utilizing general method 3 for the synthesis of amino-esters, the above compound was obtained in 64% yield from ethyl bromoacetate and 1-phenylpiperazine. The reaction mixture was refluxed for 48 hours. The product had b.p. 148-153° (0.4 mm).

IR spectrum (film): 1745 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.20 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 2.4-3.5 (m, 10, $C_4\underline{H_8}N_2\underline{CH_2}$), 4.10 (q, 2, J = 7.0 Hz, $\underline{CH_2}CH_3$), 6.6-7.5 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 182-184°.

Analysis - Found: C, 52.08; H, 6.90; N, 8.72.

$C_{14}H_{22}Cl_2N_2O_2$ requires: C, 52.34; H, 6.90; N, 8.72.

Ethyl 2-[1-(4-benzylpiperazinyl)]acetate

Following the procedure of general method 3 for amino-ester synthesis, the above compound was obtained in 98% yield from ethyl bromoacetate and 1-benzylpiperazine. The reaction mixture was refluxed for 48 hours. The product had b.p. 152-156° (0.6 mm).

IR spectrum (film): 1750 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.22 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 2.3-2.8 (m, 8, $C_4\underline{H_8}N_2$), 3.12 (s, 2, $NC\underline{H_2}CO_2$), 3.46 (s, 2, $C_6H_5\underline{CH_2}N$), 4.14 (q, 2, J = 7.5 Hz, $\underline{CH_2}CH_3$), 7.1-7.5 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the above compound had m.p. 222-223°.

Analysis - Found: C, 53.68; H, 7.28; N, 8.42.

$C_{15}H_{24}Cl_2N_2O_2$ requires: C, 53.74; H, 7.22; N, 8.35.

Ethyl 2-[1-(4-phenethylpiperazinyl)]acetate

The title compound was synthesized in 91% yield by general method 3 for the synthesis of amino-esters using ethyl bromoacetate and 1-phenethylpiperazine. The reaction mixture was refluxed for 60 hours. The product had b.p. 160-164° (0.6 mm).

IR spectrum (film): 1750 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.22 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 2.3-2.9 (m, 12, $\underline{CH_2}\underline{CH_2}C_4\underline{H_8}N_2$), 3.10 (s, 2, $NCH_2\underline{CO_2}$), 4.10 (q, 2, J = 7.0 Hz, $\underline{CH_2}CH_3$), 7.0-7.4 (m, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 207-208.5°.

Analysis - Found: C, 55.09; H, 7.55; N, 8.47.
$C_{16}H_{26}Cl_2N_2O_2$ requires: C, 55.02; H, 7.50; N, 8.02.

Ethyl 2-[1-(4-phenylpiperidino)]acetate

General method 3 for amino-ester synthesis gave the title compound in 80% yield from ethyl bromoacetate and 4-phenylpiperidine when the reaction mixture was heated at reflux for 24 hours. The product had b.p. 123-132° (0.3 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): δ 1.22 (t, 3, J = 6.5 Hz, $CH_2\underline{CH_3}$), 1.5-4.6 (m, 13, $C_5\underline{H_9}NCH_2\underline{CO_2}\underline{CH_2}CH_3$), 7.24 (s, 5, $C_6\underline{H_5}$).

The hydrochloride of the title compound had m.p. 86-88°.

Analysis - Found: C, 63.11; H, 8.00; N, 4.82.
$C_{15}H_{22}ClNO_2$ requires: C, 63.48; H, 7.82; N, 4.94.

Ethyl 1-(4-phenylpiperazinyl)formate

General method 3 for amino-ester synthesis gave the title compound in 57% yield from ethyl chloroformate and 1-phenylpiperazine when the reaction mixture was heated at reflux for 24 hours. The product was not distilled, but isolated as the hydrochloride salt by acidifying the residue with HCl gas dissolved in anhydrous ethanol. The hydrochloride of the title compound had m.p. 196-197°.

IR spectrum (Nujol mull): 1700 $cm^{-1}$ (C=O).

NMR spectrum ($D_2O$): δ 1.39 (t, 3, J = 7.0 Hz, $CH_2\underline{CH_3}$), 3.6-4.6 (m, 10, $C_4\underline{H_8}N_2CO_2\underline{CH_2}CH_3$), 7.96 (s, 5, $C_6\underline{H_5}$).

Analysis - Found: C, 58.07; H, 7.02; N, 10.62.

$C_{13}H_{19}ClN_2O_2$ requires: C, 57.67; H, 7.07; N, 10.35.

Ethyl 1-(4-benzylpiperazinyl)formate

The title compound was synthesized in 69% yield from ethyl chloroformate and 1-benzylpiperazine by general method 3 for synthesis for amino-esters with refluxing for 24 hours. The product was not distilled, but isolated as the hydrochloride salt by acidifying the reaction residue with HCl gas dissolved in anhydrous ethanol. The hydrochloride of the above compound had m.p. 219-220°.

IR spectrum (Nujol mull): 1710 $cm^{-1}$ (C=O).

NMR spectrum ($D_2O$): δ 1.36 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 3.1-4.4 (m, 10, $C_4\underline{H_8}N_2CO_2\underline{CH_2}CH_3$), 4.50 (s, 2, $C_6H_5\underline{CH_2}N$), 7.68 (s, 5, $C_6\underline{H_5}$).

Analysis - Found: C, 59.33; H, 7.83; N, 9.54.

$C_{14}H_{21}ClN_2O_2$ requires: C, 59.05; H, 7.43; N, 9.84.

Ethyl 1-(4-phenethylpiperazinyl)formate

Following the procedure of general method 3 for amino-ester synthesis, the title compound was prepared in 64% yield from ethyl chloroformate and 1-phenethylpiperazine. The reaction mixture was refluxed for 24 hours. The product was not distilled, but isolated as the hydrochloride salt by acidifying the reaction residue with HCl gas dissolved in anhydrous ethanol. The hydrochloride of the compound had m.p. 203-205°.

IR spectrum (Nujol mull): 1715 $cm^{-1}$ (C=O).

NMR spectrum ($D_2O$): δ 1.36 (t, 3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 3.0-4.6 (m, 14, $\underline{CH_2}\underline{CH_2}C_4\underline{H_8}N_2CO_2\underline{CH_2}CH_3$), 7.52 (s, 5, $\underline{C_6H_5}$).

Analysis - Found: C, 60.30; H, 7.76; N, 9.40.

$C_{15}H_{23}ClN_2O_2$ requires: C, 60.30; H, 7.76; N, 9.37.

Preparation of Purified Anhydrous Dioxane

Commercial dioxane (350 ml) was refluxed with excess sodium metal (10 gm) for four hours. The reaction solution was distilled through a 60 cm Vigreux column at atmospheric pressure (720 mm). The constant boiling fraction at 98-99° was collected. A drying tube containing Drierite was always kept between the atmosphere and the reaction or distillation system.

Synthesis of 1-Deutero-4-phenylpiperazine

1-Phenylpiperazine (0.1 mole) was dissolved in purified anhydrous dioxane (30 ml) and $D_2O$ (0.6 mole) was added. A Drierite filled drying tube was introduced between the atmosphere and the reaction mixture. The solution was stirred for one hour, the solvents removed in vacuo and the residual oil fractionally distilled under reduced pressure. The fraction boiling at 102-108° (0.1 mm) gave a 91% yield of the title compound.

IR spectrum (film): 2440 $cm^{-1}$ (C-D), NH stretching near 3280 $cm^{-1}$ was absent.

NMR spectrum ($CCl_4$): δ 1.18 (m, 1/4, N-H contamination), 2.6-3.2 (m, 8, $C_4H_8N_2$), 6.5-7.4 (m, 5, $C_6H_5$).

Synthesis of Methyl 3-methyl-2-deutero-3-[1-(4-phenylpiperazinyl)]-propionate

1-Deutero-4-phenylpiperazine (0.6 mole) was added to a solution of methyl crotonate (0.06 mole) in purified dioxane (40 ml). The reaction mixture was refluxed for 24 hours with a Drierite filled drying tube inserted between the atmosphere and the reaction system. The dioxane was removed in vacuo and the residual oil fractionally distilled under reduced pressure. The product had b.p. 120-145° (0.07-1.0 mm) and was obtained in 64% yield.

IR spectrum (film): 1735 $cm^{-1}$ (C=O), 2470 $cm^{-1}$ (N-D).

The hydrochloride of the title compound had m.p. 210-212°.

NMR spectrum ($D_2O$): δ 1.54 (d, 3, J = 7.0 Hz, $CHCH_3$), 2.7-3.2 (broad collapsed multiplet, 1, $CH(CH_3)CHD$), 3.6-4.3

(m, 12, $C_4\underline{H}_8N_2C\underline{H}(CH_3)CHDCOOC\underline{H}_3$), 7.3-7.8 (m, 5, $C_6\underline{H}_5$).

Analysis - Found: C, 53.39; H, 6.81; N, 8.34.

$C_{15}H_{23}DCl_2N_2O_2$ requires: C, 53.58; H, 7.49; N, 8.33.

## Synthesis of Amino-carboxylic Acid Hydrochlorides

### General Preparative Methods

Each of the carboxylic acids prepared in this series was characterised by its IR spectrum and by elemental analysis. IR spectra were recorded as Nujol mulls. All hydrochlorides showed strong $NH^+$ stretching bands in the 2340-2760 $cm^{-1}$ region (Thompson et al. 1965).

### Method 1

Crotonic acid or acrylic acid (0.05 mole) was added to a solution of an equimolar quantity of the appropriate amine in dioxane (50 ml). The reaction mixture was heated at reflux for 24-48 hours. The dioxane was removed in vacuo. The residue obtained was acidified with dilute HCl, then acetone added until the solution was turbid. The solid that formed upon refrigeration was recrystallized from acetone-water.

### Method 2 - (Vogel, 1956, p. 1063)

The appropriate amino-ester (0.02 mole) was cooled in an ice bath and acidified with 3N HCl. The volume of the solution was reduced in vacuo. On refrigeration a solid formed which was recrystallized from acetone-water.

### 3-[1-(4-Phenylpiperazinyl)]propionic acid monohydrochloride

General method 1 for the synthesis of amino-carboxylic acids

gave the title compound in 74% yield from acrylic acid and 1-phenylpiperazine. The reaction solution was stirred at reflux for 48 hours. The product had m.p. 213-216°.

IR spectrum (Nujol mull): 1725 $cm^{-1}$ (C=O).

Analysis - Found: C, 57.65; H, 7.19; N, 10.29.

$C_{13}H_{19}ClN_2O_2$ requires: C, 57.67; H, 7.07; N, 10.35.

3-Methyl-3-[1-(4-phenylpiperazinyl)]propionic acid monohydrochloride

The title compound was synthesized in 60% yield by general method 1 for the synthesis of amino-carboxylic acids using crotonic acid and 1-phenylpiperazine. The reaction mixture was refluxed for 24 hours. The product had m.p. 155-157°.

IR spectrum (Nujol mull): 1725 $cm^{-1}$ (C=O).

Analysis - Found: C, 58.62; H, 7.37; N, 9.31.

$C_{14}H_{21}ClN_2O_2$ requires: C, 59.05; H, 7.43; N, 9.84.

2-Methyl-3-[1-(4-phenylpiperazinyl)]propionic acid monohydrochloride

When general method 2 for the synthesis of amino-carboxylic acid was employed, the above compound was obtained in 84% yield from 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate. The reaction mixture was refluxed with stirring for four hours. The product had m.p. 218-222°.

IR spectrum (Nujol mull): 1715 $cm^{-1}$ (C=O).

Analysis - Found: C, 52.17; H, 6.81; N, 8.78.

$C_{14}H_{22}Cl_2N_2O_2$ requires: C, 52.34; H, 6.90; N, 8.72.

## Synthesis of Amino-hydroxamic Acid Hydrochlorides

### General Preparative Methods

Each of the hydroxamic acids prepared in this series gave a violet color with alcoholic ferric chloride and was characterised by its NMR and IR spectra and by elemental analysis. NMR spectra were recorded at operating temperature using a sample concentration of 100 mg/0.5 ml, in DMSO-$d_6$. Chemical shifts are quoted in $\delta$ units. IR spectra were recorded as Nujol mulls. All hydrochlorides showed strong $NH^+$ stretching bands in the 2340-2760 $cm^{-1}$ region (Thompson et al. 1965).

Method 1 - (Coutts, et al. 1969; 1971)

The appropriate amino-ester (0.05 mole) was dissolved in anhydrous methanol (50 ml) and the solution cooled in an ice bath. A solution of hydroxylamine hydrochloride (0.05 mole) in anhydrous methanol (25 ml) was then added dropwise with stirring. The reactants were allowed to stand at room temperature for from one to ten days and then at 50° for another one to seven days. Methanol was removed in vacuo and the resulting oil dried in vacuo over Drierite. The dried semi-solid was dissolved in warm absolute methanol and anhydrous ether added until the solution became turbid. The solution was refrigerated; the resulting precipitate collected and recrystallized from absolute methanol-anhydrous ether.

Method 2 - (Coutts, et al. 1969; 1971)

A cold methanolic solution of potassium hydroxide (0.12 mole

in 30 ml absolute methanol) was added to a constantly stirred solution of hydroxylamine hydrochloride (0.18 mole in 75 ml absolute methanol) cooled in an ice bath. After three minutes the precipitated potassium chloride was removed by filtration. This filtrate was added to an ice cooled solution of the appropriate ester (0.02 mole in 30 ml dry methanol). The temperature of the stirred solution was maintained at 0° to 5° for one hour, then allowed to reach room temperature. Stirring was continued for one to ten days. Methanol was removed in vacuo and the resulting oil dried in a vacuum dessicator over Drierite. The resulting semi-solid was dissolved in warm absolute methanol and acetone added to give a turbid solution. On refrigeration a solid formed which was recrystallized from absolute methanol- anhydrous ether several times to remove any potassium chloride.

Method 3 - (Fishbein, et al. 1969)

Cold aqueous 10N NaOH (20 ml, 0.2 mole) was slowly added with stirring to a solution of hydroxylamine hydrochloride (0.1 mole) in a mixture of water (15 ml) and ethanol (12 ml) cooled on an ice bath. The appropriate amino-ester (0.1 mole) was added dropwise with continued stirring. The reaction mixture was stirred at room temperature for two hours. The solution was next cooled on an ice bath and concentrated hydrochloric acid (7.5 ml, 0.09 mole) slowly added with stirring. Further small additions of hydrochloric acid were made to reach an end point at pH 6 (Universal pH paper). The solvents were removed in vacuo and the crystalline cake obtained

recrystallized several times from absolute ethanol to obtain an analytically pure product.

Attempted preparation of 2-methyl-3-(1-piperazino)propionohydroxamic acid hydrochloride

General method 1 and 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized or characterized.

2-Methyl-3-[1-(4-methylpiperazino)]propionohydroxamic acid monohydrochloride

The title compound was prepared in 38% yield using general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for two days at room temperature before keeping it at 5° for seven days. The product had m.p. 157-158.5°. Reported (Midha, 1969), m.p. 177-178°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at $\tau$ 8.3-11.0; the combined signal integrating for three protons ($\overset{+}{N}\underline{H}CH_2CHCH_3CO\underline{N}H\underline{O}H$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 45.54; H, 8.46; N, 17.45.

$C_9H_{20}ClN_3O_2$ requires: C, 45.47; H, 8.48; N, 17.68.

2-Methyl-3-[1-(4-ethylpiperazino)propionohydroxamic acid monohydrochloride

Following the procedure of general method 1 for the synthesis

of amino-hydroxamic acids, the above compound was obtained in 50% yield. The reaction solution was stirred at room temperature for one day then at 50° for six days. The product had m.p. 169.5-170°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.66 overlapping a broad multiplet ranging from δ 9.3-11.0; the combined signal integrating for three protons ($N\underline{H}CH_2CHCH_3CON\underline{H}O\underline{H}$). These protons exchanged in $D_2O$.

Analysis - Found: C, 47.52; H, 8.95; N, 16.69.
$C_{10}H_{22}ClN_3O_2$ requires: C, 47.52; H, 8.81; N, 16.69.

2-Methyl-3-[1-(4-n-propylpiperazino)]propionohydroxamic acid monohydrochloride

General method 1 for the synthesis of amino-hydroxamic acids gave the title compound in 51% yield. The reaction mixture was let stand at room temperature for seven days. The product had m.p. 188-189°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.78, with a broad collapsed multiplet at δ 8.3-10.4; the combined signals integrating for three protons ($N\underline{H}CH_2CHCH_3CON\underline{H}O\underline{H}$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 49.63; H, 9.44; N, 15.54.
$C_{11}H_{24}ClN_3O_2$ requires: C, 49.71; H, 9.48; N, 15.81.

2-Methyl-3-[1-(4-n-butylpiperazino)]propionohydroxamic acid dihydrochloride

When general method 1 for the synthesis of amino-hydroxamic acids was employed, an oily residue was obtained. The title compound was obtained in 32% yield by acidifying a methanolic solution of the oil with HCl gas dissolved in methanol. The reaction mixture was let stand at room temperature for seven days. The product had m.p. 175-175.5°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Broad singlet centered at δ 9.62, overlapping a broad collapsed multiplet ranging from δ 8.82-10.12; the combined signal integrating for four protons ($\overset{+}{N}\underline{H}CH_2CH_2$) and ($\overset{+}{N}\underline{H}CH_2CHCH_3CONH\underline{O}\underline{H}$). These protons exchanged with $D_2O$.

Analysis - Found: C, 45.54; H, 8.46; N, 13.31.
$C_{12}H_{27}Cl_2N_3O_2$ requires: C, 45.57; H, 8.61; N, 13.29.

2-Methyl-3-[1-(4-phenylpiperazino)]propionohydroxamic acid monohydrochloride

Employing general method 1 for the synthesis of amino-hydroxamic acids, the title compound was prepared in 30% yield. The reaction mixture was kept at room temperature for seven days. The product had m.p. 182-183°.

IR spectrum (Nujol mull): 1645 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ

10.70 overlapping a broad multiplet at δ 8.3-11.0; the combined signal integrating for three protons ($\underline{NH}CH_2CHCH_3CON\underline{HO}\underline{H}$). These protons exchanged in $D_2O$.

Analysis - Found: C, 56.09; H, 7.59; N, 14.55.

$C_{12}H_{22}ClN_3O_2$ requires: C, 56.09; H, 7.40; N, 14.02.

2-Methyl-3-[1-(4-benzylpiperazino)]propionohydroxamic acid monohydrochloride

The above compound was prepared in 51% yield by general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was kept at room temperature for seven days. The product had m.p. 199-200°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.08 overlapping a broad multiplet ranging from δ 10.0-11.6; the combined signal integrating for three protons ($\underline{NH}CH_2CHCH_3CON\underline{HO}\underline{H}$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 57.71; H, 8.16; N, 13.28.

$C_{15}H_{24}ClN_3O_2$ requires: C, 57.41; H, 7.71; N, 13.39.

2-Methyl-3-[1-(4-phenethylpiperazino)]propionohydroxamic acid monohydrochloride

The title compound was obtained in 37% yield using general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for two days at room temperature before keeping

it at 50° for ten days. The product m.p. 185-186°.

IR spectrum (Nujol mull): 1655 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.12 overlapping a broad multiplet ranging from δ 8.3-11.4; the combined signal integrating for three protons ($\underline{NH}CH_2CHCH_2CON\underline{H}\underline{O}H$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 58.72; H, 7.87; N, 12.81.

$C_{16}H_{26}ClN_3O_2$ requires: C, 58.62; H, 7.99; N, 12.81.

2-Methyl-3-[1-(4-benzylpiperidino)]propionohydroxamic acid monohydrochloride

The title compound was obtained in 75% yield using general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for one day at room temperature before keeping it at 50° for two days. The product had m.p. 195-196°.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.02 overlapping a broad multiplet ranging from δ 8.7-11.2; the combined signal integrating for three protons ($\underline{NH}CH_2CHCH_3CON\underline{H}\underline{O}H$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 61.69; H, 7.97; N, 8.99.

$C_{16}H_{25}ClN_2O_2$ requires: C, 61.43; H, 8.06; N, 8.95.

Attempted preparation of 3-methyl-3-(1-piperazino)propionohydroxamic acid hydrochloride

General method 1 and 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized or purified.

3-Methyl-3-[1-(4-methylpiperazino)]propionohydroxamic acid monohydrochloride

When general method 1 for the synthesis of aminohydroxamic acids was employed, the title compound was obtained in 38% yield. The reaction mixture was kept at room temperature for two days then at 50° for seven days. The product had m.p. 154-156°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

Analysis - Found: C, 45.11; H, 8.34; N, 17.65.
$C_9H_{20}ClN_3O_2$ requires: C, 45.47; H, 8.48; N, 17.68.

3-Methyl-3-[1-(4-ethylpiperazino)]propionohydroxamic acid monohydrochloride

General method 1 for the synthesis of amino-hydroxamic acids gave the title compound in 45% yield. The reaction mixture was let stand at room temperature for nine days. The product had m.p. 151-152°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.60 overlapping a broad collapsed multiplet at δ 8.3-11.1; the

combined signal integrating for three protons ($\underline{NH}^{+}CHCH_3CH_2CONH\underline{OH}$). The protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 47.80; H, 9.02; N, 16.72.
$C_{10}H_{22}ClN_3O_2$ requires: C, 47.71; H, 9.21; N, 16.69.

3-Methyl-3-[1-(4-n-propylpiperazino)]propionohydroxamic acid monohydrochloride

Following the procedure of general method 1 for the synthesis of amino-hydroxamic acids, the title compound was prepared in 42% yield. The reaction mixture was let stand at room temperature for seven days. The product had m.p. 158.5-159.5°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.6 overlapping a broad collapsed multiplet ranging from δ 8.3-11.1; the combined signal integrating for three protons ($\underline{NH}^{+}CHCH_3CH_2CONH\underline{OH}$). These protons exchanged in $D_2O$.

Analysis - Found: C, 49.74; H, 9.35; N, 15.69.
$C_{11}H_{24}ClN_3O_2$ requires: C, 49.71; H, 9.48; N, 15.81.

3-Methyl-3-[1-(4-n-butylpiperazino)]propionohydroxamic acid monohydrochloride

The above compound was obtained in 48% yield by general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was kept at room temperature for four days. The product had m.p. 175.5-176°.

IR spectrum (Nujol mull): 1635 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.72 overlapping a broad collapsed multiplet ranging from δ 8.3-11.0: the combined signal integrating for three protons ($\underline{NH}^{+}CHCH_3CH_2CONH\underline{OH}$). These protons exchanged with the addition of $D_2O$.

Analysis - Found: C, 51.67; H, 9.28; N, 15.22; Cl, 12.01. $C_{12}H_{26}ClN_3O_2$ requires: C, 51.51; H, 9.37; H, 15.08; Cl, 12.67.

3-Methyl-3-[1-(4-phenylpiperazino)]propionohydroxamic acid dihydrochloride

When general method 1 for the synthesis of amino-hydroxamic acids was used, only an oily residue was obtained. The title compound was obtained in 45% yield by acidifying a methanolic solution of this residue with HCl gas dissolved in methanol. The reaction mixture was let stand at room temperature for two days then kept at 50° for seven days. The product had m.p. 171-173°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.32 overlapping a broad collapsed multiplet at δ 8.0-11.0; the combined signal integrating for four protons ($\underline{NH}^{+}(CH_2CH_2)_2\underline{NH}^{+}$-$CHCH_3CH_2CONH\underline{OH}$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 49.79; H, 6.97; N, 12.17. $C_{14}H_{23}Cl_2N_3O_2$ requires: C, 50.01; H, 6.89; N, 12.50.

3-Methyl-3-[1-(4-benzylpiperazino)]propionohydroxamic acid monohydrochloride

General method 1 for the synthesis of amino-hydroxamic acids gave the title compound in 33% yield. The reaction mixture was let stand at room temperature for ten days. The product had m.p. 178-179°.

IR spectrum (Nujol mull): 1625 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.86 overlapping a broad collapsed multiplet ranging from δ 8.3-11.3; the combined signal integrating for three protons ($\underline{NH}CHCH_3CH_2CO\underline{NH}O\underline{H}$). These protons exchanged in $D_2O$.

Analysis - Found: C, 57.43; H, 7.62; N, 13.29.
$C_{15}H_{24}ClN_3O_2$ requires: C, 57.41; H, 7.71; N, 13.39.

3-Methyl-3-[1-(4-phenethylpiperazino)]propionohydroxamic acid monohydrochloride

Following the procedure of general method 1 for the synthesis of amino-hydroxamic acids, the title compound was obtained in 44% yield. The reaction solution was stirred at 50° for four days. The product had m.p. 174-175°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.72 overlapping a broad multiplet from δ 8.3-1.1; the combined signal integrating for three protons ($\underline{NH}CHCH_3CH_2CO\underline{NH}O\underline{H}$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 58.60; H, 8.00; N, 12.63.
$C_{16}H_{26}ClN_3O_2$ requires: C, 58.62; H, 7.99; N, 12.81.

4-[1-(4-Phenylpiperazino)]butyrohydroxamic acid monohydrochloride

Employing general method 1 for the synthesis of amino-hydroxamic acids, the title compound was obtained in 62% yield. The reaction mixture was kept at room temperature for seven days. The product had m.p. 198-199°.

IR spectrum (Nujol mull): 1670 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.78 overlapping a broad collapsed multiplet ranging from δ 8.3-11.0; the combined signal integrating for three protons ($\overset{+}{N}\underline{H}CH_2CH_2CH_2CONH\underline{O}H$). These protons exchanged with $D_2O$.

Analysis - Found: C, 55.80; H, 7.71; N, 14.12.
$C_{14}H_{22}ClN_3O_2$ requires: C, 56.09; H, 7.40; N, 14.02.

4-[1-(4-Benzylpiperazino)]butyrohydroxamic acid dihydrochloride

The above compound was prepared in 40% yield by general method 2 for the synthesis of amino-hydroxamic acids. The reaction mixture was allowed to stand at room temperature for ten days. The product had m.p. 193-197°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

Analysis - Found: C, 48.94; H, 7.84; N, 11.23.
$C_{15}H_{27}Cl_2N_3O_3$ requires: C, 48.92; H, 7.39; N, 11.41.

4-[1-(4-Phenethylpiperazino)]butyrohydroxamic acid dihydrochloride

General method 2 for the synthesis of amino-hydroxamic acids gave the title compound in 42% yield. The reaction mixture was kept at room temperature for ten days. The product had m.p. 202-203°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

Analysis - Found: C, 52.93; H, 7.73; N, 11.53.
$C_{16}H_{27}Cl_2N_3O_2$ requires: C, 52.75; H, 7.47; N, 11.53.

Attempted preparation of 4-[1-(4-phenylpiperidino)]-butyrohydroxamic acid monohydrochloride

General method 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized or purified.

4-[1-(4-Phenyl-1,2,5,6-tetrahydropyridino)]butyrohydroxamic acid monohydrochloride

Following the procedure of general method 2 for the synthesis of amino-hydroxamic acids, the above compound was prepared in 88% yield. The reaction mixture was let stand at room temperature for ten days. The product had m.p. 186-187°.

IR spectrum (Nujol mull): 1695 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 10.88 overlapping a broad collapsed multiplet at δ 8.3-11.3; the combined signal integrating for three protons ($\overset{+}{N}\underline{H}CH_2CH_2CH_2CONH\underline{O}\underline{H}$).

The protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 60.90; H, 7.46; N, 9.68.

$C_{15}H_{22}ClN_2O_2$ requires: C, 60.60; H, 7.45; N, 9.41.

3-[1-(4-Phenylpiperazino)]propionohydroxamic acid monohydrochloride

The title compound was prepared in 36% yield using general method 1 for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for three days at room temperature before keeping it at 50° for seven days. The product had m.p. 152-153°.

IR spectrum (Nujol mull): 1665 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.06 overlapping a broad collapsed multiplet ranging from δ 8.8-11.5; the combined signal integrating for three protons ($\underline{NH}^{+}CH_2CH_2CON\underline{HOH}$). These protons exchanged with the addition of $D_2O$.

Analysis - Found: C, 54.43; H, 7.01; N, 14.67.

$C_{13}H_{20}ClN_3O_2$ requires: C, 54.64; H, 7.05; N, 14.70.

3-[1-(4-Benzylpiperazino)]propionohydroxamic acid monohydrochloride

Following the procedure of general method 3 for the synthesis of amino-hydroxamic acids, the above compound was prepared in 39% yield. The reaction was stirred at room temperature for two hours. The product had m.p. 154-155.5°.

IR spectrum (Nujol mull): 1640 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad collapsed multiplet

centered at δ 10.86 overlapping a broad collapsed multiplet ranging from δ 8.0-11.2; the combined signal integrating for three protons ($\underline{NH}^{+}CH_2CH_2CON\underline{H}O\underline{H}$). These protons exchanged upon the addition of $D_2O$.

Analysis - Found: C, 56.36; H, 7.56; H, 14.00.

$C_{14}H_{22}ClN_3O_2$ requires: C, 56.09; H, 7.40; N, 14.02.

3-[1-(4-Phenethylpiperazino)]propionohydroxamic acid monohydrochloride

General method 3 for the synthesis of amino-hydroxamic acids gave the above compound in 29% yield. The reaction mixture was stirred for two hours. The product had m.p. 160.5-162°.

IR spectrum (Nujol mull): 1645 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad collapsed multiplet centered δ 10.85 overlapping a broad collapsed multiplet ranging from δ 8.0-11.2; the combined signal integrating for three protons ($\underline{NH}^{+}CH_2CH_2CON\underline{H}O\underline{H}$). The protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 57.24; H, 7.71; N, 13.50

$C_{15}H_{22}ClN_3O_2$ requires: C, 57.41; H, 7.71; N, 13.39.

3-Methyl-3-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]propionohydroxamic acid monohydrochloride

The title compound was obtained in 56% yield by general method 2 for the synthesis of amino-hydroxamic acids. The reaction mixture was kept at room temperature for 13 days. The product had m.p. 185-186°.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.06 overlapping a broad collapsed multiplet ranging from δ 8.3-11.3; the combined signal integrating for three protons ($N\underline{H}CHCH_3CH_2CONH\underline{O}H$). These protons exchanged with the addition of $D_2O$.

Analysis - Found: C, 60.83; H, 7.47; H, 9.44.

$C_{15}H_{21}ClN_2O_2$ requires: C, 60.70; H, 7.13; N, 9.44.

2-[1-(4-Phenylpiperazino)]acetohydroxamic acid monohydrochloride

The title compound was obtained in 83% yield by general method 2 for the synthesis of amino-hydroxamic acids. The reaction mixture was kept at room temperature for ten days. The product had m.p. 201.5-202°.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad singlet centered at δ 11.70 overlapping a broad collapsed multiplet ranging from δ 8.3-12.0; the combined signal integrating for three protons ($N\underline{H}CH_2CONH\underline{O}H$). These protons exchanged with $D_2O$.

Analysis - Found: C, 52.61; H, 6.92; N, 15.01.

$C_{12}H_{18}ClN_3O_2$ requires: C, 53.04; H, 6.68; N, 15.46.

Attempted preparation of 2-[1-(4-benzylpiperazino)]acetohydroxamic acid monohydrochloride

General method 2 for aminohydroxamate synthesis gave an oil which could not be recrystallized or purified.

2-[1-(4-Phenethylpiperazino)]acetohydroxamic acid dihydrochloride

General method 2 for the synthesis of amino-hydroxamic acids gave the title compound in 67% yield. The reaction mixture was let stand at room temperature for ten days. The product had m.p. 200-202°.

IR spectrum (Nujol mull): 1690 $cm^{-1}$ (C=O).

NMR spectrum (DMSO-$d_6$): Very broad collapsed multiplet centered at δ 11.06 overlapping a broad collapsed multiplet at δ 8.3-11.6; the combined signal integrating for four protons ($\overset{+}{N}\underline{H}C_4H_8\overset{+}{N}\underline{H}CH_2CON\underline{H}\underline{O}H$). These protons exchanged upon addition of $D_2O$.

Analysis - Found: C, 49.79; H, 6.96; N, 13.27.
$C_{14}H_{23}Cl_2N_3O_2$ requires: C, 50.01; H, 6.89; N, 12.50.

## Synthesis of o-Nitroesters

Each of the o-nitroesters prepared in the present series was characterized by its NMR and IR spectrum as well as by its mass spectrum and elemental analysis. Since most syntheses included a primary amine as one reactant, the presence of two peaks within the range of 3300-3500 $cm^{-1}$ (due to $NH_2$ stretching) in the IR spectrum of the crude reaction residue was considered positive indication that the reaction had failed.

The commerically unavailable reactants ethyl glycinate and diethyl bromomalonate were prepared by the methods described below.

(i) Synthesis of ethyl glycinate - (Vogel, 1956, p. 384)

Glycine (0.5 mole) was dissolved in absolute ethanol (700 ml). Concentrated sulfuric acid (27 ml) was cautiously added to the stirred solution. The reaction mixture was refluxed for eight hours, cooled and the ethanol removed in vacuo. The residue obtained was dissolved in distilled water (100 ml), basified with sodium carbonate then extracted with chloroform (3 x 50 ml). The chloroform extracts were combined, dried over anhydrous sodium sulfate, filtered and the chloroform removed from the filtrate in vacuo. The fraction with b.p. 51-53° (12 mm) gave the title compound in 57% yield. Reported (Handbook of Chemistry and Physics, 1970), 57.6° (18 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O) and 3380 $cm^{-1}$ ($NH_2$).

The hydrochloride of the title compound had m.p. 141-143°. Reported (Handbook of Chemistry and Physics, 1970), 144°.

Analysis - Found: C, 46.23; H, 8.50; N, 13.56. $C_4H_9NO_2$ requires: C, 46.59; H, 8.80; N, 13.59.

(ii) Synthesis of diethyl bromomalonate - (Palmer and McWherter, 1941)

Diethyl malonate (1 mole) was dissolved in carbon tetrachloride (150 ml) in a 3-neck flask fitted with a mechanical stirrer, a dropping funnel and a reflux condenser with plastic tubing leading into a flask of water (500 ml). Commercial liquid bromine (1.03 mole) was dried by shaking with an equal volume of concentrated sulfuric acid in a separatory funnel. The bromine was then gradually added to the reaction mixture at a rate sufficient to maintain a gentle reflux. Once all the bromine was added, the solution was washed with 5% sodium carbonate (5 x 50 ml), then distilled under reduced pressure. The fraction boiling at 126-128° (15 mm) gave a 75% yield of the title compound. Reported (Palmer and McWherter, 1941), 132-136° (33 mm).

IR spectrum (film): 1740 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): $\delta$ 1.31 (t,6, J = 7.0 Hz, $(-CH_2\underline{CH_3})_2$), 4.22 (q,4, J = 7.0 Hz, $(-\underline{CH_2}CH_3)_2$), 4.70 (s,1, Br-$\underline{CH}$-).

Method 1 - (King and Clark-Lewis, 1951)

A mixture of the appropriate 2-bromoester (0.25 mole) and o-nitroaniline (0.5 mole) was heated at 120-135° for five hours,

then cooled. Anhydrous ether (200 ml) was added, the reaction suspension filtered and the ether removed from the filtrate in vacuo. The residue obtained was recrystallized from absolute ethanol.

Method 2 - (Zaugg et al. 1961)

Sodium hydride (0.1 mole of a 60% dispersion in mineral oil) was suspended in anhydrous solvent (200 ml) in a 3-neck flask fitted with a thermometer, stirrer, reflux condenser (drying tube), nitrogen inlet and dropping funnel. o-Nitroaniline (0.1 mole) dissolved in an anhydrous solvent (100 ml) was added dropwise with stirring under a stream of dry nitrogen gas. The internal temperature was maintained below 40-50° by regulation of the rate of o-nitroaniline addition. After cessation of hydrogen gas evolution, the appropriate 2-bromoester (0.1 mole) was added portionwise. The reaction mixture was stirred at reflux for 7 to 24 hours. After cooling, the solvent was removed in vacuo and anhydrous ether (2 x 150 ml) was added. After filtration, the ether was removed in vacuo and the product was isolated by suitable means.

Method 3

o-Nitrochlorobenzene (0.1 mole) and the appropriate amino-acid ethyl ester were refluxed together in absolute ethanol (150 ml) for one day to two weeks. After cooling, the ethanol was removed in vacuo and the residue recrystallized from absolute ethanol.

Ethyl N-o-nitrophenylglycinate

Following the procedure of general method 1 for the synthesis of o-nitroester, the title compound was obtained in 20% yield from ethyl bromoacetate and o-nitroaniline. The product had m.p. 78.5-79.5°. Reported (King and Clark-Lewis, 1951), 77-78°.

IR spectrum (Nujol mull): 1725 $cm^{-1}$ (C=O), 3370 $cm^{-1}$ (NH).

NMR spectrum ($CDCl_3$): δ 1.30 (t,3, J = 7.5 Hz, $CH_2\underline{CH_3}$), 4.05 (s,2, $NHCH_2$), 4.28 (q,2, J = 7.5 Hz, $\underline{CH_2}CH_3$), 8.22 (s,1, NH, absent after addition of $D_2O$), 6.5-8.4 (m,4, $C_6H_4$).

Analysis - Found: C, 53.40; H, 5.39; N, 12.75.
$C_{10}H_{12}N_2O_4$ requires: C, 53.57; H, 5.40; N, 12.49.

Attempted preparation of ethyl N-o-nitrophenyl-2-methylglycinate

(i) The IR spectrum (film) of the ether extraction residue obtained from ethyl 2-bromopropionate and o-nitroaniline by using general method 1 for the synthesis of o-nitroesters had significant absorbances at 1735 $cm^{-1}$ (C=O), 3370 $cm^{-1}$ and 3495 $cm^{-1}$ ($NH_2$). The reaction mixture was heated for five hours.

(ii) Utilizing general method 2 for the synthesis of o-nitroesters, o-nitroaniline and ethyl 2-bromopropionate were reacted in anhydrous dimethyl sulfoxide. After stirring at room temperature for 24 hours, the ether extraction had IR spectrum (film): 1740 $cm^{-1}$ (C=O), 3380 $cm^{-1}$ and 3495 $cm^{-1}$ ($NH_2$).

(iii) Reaction of o-nitroaniline and ethyl 2-bromopropionate by general method 2 for the synthesis of o-nitroesters using anhydrous dioxane as solvent in place of anhydrous dimethyl sulfoxide, gave an ether extraction residue which had IR spectrum (film): 1740 $cm^{-1}$ (C=O), 3370 $cm^{-1}$ and 3485 $cm^{-1}$ ($NH_2$). The reaction mixture was refluxed for 24 hours.

(iv) General method 3 for the synthesis of o-nitroesters with o-nitrochlorobenzene and ethyl glycinate refluxed in chloroform for 24 hours gave a reaction residue which had IR spectrum (film): 1735 $cm^{-1}$ (C=O), 3320 $cm^{-1}$ and 3390 $cm^{-1}$ ($NH_2$). The reaction residue was redissolved in 70% water in ethanol (50 ml), sodium carbonate (0.03 mole) was added and the reaction mixture refluxed for five days. The IR spectrum of the reaction residue was unchanged.

(v) o-Nitrochlorobenzene, ethyl glycinate and triethylamine were refluxed in absolute ethanol for 24 hours according to general method 3 for the synthesis of o-nitroesters to give a reaction residue with IR spectrum (film): 1735 $cm^{-1}$ (C=O), 3320 $cm^{-1}$ and 3390 $cm^{-1}$ ($NH_2$).

Attempted synthesis of ethyl N-o-nitrophenyl-2-ethylglycinate

With ethyl 2-bromobutyrate and o-nitroaniline, general method 1 for the synthesis of o-nitroesters gave a yellow solid having an IR spectrum and melting point (69-71°) identical to that of o-nitroaniline. The reaction mixture was heated for five hours.

Attempted synthesis of ethyl N-o-nitrophenyl-2-n-butylglycinate

Heating ethyl 2-bromohexanoate and o-nitroaniline for five hours under the conditions of general method 1 for the synthesis of o-nitroesters gave a yellow solid having an IR spectrum (Nujol mull) and melting point (69-71°) identical to that for o-nitroaniline. Increasing the reaction time to four days did not alter the IR spectrum of the ether extraction residue.

Attempted synthesis of ethyl N-o-nitrophenyl-2,2-dimethylglycinate

(i) General method 2 for the synthesis of o-nitroesters with o-nitroaniline and ethyl 2-bromoisobutyrate refluxed in anhydrous xylene for seven hours gave an ether extraction residue which had an IR spectrum (film) with the following significant absorbances: 1725 $cm^{-1}$ (C=O), 3350 $cm^{-1}$ and 3490 $cm^{-1}$ ($NH_2$).

(ii) Repetition of the reaction under the conditions of general method 2 for the synthesis of o-nitroesters using anhydrous dimethyl sulfoxide as solvent in place of anhydrous xylene gave an ether extraction residue with IR spectrum (film): 1725 $cm^{-1}$ (C=O), 3370 $cm^{-1}$ and 3485 $cm^{-1}$ ($NH_2$). The reaction mixture was refluxed for five hours.

Attempted synthesis of ethyl N-o-nitrophenyl-2-ethoxycarbonylglycinate

(i) With diethyl bromomalonate and o-nitroaniline, general

method 1 for the synthesis of o-nitroesters gave an ether extraction residue with IR spectrum (film): 1740 $cm^{-1}$ (C=O), 3380 $cm^{-1}$ and 3500 $cm^{-1}$ ($NH_2$). The reaction mixture was refluxed for three days.

(ii) Reaction of diethyl bromomalonate and o-nitroaniline for 18 hours at room temperature by general method 2 for the synthesis of o-nitroesters, gave an ether extraction residue which had IR spectrum (film): 1735 $cm^{-1}$ (C=O), 3320 $cm^{-1}$ and 3470 $cm^{-1}$ ($NH_2$). Refrigeration of this residue gave pale yellow prisms m.p. 48-52°. Several recrystallizations from absolute ethanol gave shiny white prisms m.p. 53-54°. The yield of pure 1,1,2,2-tetraethoxycarbonylethylene was 47%.

IR spectrum (Nujol mull): 1725 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): $\delta$ 1.31 (t,12, J = 7.0 Hz, $(-CH_2\underline{CH_3})_4$), 4.26 (q,8, J = 7.0 Hz, $(-\underline{CH_2}CH_3)_4$).

Analysis - Found C, 53.17; H, 6.44.

$C_{14}H_{20}O_8$ requires: C, 53.16; H, 6.37.

1,1,2,2-Tetraethoxycarbonylethylene

The title compound was obtained in 52% yield by general method 2 for the synthesis of o-nitroesters from diethyl bromomalonate without the presence of o-nitroaniline. The reaction mixture was stirred at room temperature for 16 hours. The product had m.p. 52-54°. Reported (Eberson, 1956) 52-53°, (Krapcho and Huyffer, 1963) 52-53°.

IR spectrum (Nujol mull): 1725 $cm^{-1}$ (C=O).

NMR spectrum ($CCl_4$): $\delta$ 1.31 (t,12, J = 7.0 Hz, $(-CH_2\underline{CH}_3)_4$), 4.26 (q,8, J = 7.0 Hz, $(-\underline{CH}_2CH_3)_4$).

Analysis - Found: C, 52.95; H, 6.32.

$C_{14}H_{20}O_8$ requires: C, 53.16; H, 6.37.

Synthesis of 1-Hydroxyquinoxalin-2-one 4-Oxides

General Preparative Method

Each of the 1-hydroxyquinoxalin-2-one 4-oxides prepared in the present series was characterized by its NMR and IR spectrum as well as by its mass spectrum and elemental analysis. All gave a violet color with alcoholic ferric chloride. Glyoxal and methyl glyoxal were purchased as 40% aqueous solutions from Aldrich Chemical Company. Phenyl glyoxal, benzofuroxan and o-quinone dioxime were synthesized as described below.

(i) Synthesis of phenyl glyoxal - (Vogel, 1956, p. 866)

Selenium dioxide (0.5 mole) was dissolved in a solution of dioxane (300 ml) and water (10 ml). To this stirred solution acetophenone (0.5 mole) was added in one lot. The reaction mixture was refluxed with stirring for four hours. The hot solution was decanted from the precipitated selenium through a fluted filter paper and the solvents were removed in vacuo. The residue was fractionally distilled under reduced pressure. One fraction was collected at b.p. 94-102° (23 mm) to give a yield of 71%. Reported (Vogel, 1956), b.p. 95-97° (25 mm). The title compound was stored in the form of the more stable hydrate which was prepared by dissolving the oil in 200 ml of hot water and allowing the compound to crystallize. The hydrate had m.p. 83-85°.

IR spectrum (Nujol mull): 1690 $cm^{-1}$ (C=O), 3280 $cm^{-1}$ (OH). The phenyl glyoxal thus prepared was used in subsequent reactions

without further characterization or purification.

(ii) Synthesis of benzofuroxan - (Mallory, 1957)

A mixture of sodium hydroxide (1.25 moles) and water (200 ml) was swirled until all the solid dissolved. The solution was cooled to 0° then 100 gm of crushed ice added. The solution was placed on an ice bath and chlorine gas bubbled through until 0.58 mole was absorbed. An excess of chlorine gas was avoided. The sodium hypochlorite solution thus prepared was stored in the dark at 0° until needed.

Potassium hydroxide (0.32 mole) was dissolved in 95% ethanol (250 ml) with heating on a steam bath. o-Nitroaniline (0.29 mole) was dissolved in this warm alkali solution. The deep red solution so obtained was cooled to 0°, then the sodium hypochlorite solution previously prepared was slowly added over the course of ten minutes with vigorous stirring such that the temperature did not rise above 10°. The precipitate formed was collected by suction filtration, washed with water (200 ml) and air dried. The product was recrystallized from ethanol-water. The title compound obtained in 70% yield had m.p. 71-73°. Reported (Mallory, 1957) m.p. 72-73°. IR spectrum (Nujol mull): 1615 $cm^{-1}$ (C-N). The benzofuroxan so prepared was used without any further characterization or purification.

(iii) Synthesis of o-quinone dioxime - (Ghosh and Whitehouse, 1968)

To a solution of benzofuroxan (0.1 mole) in ethanol (400 ml) was added a solution of hydroxylamine hydrochloride (0.12 mole) in water (25 ml). The mixture was cooled to 0° on an ice bath and potassium hydroxide (0.35 mole) in water (50 ml) was added with stirring. The solution was stirred at room temperature for one hour, then the bulk of the ethanol was removed in vacuo. The residue was acidified with 2N HCl. The product collected by suction filtration was recrystallized from ethanol-water. o-Quinone dioxime obtained in 60% yield had m.p. 142-144°. Reported (Zincke and Schwarz, 1899) m.p. 142°. IR spectrum (Nujol mull): 1623 $cm^{-1}$ (C-N), 3110 $cm^{-1}$ (OH). The o-quinone dioxime thus prepared was used without any further purification or characterization.

Method 1 - (Abushanab, 1970)

Glyoxal, methyl glyoxal or phenyl glyoxal (0.1 mole dissolved in 15 ml of water) was added to a suspension of o-quinone dioxime (0.05 mole) in water (50 ml). The suspension was heated on a steam bath for five minutes. After cooling, the precipitate was collected by filtration. The product was decolorized with activated charcoal during recrystallization from absolute methanol.

1-Hydroxyquinoxalin-2-one 4-oxide

General method 1 for the synthesis of 1-hydroxyquinoxalin-2-one 4-oxides gave the title compound in 65% yield from glyoxal

and o-quinone dioxime. The product had m.p. 239-241°. Reported (Abushanab, 1970). m.p. 255-257°. Thin-layer chromatography using silica gel G with $CHCl_3$(9): (MeOH(1) as developing solvent gave one spot after iodine vapor treatment with Rf = 0.44.

IR spectrum (Nujol mull): 1260 $cm^{-1}$ (NO), 1635 $cm^{-1}$ (C=O and C=N) and 2200-3150 $cm^{-1}$ (OH).

NMR spectrum (DMSO-$d_6$): broad collapsed multiplet centered at δ 11.88 (m, 1, OH, absent after $D_2O$ exchange), 7.3-8.6 (m, 5, remaining protons).

Mass spectrum: m/e (% relative abundance), 180 (1.05), 179 (6.47), 178 $[C_8H_6N_2O_3]^+$ (100.0), 162 (11.3), 161 (0.2), 151 (4.7), 150 (63.2), 133 (1.6), 106 (11.1), 105 (10.5), 94 (10.5), 92 (16.8), 90 (23.7), 80 (4.74, 79 (40.5), 78 (36.8), 77 (17.4), 76 (34.2), 75 (12.6), 74 (6.3), 65 (8.4), 64 (29.5), 63 (25.3), 62 (7.9), 53 (10.0), 52 (24.7), 51 (28.9), 50 (27.9), 39 (33.2), 38 (11.6), 30 (10.3), 29 (5.3), 28 (17.9), 27 (7.9), 18 (12.1).

Analysis - Found: C, 54.06; H, 3.31; N, 16.22.
$C_8H_6N_2O_3$ requires: C, 53.94; H, 3.40; N, 15.72.

3-Methyl-1-hydroxyquinoxalin-2-one 4-oxide

The title compound was synthesized in 42% yield by general method 1 for the synthesis of 1-hydroxyquinoxalin-2-one 4-oxides from ethyl glyoxal and o-quinone dioxime. The product had m.p. 218-220°. Reported (Abushanab, 1970), m.p. 231-232°. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) as developing solvent gave a single spot after iodine vapor treatment with Rf = 0.46.

IR spectrum (Nujol mull): 1250 $cm^{-1}$ (NO), 1640 $cm^{-1}$ (C=O, C=N)

and 2300-2750 $cm^{-1}$ (OH).

NMR spectrum (DMSO-$d_6$): δ 2.46 (s, 3, $CCH_3$), 7.3-8.4 (m, 4, $C_6H_4$), a broad collapsed multiplet centered at δ 9.5 (m, 1, OH, absent after $D_2O$ exchange).

Mass spectrum: m/e (% relative abundance), 194 (3.40), 193 (11.5), 192 $[C_9H_8N_2O_3]^+$ (100.0), 176 (10.2), 175 (32.3), 164 (0.1), 159 (4.3), 148 (4.7), 147 (38.3), 131 (5.7), 130 (5.5), 119 (3.8), 117 (4.3), 106 (6.8), 105 (13.6), 103 (5.1), 92 (13.4), 91 (4.7), 90 (27.0), 89 (4.7), 82 (4.7), 79 (8.1), 78 (11.1), 77 (17.4), 76 (15.9), 75 (5.1), 65 (8.5), 64 (12.3), 63 (14.0), 62 (5.1), 55 (4.7), 53 (4.7), 52 (11.9), 51 (14.0), 50 (13.2), 43 (55.3), 42 (6.3), 39 (14.9), 38 (6.2), 28 (12.3), 27 (8.7), 26 (4.3), 18 (19.8), 17 (4.3).

Analysis - Found: C, 55.95; H, 4.47; N, 14.20.
$C_9H_8N_2O_3$ requires: C, 56.25; H, 4.19; N, 14.58.

3-Phenyl-1-hydroxyquinoxalin-2-one 4-oxide

Utilizing general method 1 for the synthesis of 1-hydroxyquinoxalin-2-one 4-oxides, the above compound (vacuum dried) was prepared in 77% yield from phenyl glyoxal and o-quinone dioxime. The product recrystallized from methanol and dried under vacuum in an acetone reflux heated pistol dessicator had m.p. 196-198°. The compound air dried changed from bright yellow to the pale yellow color of the vacuum dried compound at 85-90° without melting, then melted at 196-198°. Thin-layer

chromatography on silica gel G of both the air-dried and vacuum-dried derivatives with $CHCl_3$(9): MeOH(1) as developing solvent gave one spot after iodine vapor treatment with Rf = 0.64.

IR spectrum of the vacuum dried compound (Nujol mull): 1240 $cm^{-1}$ (NO), 1610 $cm^{-1}$ (C=O C=N), 2300-3200 $cm^{-1}$ (OH).

IR spectrum of air-dried compound (Nujol mull): 1225 $cm^{-1}$ (NO), 1610 $cm^{-1}$ (C=O), [illegible] $cm^{-1}$ (C=O), 2300-4300 $cm^{-1}$ (OH - including two distinct broad peaks centered at 3110 $cm^{-1}$ and 3250 $cm^{-1}$).

IR spectrum of air-dried compound after standing for two months (Nujol mull): 1240 $cm^{-1}$ (NO), 1610 $cm^{-1}$ (C=O and C=N), 2300-3200 $cm^{-1}$ (OH).

NMR spectrum of vacuum-dried compound (DMSO-$d_6$): δ 7.3-8.7 (m, 9, $C_{14}\underline{H}_9N_2O_3H$), a broad collapsed multiplet centered at δ 11.8 (m, 1, NO$\underline{H}$, absent after $D_2O$ exchange).

NMR spectrum of air-dried compound (DMSO-$d_6$): δ 3.22 (d, 3, $C\underline{H}_3O$), 7.3-8.6 (m, 9, $C_{14}\underline{H}_9N_2O_3H$).

Mass spectrum of vacuum-dried compound: m/e (% relative abundance), 256 (1.90), 255 (15.9), 254 $[C_{14}N_{10}N_2O_3]^+$ (100.0), 253 (7.1), 238 (21.5), 237 (62.0), 236 (12.4), 226 (2.5), 220 (24.0), 213 (9.4), 210 (8.9), 209 (31.6), 208 (16.6), 193 (29.1), 192 (10.8), 181 (34.2), 180 (9.4), 121 (15.4), 105 (49.4), 91 (3.5), 90 (40.5), 78 (11.5), 77 (70.9), 76 (21.5), 75 (7.3), 65 (12.8), 64 (14.2), 63 (24.7), 62 (7.6), 52 (9.7), 51 (30.4), 50 (18.9), 39 (25.3), 32 (2.3), 28 (14.4), 18 (20.3).

Mass spectrum of air-dried compound: m/e (% relative abundance), 256 (7.83), 255 (15.3), 254 $[C_{14}H_{10}N_2O_3]^+$ (100.0), 253 (7.0), 238 (18.7), 237 (68.3), 236 (13.2), 220 (24.7), 213 (10.0), 210 (9.0), 209 (35.0), 208 (18.5), 193 (34.3), 192 (13.0), 180 (11.7), 121 (24.2), 105 (90.0), 91 (10.2), 90 (68.3), 89 (12.2), 78 (15.8), 77 (96.7), 76 (29.2), 75 (10.0), 65 (18.8), 64 (19.8), 63 (35.0), 62 (10.3), 52 (14.2), 51 (41.7), 50 (25.0), 39 (33.3), 32 (6.0), 28 (18.2), 18 (28.3).

Analysis of vacuum-dried compound - Found: C, 66.19; H, 4.08; N, 10.91. $C_{14}H_{10}N_2O_3$ requires: C, 66.14; H, 3.96; N, 11.02.

Analysis of air-dried compound - Found: C, 62.96; H, 4.97; N, 9.46. $C_{14}H_{10}N_2O_3 \cdot CH_3OH$ requires: C, 62.93; H, 4.93; N, 9.78.

## Synthesis of 1-Hydroxyquinoxalin-2-ones

Each of the 1-hydroxyquinoxalin-2-ones prepared in the present series was characterized by its NMR and IR spectrum as well as by its mass spectrum and elemental analysis. The preparation of commerically unavailable glycine hydroxamic acid and o-benzoquinone are described below.

(i) Synthesis of glycine hydroxamic acid - (Safir and Williams, 1952)

To an ice-cooled stirred solution of ethyl glycinate hydrochloride (0.03 mole) and hydroxylamine hydrochloride (0.03 mole) in 50% water ethanol (10 ml) was added 12N sodium hydroxide (8.8 ml, 0.33 mole). The solution was stirred at room temperature for one-half hour, then neutralized with concentrated hydrochloric acid (about 5 ml). Upon refrigeration, the product crystallized as a white solid. The title compound was obtained in 56% yield and had m.p. 132-133° (dec). Reported (Safir and Williams, 1952) 142-143° (dec).

IR spectrum (Nujol mull): 1610 $cm^{-1}$ (C=O).

NMR spectrum ($CF_3COOH$): $\delta$ 3.8-4.8 (broad two-proton multinlet, $-CH_2-$), 6.9-8.3 (broad four-proton multiplet which largely exchanged upon addition of $D_2O$, $\underline{NH_2}CH_2CON\underline{HOH}$).

Analysis - Found: C, 26.61; H, 6.52.

$C_2H_6N_2O_2$ requires: C, 26.66; H, 6.72.

(ii) Synthesis of o-benzoquinone - (Willstatter and Pfannenstiel, 1904)

Silver nitrate (0.1 mole) was dissolved in distilled water (25 ml) and alkalinized with 3N sodium hydroxide (about 35 ml). The precipitated silver oxide was washed by decantation with distilled water (12 x 100 ml), then with acetone (6 x 100 ml) and finally with anhydrous ether (6 x 100 ml). Anhydrous sodium sulfate (0.1 mole) and a solution of catechol (0.03 mole) in anhydrous ether (80 ml) were added to the suspension of silver oxide in anhydrous ether. The reaction mixture was shaken for five minutes, filtered and the ether removed from the filtrate in vacuo. The crude residue was used in subsequent reactions without further purification.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O).

NMR spectrum ($CDCl_3$): δ 6.2-8.4 (m, 4, $C_6H_4O_2$).

Method 1 - (Ochiai, 1967, p. 195)

To a solution of the appropriate 1-hydroxyquinoxalin-2-one 4-oxide (0.006 mole) dissolved in chloroform (20 ml), (while chilled in ice) phosphorus trichloride (0.02 mole) was added dropwise. The mixture was refluxed for one hour then neutralized with 3N sodium hydroxide. After separating the chloroform layer and extracting the aqueous solution with chloroform (2 x 25 ml), the combined chloroform extracts were dried over anhydrous sodium sulfate and the chloroform removed in vacuo. The residue was recrystallized from absolute methanol.

Method 2

Anhydrous dioxane (200 ml) was cautiously added to palladium-charcoal (100 mg) in a Parr glass hydrogenation bottle. After the appropriate 1-hydroxyquinoxalin-2-one 4-oxide (0.005 mole) was added, the reaction suspension was shaken on a Parr hydrogenation apparatus for two to four hours at 40 p.s.i. of hydrogen gas pressure. The pressure was released, the reaction suspension filtered and the dioxane removed from the filtrate *in vacuo*. The product obtained was recrystallized from anhydrous ethanol.

Attempted synthesis of 1-hydroxyquinoxalin-2-one from glycine hydroxamic acid and o-benzoquinone.

Glycine hydroxamic acid (0.01 mole) and o-benzoquinone (0.01 mole) were dissolved in distilled water (100 ml). The reaction mixture was kept at room temperature for three days, then the water removed *in vacuo*. No pure product was isolated from the black residue.

IR spectrum (Nujol mull): 1605 $cm^{-1}$ and 1630 $cm^{-1}$ (C=O), 2000-3400 $cm^{-1}$ (OH,NH).

Attempted synthesis of 1-hydroxyquinoxalin-2-one by reductive cyclization of ethyl N-o-nitrophenylglycinate - (Coutts *et al.* 1964).

A solution of ethyl N-o-nitrophenylglycinate (0.01 mole) in

dioxane (25 ml) was added over five minutes to a suspension of palladised charcoal (0.2 g) in 5% sodium hydroxide solution (20 ml) containing sodium borohydride (0.025 mole). A stream of dry nitrogen was passed through the stirred mixture for 30 minutes, the catalyst removed by filtration, and the filtrate acidified with dilute hydrochloric acid. The acidified filtrate was extracted with chloroform (3 x 50 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate and the chloroform removed *in vacuo*. Recrystallization of the residue from absolute methanol gave 1.6 g of product melting at 148-155°. Repeated recrystallization from absolute methanol did not narrow the melting point range. Reported (Coutts *et al.* 1964), 156°. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) gave two spots after iodine vapor treatment with Rf = 0.36 and Rf = 0.43. The product gave a dark blue color with alcoholic ferric chloride.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O), 3200 $cm^{-1}$ and 3270 $cm^{-1}$ (OH,NH).

Attempted synthesis of 1-hydroxyquinoxalin-2-one

General method 1 for the synthesis of 1-hydroxyquinoxalin-2-ones, gave 0.8 g of product which did not melt up to 300° from 1-hydroxyquinoxalin-2-one 4-oxide. The product did not give any color change with alcoholic ferric chloride. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) as developing solvent gave a single spot after iodine vapor treatment with Rf = 0.28.

IR spectrum (Nujol mull): 1670 $cm^{-1}$ (C=O), 3040 $cm^{-1}$ and 3160 $cm^{-1}$ (NH,OH).

Mass spectrum: m/e (% relative abundance), 215 (0.18), 222 (0.32), 180 (0.02), 163 (9.9), 162 (100.0), 135 (5.9), 134 (70.6), 133 (5.5), 107 (6.7), 106 (92.4), 105 (35.3), 90 (8.2), 80 (8.1), 79 (67.2), 78 (26.6), 77 (8.4), 76 (8.9), 75 (5.5), 67 (12.9), 66 (1.5), 65 (6.0), 64 (17.5), 63 (21.5), 62 (8.7), 61 (4.5), 54 (5.2), 53 (19.5), 52 (60.5), 51 (41.5), 50 (21.5), 41 (3.9), 40 (5.7), 39 (19.7), 38 (18.3), 37 (10.6), 29 (3.5), 28 (13.8), 27 (11.4), 26 (7.9).

Analysis - Found variable: C, 63.12; H, 4.98 and C, 59.05; H, 4.16.

Attempted synthesis of 3-methyl-1-hydroxyquinoxalin-2-one

Following general method 1 for the synthesis of 1-hydroxyquinoxalin-2-ones gave 0.7 g of a compound which melted at 252-258° from 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide. The product did not give any color change with alcoholic ferric chloride. Thin-layer chromatography on silica gel G with $CHCl_3$(9):MeOH(1) as developing solvent gave one spot after iodine vapor treatment with Rf = 0.66.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O).

Mass spectrum: m/e (% relative abundance), 230 (4.4), 229 (0.8), 228 (7.2), 202 (6.6), 201 (3.4), 200 (10.4), 196 (22.4), 195 (8.2), 194 (72.0), 168 (32.0), 167 (25.0), 166 (100.0), 165 (52.0), 160 (6.8), 138 (6.0), 132 (12.2), 131 (38.0), 126 (7.2),

124 (15.4), 104 (7.0), 103 (4.8), 102 (5.2), 101 (3.4), 100 (5.2), 99 (8.2), 98 (6.6), 97 (9.0), 90 (15.2), 89 (6.8), 88 (10.0), 83 (8.6), 77 (7.2), 76 (12.8), 75 (14.2), 74 (8.6), 73 (9.0), 65 (10.2), 64 (12.4), 63 (42.0), 62 (20.0), 61 (9.4), 52 (16.4), 51 (10.8), 50 (12.0), 49 (4.2), 42 (11.6), 39 (9.0), 38 (12.2), 37 (7.2), 28 (13.6), 27 (7.8), 26 (6.4), 18 (3.4), 15 (7.2).

Analysis - Found: C, 54.70; H, 3.93; N, 14.59.

$C_9H_7ClN_2O$ requires: C, 55.54; H, 3.63; N, 14.39.

Attempted synthesis of 3-phenyl-1-hydroxyquinoxalin-2-one

General method 1 for the synthesis of 1-hydroxyquinoxalin-2-ones gave 0.8 g of a product from 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide which melted at 225-228°. The compound gave no color change with alcoholic ferric chloride. Thin-layer chromatography on silica gel G with $CHCl_3$(9); MeOH(1) as developing solvent gave a single spot after iodine vapor treatment with Rf = 0.70.

IR spectrum (Nujol mull): 1655 $cm^{-1}$ (C=O).

Mass spectrum: m/e (% relative abundance), 292 (0.34), 291 (0.09), 290 (0.51), 258 (8.7), 257 (4.6), 256 (26.5), 230 (14.7), 229 (7.8), 228 (45.6), 223 (9.6), 222 (60.3), 195 (14.6), 194 (100.0), 193 (20.0), 192 (7.6), 167 (6.0), 166 (8.2), 124 (5.7), 114 (4.0), 111 (6.3), 104 (14.1), 103 (7.6), 97 (6.2), 91 (11.6), 90 (27.2), 89 (10.4), 88 (4.4), 78 (4.1), 77 (25.6), 76 (13.5), 75 (8.1), 74 (4.3), 65 (8.4), 64 (23.5), 63 (38.2), 62 (11.8), 52 (12.3), 51 (20.7), 50 (14.1), 41 (3.2), 39 (17.5), 38 (8.7), 28 (2.2), 27 (3.7).

Analysis - Found: C, 69.38; H, 4.42; N, 11.42.
$C_{14}H_9ClN_2O$ requires: C, 65.51; H, 3.53; N, 10.91.

3,4-Dihydro-1-hydroxyquinoxalin-2-one

General method 2 for the synthesis of 1-hydroxyquinoxalin-2-ones, gave the title compound in 89% yield from 1-hydroxyquinoxalin-2-one 4-oxide. The reaction suspension was shaken for four hours. The product had m.p. 161-162° and gave a dark blue color with alcoholic ferric chloride. Reported (Coutts et al. 1964), m.p. 156°. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) as developing solvent gave a single spot after iodine vapor treatment with Rf = 0.39.

IR spectrum (Nujol mull): 1660 $cm^{-1}$ (C=O), 2300-2700 $cm^{-1}$ (OH), 3200 $cm^{-1}$ (NH).

NMR spectrum (DMSO-$d_6$): δ 3.94 (m, 2, $NHCH_2CO$), 6.04 (s, 1, NH, absent after $D_2O$ exchange), 6.5-7.8 (m, 4, $C_6H_4$), 10.42 (s, 1, OH, absent after $D_2O$ exchange).

Mass spectrum: m/e (% relative abundance), 116 (0.73), 165 (6.34), 164 $[C_8H_8N_2O_2]^+$ (68.3), 148 (6.8), 147 (14.2), 146 (9.5), 136 (0.2), 120 (8.3), 119 (100.0), 118 (13.2), 92 (19.0), 91 (6.8), 90 (4.9), 78 (5.4), 77 (5.4), 68 (4.9), 65 (12.2), 64 (6.3), 63 (5.9), 53 (4.9), 52 (6.1), 39 (5.9), 28 (8.3), 18 (6.3).

Analysis - Found: C, 58.56; H, 4.99; N, 17.06.
$C_8H_8N_2O_2$ requires: C, 58.53; H, 4.91; N, 17.06.

3-Phenyl-1-hydroxyquinoxalin-2-one

Following general method 2 for the synthesis of 1-hydroxyquinoxalin-2-ones, the title compound was obtained in 95% yield from 3-phenyl-1-hydroxyquinoxalin-2-one 4-oxide. The reaction suspension was shaken for two hours. The product had m.p. 157.5-158° and gave a dark blue color with alcoholic ferric chloride. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) gave one spot after treatment with iodine vapor having Rf = 0.62.

IR spectrum (Nujol mull): 1620 $cm^{-1}$ (C=O), 2200-3200 $cm^{-1}$ (OH).

NMR spectrum ($CDCl_3$): δ 10.87 (s, 1, NO<u>H</u>, absent after $D_2O$ exchange), 7.1-8.6 (m, 9, remaining protons).

Mass spectrum: m/e (% relative abundance), 240 (1.82), 239 (15.8), 238 $[C_{14}H_{10}N_2O_2]^+$ (84.8), 222 (29.1), 221 (20.0), 210 (33.9), 194 (32.1), 193 (23.6), 106 (9.1), 105 (100.0), 90 (29.1), 77 (24.2), 64 (8.5), 63 (12.1), 51 (85), 39 (8.5), 28 (13.9), 18 (36.9), 17 (7.3).

Analysis - Found: C, 70.32; H, 4.44; N, 11.73.
$C_{14}H_{10}N_2O_2$ requires: C, 70.58; H, 4.23; N, 11.76.

3-Methyl-1-hydroxyquinoxalin-2-one

The title compound was synthesized in 78% yield by general method 2 for the synthesis of 1-hydroxyquinoxalin-2-ones from 3-methyl-1-hydroxyquinoxalin-2-one 4-oxide. The reaction suspension was shaken for three hours. The product had m.p. 185-186° and gave a dark blue color with alcoholic ferric chloride. Thin-layer chromatography on silica gel G with $CHCl_3$(9): MeOH(1) as developing solvent gave one spot after iodine vapor treatment with Rf = 0.57.

IR spectrum (Nujol mull): 1630 $cm^{-1}$ (C=O), 2300-3200 $cm^{-1}$ (OH).

NMR spectrum ($CDCl_3$): δ 2.12 (s, 3, $CH_3$), 11.2 (s, 1, NOH), 7.3-8.1 (m, 4; remaining protons).

Mass spectrum: m/e (% relative abundance), 178 (0.94), 177 (12.5), 176 $[C_9H_8N_2O_2]^+$ (100.0), 160 (7.5), 159 (14.7), 148 (31.3), 132 (6.9), 131 (14.1), 107 (5.6), 106 (71.9), 105 (56.3), 90 (31.3), 79 (16.9), 78 (10.9), 77 (12.2), 76 (9.7), 75 (3.8), 74 (10.3), 65 (3.8), 64 (13.4), 63 (16.9), 62 (5.3), 52 (10.3), 51 (10.0), 50 (9.1), 43 (25.0), 39 (11.6), 28 (4.7), 18 (10.6).

Analysis - Found: C, 61.32; H, 4.77; N, 16.02. $C_9H_8N_2O_2$ requires: C, 61.36; H, 4.58; N, 15.90.

# PHARMACOLOGY

## I. EXPERIMENTAL

Male rats weighing approximately 200 gm were anesthetized with urethane (1.99 g/kg) injected intraperitoneally. The trachea was cannulated and the arterial blood pressure recorded through a polyethylene cannula inserted in a carotid artery using an E and M pressure transducer (type P-1000A) connected to an E and M physiograph (type DMP-4A). The vagus nerve on the same side as the carotid cannulation was cut. The vagus on the opposite side was carefully dissected free from its carotid sheath, tied and cut cephalad, then gently placed on a pair of platinum electrodes connected to a stimulator. Several drops of heavy liquid paraffin were placed on the nerve-electrode junction. Drugs were dissolved in 0.9% saline and injected through a polyethylene cannula inserted into a femoral vein. After each injection, the drug was washed into the animal with 0.2 ml 0.9% saline. The blood pressure response to two doses each of 0.9% saline (0.2 ml), noradrenaline bitartrate (5 µg/kg), acetylcholine chloride (0.5 µg/kg), vagal stimulation (15 volts at 25 Herz and 0.5 millisecond duration for five seconds) and 1,1-dimethyl-4-phenylpiperazine (125 µg/kg) or nicotine (20-50 µg/kg) were recorded with adequate time intervals for blood pressure recovery between doses. The concentration of each drug was calculated such that the correct dosage was administered in 0.1 ml of a 0.9% saline solution of the drug. The quinoxaline derivatives were given as solutions in 0.1 ml of N/10 NaOH.

The order of administration of these "standard drug" dosages was randomized among the experiments, but a particular sequence was maintained within an experiment. Following the recording of the "standard drug" responses, the "test drug" was given at the desired dosage level (0.1 ml of a 0.9% saline solution) and the "standard drug" responses repeated. The "standard drug" regimen was repeated until either the blood pressure recovered to its level before the administration of "test drug", or the blood pressure showed no indication of making a recovery even after a prolonged period of time, or the rat died.

II. RESULTS

Examples of Recordings of the Changes in Arterial Blood Pressure of the Anesthetized Rat Caused by Various Drugs.

Figures VII to IX are carotid blood pressure recordings of anesthetized rats for three compounds obtained in the present investigation. These recordings were chosen to illustrate the responses of the arterial blood pressure to the screening regimen used throughout this study to evaluate hypotensive activity. In each figure, the following code was used:

V = vagal stimulation at 15 volts, 25 Hz and 0.5 millisecond for 5 seconds.

S = 0.2 ml of 0.9% saline

A = 0.5 µg/kg of acetylcholine chloride

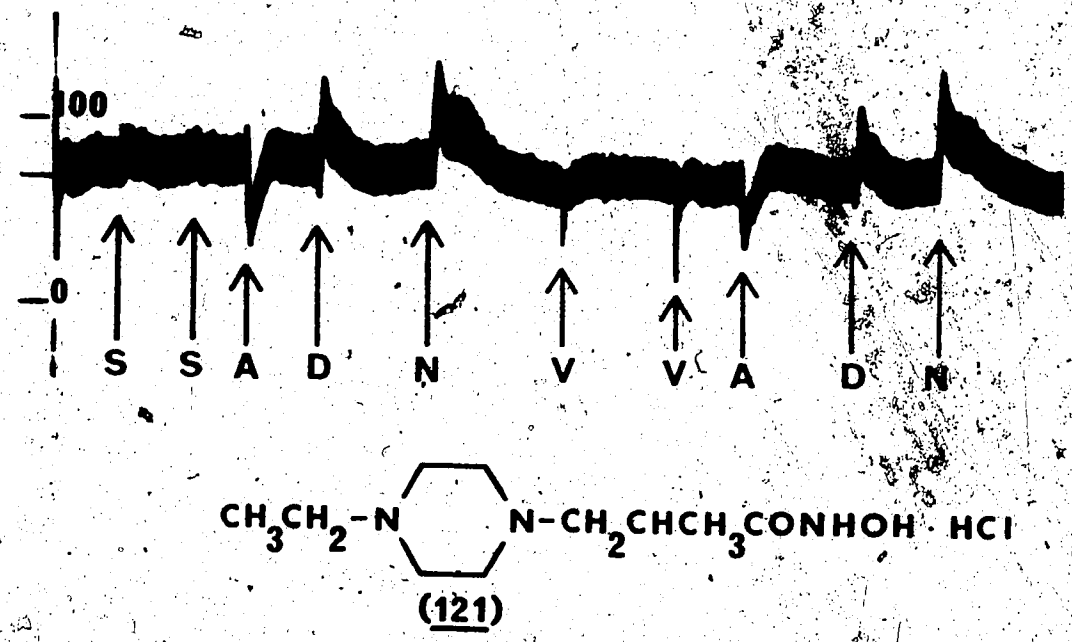
D = 125 µg/kg of 1,1-dimethyl-4-phenylpiperazine

N = 5 µg/kg of noradrenaline as the bitartrate

T = test drug at dosage shown in 0.9% saline

A, D, N, V = "standard drugs".

An evaluation of 2-methyl-3-[1-(4-ethylpiperazino)]-propionohydroxamic acid monohydrochloride (121) at 5 mg/kg is presented in Figure VII. This compound produced a transient rise in blood pressure - not a fall. Furthermore, the blood pressure responses to the "standard drugs" before and after administration of the test drug were unchanged. Thus, the conclusion was drawn that compound (121) was inactive as a hypotensive agent at the dosage of 5 mg/kg. Inactive compounds such as this were common and served as useful control recordings




$CH_3CH_2-N$ ⟨ ⟩ $N-CH_2CHCH_3CONHOH \cdot HCl$

(121)

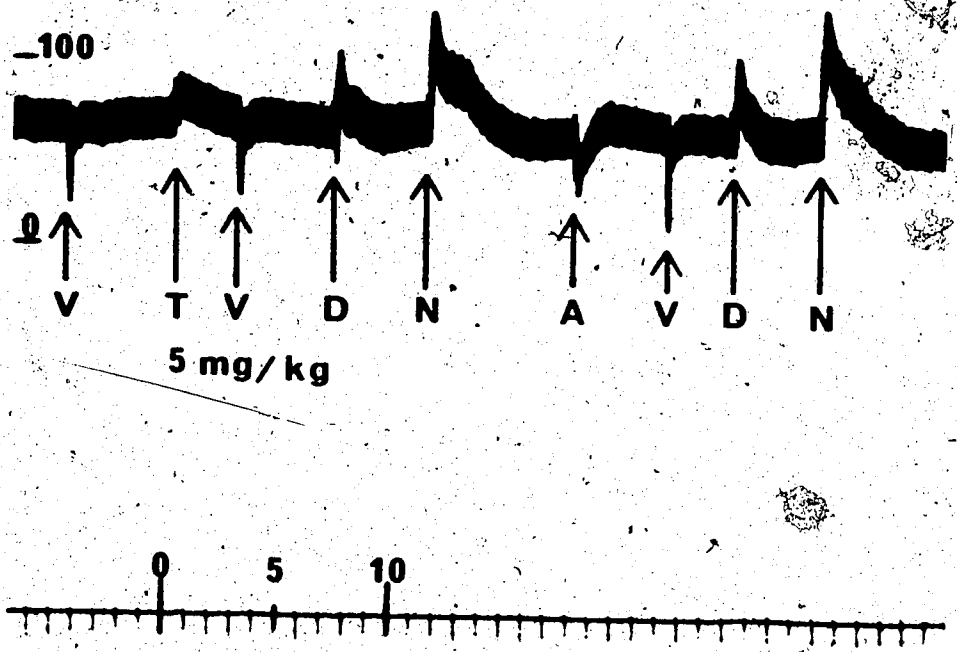




**Figure VII : Hypotensive evaluation of 2-methyl-3-[1-(4-ethylpiperazino)]propionohydroxamic acid monohydrochloride (121).**

to verify the validity of the screening regimen.

Figure VIII depicts a blood pressure recording obtained for methyl 3-methyl-3-[1-(4-phenethylpiperazinyl)]propionate dihydrochloride (89) at 5 mg/kg. This compound produced a significant fall in blood pressure (53%) for a prolonged period of time (> 1 1/2 hours). In addition, the blood pressure responses to vagal stimulation (V), 1,1-dimethyl-4-phenylpiperazine (D) and acetylcholine (A) were all reduced after administration of the test drug relative to responses before the test drug was given. The response to noradrenaline (N) after test drug administration was similar before and after the test drug. Such a pattern of effects suggests that compound (89) may be producing its effect on blood pressure by a ganglionic blocking mechanism. Although such a conclusion based on this data must remain tentative, the information provided would be useful in directing future investigation of specific mechanisms of action.

Figure IX illustrates the blood pressure responses recorded for 3-[1-(4-phenylpiperazino)]propionohydroxamic acid monohydrochloride (136) at 5 mg/kg. In this recording, compound (136) caused a profound depression of the blood pressure (68%) for longer than three hours. Responses to vagal stimulation (V), 1,1-dimethyl-4-phenylpiperazine (D), noradrenaline (N) and acetylcholine (A) were all reduced after administration of the test drug. This was taken to indicate a mixed ganglionic blocking and α-adrenergic blocking action. As with other compounds, this result is merely suggestive and mainly of value in indicating future lines of study.

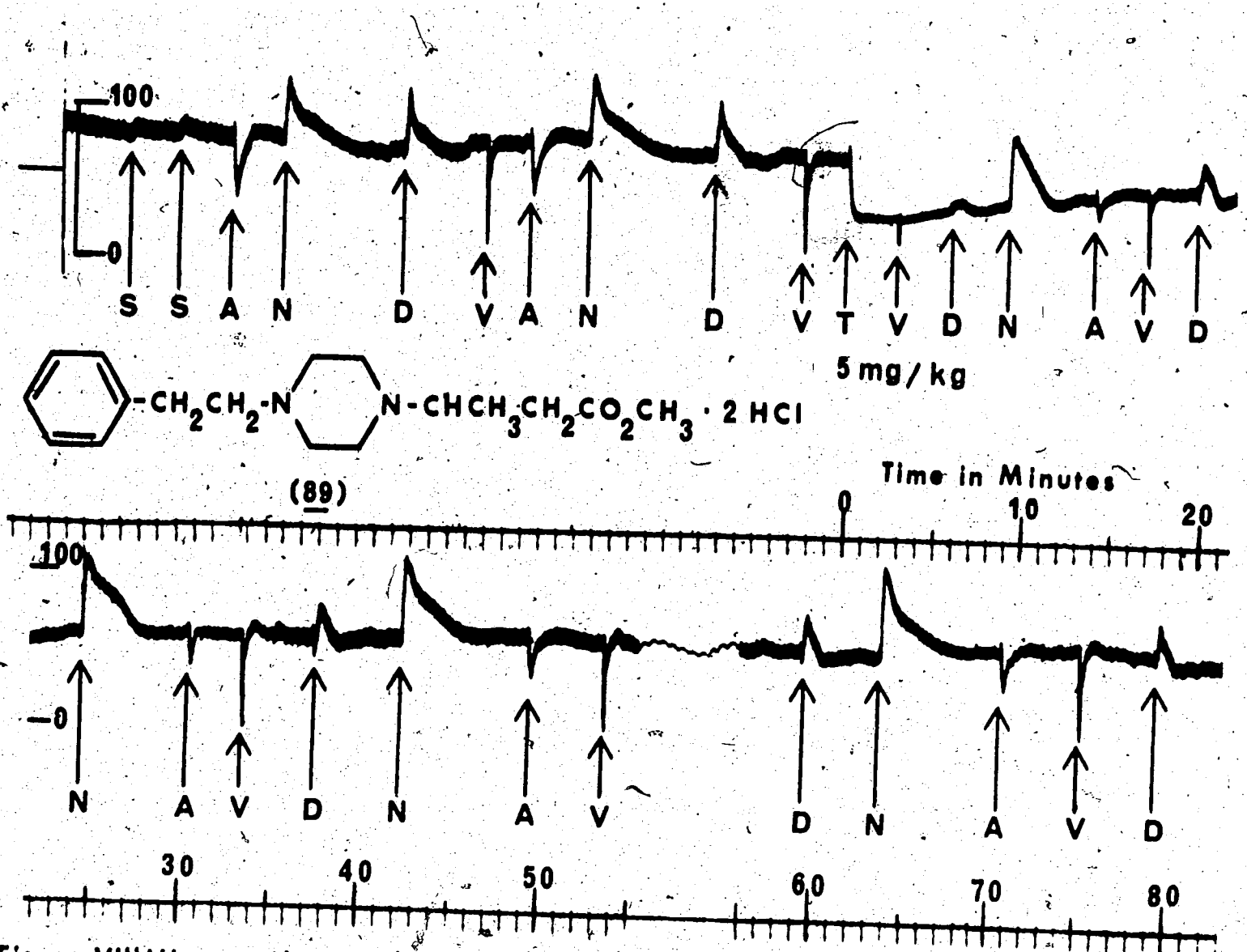



Figure VIII: Hypotensive evaluation of methyl 3-methyl-3-[1-(4-phenethylpiperazinyl)]-propionate dihydrochloride (89).

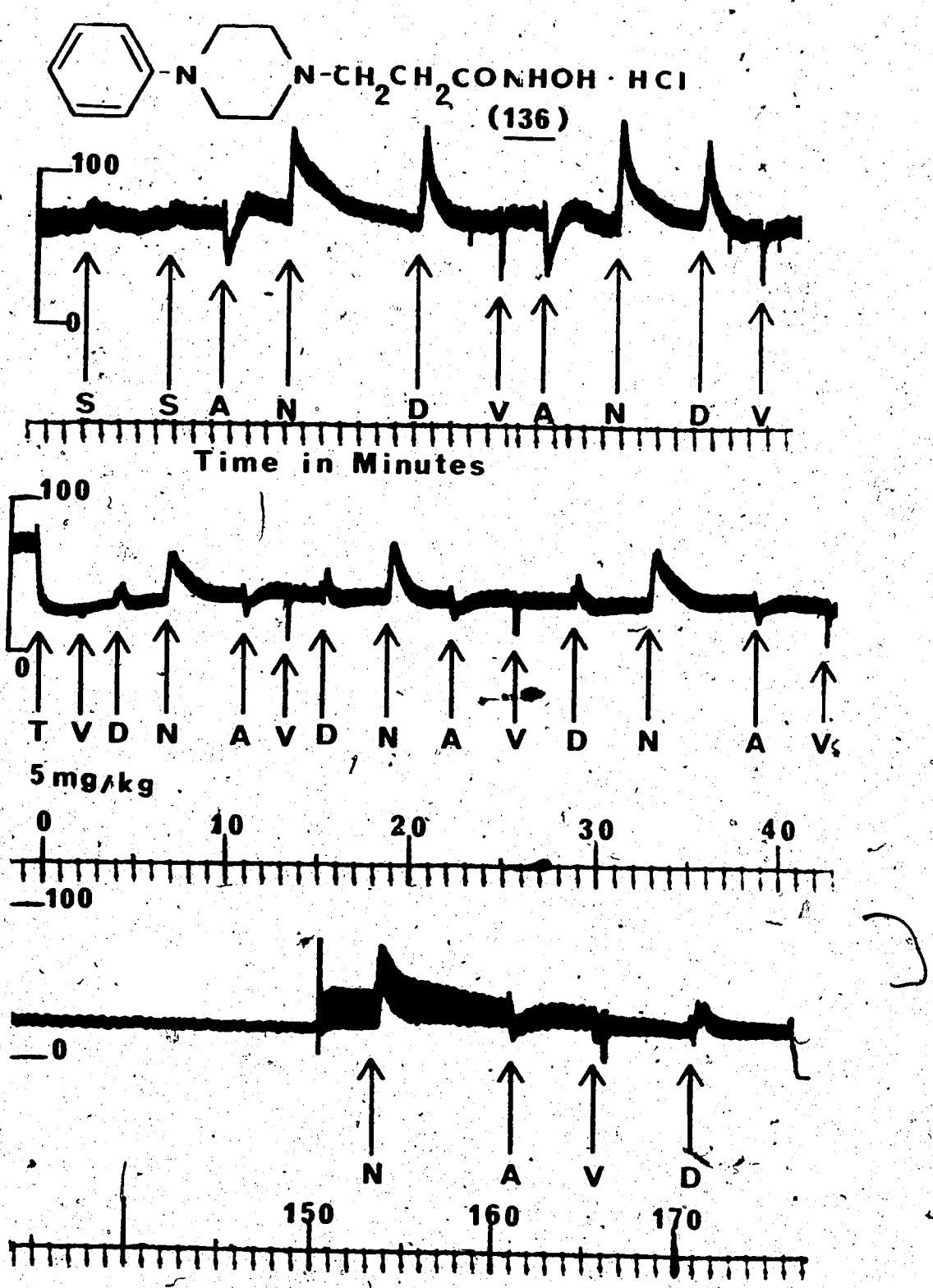



Figure IX : Hypotensive evaluation of 3-[1-(4-phenyl-piperazino)]propionohydroxamic acid monohydrochloride(136).

Pharmacological Evaluation of Aminoester Hydrochlorides, Aminocarboxylic Acid Hydrochlorides, Aminohydroxamic Acid Hydrochlorides, 1-Hydroxyquinoxalin-2-one 4-oxides and 1-Hydroxyquinoxalin-2-ones.

All the compounds synthesized in this study were evaluated for their effect on the arterial blood pressure of urethane-anesthetized rats. At each dosage tested, every compound was evaluated at least twice in separate animals. The data obtained is shown in Tables XVII B, XVIII, XIX, XX, XXI, XXII, XXIII and XXIV.

For Tables XVIII, XX, XXII and XXIV, where the effect of the test drug upon the "standard drug" blood pressure responses of the anesthetized rat are recorded, the following symbols are used:

+++ to indicate a blood pressure response to the "standard drug" after test drug administration equal to 2/3 or greater of the response before the test drug was given.

++ to indicate a blood pressure response to the "standard drug" after test drug administration from 1/3 to 2/3 of the response before the test drug was given.

\+ to indicate a blood pressure response to the "standard drug" after test drug administration less than 1/3 of the response before the test drug was given.

\- indicates a complete absence of any blood pressure response to the "standard drug" after test drug administration.

TABLE XVII B

Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 74 | 2 | 5 | -6 | -8 | 1.5 |
| 75 | 2 | 5 | 11 | 17 | 2.5 |
| | 2 | 25 | 14 | 20 | 10 |
| 76 | 2 | 5 | 13 | 16 | 5 |
| | 2 | 25 | 22 | 25 | 27 |
| 77 | 2 | 5 | 4 | 3 | 14 |
| | 2 | 25 | 28 | 24 | 30 |
| 78 | 2 | 5 | 6 | 9 | 7 |
| | 3 | 25 | 7 | 12 | 7 |
| 79 | 2 | 1 | 10 | 22 | 8.5 |
| | 3 | 5 | 41 | 51 | 20 |
| | 11 | 25 | 47 | 56 | 41 |
| 80 | 2 | 1 | 2 | 4 | 1.5 |
| | 6 | 5 | 5 | 9 | 7.5 |
| | 7 | 25 | 19 | 25 | 14 |

TABLE XVII B cont'd

Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 81 | 2 | 1 | 8 | 18 | 7 |
| | 3 | 5 | 36 | 43 | 9 |
| | 2 | 25 | 48 | 58 | 19 |
| 97 | 2 | 5 | 39 | 58 | >94 |
| 96 | 2 | 1 | 6 | 10 | 1 |
| | 2 | 5 | 16 | 36 | >106 |
| 82 | 2 | 5 | -16 | -30 | 4.5 |
| 83 | 2 | 5 | 2 | 2 | 0.5 |
| | 2 | 25 | 11 | 12 | 21 |
| 84 | 2 | 5 | -3 | -4 | 4 |
| | 2 | 25 | -2 | -3 | 3.5 |
| 85 | 2 | 5 | 10 | 14 | 2 |
| | 3 | 25 | 20 | 19 | 12 |
| 86 | 2 | 5 | 18 | 31 | 10 |
| | 2 | 25 | 34 | 44 | 16 |

TABLE XVII B cont'd

Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 87 | 2 | 1 | 31 | 58 | 30 |
| | 2 | 5 | 44 | 49 | 16 |
| | 2 | 25 | 46 | 49 | >172 |
| 88 | 2 | 1 | 13 | 21 | 7 |
| | 5 | 5 | 18 | 28 | 17 |
| | 8 | 25 | 36 | 37 | 50 |
| 89 | 2 | 1 | 12 | 22 | 14 |
| | 3 | 5 | 29 | 44 | >44 |
| | 3 | 25 | 18 | 40 | >102 |
| 99 | 2 | 5 | 20 | 32 | 69 |
| 100 | 2 | 5 | 26 | 42 | >140 |
| 98 | 2 | 5 | 5 | 8 | 4 |
| 90 | 2 | 5 | 36 | 60 | 10.5 |
| | 2 | 25 | 28 | 62 | >130 |
| 91 | 3 | 5 | 17 | 28 | 11 |
| | 2 | 25 | 17 | 30 | 10 |

TABLE XVII B cont'd

Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 92 | 2 | 1 | 32 | 50 | 7 |
| | 3 | 5 | 29 | 45 | >40 |
| 93 | 2 | 5 | 16 | 36 | >85 |
| | 2 | 25 | 24 | 51 | >53 |
| 94 | 2 | 5 | 11 | 19 | 4 |
| | 2 | 25 | 23 | 39 | 5.5 |
| 95 | 3 | 5 | 16 | 28 | 5 |
| | 2 | 25 | 13 | 28 | 15 |
| 101 | 2 | 5 | 6 | 9 | 2 |
| 102 | 4 | 5 | 10 | 20 | 4 |
| 103 | 2 | 5 | 9 | 13 | 7 |
| 104 | 2 | 5 | 12 | 26 | 3.5 |
| 105 | 2 | 5 | 6 | 8 | 6.5 |
| 106 | 2 | 5 | 24 | 32 | >79 |
| 107 | 2 | 5 | 14 | 26 | 16.5 |
| 108 | 2 | 5 | 34 | 68 | >142 |

TABLE XVII B cont'd

Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mm/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 109 | 2 | 5 | 26 | 32 | 19 |
| 110 | 2 | 5 | 26 | 40 | >87 |
| 111 | 2 | 5 | 28 | 55 | >87 |
| 112 | 3 | 1 | 18 | 36 | >54 |
| | 2 | 5 | 42 | 68 | >35 |

TABLE XVIII

Effect of Aminoester Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 74 | 5 | +++ | +++ (D) | +++ | +++ |
| 75 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | ++ (N) | +++ | ++ |
| 76 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | + | ++ (N) | ++ | ++ |
| 77 | 5 | ++ | ++ (N) | +++ | +++ |
| | 25 | + | +++ (D) | +++ | ++ |
| 78 | 5 | +++ | ++ (D) | +++ | +++ |
| | 25 | + | ++ (D) | +++ | +++ |
| 79 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | +++ | +++ (N) | ++ | ++ |
| | 25 | +++ | + (D) | ++ | ++ |
| 80 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | +++ | ++ (N) | +++ | +++ |
| | 25 | ++ | ++ (D) | +++ | +++ |
| 81 | 1 | +++ | ++ (D) | +++ | +++ |
| | 5 | + | + (D) | +++ | +++ |
| | 25 | + | + (D) | +++ | +++ |

TABLE XVIII cont'd

Effect of Aminoester Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 97 | 5 | +++ | - (D) | ++ | + |
| 96 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | +++ | + (D) | ++ | + |
| 82 | 5 | +++ | +++ (D) | +++ | +++ |
| 83 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | +++ (N) | +++ | ++ |
| 84 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | +++ (N) | +++ | +++ |
| 85 | 5 | +++ | ++ (D) | +++ | ++ |
| | 25 | ++ | ++ (D) | ++ | ++ |
| 86 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | - (D) | +++ | +++ |
| 87 | 1 | +++ | ++ (D) | +++ | ++ |
| | 5 | +++ | + (N) | ++ | ++ |
| | 25 | +++ | + (N) | ++ | + |
| 88 | 5 | ++ | ++ (N) | +++ | +++ |
| | 25 | + | + (N) | ++ | + |

TABLE XVIII cont'd

Effect of Aminoester Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 89 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | + | + (D) | +++ | ++ |
| | 25 | + | + (D) | ++ | + |
| 99 | 5 | +++ | + (D) | + | + |
| 100 | 5 | +++ | + (D) | ++ | + |
| 98 | 5 | +++ | +++ (D) | +++ | +++ |
| 90 | 5 | +++ | + (D) | ++ | +++ |
| | 25 | +++ | - (D) | ++ | ++ |
| 91 | 5 | ++ | + (D) | +++ | +++ |
| | 25 | ++ | - (D) | ++ | ++ |
| 92 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | +++ | - (D) | +++ | ++ |
| 93 | 5 | +++ | + (D) | ++ | ++ |
| | 25 | +++ | + (D) | + | - |
| 94 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | ++ (D) | ++ | ++ |

TABLE XVIII cont'd

Effect of Aminoester Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 95 | 5 | +++ | ++ (D) | ++ | +++ |
| | 25 | +++ | - (D) | ++ | + |
| 101 | 5 | +++ | +++ (D) | +++ | +++ |
| 102 | 5 | +++ | +++ (D) | +++ | +++ |
| 103 | 5 | +++ | +++ (D) | +++ | +++ |
| 104 | 5 | +++ | +++ (D) | +++ | +++ |
| 105 | 5 | +++ | +++ (D) | +++ | +++ |
| 106 | 5 | +++ | ++ (D) | +++ | + |
| 107 | 5 | +++ | +++ (D) | +++ | +++ |
| 108 | 5 | +++ | + (D) | ++ | - |
| 109 | 5 | +++ | + (D) | ++ | + |
| 110 | 5 | +++ | ++ (D) | ++ | + |
| 111 | 5 | +++ | + (D) | ++ | + |
| 112 | 1 | +++ | - (D) | + | - |
| | 5 | +++ | - (D) | + | + |

TABLE XIX

Effect of Aminocarboxylic Acid Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 117 | 2 | 1 | 4 | 10 | 4.5 |
| | 4 | 5 | 17 | 31 | >30 |
| 118 | 2 | 5 | 7 | 12 | 4 |
| | 4 | 5 | 6 | 12 | 4 |
| 119 | 3 | 5 | 6 | 10 | 3 |

TABLE XX

Effect of Aminocarboxylic Acid Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 117 | 1 | +++ | +++ (D) | +++ | +++ |
| | 5 | +++ | ++ (D) | +++ | ++ |
| 118 | 5 | +++ | +++ (D) | +++ | +++ |
| 119 | 5 | +++ | +++ (D) | +++ | +++ |

TABLE XXI

Effect of Aminohydroxamic Acid Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 120 | 2 | 5 | -7 | -11 | 1 |
| | 2 | 25 | -7 | -11 | 2.5 |
| 121 | 3 | 5 | 0 | - | - |
| | 3 | 25 | 5 | | 2 |
| 122 | 2 | 5 | 5 | 6 | 1 |
| | 2 | 25 | 3 | 4 | 2 |
| 123 | 2 | 5 | 56 | 59 | 8 |
| | 2 | 25 | 47 | 58 | 3.5 |
| 124 | 2 | 1 | 18 | 34 | 21.5 |
| | 3 | 5 | 43 | 46 | 66 |
| | 2 | 25 | 47 | 53 | >108 |
| 125 | 3 | 5 | 13 | 26 | 22 |
| | 2 | 35 | 20 | 25 | 15 |
| 126 | 2 | 1 | 8 | 16 | 9.5 |
| | 4 | 5 | 31 | 49 | >89 |
| 134 | 2 | 5 | 31 | 66 | >123 |

TABLE XXI cont'd

Effect of Aminohydroxamic Acid Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 127 | 2 | 5 | 1 | 1 | 1 |
| | 2 | 25 | -3 | -5 | 1 |
| 128 | 2 | 5 | -4 | -6 | 1.5 |
| | 2 | 25 | -6 | -8 | 2 |
| 129 | 2 | 5 | 1 | 3 | 1 |
| | 2 | 25 | 3 | 7 | 1.5 |
| 130 | 2 | 5 | 10 | 16 | 5 |
| | 2 | 25 | 26 | 35 | 5 |
| 131 | 2 | 1 | 22 | 42 | >123 |
| | 2 | 5 | 45 | 64 | >93 |
| | 2 | 25 | 45 | 66 | >97 |
| 132 | 3 | 5 | 18 | 24 | 3 |
| | 3 | 25 | 43 | 41 | 8 |
| 133 | 2 | 1 | 13 | 22 | >68 |
| | 3 | 5 | 20 | 32 | >113 |
| | 2 | 25 | 20 | 41 | >128 |
| 142 | 2 | 5 | 32 | 64 | >140 |

TABLE XXI cont'd

Effect of Aminohydroxamic Acid Hydrochlorides on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 136 | 2 | 1 | 16 | 20 | >62 |
| | 4 | 5 | 30 | 62 | >212 |
| 143 | 2 | 5 | 13 | 22 | 3.5 |
| 144 | 2 | 5 | 22 | 33 | 30 |
| | 2 | 25 | 28 | 54 | >56 |
| 139 | 2 | 5 | 5 | 10 | >23 |
| 140 | 2 | 5 | 20 | 38 | 6 |
| 135 | 2 | 5 | 20 | 42 | >68 |
| 137 | 2 | 5 | 28 | 44 | 6.5 |
| 138 | 2 | 5 | 8 | 18 | >96 |
| 141 | 2 | 5 | 38 | 63 | >127 |

TABLE XXII

Effect of Aminohydroxamic Acid Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 120 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | +++ (N) | +++ | +++ |
| 121 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 122 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | +++ (N) | +++ | +++ |
| 123 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (N) | ++ | +++ |
| 124 | 1 | +++ | ++ (D) | ++ | + |
| | 5 | +++ | + (D) | +++ | ++ |
| | 25 | +++ | - (D) | + | - |
| 125 | 5 | +++ | ++ (D) | +++ | +++ |
| | 25 | ++ | ++ (D) | +++ | +++ |
| 126 | 1 | +++ | +++ (D) | +++ | ++ |
| | 5 | ++ | ++ (D) | +++ | ++ |
| 134 | 5 | +++ | ++ (D) | ++ | + |

TABLE XXII cont'd

Effect of Aminohydroxamic Acid Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 127 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 128 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 129 | 5 | +++ | +++ (N) | +++ | +++ |
| | 25 | +++ | +++ (N) | +++ | +++ |
| 130 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | + | +++ (D) | +++ | +++ |
| 131 | 1 | +++ | + (D) | ++ | + |
| | 5 | +++ | ++ (D) | +++ | ++ |
| | 25 | +++ | - (D) | +++ | + |
| 132 | 5 | ++ | +++ (D) | +++ | +++ |
| | 25 | + | +++ (D) | ++ | +++ |
| 133 | 1 | +++ | ++ (D) | +++ | ++ |
| | 5 | +++ | ++ (D) | +++ | ++ |
| | 25 | ++ | + (D) | ++ | + |
| 142 | 5 | +++ | + (D) | + | - |

TABLE XXII cont'd

Effect of Aminohydroxamic Acid Hydrochlorides on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 136 | 1 | +++ | ± (D) | ++ | - |
| | 5 | ++ | + (D) | ++ | + |
| 143 | 5 | +++ | +++ (D) | +++ | +++ |
| 144 | 5 | +++ | ++ (D) | +++ | +++ |
| | 25 | +++ | + (D) | +++ | - |
| 139 | 5 | +++ | ++ (D) | +++ | ++ |
| 140 | 5 | +++ | +++ (D) | +++ | +++ |
| 135 | 5 | +++ | - (D) | + | - |
| 137 | 5 | +++ | +++ (D) | +++ | +++ |
| 138 | 5 | +++ | ++ (D) | ++ | + |
| 141 | 5 | +++ | - (D) | + | - |

TABLE XXIII

Effect of 1-Hydroxyquinoxalin-2-one 4-oxides and 1-Hydroxyquinoxalin-2-ones on the Arterial Blood Pressure of the Anesthetized Rat

| Compound | Number of Experiments | Dosage (mg/kg) | Mean B.P. Fall (mm/Hg) | Mean Percentage B.P. Fall | Mean Duration (Minutes) |
|---|---|---|---|---|---|
| 159 | 2 | 5 | 0 | - | - |
| | 2 | 25 | 2 | 4 | 0.5 |
| 160 | 2 | 5 | 0 | - | - |
| | 2 | 25 | 10 | 14 | 2 |
| 161 | 2 | 5 | 0 | - | - |
| | 2 | 25 | 8 | 14 | 1 |
| 162 | 2 | 5 | 0 | - | - |
| | 1 | 25 | 32 | 42 | 2 |
| 163 | 2 | 5 | 0 | - | - |
| | 2 | 25 | 2 | 4 | 1 |
| 164 | 2 | 5 | 0 | - | - |
| | 2 | 25 | 10 | 18 | 3.5 |

TABLE XXIV

Effect of 1-Hydroxyquinoxalin-2-one 4-oxides and 1-Hydroxyquinoxalin-2-ones on the Standard Drug Blood Pressure Responses of the Anesthetized Rat

| Compound | Dose (mg/kg) | Vagal Stimulation | Nicotine (N) or DMPP (D) | Noradrenaline | Acetylcholine |
|---|---|---|---|---|---|
| 159 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | ++ |
| 160 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 161 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 162 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |
| 163 | 5 | +++ | +++ (D) | +++ | ++ |
| | 25 | +++ | +++ (D) | +++ | ++ |
| 164 | 5 | +++ | +++ (D) | +++ | +++ |
| | 25 | +++ | +++ (D) | +++ | +++ |

## III. DISCUSSION

### Effect of Aminoester Hydrochlorides on the Arterial Blood Pressure of Anesthetized Rats

The initial aminoesters investigated were the methyl and ethyl 3-aminopropionate hydrochlorides. Represented by general structure (169), the structures of the 3-aminopropionate hydrochlorides are summarized in Table XXV, while the pharmacological results obtained with these derivatives are presented in Tables XVII and XVIII.

TABLE XXV

3-Aminopropionate Hydrochlorides

$R\text{-}CHR^1CHR^2COOR^3 \cdot 2HCl$

(169)

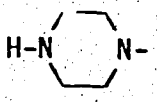
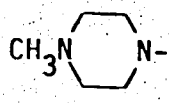
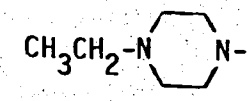
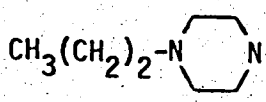
| Compound | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 74 |  | H | $CH_3$ | $CH_3$ |
| 75 |  | H | $CH_3$ | $CH_3$ |
| 76 |  | H | $CH_3$ | $CH_3$ |
| 77 |  | H | $CH_3$ | $CH_3$ |

TABLE XXV cont'd

3-Aminopropionate Hydrochlorides

$R\text{-}CHR^1CHR^2COOR^3 \cdot 2HCl$

(169)

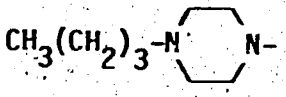
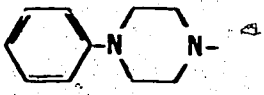
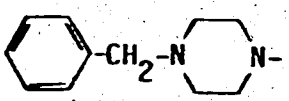
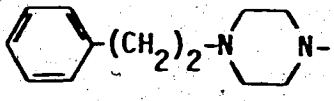
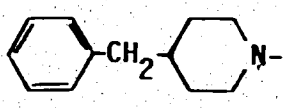
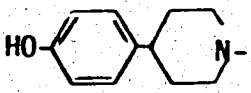
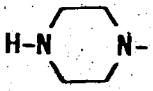
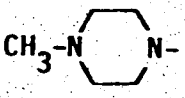
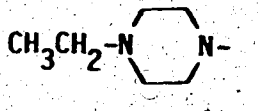
| Compound | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 78 |  | H | $CH_3$ | $CH_3$ |
| 79 |  | H | $CH_3$ | $CH_3$ |
| 80 |  | H | $CH_3$ | $CH_3$ |
| 81 |  | H | $CH_3$ | $CH_3$ |
| 97 |  | H | $CH_3$ | $CH_3$ |
| 96 |  | H | $CH_3$ | $CH_3$ |
| 82 |  | $CH_3$ | H | $CH_3$ |
| 83 |  | $CH_3$ | H | $CH_3$ |
| 84 |  | $CH_3$ | H | $CH_3$ |

TABLE XXV cont'd

3-Aminopropionate Hydrochlorides

$R\text{-}CHR^1CHR^2COOR^3 \cdot 2HCl$

(169)

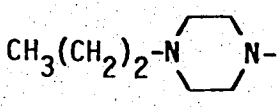
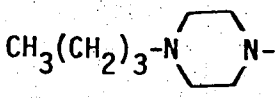
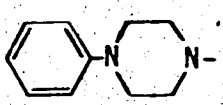
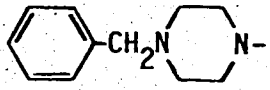
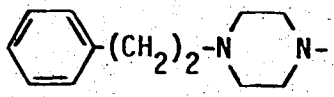
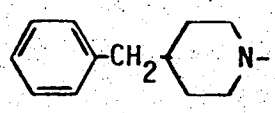
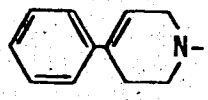
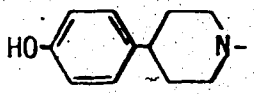
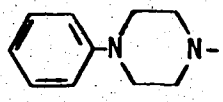
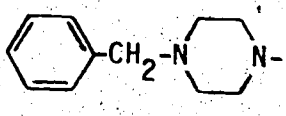
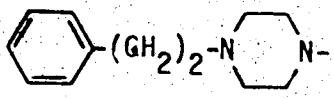
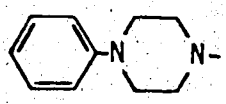
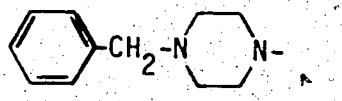
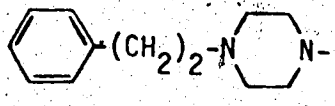
| Compound | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 85 |  | $CH_3$ | H | $CH_3$ |
| 86 |  | $CH_3$ | H | $CH_3$ |
| 87 |  | $CH_3$ | H | $CH_3$ |
| 88 |  | $CH_3$ | H | $CH_3$ |
| 89 |  | $CH_3$ | H | $CH_3$ |
| 99 |  | $CH_3$ | H | $CH_3$ |
| 100 |  | $CH_3$ | H | $CH_3$ |
| 98 |  | $CH_3$ | H | $CH_3$ |
| 90 |  | H | H | $CH_3$ |

TABLE XXV cont'd

3-Aminopropionate Hydrochlorides

$R\text{-}CHR^1CHR^2COOR^3 \cdot 2HCl$

(169)

| Compound | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| 91 |  | H | H | $CH_3$ |
| 92 |  | H | H | $CH_3$ |
| 93 |  | H | H | $CH_3CH_2$ |
| 94 |  | H | H | $CH_3CH_2$ |
| 95 |  | H | H | $CH_3CH_2$ |

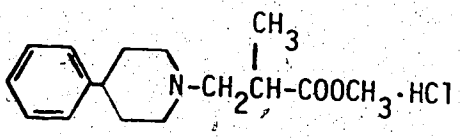
Midha (1969) has reported the evaluation of the hypotensive action of one of the above compounds, namely, methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate hydrochloride (75). The author observed that this compound (at 25 mg/Kg) gave a 43% fall (for five minutes) in the arterial blood pressure of an anesthetized cat. In the present study, the same compound at the same dosage gave a 20% fall (for ten minutes) in the arterial blood pressure of anesthetized rats.

Before commenting any further, it should be noted that most of the following discussion can only represent tentative qualitative speculation since the pharmacological data obtained for each compound is based upon a small number of experiments. Furthermore, all test drug dosages were administered on a mg/Kg bases, which should introduce a slight bias by making those derivatives with a lower molecular weight seem more potent relative to those analogues with a larger molecular weight than if all drugs had been tested at the same molar dosage. Finally, the limitation of the screening regimen employed does not permit any speculation on the possibility of pre-synaptic interactions or other possible sites of action different from those particular receptors acted upon by the "standard drugs" utilized in the screening method.

Considering the homologous series of α-methyl branched 3-aminopropionates, it would appear that the hypotensive activity was greatest for those analogues which had a 3-amino substituent incorporating a 4-position aromatic group. Thus the hydro-, methyl-, ethyl-, n-propyl- and n-butyl-piperazinyl derivatives produced only moderate falls in blood pressure for relatively short periods of time, even at dosages up to 25 mg/Kg; whereas the phenyl-, and phenethyl-piperazinyl as well as the benzyl- and p-hydroxyphenyl-piperidino derivatives gave relatively large falls in blood pressure for prolonged lengths of time. One exception was the benzylpiperazinyl compound which had a

blood pressure response similar to that for the non-aromatic containing analogues. This difference was notable because the structurally similar benzylpiperidino derivative was among the more potent hypotensive agents in the series. Methyl 2-methyl-3-[1-(p-hydroxyphenylpiperidino)]propionate hydrochloride (96) and methyl 2-methyl-3-[1-(4-benzylpiperidino)]propionate hydrochloride (97) were found to cause similar percent falls in blood pressure to the various aromatic-substituted piperazine derivatives, but maintained that fall for much longer periods of time.

In agreement with the observed importance of an aromatic function in the amino substituent for hypotensive activity in this type of structure, Midha et al. (1969) found methyl 2-methyl-3-[1-(4-phenylpiperidino)]propionate hydrochloride (4) among the most potent of the several hypotensive analogs which they examined.



(4)

This compound was found to give a 44% blood pressure fall for 30 minutes at 25 mg/Kg. The comparable piperazine derivative investigated in the present study was methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (79) which gave a 56% blood pressure fall for 41 minutes at the same dosage level.

Apparently, a cyclic aromatic-containing 3-amino function is best, since two investigations by Biggs et al. (1972a and 1972b) on comparable 3-aminopropionate esters with general structure (11) where the amino substituents were generally acyclic alkyl and ara-alkyl substituted amino groups, were found to possess only moderate degrees of hypotensive activity of very short duration at 4 mg/Kg.

$$R^1R^2\overset{\oplus}{N}HCHR^3CHR^4COOCH_3X^{\ominus}$$

(11)

All members of the α-methyl branched series of 3-amino-propionates at dosages of 5 mg/Kg or 25 mg/Kg decreased with normal pressor response to small doses of either 1,1-dimethyl-4-phenylpiperazine (DMPP) or nicotine, except for methyl 3-(1-piperazinyl)propionate hydrochloride (74) which had no affect upon any "standard drug" response. The ethyl- and phenyl-piperazinyl, benzyl- and p-hydroxyphenyl-piperidino propionates additionally interfered with the normal pressor responses to noradrenaline, thus indicating that these analogues possess an α-adrenoceptive blocking mechanism of action. Of the above four derivatives, the ethylpiperazinyl derivative also antagonized the cardiac arrest induced by vagal stimulation. This result would seem to imply a mixed ganglionic and α-adrenoceptive blocking activity for this drug. The n-propyl-, n-butyl-,

n-benzyl- and phenethyl-piperazinylpropionates attenuated the blood pressure response to vagal stimulation, but left the response to noradrenaline unaltered. The foregoing observation could be interpreted as evidence for a ganglionic blocking mechanism of action for these derivatives. A similar mechanism is likely for methyl 2-methyl-3-[1-(4-methylpiperazinyl)]-propionate hydrochloride (75) which inhibited only the responses to nicotine and acetylcholine. The failure of this methylpiperazinyl analogue to inhibit the noradrenaline response means that an α-adrenoceptive blocking activity for this drug is unlikely, while the failure to inhibit vagal stimulation responses would seem to imply that the degree of blockade at the parasympathetic ganglia was less than that at the sympathetic ganglia. Most of the α-methyl branched analogs reduced the depressor response to acetylcholine; however, little importance was attached to this occurrence since comparability before and after test drug administration was judged invalid due to the lowered blood pressure caused by the test drugs.

In summary, those 3-aminopropionates in which the 4-position of the piperazine substituent was an alkyl or aralkyl group appeared to have ganglionic blocking component in their mechanism of action. Whereas the 4-phenylsubstituted-piperazinyl and both phenyl piperidino derivatives had an α-adrenoceptive blocking action. The possibility that these latter three compounds also possess a selective sympathetic

ganglion blocking action or a presynaptic action like guanethidine could not be ruled out by the screening regimen employed.

Similarly with the β-methyl branched compounds, the presence of a 4-position aromatic group in the 3-amino substituent endowed the compound with significant activity and a prolonged duration of action. Once again, the benzylpiperazinyl analogue was observed to have less hypotensive activity than either the phenyl- or phenethyl-piperazinyl β-methyl branched propionates.

Upon examining the responses to the "standard drugs" after administration of the β-methyl branched test drugs, it was found that the hydro-, methyl- and ethyl-piperazinyl and p-hydroxyphenyl-piperidino derivatives, at a dosage of 5 mg/Kg or 25 mg/Kg had no effect upon any "standard drug" response, while the remaining analogues all interfered with either nicotine or DMPP. The n-propyl-, benzyl- and phenethyl-piperazinyl β-methyl branched compounds also antagonized both the responses to vagal stimulation and noradrenaline at 25 mg/Kg. These findings would allow the interpretation of a mixed ganglionic and α-adrenoceptive blocking action for the latter three compounds. In contrast, the corresponding three α-methyl branched propionates all seemed to possess a ganglionic blocking mechanism only. It would appear that the mechanism of action for these three of the alkyl- and ara-alkyl-substituted piperazinylpropionates had changed from ganglionic blockade in the case of the α-methyl branched esters to a mixture of ganglionic and α-adrenoceptive

blockade for the β-methyl branched analogues. The phenylpiperazinyl as well as the benzylpiperidino and phenyl-1,2,5,6-tetrahydropyridino β-methyl branched propionates at 5 mg/Kg were found to interfere with the normal pressor responses to noradrenaline without having an effect upon the vagal stimulation response. Such observations suggested an α-adrenoceptive blocking mechanism. The α-methyl esters corresponding to these three β-methyl branched analogues seemed to have the same mechanism. However, the possibility of a selective sympathetic ganglionic blocking component in addition to α-adrenoceptive blockade can not be overlooked for these latter compounds. The n-butylpiperazinyl derivative abolished only the DMPP response without altering any other "standard drug" responses or the response to vagal stimulation. As mentioned for methyl 2-methyl-3-[1-(4-methylpiperazinyl)]propionate hydrochloride (75), this pattern of results permits the interpretation of selective sympathetic ganglionic blockade. Again, most β-methyl branched esters attenuated the acetylcholine blood pressure fall - but as with the α-methyl branched compounds, the fact that the blood pressure was already lowered did not allow much significance to be attached to these observations.

Besides defining the desirable structural characteristics for the 4-position of the 3-amino substituent, the above series of compounds were prepared with the objective of examining the effect of α- and β-methyl substitution upon the hypotensive

response. In comparing the magnitude and duration of blood pressure fall of the α-methyl branched analogues with the corresponding β-methyl esters, any differences observed were not consistently in favor of either series for those derivatives acting via a ganglionic blocking or a mixed ganglionic and α-adrenoceptive receptor blocking action. However, for the phenylpiperazinyl and benzylpiperidino substituents, which appeared to act predominately by an α-adrenoceptive receptor blocking mechanism, the α-methyl derivatives had a somewhat greater magnitude of hypotensive response than the β-methyl branched propionates. Those methyl 3-aminopropionates with no α- or β-methyl group, namely, methyl 3-[-1(4-phenylpiperazinyl)]-propionate hydrochloride (90), methyl 3-[1-(4-benzylpiperazinyl)]-propionate hydrochloride (91), and methyl 3-[1-(4-phenethyl-piperazinyl)]propionate hydrochloride (92), had similar magnitudes, durations and mechanisms of hypotensive action to the corresponding methyl esters with an α- or β-methyl branch. Taken together, these observations would seem to suggest that the presence or absence of either an α- or β-methyl group was not crucial to the ability of this type of 3-aminopropionate to lower the blood pressure of anesthetized rats for a period of time.

To compare with the methyl 3-aminopropionates lacking an α- or β-methyl group, three unbranched ethyl 3-aminopropionates were synthesized. Ethyl 3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (93) and ethyl 3-[1-(4-benzylpiperazinyl)]propionate

hydrochloride (94) had similar magnitudes and durations of action as the corresponding methyl esters. However, ethyl 3-[1-(4-phenethylpiperazinyl)]propionate hydrochloride (95) lowered the blood pressure only 28% for five minutes at a dosage of 5 mg/Kg, while methyl 3-[1-(4-phenethylpiperazinyl)]propionate hydrochloride (92) gave a blood pressure fall of 45% for longer than 40 minutes at 5 mg/Kg.

Although the three methyl esters and the three ethyl esters all interfered with the pressor response to DMPP as well as the depressor response to acetylcholine at 5 mg/Kg or 25 mg/Kg, their effects upon the other two "standard drug" responses were variable. Of the six esters, methyl 3-[1-(4-phenethylpiperazinyl)]propionate hydrochloride (92) did not interfere with the pressor response to noradrenaline or with the response to vagal stimulation, thus indicating a possible selective sympathetic ganglionic blocking mechanism of action for this compound. Suggestive of a mixed ganglionic and α-adrenoceptive blocking action, methyl 3-[1-(4-benzylpiperazinyl)]-propionate hydrochloride (91) was the only derivative to additionally attenuate both vagal stimulation and noradrenaline responses. The remaining four unbranched esters besides interfering with DMPP and acetylcholine responses also antagonized the noradrenaline response, but did not alter the vagal stimulation response. This was consistent with an α-adrenoceptive receptor blocking action.

Once evidence of the importance of the aromatic amino functional group and the relative unimportance of the presence or absence of α- or β-methyl substituent was obtained, the effect of increasing or decreasing the number of methylene groups inserted between the amino function and the ester group on the degree and duration of hypotensive activity were examined. This was approached by preparing several ethyl formate, ethyl acetate and ethyl butyrate derivatives containing the aromatic-substituted amino groups previously found to be associated with highly active hypotensive 3-aminopropionates. The structures of this new series of compounds represented by general formula (170) are represented in Table XXVI, while the pharmacological data is summarized in Tables XVII and XVIII.

TABLE XXVI

Ethyl Amino-ester Hydrochlorides

$R\text{-}(CH_2)_nCOOCH_2CH_3 \cdot xHCl$

(170)

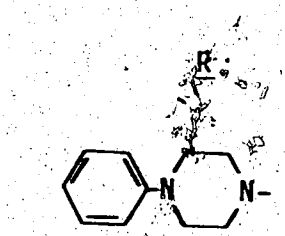
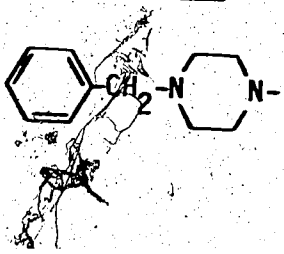
| Compound | | n | x |
|---|---|---|---|
| 101 |  | 0 | 1 |
| 102 |  | 0 | 1 |

TABLE XXVI cont'd

Ethyl Amino-ester Hydrochlorides

$R\text{-}(CH_2)_nCOOCH_2CH_3 \cdot xHCl$

(170)

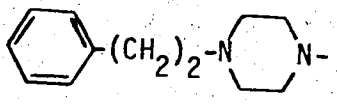
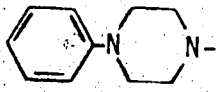
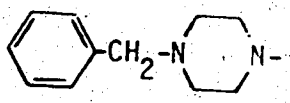
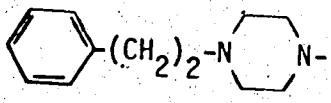
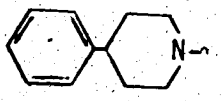
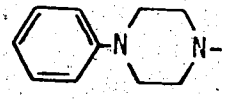
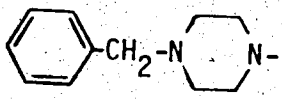
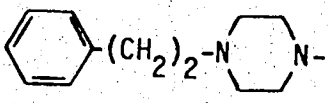
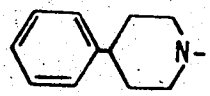
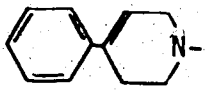
| Compound | R | n | x |
|---|---|---|---|
| 103 |  | 0 | 1 |
| 104 |  | 1 | 2 |
| 105 |  | 1 | 2 |
| 106 |  | 1 | 2 |
| 107 |  | 1 | 2 |
| 108 |  | 3 | 2 |
| 109 |  | 3 | 2 |
| 110 |  | 3 | 2 |
| 111 |  | 3 | 2 |

TABLE XXVI cont'd

Ethyl Amino-ester Hydrochlorides

$R\text{-}(CH_2)_nCOOCH_2CH_3 \cdot xHCT$

(170)

| Compound | R | n | x |
|---|---|---|---|
| 112 |  | 3 | 2 |

Of the three formate derivatives, ethyl 1-(4-benzyl-piperazinyl)formate hydrochloride (102) had a similar magnitude and duration of action, while the phenyl- and phenethyl-piperazinyl-formates were much weaker hypotensives than the corresponding ethyl 3-aminopropionate hydrochlorides (all derivatives were compared at the 5 mg/Kg dosage level). Examination of the ethyl acetates revealed that ethyl 2-[1-(4-phenethylpiperazinyl)]acetate hydrochloride (106) had a hypotensive response at 5 mg/Kg comparable to the similarly substituted ethyl 3-aminopropionate, whereas the phenyl- and benzyl-piperazinyl acetates compared with their respective ethyl 3-aminopropionates exhibited much less hypotensive activity. Unfortunately, no ethyl 3-aminopropionate was prepared which would be comparable with ethyl 2-[1-(4-phenylpiperidino)]-acetate hydrochloride (107). These findings would seem to indicate that generally speaking, shortening the distance between the ester and amino function by either one or two methylene groups results

in diminished hypotensive activity.

Due to the evanescent effects of all three ethyl formates, it is not surprising that none were found to have an affect upon any of the "standard drug" responses. Similarly, for the phenyl- and benzyl-acetates, the duration of action was short and no effects upon the "standard drug" responses were detected. However, ethyl 2-[1-(4-phenethylpiperazinyl)]acetate hydrochloride (106), which produced a substantial hypotensive response of long duration, was found to inhibit the pressor response to DMPP and to attenuate the depressor response to acetylcholine, while leaving the vagal stimulation and noradrenaline responses unaffected. As previously discussed, this could suggest a selecti sympathetic ganglionic blocking action.

With regards to the butyrate derivatives, ethyl 4-[1-(4-phenylpiperazinyl)]butyrate hydrochloride (108), ethyl 4-[1-(4-benzylpiperazinyl)]butyrate hydrochloride (109) and ethyl 4-[1-(4-phenethylpiperazinyl)]butyrate hydrochloride (110) gave hypotensive responses at 5 mg/Kg which were substantially greater in magnitude and longer in duration of action than were the corresponding ethyl 3-aminopropionates compared at the same dosage level. Thus it would seem that lengthening the ester-amino group distance by one methylene group is certainly not disadvantageous and probably advantageous for potent hypotensive action.

Although no butyrate was found to affect the response to vagal stimulation in any way, all these derivatives did inhibit the pressor responses to noradrenaline and to low doses of DMPP as well as reducing the depressor response to acetylcholine. Consequently, an α-adrenoceptive blocking mechanism of action was again implicated, with the possibility of a selective ganglionic blocking component unresolved.

Effect of 3-[1-(4-Phenylpiperazinyl)]propionic Acid Hydrochlorides on the Arterial Blood Pressure of Anesthetized Rats.

Coutts et al. (1971) found that 3-aminopropionic acids with general structure (28), where R was piperidino, 3-methylpiperidino, homopiperidino or ethylamino and $R^1$ was a hydrogen atom or a methyl group, had no effect upon the blood pressure of anesthetized cats, whereas the corresponding esters and hydroxamates had significant hypotensive properties.

$$R\text{-}CH_2CHR^1COOH$$

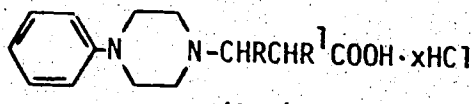
(28)

Consequently, in the present investigation, three 3-[1-(4-phenylpiperazinyl)]propionic acid hydrochlorides, one with an α-methyl group, 2-methyl-3-[1-(4-phenylpiperazinyl)]propionic acid hydrochloride (117), one with a β-methyl group, 3-methyl-3-[1-(4-phenylpiperazinyl)]propionic acid hydrochloride (118)

and one without a methyl branch, 3-[1-(4-phenylpiperazinyl)]-propionic acid hydrochloride (119) were prepared. These aminocarboxylic acids whose structures may be represented by general formula (171) and are summarized in Table XXVII were examined for their hypotensive activity because their substituents coincided with three of the more potent aminoester hypotensives previously discussed, namely, methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (79), methyl 3-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (87) and methyl 3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (90), respectively. The pharmacological findings for the three aminocarboxylic acids are tabulated in Table XIX and XX.

TABLE XXVII

3-[1-(4-Phenylpiperazinyl)]propionic Acid Hydrochlorides



(171)

| Compound | R | $R^1$ | x |
|---|---|---|---|
| 117 | H | H | 1 |
| 118 | $CH_3$ | H | 1 |
| 119 | H | $CH_3$ | 2 |

The unbranched carboxylic acid was found to give a fall in blood pressure of 31% for over 30 minutes at a dosage of 5 mg/Kg in the anesthetized rat, while the unbranched ester gave a 60% fall for ten minutes under the same conditions. The β-methyl branched acid gave a 12% fall in anesthetized rat blood pressure for four minutes at 5 mg/Kg and the corresponding ester gave a 49% fall for 16 minutes at the same dosage. A 10% fall in the arterial blood pressure for three minutes at 5 mg/Kg was recorded in the anesthetized rat for the α-methyl branched carboxylic acid, wherease the analogous ester gave a 51% fall for 20 minutes under the same circumstances. It is thus apparent that the aminocarboxylic acids are substantially weaker hypotensives than the corresponding esters. No effects upon the "standard drug" responses were observed with these two compounds - likely attributable to the very short duration of action for these α- and β-methyl branched aminocarboxylic acids. On the other hand, 3-[1-(4-phenylpiperazinyl)]propionic acid hydrochlorde (119) was found to antagonise the pressor response to DMPP as well as the depressor response to acetylcholine. No effects were observed on vagal stimulation or noradrenaline responses for the latter compound. This pattern seemed suggestive of a mainly ganglionic blocking action with a possible selective sympathetic ganglion blocking action as discussed previously.

Effect of Aminohydroxamic Acid Hydrochlorides on the Arterial Blood Pressure of Anesthetized Rats.

Although corresponding esters and hydroxamic acids had similar magnitudes of hypotensive activity, Coutts et al. (1969) noted that the duration of action of the hydroxamates were more prolonged than that of the esters.

In the present study, most of the previously prepared and tested 3-aminopropionates were converted to the corresponding hydroxamic acid hydrochlorides. The structures of the β-amino-propionohydroxamic acid hydrochlorides given by general structure (172)are summarized in Table XXVIII, while pharmacological findings are presented in Tables XXI and XXII.

TABLE XXVIII

3-Aminopropionohydroxamic Acid Hydrochlorides

$R\text{-}CHR^1CHR^2CONHOH\cdot HCl$

(172)

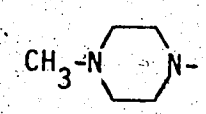
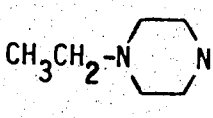
| Compound | R | $R^1$ | $R^2$ |
|---|---|---|---|
| 120 |  | H | $CH_3$ |
| 121 |  | H | $CH_3$ |

TABLE XXVIII cont'd

3-Aminopropionohydroxamic Acid

Hydrochlorides

$R\text{-}CHR^1CHR^2CONHOH\cdot HCl$

(172)

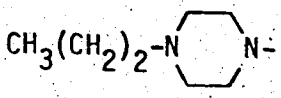
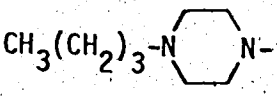
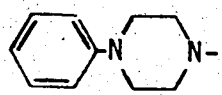
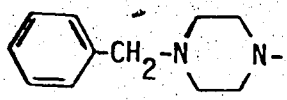
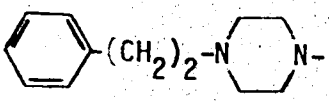
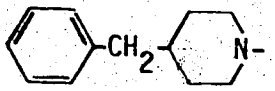
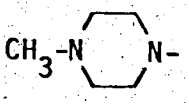
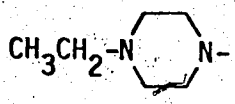
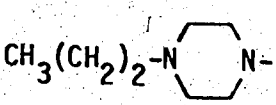
| Compound | R | $R^1$ | $R^2$ |
|---|---|---|---|
| 122 |  | H | $CH_3$ |
| 123 |  | H | $CH_3$ |
| 124 |  | H | $CH_3$ |
| 125 |  | H | $CH_3$ |
| 126 |  | H | $CH_3$ |
| 134 |  | H | $CH_3$ |
| 127 |  | $CH_3$ | H |
| 128 |  | $CH_3$ | H |
| 129 |  | $CH_3$ | H |

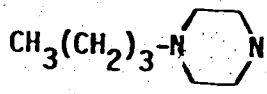
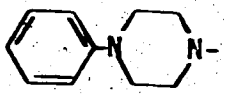
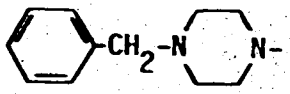
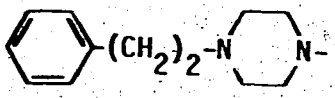
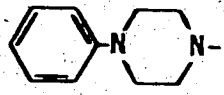
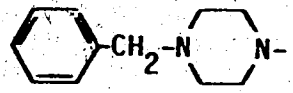
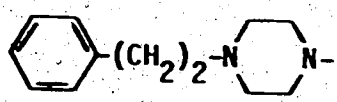
TABLE XXVIII cont'd

3-Aminopropionohydroxamic Acid

Hydrochlorides

$R\text{-}CHR^1CHR^2CONHOH\cdot HCl$

(172)

| Compound | R | $R^1$ | $R^2$ |
|---|---|---|---|
| 130 |  | $CH_3$ | H |
| 131 |  | $CH_3$ | H |
| 132 |  | $CH_3$ | H |
| 133 |  | $CH_3$ | H |
| 142 |  | $CH_3$ | H |
| 136 |  | H | H |
| 143 |  | H | H |
| 144 |  | H | H |

The hypotensive evaluation of one of the above compounds, namely, 2-methyl-3-[1-(4-methylpiperazinyl)]propionohydroxamic acid hydrochloride (120), has been reported by Midha (1969). The author found that this compound (at 25 mg/Kg) gave a 43% fall in anesthetized cat blood pressure for ten minutes. The same compound in the current investigation gave an 11% rise in blood pressure for 2.5 minutes at 25 mg/Kg in the anesthetized rat.

Within the series of α-methyl branched hydroxamic acids, as with the parallel series of substituted esters, those derivatives with an aromatic group in the 4-position of the cyclic 3-amino function are the most noteworthy hypotensives. As with the esters, the benzylpiperazinyl hydroxamate was much weaker a hypotensive than the phenyl- or phenethyl-piperazinyl hydroxamates.

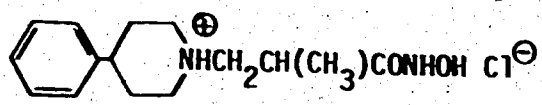
Upon comparing the α-methyl branched hydroxamic acids with the similarly substitued esters, the methyl, ethyl, and n-propyl hydroxamic acids had noticeably smaller falls in blood pressure and briefer durations of action at all dosages than did the esters. But the n-butylhydroxamate produced a much greater hypotensive response than the n-butyl ester. In the remaining α-methyl branched hydroxamic acids of the series, all of which contained an aromatic group at the 4-position of the 3-amino substituent, the magnitude of blood pressure fall was quite similar at all dosages tested to that produced by the corresponding

α-methyl branched esters. However, in each of these cases, the duration of action of the aromatic-containing hydroxamates was longer than for the similarly substituted esters.

Of the α-methyl branched hydroxamic acids, the methyl, ethyl and n-propyl derivatives at each dosage examined caused no change in any of the "standard drug" responses. The apparent difference from the corresponding α-methyl branched esters all of which reduced the response to nicotine or DMPP. (as well as antagonized the vagal stimulation responses in the case of the ethyl and n-propyl analogues) could be explained on the basis of the weaker and shorter hypotensive action of the hydroxamates. However, the n-butyl hydroxamate slightly reduced only the response to noradrenaline while the n-butyl ester antagonized the DMPP and vagal stimulation responses with no effect upon the noradrenaline response. This difference might be taken to infer a possible change in mechanism of action from ganglionic blockade in the case of the n-butyl ester to a degree of α-adrenoceptive blockade in the case of the n-butylhydroxamate. As occurred with the analogous α-methyl branched esters, the benzyl- and phenethyl-piperazinyl hydroxamic acids inhibited both the DMPP and vagal stimulation responses without altering the noradrenaline response. Such an occurrence was taken as evidence for a ganglionic blocking mechanism of action. Similarly, both the phenylpiperazinyl and the benzylpiperidino α-methyl branched esters and hydroxamates interfered with both the DMPP

and noradrenaline responses, but not with the vagal stimulation - indicative of an α-adrenoceptive blocking action, once again with the possibility of an accompanying selective sympathetic ganglionic blockade component. For the reasons mentioned earlier, alterations in acetylcholine response were not emphasized.

The possibility of a degree of selective sympathetic ganglionic blocking action for the above compounds which inhibited the response to DMPP, but not to vagal stimulation is realistic since Midha et al. (1971), after extensive investigation, suggested that 3-[4-phenylpiperidino]propionohydroxamic acid hydrochloride (4) effected its hypotensive action via a sympathetic ganglionic blockade. Of course, the simple crude screening



(4)

regimen followed in the present study does not allow a similar conclusion to be made concerning those compounds which antagonize DMPP, but have no effect upon vagal stimulation. This is especially true for those compounds which were also found to definitely possess α-adrenoceptive blocking action. Clearly, a variety of pharmacological techniques and procedures would have to be applied to eliminate other possible mechanisms as well as to confirm the postulated mechanism before any compounds in the present study could be validly implicated as selective

sympathetic ganglionic blocking agents. Such studies were judged beyond the scope of the present work, but could form the basis of interesting and potentially rewarding future investigations.

Analysis of the pharmacological data for β-methyl branched hydroxamates once more illustrated the significance of an appropriately placed aromatic substituent for hypotensive activity. As before, the benzylpiperazinyl-hydroxamic acid was much less potent than the phenyl- or phenethyl-piperazinyl derivatives.

Comparisons between the α-methyl branched and β-methyl branched hydroxamates as was found with the α- and β-methyl branched esters, revealed no consistent advantage for either substituent throughout the series. Thus the methyl-, ethyl- and n-propyl- hydroxamates whether α- or β-methyl branched are comparable both in magnitude and duration of weak blood pressure response. The 3-methyl-3-[1-(4-n-butylpiperazinyl)]propionohydroxamic acid hydrochloride (130) gave a 16% fall in blood pressure for five minutes, at 5 mg/Kg in anesthetized rats, while the corresponding α-methyl hydroxamate gave a 59% fall for eight minutes at the same dose. Likewise, the β-methyl branched phenethyl piperazinyl hydroxamate was weaker than the α-methyl analogue, but the β-branched phenyl- and benzyl-piperazinyl-hydroxamates were stronger hypotensives than the comparable α-branched hydroxamates.

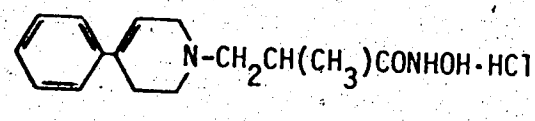
For the most part, the magnitudes of blood pressure fall throughout the series of β-methyl branched hydroxamates were

fairly similar to those for the comparably substituted esters. Contrary to the findings in the several previous series of compounds, the duration of action of these hydroxamic acids were usually similar to those for the corresponding ester or were slightly less.

The effects of the β-methyl branched hydroxamates upon the "standard drug" responses were virtually identical to those for the α-methyl branched hydroxamates in the case of the methyl-, ethyl-, n-propyl-, phenyl- and phenethyl-piperazinyl derivatives. 3-Methyl-3-[1-(4-n-butylpiperazinyl)]propionohydroxamic acid hydrochloride (130) attenuated only the vagal stimulation response, without affecting any other standard responses at 25 mg/Kg and 3-methyl-3-[1-(4-benzylpiperazinyl)]-propionohydroxamic acid hydrochloride (132) antagonized the vagal stimulation as well as the pressor response to noradrenaline. The occurrence of antagonism to vagal stimulation, without an alteration of the blood pressure response to DMPP could be interpreted as suggestive of a degree of selective parasympathetic blockade. However, for the latter compound, this possibility seems complicated by an overlapping α-adrenoceptive blocking activity.

Biggs et al (1972c) conducted an exhaustive investigation of the mechanism of action of 2-methyl-3-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]propionohydroxamic acid hydrochloride (173) in anesthetized cats, rats, dogs and rabbits. The authors

concluded that this α-methyl branched hydroxamate showed α-adrenoceptive receptor blocking properties in all the species studied. This compound was found to give a fall of 46 ± 5% for 38 ± seven minutes at 5 mg/Kg in the blood pressure of anesthetized cats. 3-Methyl-3-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]-propionohydroxamic acid hydrochloride (142) prepared in the present study caused a 64% fall in blood pressure for greater than 140 minutes in the anesthetized rat at the dosage level of 5 mg/Kg. This β-methyl branched hydroxamate attenuated the responses to DMPP, noradrenaline, and acetylcholine but did not alter the vagal stimulation - a pattern of effects also consistent with an α-adrenoceptive blocking mechanism of action.




(173)

As occurred within the methyl 3-aminopropionate series, those hydroxamic acids with no α- or β-methyl branch namely, 3-[1-(4-phenylpiperazinyl)]propionohydroxamic acid hydrochloride (136), 3-[1-(4-benzylpiperazinyl)]propionohydroxamic acid hydrochloride (143) and 3-[1-(4-phenethylhydroxamic acid hydrochloride (144) had almost identical magnitudes, durations and mechanisms of hypotensive action as the corresponding hydroxamates with either an α- or β-methyl branch (all derivatives were compared at the same dosage levels). This information seems to

strengthen the hypothesis that the presence or absence of an α- or β-methyl group in this particular type of structure was entirely irrelevant to the duration or magnitude of hypotension produced.

Although none of the ethyl formates were converted to the hydroxamic acid, several of the ethyl acetates and ethyl butyrates were reacted with hydroxylamine to give the corresponding hydroxamate. The structures of those derivatives which were successfully prepared are presented in Table XXIX. The pharmacological results obtained for the compounds represented by general structure (174) are contained in Tables XXI and XXII.

TABLE XXIX

Aminohydroxamic Acid

Hydrochlorides

$R\text{-}(CH_2)_n\text{-}CONHOH\cdot HCl$

(174)

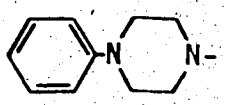
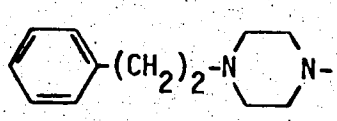
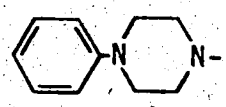
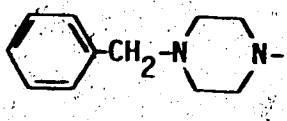
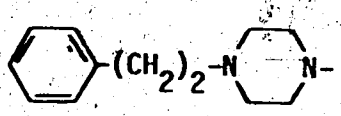
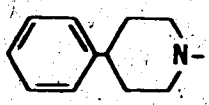
| Compound | R | n |
|---|---|---|
| 139 |  | 1 |
| 140 |  | 1 |
| 135 |  | 3 |

TABLE XXIX cont'd

Aminohydroxamic Acid Hydrochlorides

$R-(CH_2)_n-CONHOH\cdot HCl$

(174)

| Compound | R | n |
|---|---|---|
| 137 |  | 3 |
| 138 |  | 3 |
| 141 |  | 3 |

The phenyl- and phenethyl-piperazinylacetohydroxamic acids gave much shorter durations and smaller magnitudes of fall in the arterial blood pressure of anesthetized rats at 5 mg/Kg than did the phenyl- and phenethyl-piperazinylpropionohydroxamates. This served to confirm the finding with the appropriate ester derivatives that reduction of the distance between the amino and ester functions resulted in decreased hypotensive activity. The phenylpiperazinylacetohydroxamate was found to inhibit the responses to DMPP and to acetylcholine, but not to vagal stimulation or noradrenaline. Once again, the possibility of selective sympathetic ganglionic blocking activity was implicated. The

phenethylpiperazinylacetohydroxamic acid left all "standard drug" responses unaltered.

With the butyrohydroxamic acids, the phenylpiperazinyl derivatives generally speaking caused a blood pressure fall and duration of response similar to that observed for the corresponding 3-[1-(4-phenylpiperazinyl)]propionohydroxamates. At the same time, in comparison to the benzyl- and phenethyl-piperazinyl-propionohydroxamates, benzylpiperazinyl-butyrohydroxamate had a larger percentage fall in blood pressure but shorter duration, while the phenethylpiperazinyl-butyrohydroxamate had a smaller percentage fall in blood pressure but an equal or longer duration of action.

Comparisons between the hypotensive activity of the aceto- and butyro-hydroxamic acids and the corresponding ethyl esters revealed no consistent dramatic differences between their magnitudes of blood pressure fall.

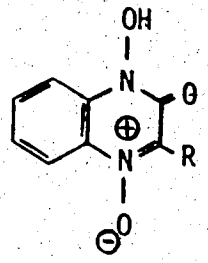
The phenyl- and phenethyl-piperazinylbutyrohydroxamates as well as the phenyl-1,2,5,6-tetrahydropyridinobutyrohydroxamate all antagonized the responses to DMPP, noradrenaline, but not to vagal stimulation at doses of 5 mg/Kg. As was the case with the similarly substituted ethyl butyrates, these observations were taken to suggest an α-adrenoceptive blocking action with the possibility of a selective sympathetic blocking action overlapping. 3-[1-(4-Benzylpiperazinyl)]butyrohydroxamic acid hydrochloride (137) left all the "standard drug" responses virtually unchanged, unlike the corresponding ethyl ester.

Effect of 1-Hydroxyquinoxalin-2-one 4-Oxides on the Arterial Blood Pressure of Anesthetized Rats.

Summarized in Table XXX are the structures of the 1-hydroxyquinoxalin-2-one 4-oxides with general formula (175) prepared in the present study. The pharmacological results obstained with these compounds are presented in Tables XXIII and XXIV.

TABLE XXX

1-Hydroxyquinoxalin-2-one 4-Oxides



(175)

| Compound | R |
|---|---|
| 159 | H |
| 160 | $CH_3$ |
| 161 | Ph |

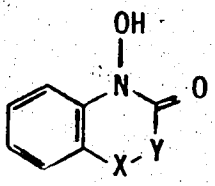
All three 1-hydroxyquinoxalin-2-one 4-oxides produced no fall in the blood pressure at a dosage of 5 mg/Kg and gave only a slight, transient fall in blood pressure at 25 mg/Kg. In no case were any effects upon the "standard drug" responses observed at either dosage.

Effect of 1-Hydroxyquinoxalin-2-ones on the Arterial Blood Pressure of Anesthetized Rats.

Table XXXI tabulates the structures of the 1-hydroxyquinoxalin-2-ones with general formula (176) which were examined. The pharmacological data for these compounds are contained in Table XXIII and XXIV.

TABLE XXXI

1-Hydroxyquinoxalin-2-ones

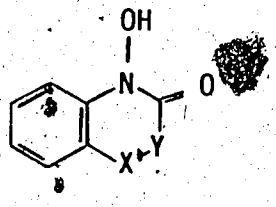



(176)

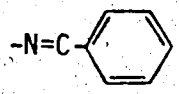
| Compound | -x-y- |
|---|---|
| 162 | $-NH-CH_2-$ |
| 163 | $-N=C-CH_3$ |

TABLE XXXI cont'd

1-Hydroxyquinoxalin-2-ones



(176)

| Compound | -x-y- |
|---|---|
| 164 |  |

As with the corresponding 4-oxides, no 1-hydroxyquinoxalin-2-one gave any fall in blood pressure at a dosage of 5 mg/Kg, but all did give a transient fall in blood pressure of different magnitudes at 25 mg/Kg. Also, none of the "standard drug" responses were altered at either dosage for any of the 1-hydroxyquinoxalin-2-ones.

## IV. CONCLUSION

From the foregoing discussion of results obtained in the present investigation, a number of tentative conclusions may be drawn regarding the hypotensive activity of the aminoesters, the aminohydroxamic acids, the aminocarboxylic acids and the cyclic quinoxaline hydroxamic acids studied.

They are as follows:

1. The presence of an aromatic group in the 4-position on a cyclic 3-amino function provides optimal hypotensive activity in the aminoesters and the aminohydroxamic acid series with general structures (169) and (172), respectively.

$$R\text{-}CHR^1CHR^2COOR^3 \cdot 2HCl$$

(169)

$$R\text{-}CHR^1CHR^2CONHOH \cdot HCl$$

(172)

2. The presence or absence of an α- or β-methyl substituent has no consistent effect on hypotensive activity in either the aminoester or aminohydroxamic acid series.
3. The aminohydroxamic acids generally show similar activity as hypotensive agents, but possess a longer duration of action than the corresponding aminoesters.
4. The corresponding methyl and ethyl 4-phenyl- and 4-benzylpiperazinyl 3-substituted-propionates have similar magnitudes and durations of

action as hypotensive agents.

5. Decreasing the length of the propionate function by one or two methylene groups results in diminishec hypotensive activity, while lengthening the ester-amino group distance by one methylene group enhances hypotensive activity.

6. The aminocarboxylic acids are substantially less active hypotensives than the corresponding aminoesters or aminohydroxamic acids.

7. The 1-hydroxyquinoxalin-2-ones and the corresponding 4-oxides were virtually devoid of hypotensive activity.

8. Within the aminoester and aminohydroxamic acid series, most of the active 4-alkyl substituted cyclic 3-amino compounds exhibited a ganglion blocking mechanism of action; whereas, the 4-aralkyl substituted derivatives often had a mixed ganglionic blocking and α-adrenoceptive blocking mechanism and the 4-aryl substituted analogues usually possessed a purely α-adrenoceptive blocking mechanism of action.

However, it must be emphasized once more that all these conclusions are based on a limited number of qualitative experimen-- which employed a restricted routine screening regimen.

# HYDROLYSIS

# I. EXPERIMENTAL RESULTS

## Apparatus

A Beckmann Model B spectrophotometer with 1 cm glass cells was used for measuring all absorbances.

## Reagents

(i) Hydroxylamine hydrochloride (12.5%) in methanol.

(ii) Sodium hydroxide (12.5%) in methanol.

(iii) Alkaline hydroxylamine reagent.

Equal volumes of 12.5% methanolic hydroxylamine hydrochloride and 12.5% methanolic sodium hydroxide were mixed together and the precipitated sodium chloride filtered off. This solution was prepared immediately prior to use.

(iv) Ferric Perchlorate.

(a) Stock Solution

Ferric perchlorate (5.0 gm) was dissolved in 70% perchloric acid (10 ml) and distilled water (10 ml). This solution was diluted to 100 ml with anhydrous ethanol, and cooled under a cold water tap as the alcohol was added.

(b) Reagent Solution

Stock solution (40 ml) was added to a 1-liter volumetric flask, then 70% perchloric acid (12 ml) was added and the solution diluted to volume with anhydrous ethanol. The dilution was carried out by adding the ethanol in 100 ml increments and cooling between each addition under a cold water tap.

Wavelength of Maximum Absorbance of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Ferric Ion Chelate

A 0.01 M solution 100 ml of methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate dihydrochloride was prepared in anhydrous ethanol. Five milliliters of this sample solution was pipetted into a round-bottom flask (25 ml) with a ground glass joint. Three milliliters of the filtered alkaline reagent solution was added to the sample flask and to a blank which contained anhydrous ethanol only (5 ml). After reflux condensers were attached, both solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents washed into volumetric flasks (50 ml) with the ferric perchlorate reagent solution, then diluted to volume with the reagent. The wavelength of maximum absorbance was determined to the nearest 10 mμ by scanning the absorbance of the sample solution against the blank solution. The above procedure was repeated three times (Table XXXII).

TABLE XXXII

Wavelength of Maximum Absorbance of 2-Methyl-3-[1-(4-phenylpiperazinyl)]-propionohydroxamic Acid Ferric Ion Chelate

| Wavelenght | Absorbance Sample 1 | Absorbance Sample 2 | Absorbance Sample 3 | Absorbance Average |
|---|---|---|---|---|
| 420 | 0.36 | 0.47 | 0.44 | 0.42 |
| 430 | 0.43 | 0.54 | 0.46 | 0.48 |
| 440 | 0.50 | 0.62 | 0.55 | 0.56 |
| 450 | 0.65 | 0.68 | 0.64 | 0.66 |
| 460 | 0.72 | 0.75 | 0.66 | 0.71 |
| 470 | 0.77 | 0.79 | 0.70 | 0.75 |
| 480 | 0.80 | 0.83 | 0.74 | 0.79 |
| 490 | 0.83 | 0.86 | 0.78 | 0.82 |
| 500 | 0.78 | 0.86 | 0.76 | 0.80 |
| 510 | 0.77 | 0.83 | 0.78 | 0.79 |
| 520 | 0.75 | 0.80 | 0.73 | 0.76 |
| 530 | 0.71 | 0.78 | 0.70 | 0.73 |
| 540 | 0.66 | 0.75 | 0.67 | 0.69 |
| 550 | 0.62 | 0.68 | 0.64 | 0.65 |
| 560 | 0.57 | 0.60 | 0.56 | 0.58 |
| 570 | 0.51 | 0.53 | 0.52 | 0.52 |
| 580 | 0.46 | 0.46 | 0.48 | 0.47 |
| 590 | 0.43 | 0.42 | 0.44 | 0.43 |
| 600 | 0.36 | 0.36 | 0.32 | 0.35 |

Optimal Time for Absorbance Stability of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Ferric Ion Chelate

A 0.01 M solution (100 ml) of 2-methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic acid hydrochloride was prepared in anhydrous ethanol. Five milliliters of this sample solution was pipetted into a round-bottom flask (25 ml) with a ground glass joint. Three milliliters of the filtered alkaline reagent solution was added to the sample flask and to a blank which contained anhydrous ethanol (5 ml) only. After reflux condensers were attached, both solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents washed into volumetric flasks (50 ml) with ferric perchlorate reagent solution, then diluted to volume with the reagent. The absorbance of the sample solution was read against the blank solution at intervals of 15, 30, 60, 90 and 120 minutes at 500 mμ. The above procedure was repeated three times. The results are listed in Table XXXIII.

TABLE XXXIII

Optimal Time for Absorbance Stability of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Ferric Ion Chelate

| Time | Absorbance Sample 1 | Absorbance Sample 2 | Absorbance Sample 3 | Absorbance Average |
|---|---|---|---|---|
| 15 | 0.85 | 0.85 | 0.88 | 0.86 |
| 30 | 0.84 | 0.82 | 0.85 | 0.84 |
| 60 | 0.81 | 0.82 | 0.83 | 0.82 |
| 90 | 0.81 | 0.80 | 0.83 | 0.81 |
| 120 | 0.85 | 0.80 | 0.82 | 0.82 |

Calibration Curve for Methyl 2-Methyl-3-[1-(4-phenylpiperazinyl)]-propionate Dihydrochloride

Solutions (100 ml) of accurately known molarity of the ester varying from 0.0025 to 0.0150 M were prepared in anhydrous ethanol. Immediately, 5 ml of each sample solution was pipetted into round-bottom flasks (25 ml) with groundglass joints. Three milliliters of the filtered alkaline reagent solution was added to each of the sample flasks and to a blank which contained anhydrous ethanol only (5 ml). After reflux condensers were attached, all solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents of each were washed into volumetric flasks (50 ml) with the ferric perchlorate reagent solution, then diluted to volume with the reagent. The absorbance of each sample

solution was recorded at [redacted] against the blank solution after allowing 90 minutes for the color intensities to stabilize. The above procedure was repeated three times at each concentration (the results are listed in Table XXXIV).

TABLE XXXIV

Calibration Curve of Methyl 2-Methyl-3-[1-(4-phenylpiperazinyl)]-propionate Dihydrochloride at 500 mμ after Ferric Chelate Formation

| Molarity | Absorbance Sample 1 | Absorbance Sample 2 | Absorbance Sample 3 | Absorbance Average |
|---|---|---|---|---|
| 0.0150 | 0.592 | 0.602 | 0.589 | 0.594 |
| 0.0125 | 0.549 | 0.539 | 0.545 | 0.544 |
| 0.0100 | 0.433 | 0.448 | 0.485 | 0.446 |
| 0.00752 | 0.321 | 0.320 | 0.314 | 0.318 |
| 0.00502 | 0.214 | 0.216 | 0.191 | 0.207 |
| 0.00251 | 0.086 | 0.089 | 0.088 | 0.088 |

Calibration Curve for 2-Methyl-3-[1-(4-phenylpiperazinyl)]-propionohydroxamic Acid Hydrochloride.

Solutions (100 ml) of accurately known molarity of the hydroxamic acid varying from 0.0010 to 0.0080 M were prepared in anhydrous ethanol. Immediately, 5 ml of each sample solution was pipetted into round-bottom flasks (25 ml) with ground-glass joints. Three milliliters of the filtered alkaline reagent solution

was added to each of the sample flasks and to a blank which contained anhydrous ethanol only (5 ml). After reflux condensers were attached, all solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents of each were washed into volumetric flasks (50 ml) with the ferric perchlorate reagent solution, then diluted to volume with the reagent. The absorbance of each sample solution was recorded at 500 mμ against the blank solution after allowing 90 minutes for the color intensities to stabilize. The above procedure was repeated three times at each concentration (Table XXXV).

TABLE XXXV

Calibration Curve of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Dihydrochloride at 500 mμ after Ferric Chelate Formation

| Molarity | Absorbance Sample 1 | Absorbance Sample 2 | Absorbance Sample 3 | Absorbance Average |
|---|---|---|---|---|
| 0.00762 | 0.800 | 0.810 | 0.830 | 0.813 |
| 0.00611 | 0.678 | 0.682 | 0.689 | 0.683 |
| 0.00503 | 0.552 | 0.554 | 0.564 | 0.557 |
| 0.00407 | 0.445 | 0.455 | 0.430 | 0.443 |
| 0.00300 | 0.326 | 0.336 | 0.332 | 0.331 |
| 0.00200 | 0.218 | 0.210 | 0.212 | 0.213 |
| 0.00100 | 0.111 | 0.094 | 0.101 | 0.103 |

Hydrolysis of Methyl 2-Methyl-3-[1-(4-phenylpiperazinyl)]-propionate Dihydrochloride in Distilled Water.

A 0.01 M solution (100 ml) of the ester was prepared in distilled water. At time intervals of 0, 30, 60, 90, 300 minutes and 24 hours, five milliliters of the sample solution was pipetted into round-bottom flasks (25 ml) with ground-glass joints. Three milliliteres of filtered alkaline reagent solution was added to the sample flask and to a blank which contained anhydrous ethanol only (5 ml). After reflux condensers were attached, both solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents washed into a volumetric flask (50 ml) with the ferric perchlorate reagent solution, then diluted to volume with the reagent. The absorbance of the sample solution was recorded at 500 mμ against the blank after allowing 90 minutes for the color intensity to stabilize (the results are listed in Table XXXVI).

TABLE XXXVI

Hydrolysis of Methyl 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionate Dihydrochloride in Distilled Water. Quantitative Determination of Unhydrolyzed Ester

| Time | Absorbance | Concentration (Calculated) |
|---|---|---|
| 0 minutes | 0.422 | 0.0098 M |
| 30 minutes | 0.364 | 0.0085 M |
| 60 minutes | 0.334 | 0.0081 M |
| 90 minutes | 0.298 | 0.0071 M |
| 300 minutes | 0.251 | 0.0061 M |
| 24 hours | 0.239 | 0.0058 M |

Hydrolysis of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Hydrochloride in Distilled Water.

A 0.006 M solution (100 ml) of the hydroxamic acid was prepared in distilled water. At time intervals of 5, 15, 60, 75 minutes and 24 hours, five milliliters of the sample solution was pipetted into round-bottom flasks (25 ml) with a ground-glass joint. Three milliliters of the filtered alkaline reagent solution was added to the sample flask and to a blank which contained anhydrous ethanol only (5 ml). After reflux condensers were attached, both solutions were refluxed for five minutes. The flasks were cooled to room temperature and the contents washed into a volumetric

flask (50 ml) with the ferric perchlorate reagent solution, then diluted to volume with the reagent. The absorbance of the sample solution was recorded at 500 mμ against the blank after allowing 90 minutes for the color intensity to stabilize (Table XXXVII).

TABLE XXXVII

Hydrolysis of 2-Methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic Acid Hydrochloride in Distilled Water. Quantitative Determination of Unhydrolyzed Hydroxamic Acid.

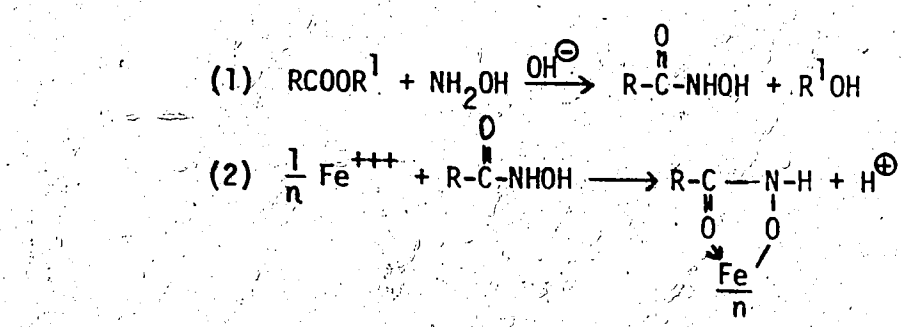
| Time | Absorbance | Concentration (Calculated) |
|---|---|---|
| 0 minutes | 0.661 | 0.0059 M |
| 15 minutes | 0.662 | 0.0060 M |
| 60 minutes | 0.662 | 0.0060 M |
| 75 minutes | 0.660 | 0.0057 M |
| 24 hours | 0.661 | 0.0059 M |

## II. DISCUSSION

Pharmacological studies (Coutts et al. (1969)) revealed that hydroxamic acids have a longer duration of action as hypotensive agents than do the corresponding esters. One reason for this could be that the latter hydrolyze more rapidly to inactive carboxylic acids than do the hydroxamates. To test this theory, a suitable method of assay was sought.

In a review of the analytical applications of hydroxamic acids, Brandt (1960) pointed out that several metal ions in dilute acidic solution form intensely colored solutions with hydroxamic acids. Of the many potentially analytically useful chelates, the author found that those formed between hydroxamic acids and iron (+3), titanium (+4), molybdenum (+6) or uranium (+6) had been used in colorimetric quantifications of either hydroxamic acids or metal ions.

Goddu et al. (1955) established the optimal conditions for the colorimetric analysis of esters and anhydrides by reacting them with hydroxylamine in alkaline solution to give the hydroxamic acid which gave quantifiable highly colored chelate complexes with ferric ion. The authors outlined this procedure with the following reaction sequence.



Utilizing Goddu's method of analysis, a study of the rate of hydrolysis of methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]-propionate hydrochloride (79) and 2-methyl-3-[1-(4-phenylpiperazinyl)]-propionohydroxamic acid hydrochloride (124) in water was undertaken.

First, the wavelength of maximum absorbance of the 2-methyl-3-[]-(4-phenylpiperazinyl)]propionohydroxamic acid ferric ion chelate was determined by scanning the abosrbance of a given concentration of chelate at 10 mμ intervals from 420 mμ to 600 mμ against a blank solution. After three such scans, 500 mμ was chosen as the wavelength for future colorimetric analysis of the chelate complex concentration (Table XXXII).

The optimal time for color stability of the chelate was found by reading the absorbance at 500 mμ of the same solution of chelate complex against a blank solution at various time intervals after having added the ferric ion. Three determinations of the optimal time indicated that after 90 minutes the absorbance was fairly stable (Table XXXIII).

A calibration curve was prepared for methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (79) by

analyzing various known concentrations of the ester with the method described by Goddu et al. (1955) and reading the absorbance at 500 mμ 90 minutes after addition of the ferric salt (Table XXXIV). Similarly, a calibration curve was prepared for 2-methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic acid hydrochloride (Table XXXV).

To study the hydrolysis of the ester and hydroxamic acid in water, a solution of known concentration of each was prepared in distilled water. At time intervals of 0, 30, 60, 300 minutes and 24 hours, aliquots from each sample solution were analysed under the conditions previously established (Tables XXXVI and XXXVII). From the respective calibration curves, the concentration of ester and hydroxamic acid remaining in the respective sample solutions at these time intervals was determined. A 0.01 M solution of methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (79) was prepared, and a 0.006 M solution of the corresponding hydroxamic acid was used. In order for the analyzed aliquots to possess absorbances within the ranges of the respective calibration curves, this difference in initial concentrations was necessary. However, valid conclusions on differences in rates of hydrolysis can still be drawn by comparing the relative proportion of hydrolysis which occurs with each compound. A comparison of the concentration of intact ester or hydroxamic acid remaining in aqueous solution at each time interval revealed that the ester was hydrolyzed at such a rate that only about 60%

of the initial ester concentration remained after 24 hours; whereas the concentration of hydroxamic acid after 24 hours was about 95% of the initial concentration (Figure X). Clearly, methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate hydrochloride (79) was more rapidly hydrolyzed in distilled water than was 2-methyl-3-[1-(4-phenylpiperazinyl)propiono-hydroxamic acid hydrochloride (124). This finding may account for the fact that methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]-propionate hydrochloride (79) gave a 51% fall in blood pressure for 20 minutes at 5 mg/kg in the anesthetized rat, while 2-methyl-3-[1-(4-phenylpiperazinyl)]propionohydroxamic acid hydrochloride (124) gave a 46% fall for 66 minutes under the same conditions. Similar differences between the magnitudes and durations of hypotension were found for most of the more potent structurally related esters and hydroxamic acids. Coutts et al. (1969) also observed that corresponding hydroxamic acids and esters gave comparable degrees of hypotension, but the hydroxamic acid acted for a much longer period of time. Thus it appears that one important reason for the longer duration of action for hydroxamic acids relative to the corresponding methyl esters may be the much slower rate of hydrolysis of the hydroxamic acids. Concievably, this difference noted in vitro could be accentuated in vivo by the presence of enzymes in the blood to catalyse ester hydrolysis but not hydroxamate hydrolysis. Of course, other factors such as differences in receptor affinity may also play

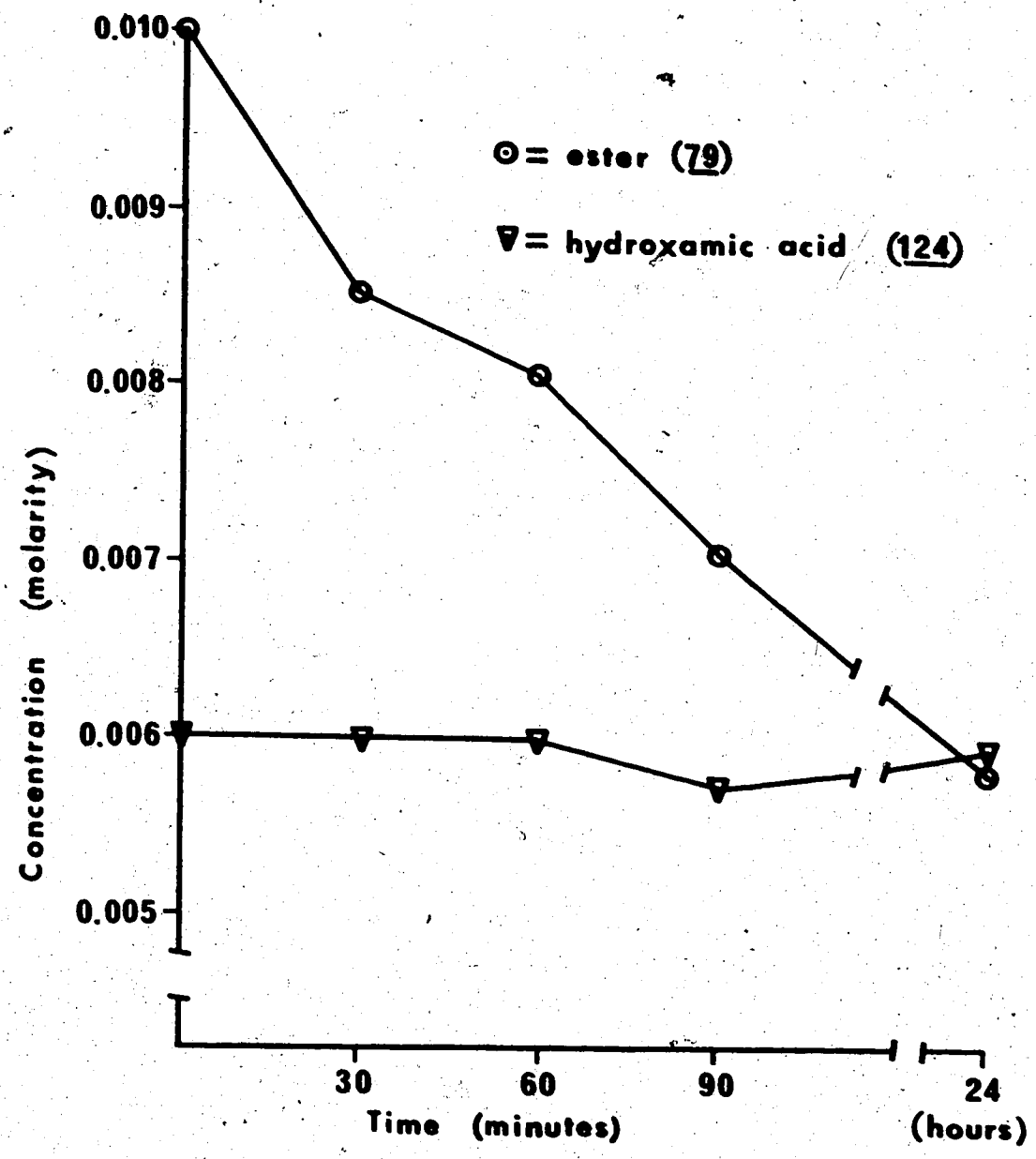



Figure X : Hydrolysis of methyl 2-methyl-3-[1-(4-phenylpiperazinyl)]propionate dihydrochloride (79) and 2-methyl-3-[1-(4-phenylpiperazinyl)]propiono-hydroxamic acid monohydrochloride (124) in distilled water.

a role in accounting for the longer hydroxamate action.

That the hydrolysis product (the corresponding carboxylic acid) be much less active as a hypotensive is a necessary corollary to the hypothesis that the differences in duration of hypotension between esters and hydroxamates is attributable to the more rapid hydrolysis rate of the esters. This was indeed found to be the case for three carboxylic acid derivatives previously discussed, namely, 3-[1-(4-phenylpiperazinyl)]propionic acid hydrochloride (117), 3-methyl-3-[1-(4-phenylpiperazinyl)]propionic acid hydrochloride (118), and 2-methyl-3-[1-(4-phenylpiperazinyl)]-propionic acid hydrochloride (119), which were structurally similar to potent hypotensive esters and hydroxamic acids, yet were found to give much smaller falls in anesthetized rat blood pressure for much shorter lengths of time than either of the correspondingly substituted esters or hydroxamic acids.

# BIBLIOGRAPHY

ABUSHANAB, E. (1970). One Step Synthesis of Quinoxaline 1,4-dioxides and Related Compounds. J. Org. Chem., 35: 4279-4280.

ADAMSON, D.W. (1954). Tertiary Allylamines of Therapeutic Value. British Patent 702, 269; Chem. Abstr., 49: 4026h (1955).

AHMAD, A. (1967). Pharmacological Study of 4-(m-Trifluoromethylphenyl)-1-(3'-amino-4'-pyridyl)piperazine: a New Anticonvulsant. Indian J. Med. Res. 55: 994-1002; Chem. Abstr. 67: 98,840e (1967).

ARNOLD, H., BROCK, N., LENKE, H.D., MOLGE, K., REBLING, R. and VOGELSANG, D. (1969). Coronary Vasodilating Bis-esters of Piperazine and Homopiperazine. S. African Patent 68 07,505; Chem. Abstr. 72: 90515K (1970).

ASHTON, B.W. and SUSCHITZKY, H. (1957). The Graebe-Ullmann Carbazole Synthesis. J. Chem. Soc., 4559-4562.

BACH, F.L., Jr., BARBANDER, H.J., and KUSHNER, S. (1958). Substituted Alkylenedipiperazines. U.S. Patent 2,837,522; Chem. Abstr., 52: 20214a (1958).

BACH, F.L., Jr., BARBANDER, H.J. and KUSHNER, S. (1959). Substituted Piperazines. U.S. Patent 2,909,523; Chem. Abstr., 54: 7747b (1960).

BALTZLY, R., BUCK, J.S., LORZ, E. and SCHON, W. (1944). The Preparation of N-Mono-substituted and Unsymmetrically Disubstituted Piperazines. J. Am. Chem. Soc., 66: 263-266.

BALTZLY, R., DVORKOVITZ, V. and PHILLIPS, A.P. (1949a). Synthetic Analogs of Oxytocic Drugs. I. Phenethyl β-Alanine Derivatives. ibid., 71: 1162-1164.

BALTZLY, R. and PHILLIPS, A.P. (1949b). Synthetic Analogs of Oxytocic Drugs. II. β-Hydroxyphenethyl-β-alanine Esters. ibid., 71: 3419-3421.

BALTZLY, R. and PHILLIPS, A.P. (1949c). Synthetic Analogs of Oxytocic Drugs. III. Homologs of the Phenethyl β-Alanine Type. ibid. 71: 3421-3422.

BAPAT, J.B., BLACK, D.ST.C. and BROWN, R.F.C. (1969). Cyclic Hydroxamic Acids. Adv. Het. Chem., 10: 199-232.

BARRASS, B.C., BRIMBLECOMBE, R.W. PARKES, D.C. and RICH, P. (1968). The Cholinergic Properties of Some Amino-acid Esters and Amides. Brit. J. Pharmacol., 34: 345-357.

BARRON, D.I., HALL, G.H., NATOFF, I.L., RIDLEY, H.F., SPICKETT, R.G.W. and VALLANCE, D.K. (1965). Compounds Affecting the Central Nervous System. III. Substituted 1,1-Diaryl-t-aminopropanols and Related Compounds. J. Med. Chem., 8: 836-841.

BARRON, D.I., HALL, G.H., NATOFF, I.L. and VALLANCE, D.K. (1965). Pharmacological Properties of 3-Dimethylamino-2-methyl-1-phenyl-1-o-tolylpropanol (SKF 70463-A). J. Pharm. Pharmacol., 17: 509-516.

BASS, W.B., SCHUELER, F.W. FEATHERSTONE, R.M. and GROSS, E.G. (1950). Preliminary Studies on the "Reversed Carboxyl" Analogue of Acetylcholine. J. Pharmacol. Exptl. Therap., 100: 465-481.

BEREGI, L., HUGON, P. and LAUBIE, M. (1968). 1-Piperonyl-4-(benzoylalkyl)piperazines with Pharmacological Activity. French Patent M.6226; Chem. Abstr., 74: 88056j (1971).

BIEBER, P. (1954). Studies in the Methacrylic Acid Series. Ann. Chim. (Paris), 9: 674-709: Chem. Abstr., 50: 169c (1956).

BIEL, J.H. (1965). Aralkoxyamides of 4-phenyl-1,2,5,6-tetrahydropyridinoalkanoic Acids. U.S. Patent 3,221,017; Chem. Abstr., 64: 5053b (1966).

BIEL, J.H. and HOPPS, H.B. (1965). N-alkynyl and N-alkynyloxy Amides of 4-Phenyl-1,2,5,6-tetrahydropyridino Alkanoic Acids. U.S. Patent 3,221,019; Chem. Abstr., 64: 5053e (1966).

BIGGS, D.F., COUTTS, R.T. and HENDERSON, D.B. (1972a). Synthesis and Pharmacological Evaluation of Some N,N-dialkylamino-proprionic, -Butyric and -Acetic Acid Esters and Several of Their Derivatives, Canadian Journal of Pharmaceutical Sciences, 7: 92-96 No. 3.

BIGGS, D.F., COUTTS, R.T., SELLEY, M.L. and TOWILL, G.A. (1972b). Potential Hypotensive Compounds: Substituted 3-Aminopropionates and 3-Aminopropionohydroxamic Acids. Journal of Pharmaceutical Sciences. 61, No. 11: 1739-1745.

BIGGS, D.F., COUTTS, R.T., HUBBARD, J.W., MIDHA, K.K. and PRASAD, K. (1972c). The mechanism of the hypotensive action of 2-methyl-3-[1-(4-phenyl-1,2,5,6-tetrahydropyridino)]-propionohydroxamic acid hydrochloride. Submitted for publication.

BIGGS, D.F., COUTTS, R.T. and HENDERSON, D.B. (1971). Muscarinic and nicotinic activities of some esters related to acetylcholine. J. Pharm. Pharmacol., 23: 628-9.

BOISSIER, J.R., DUMONT, C., RATOUIS, R. and PAGNY, R. (1961). Tentative de Pharmacologie Prévisionnelle dans le Domaine des Neuroteptiques: Actions Sédative Centrale et Adrenolytique de la N-(dimethoxy-3,4-phenethyl)-N'-(chloro-2-phenyl)pipérazine. Arch. Int. Pharmacodyn., 133: 29-49.

BOISSIER, J.R. and RATOUIS, R. (1963a). N-Phenyl-N'-(3,4-dimethoxy-phenethyl)piperazines. French Patent M1749; Chem. Abstr., 59: 10076d (1963).

BOISSIER, J.R. and RATOUIS, R. (1963b). N-Aryl-N'-(2-pyridylethyl)-piperazines. French Patent M1769; Chem. Abstr., 59: 11521a (1963).

BOISSIER, J.R. and RATOUIS, R. (1963c). N-(3,4-Dimethoxyphenyl)-N'-phenylpiperazines. French Patent M1784; Chem. Abstr., 59: 12824d (1963.

BOISSIER, J.R. and RATOUIS, R. (1963d). N-Phenyl-N'-(3,4-dimethoxy-phenethyl)piperazines. French Patent M1806; Chem. Abstr., 59: 14003g (1963).

BOISSIER, J.R. and RATOUIS, R. (1963e). 1,4-Disubstituted Piperazines. French Patent 1,318,449; Chem. Abstr., 59: 12825e (1963).

BOISSIER, J.R. and RATOUIS, R. (1963f). N-(3,4-Dimethoxyphenethyl)-N'-phenylpiperazines. French Patent 1,320,295; Chem. Abstr., 59: 10083f (1963).

BOISSIER, J.R., RATOUIS R., and DUMONT, C. (1963g). Synthesis and Pharmacological Study of New Piperazine Derivatives. I. Benzyl-piperazines. J. Med. Chem., 6: 541-544.

BOVET, D. and BOVET-NITTI, F. "Médicaments du Système Nerveux Végétatif", S. Karger, Basel, Switzerland (1948).

BOWIE, J.H., HEARN, M.T.W. and WARD, A.D. (1969). Hydroxamic Acids. III. Mass Spectra of Substituted Hydroxamic Acids and Cyclic Imides. Aust. J. Chem., 22: 175-184.

BRANDT, W.W. (1960). Analytical Applications of Hydroxamic Acids. Rec. Chem. Prog., 21: 159-177.

BRIMBLECOMBE, R.W. and SUTTON, J.V. (1968). The Ganglion Stimulating Effects of Some Amino Acid Esters. Brit. J. Pharmacol. 34: 358-369.

BROWN, H.C. and TSUKAMOTO, A. (1961). Selective Reductions. I. The Partial Reduction of Tertiary Amides With Lithium Aluminum Hydride. A New Aldehyde Synthesis Via the 1-Acylaziridines. J. Am. Chem. Soc., 83: 4549-4552.

BUCK, J.S. and BALTZLY, R. (1947). Unsymmetrically Substituted Piperazines. U.S. Patents 2,415,785; 2,415,786; 2,415,787; Chem. Abstr., 41: 3132. (1947).

CAMBAR, P.J. and AVIADO, D.M. (1970). Pharmacology of New Antimalarial Drugs. A Piperazine Which Exerts An Unusual Type of Adrenergic Blockade. Arch. Int. Pharmacodyn., 183: 107-126.

CIBA LTD. (1970). Physiologically Active 2-Aminomethyl-2,3-dihydrobenzofuran. French Patent 1,588,498; Chem. Abstr., 75: 5951μ (1971).

COE, D.G. (1959). Quaternary Hydroxamic Acids Derived From Pyridine. J. Org. Chem., 24: 882-884.

COCCIA, L., CAUSA, P. and DE NATALE, G. (1968) Local Anesthetic Activity of Aryl Esters of N-N-Disubstituted α-Amino Acids. Boll. Chim. Farm., 107: 310-319; Chem. Abstr., 69: 58253a (1968).

COUTTS, R.T. NOBLE, D. and WIBBERLEY, D.G. (1964). Some Cyclic Hydroxamic Acids. J. Pharm. Pharmacol., 16: 773-778.

COUTTS, R.T. (1967). Hydroxamic Acids. Part II: Cyclic Hydroxamic Acids. Can. J. Pharm. Sci., 2: 27-36.

COUTTS, R.T. and HINDMARSH, K.W. (1969). Mass Spectra of Some Quinoline Hydroxamic Acids and Related Compounds. Org. Mass Spect., 2: 681-695.

COUTTS, R.T., MIDHA, K.K. and PRASAD, K. (1969): β-Aminopropionohydroxamic Acids and β-Aminopropionic Esters with Hypotensive Properties. J. Med. Chem., 12: 940-941.

COUTTS, R.T. and HINDMARSH, K.W. (1970). Mass Spectra of 2H-1,4-Benzoxazine and -Benzothiazine Hydroxamic Acids. Org. Mass Spect., 3: 105-118.

COUTTS, R.T., HUBBARD, J.W., MIDHA, K.K. and PRASAD, K. (1971). Synthesis and Properties of Some Hypotensive N-Alkylaminopropionic Esters and N,N-Dialkylaminopropionic Esters and Their Hydroxamic Acids. J. Pharm. Sci., 68: 28-33.

COVERDALE, C.E. (1971). Hypotensive p-(7-Trifluoromethyl-4-quinolylamino-N,N-(3-methyl-3-azapentamethylene)benzamide. Ger. Offen. 2,034,640; Chem. Abstr., 74: 100,099k (1971).

DA COSTA, F.M. and SPECTOR, S. (1963). Catecholamine Depleting, Adrenolytic and Anti-arrhythmic Properties of 1-[5,6-Dimethoxy-2-methyl-(3-indole)ethyl]-4-phenylpiperazine. Fed. Proc., 22: 447.

DAMILWICZ, J.C. and KEMP, J.E.G. (1971). Antihypertensive 1-(2-hydroxy-3-phenoxypropyl)-4-phenylpiperazines. Ger. Offen. 2,040,438; Chem. Abstr., 74: 141,874c (1971).

DAS, P.C., PATRA, B.B., CHAUDHURI, S.B., BASU, U.P., KOLAY, B.N. and MAITRA, S.R. (1971). Synthesis of Some 1-Piperazine-carboxylic Acid Ethyl Ester Derivatives as Possible Anti-filarial and Antihypertensive Agents. J. Med. Chem., 14: 890-891.

DE MARCHI, F. and TORRIELLI, M.V. (1968). Synthesis and Preliminary Pharmacological Study of 1-(p-Chlorobenzhydryl)-4-[2-[-(3,4,5-trimethoxybenzoyloxy)ethoxy] ethyl]piperazine. Chim. Ther., 3: 210-213; Chem. Abstr., 69: 106,662g (1968).

DE STEVENS, G. and MULL, R.P. (1967a). 1-(3-Ethoxy-3-phenylpropyl)-4-(3-methylphenyl)piperazine. Switz. Patent 433,337; Chem. Abstr., 68: 114647a (1967).

DE STEVENS, G. and MULL, R.P. (1967b). 1-[3-(4-Methylphenyl)-3-alkoxypropyl]-4-(2-substitutedphenyl)piperazines. Swiss Patent 433,340; Chem. Abstr., 68: 105,246s (1967).

DRAKE, W.V. and MCELVAIN, S.M. (1934). The Reaction of Organic Halides with Piperidine. IV. Bromo Esters. J. Am. Chem. Soc., 56: 697-700.

DUERCKHEIMER, W., HARTUNG, H. and SCHRINNER, E. (1971). 2-(Alkylidene-hydrazinocarbonyl)quinoxaline 1,4-dioxides. Ger. Offen. 2,002,712; Chem. Abstr., 75: 98595μ (1971).

DYER, J.R. Applications of Absorption Spectroscopy of Organic Compounds, Prentice-Hall, Englewood Cliffs, N.J., 1965, pp.84.

EBERSON, L. (1965). Diethyl Trimethylsilylmethylhalogenomalonates. Acta Chem. Scand., 10: 633-7; Chem. Abstr. 51: 8002d (1957).

ERB-DEBRUYNE, F. (1964). N-Amino Derivatives of 1-methylpiperazine, Piperidine and Morpholine. Ann. Chim. (Paris), 9: 73-93; Chem. Abstr., 61: 5646h (1964).

FAURAN, C., TURIN, M., RAYNAUD, G. and POURRIAS, B.N. (1970). Hypotensive and Vasodilatory N-(3-Phenylpropyl)piperazines. Ger. Offen. 1,943,171; Chem. Abstr. 72: 100,751a (1970).

FAURAN, C., TURIN, M., RAYNAUD, G. and POURRIAS, B.N. (1971). 1-(3,4,5,-Trimethoxycinnamoyl)-4-(alkoxycarbonylethyl)-piperazine Maleates. Ger. Offen. 2,127,129; Chem. Abstr., 76: 59653n (1972).

FELDMANN, E.G. and FOYE, W.O. (1959). Comparative Activities and Toxicities. III. D-Glucamine and Glycine Derivatives of Nitro and Halogenated Benzenes. J. Am. Pharm. Assoc., 48: 419-422.

FISHBIEN, W.N., DALY, J. and STREETER, C.L. (1969). Preparation and Some Properties of Stable Carbon-14 and Tritium Short-chain Aliphatic Hydroxamic Acids. Anal. Biochem., 28: 13-24.

FOURNEAU, M.E. and BARELET, C.E. (1929). Contribution à l'étude des Anesthésiques Locaux Dérivés de la Pipérazine. Bull. Soc. Chim. France, 45: 1172-1189.

FOYE, W.O. and FELDMANN, E.G. (1957). Reaction of D-glucamine With Aromatic Nitro and Halogen Compounds. J. Org. Chem., 22: 475-478.

FRIEDMANN, M. and WALL, J.S. (1966). Additive Linear Free-energy Relationships in Reaction Kinetics of Amino Groups with α,β-Unsaturated Compounds. J. Org. Chem., 31: 2888-2894.

GALT, R.H.B. and PEARCE, R.J. (1971). Antibacterial Quinoxaline Derivatives. British Patent 1,237,438; Chem. Abstr., 75: 110,340s (1971).

GHOSH, P.B. and WHITEHOUSE, M.W. (1968). Potential Antileukemic and Immunosuppressive Drugs. Preparation and in vitro Pharmacological Activity of Some Benzo-2,1,3-oxadiazoles (Benzofurazans) and Their N-oxides (Benzofuroxans). J. Med. Chem., 11: 305-311.

GHOURI, M.S.K. and HALEY, T.J. (1969). Structure-activity Relationships in the Adrenergic-blocking Agents. J. Pharm. Sci., 58: 511-538.

GILBERT, G., WAGNER-JAUREGG, T. and STEINBERG, G.M. (1961). Hydroxamic Acids: Relationship Between Structure and Ability to Reactivate Phosphonate-inhibited Acetylcholinesterase. Arch. Biochem. Biophys. 93: 469-475.

GOODU, R.F. LEBLANC, N.F. and WRIGHT, C.M. (1955). Spectrophotometric Determination of Esters and Anhydrides by Hydroxamic Acid Reaction. Anal. Chem., 27: 1251-1255.

GIULIANO, R. and STEIN, M.L. (1956). Use of Triethylamine in the Preparation of Esters of Diethylaminoethyl. Il Farmaco (Pavia), Ed. Sci., 11: 3-9; Chem. Abstr., 50: 12986d (1956).

HALVERSTADT, I.F., HARDIE, W.R. and WILLIAMS, A.R. (1959). Hypotensors: 2-Ammionioalkyl 3-Ammonioalkanoate Salts. J. Am. Chem. Soc., 81: 3618-3628.

HAMANA, M. (1955). Reduction of Aromatic Tertiary Amine Oxide by Triphenyl Phosphite. J. Pharm. Soc. Japan, 75: 139-145.

HAMLIN, K.E., WESTON, A.W., FISCHER, F.E. and MICHAELS, R.J. (1949). Histamine Antagonists. II. Unsymmetrical 1,4-Disubstituted Piperazines. J. Am. Chem. Soc., 71: 2731-2734.

HAYAO, S., SCHUT, R.N. and STRYCKER, W.G. (1963). New Sedative and Hypotensive Phenylpiperazine Esters and Carbamates. J. Med. Chem., 6: 133-135.

HEACOCK, R.A. and MARION, L. (1956). The Infrared Spectra of Secondary Amines and Their Salts. Can. J. Chem., 34: 1782-1795.

HORII, Z., IKEDA, M., TAMURA, Y. and WATANABE, T. (1962). Synthesis of oxytocics. III. Synthesis of β-Alanine and 2-Methyl-β-alanine Derivatives. Yakugaku Zasshi, 82: 706-710; Chem. Abstr., 58: 4643 (1963).

HOWELL, H., POLLARD, C.B., KIER, L.B. and SISLER, H.H. (1963). Derivatives of 1-Phenyl-4-(2-hydroxy-3-methoxypropyl)-piperazine. J. Med. Chem., 6: 604-607.

HOWTON, D.R. (1945). 1,3-Dimethylpiperidone. J. Org. Chem., 10. 277-282.

INGOLD, C.K. (1953). Structure and Mechanism in Organic Chemistry. Cornell University Press, Ithaca, N.Y. pp. 752-754.

JACKMAN, L.M. and STERNHELL, S. (1969). Applications of Nuclear Magnetic Responance Spectroscopy in Organic Chemistry. Pergamon Press, pp. 134 and 368-379.

JACOBI, J. (1933). Zur Halbseitigen Substitution am Piperazin: Halogenwasserstoff - abspaltende Substitutionsmittel. Ber., 66: 113-116.

JAIN, P.C., KAPOOR, V., ANAND, N., AHMAD, A. and PATNAIK, G.K. (1967). Compounds Acting on the Central Nervous System. VII. Studies in 1-Pyridyl-4-substituted Piperazines. A New Class of Anticonvulsants. J. Med. Chem., 10: 812-818.

KING, F.E. and CLARK-LEWIS, J.W. (1951). The Structures of Some Supposed Azetid-2:4-diones. Part III. The "Alloxan-5-O-dimethylaminoanil" of Rudy and Cramer and its Alkali Hydrolysis Product. J. Chem. Soc., 3080-3085.

KRAPCHO, P.A. and KUYPER, P.S. (1963). The Thermal Decarboxylation of Sodium Trichloroacetate in the Presence of Active Hydrogen and Halogen Compounds. J. Org. Chem., 28: 2904-2905.

KUMEI, K. (1961). Effect of Derivatives of γ-aminobutyric Acid on the Mammalian Ileum, Nippon Seirigaku Zasshi, 23: 252-260; Chem. Abstr., 55: 20229h (1961).

LANYI, K., ERDELYI, I., INSTITORIS, E., TARDOS, L. and SZABO, Z. (1968). Piperazine Derivatives. II. Effect of Piperazine Derivatives. Arzneim.-Forsch., 18: 1431-1435; Chem. Abstr., 70: 27434b (1970).

LEPETIT, S.p.A. (1967). Preparation of Pharmacologically Active Substituted Piperazines. Neth. Appl. 6,611,479; Chem. Abstr., 68: 59615n (1968).

LESPAGNOL, A., LESPAGNOL, C., BONVARLET, J.C. CAZIN, J.C., and CAZIN, M. (1969). Chemical and Pharmacodynamic Study of Esters of α,α,α-Trisubstituted acids and of Various Amino Alcohols. Chim. Ther., 4: 103-111; Chem. Abstr., 71: 29055y (1969).

LUTZ, R.E. and SHEARER, N.H. (1947). Antimalarials. Some Piperazine Derivatives. J. Org. Chem., 12: 771-775.

MALLORY, F.B. (1957). Benzofurazan Oxide. Org. Syn., 37: 1-2.

MATKOVICS, B., FOLDEAK, S., PORSZASZ, J. and SIPOS, G. (1961). The Synthesis of Esters of Some Amino Acids Having Pharmacological Importance. I. The Synthesis of Esters of Piperidino Carboxylic Acids. Acta Pharm. Hung., 31: 113-121; Chem. Abstr., 55: 22321g (1961).

MATKOVICS, B., FOLDEAK, S. and PORSZASZ, J. (1961). Physiological Activity and the Chemical Structure of New Drugs Acting on the Central Nervous System. II. Synthesis of Cyclic Tertiary Aminopropionic Esters and "Reversed" Esters. Acta Phys. et Chem., 7: 60-65; Chem. Abstr., 56: 1535h (1962).

MIDHA, K.K. (1969). New Hypotensive Agents. Ph.D. Thesis, Edmonton.

MIDHA, K.K., COUTTS, R.T., HUBBARD, J.W. and PRASAD, K. (1970). 2-Methyl-3-(4-phenylpiperidino)propionohydroxamic Acid Hydrochloride - a New Hypotensive Agent. Eur. J. Pharmacol. 11: 48-55.

MOFFIT, R.L. and LIM, R.K.S. (1956). New Hypotensives with Central and Peripheral Actions. Fed. Proc., 15: 461-462.

MOORE, T.S. BOYLE, M. and THORN, V.M. (1929). N-Substituted Derivatives of Piperazine and Ethylenediamine. Part I. The Preparation of N-Monosubstituted Derivatives. J. Chem. Soc., 39-51.

MOREN, M., DENAYER, R. and LINZ, R. (1950). Contribution à l'étude de la pipérazine et de ses derivés. Obtention de la N. Monométhylpipérazine. Bull. Soc. Chim. Belg., 59: 223-227.

MORPHIS, B.B., ROTH, L.W. and RICHARDS, R.K. (1959). Pharmacologic Studies of a New Antihypertensive Compound N-(o-Methoxyphenyl)-N'-(3-methoxypropyl)piperazine Phosphate. Proc. Soc. Exp. Biol. N.Y., 101: 174-177.

MOSHER, H.S., CORNELL, J., STAFFORD, O L. and ROE, R. (1953). Synthesis of Piperazines By Reductive Cyclization. J. Am. Chem. Soc., 75: 4949-4951.

NAGATA, M., IMAMURA, I. and SUGIMOTO, J. (1969). Anti-nicotinic Action of Vastarel ® on Guinea-pig Atria. Jap. J. Pharmacol. 19: 628-629.

NAST, R., LEG, K., EHOLZER, M., METZGER, K.G. and FRITSCHE, D. (1970). Physiologically Active Di-N-oxides of 3-Carbamoyl-quinoxaline Derivatives. S. African Patent 6,905,597; Chem. Abstr., 73: 120671y (1970).

NIKOLOVA, M., DELEVA, L. and MARINOVA, V. (1969). Preliminary Pharmacological Investigation of Some Piperazine Derivatives. Farmatsiya (Sofia), 19: 31-37; Chem. Abstr., 71: 59406f (1969).

OCHIAI, E., "Aromatic Amine Oxides", Elsevier Publishing Company, 1967, pp. 184-206.

O'LEARY, J.F. (1953). Cardiovascular Actions of 1,4-bis-(1,4-Benzodioxan-2-yl-methyl)piperazine, a New Adrenergic Blocking Agent. Fed. Proc., 12: 355-356.

OLIN, S.M. (1958). Piperazine Derivatives. U.S. Patent 2,858,313s Chem. Abstro., 53: 6273g (1959).

PACHEO, M., DREUX, M. and BEAUVILLAIN, A. (1962). Synthesis and Anti-spasmodic Activity of Compounds Related to 3-Amino-3-phenylpropionic Acid: Bull. Soc. Chim. France, 1379-1387; Chem. Abstr., 58: 7933h (1963).

PADEISKAYA, E.N., PERSHIN, O.N. and RADKEVICH, T.P. (1967). Chemotherapeutic Effect of Quinoxaline Derivatives in Acute Bacterial Infections. Int. Congr. Chemotherap., 5th, 1: 825-830.

PAGE, I.H., WOLFORD, R.W. and CORCORAN, A.C. (1959). Pharmacological and Clinical Observations on 1-(2-Methoxyphenyl)piperazine. Arch. Int. Pharm., 119: 214-224.

PALMER, C.S. and MCWHERTER, P.W. (1941). Ethyl Bromomalonate. Org. Syn., 1: 240-241.

PARKE-DAVIS and Co. (1958). 4-(o-Allyloxy or Alkoxyphenyl)-1-piperazine Alkanols and Esters. British Patent 803,403; Chem. Abstr., 53: 12314h (1959).

PFAU, M. (1967). Préparation de Dérivés N-substitués d'esters Aspartiques et de β-Aminoesters Ainsi Que des Acides Correspondants. Bull. Soc. Chim. France. 1117-1125.

PHILLIPS, A.P. (1950). Synthetic Analogs of Oxytocic Drugs. IV. Miscellaneous Substituted-β-alanine Esters. J. Am. Chem. Soc., 72: 3298-3299.

POLLARD, C.B. and MACDOWELL, L.G. (1934), A New Synthesis of n-Monophenylpiperazine. J. Am. Chem. Soc., 56: 2199-2200.

PORSZARZ, J., FOLDEAK, S., MATKOVICS, B., BARANKAY, T., and GIBISZER-PORSZASZ, K. (1961). Comparative Pharmacology of N-Substituted Teriary and Quaternary Amino Esters of Acetic and Propionic Acid. Acta Physiol. (Acad. Sci. Hung.), 19: 235-238.

PRASAD, R.N., HAWKINS, L.R. and TIETJE, K. (1968). Potential Antihypertensive Agents. II. Unsymmetrically 1,4-Disubstituted Piperazines. J. Med. Chem., 11: 1144-1150.

PRASAD, R.N. and TIETJE, K. (1969). Potential Antihypertensive Agents. IV. Unsymmetrically 1,4-Disubstituted Piperazines. J. Med. Chem., 12: 551-552.

PRELOG, V. and BLAZEK, Z. (1934). Sur les Pipérazines N-Monoarylées et leurs Dérivés. Collection Czechoslov. Chem. Communs., 6: 211-224.

PRELOG, V. and DRIZA, G.J. (1933). Sur la N-Phenylpiperazine (Recherches sur les bis-[β-Halogenoethyl]amines III). ibid., 5: 497-502.

PRELOG, V. and STEPHAN, V. (1935). Nouvelle Synthése des Pipérazines N-Monoalcoylées (VII ieme Communication sur les bis-[β-Halogenoethyl]-amines). ibid., 7: 93-102.

QUESUEL, G., CHALAUST, R., SCHMITT, H., KRONEBERG, G. and SCHMITT, M.H. (1960). Un Nouveau Dérivé Vasodilatateur de la Pipérazine: la 3-4-Diméthoxybenzylpiperazine. Arch. Int. Pharmacodyn., 128: 17-30.

RATOUIS, R., BOISSIER, J.R. and DUMONT, C. (1965). Synthesis and Pharmacological Study of New Piperazine Derivatives. II. Phenethylpiperazines. J. Med. Chem., 8: 104-107.

RATOUIS, R., BOISSIER, J.R. and DUMONT, C. (1965). Synthesis and Pharmacological Study of New Piperazine Derivatives. III. Phenoxyalkylpiperazines. J. Med. Chem., 8: 271-273.

RODRIGUEZ, R., HONG, E., VIDRIO, H. and PARDO, E.G. (1965). Pharmacology of a Group of Phenylpiperazine Tetrazole Derivatives with Adrenergic Blocking Actions. J. Pharmacol. Exp. Therap., 148: 54-65.

ROTH, L.W. (1954). Blocking Effects of a Series of Substituted Piperazines. J. Pharmacol. Exp. Therap., 110: 157-165.

RUDNER, B. (1961). Piperazinium Salts. U.S. Patent 2,967,865; Chem. Abstr., 55: 21152e (1961).

RUSHIG, H., SCHMITT, K., MOHLER, H., GOEBEL, A. and LINDNER, E. (1971). α-Sympatholytic Heterocyclic 2-(4-Phenyl-1-piperazinyl)ethyl Ethers. Ger. Offen. 1,946,172; Chem. Abstr., 74: 141866b (1971).

SAFIR, S.R. and WILLIAMS, J.H. (1952). Synthesis of Cyclic Hydroxamic Acid Derivatives of Pyrazine. J. Org. Chem., 17: 1298-1301.

SCHEULER, F.W. and KEASLING, H.H. (1951). Further Studies on the (RC) Analogue of Acetylcholine and Some Additional Derivatives. J. Pharmacol. Exptl. Therap., 103: 222-235.

SCHWEIZER, E. and EGLI, C. (1971). Antibacterial Quinoxaline 1,4-dioxides Containing a Heterocyclic Ring in the 2-position. Ger. Offen. 2,107,301; Chem. Abstr., 76: 3901z (1972).

SELLEY, M.L. (1971). Studies on Hypotensive Agents. M.Sc. Thesis, Edmonton.

SENG, F., LEY, K. and METZGER, K.G. (1971). Antimicrobial 2-Iminomethyl-3-carboxyquinoxaline 1,4-dioxides. Ger. Offen. 2,015,676; Chem. Abstr., 76: 34292f (1972).

SOCIÉTÉ des Usines Chimiques Rhône-Poulenc (1958). 9-(1-Piperazinylalkyl)carbazoles. French Patent 1,167,510; Chem. Abstr., 55: 8443e (1961).

SONNTAG, N.O.V. (1953). The Reactions of Aliphatic Acid Chlorides. Chem. Rev., 52: 237-416.

STEWART, H.W., TURNER, R.J. DENTON, J.J., KUSHNER, S., BRANCONE, L.M., MCEWEN W.L., HEWITT, R.I. and SABBAROW, Y. (1948). Experimental Chemotherapy of Filariasis. IV. The Preparation of Derivatives of Piperazine. J. Org. Chem., 13: 134-143.

TAKAHASHI, H., UCHIKURA, K., TAKAHASHI, H. and IKEDA, O. (1961a). Relationship Between Pharmacological Actions on the Mammalian Ileum and Chemical Structure of γ-Aminobutyric Acid. Japan. J. Physiol., 11: 229-237.

TAKAHASHI, H., IKEDA, O., ITAYA, T. and KOSHINO, F. (1961b). Anti-stimulant Actions of γ-Aminobutyric Acid and its Derivatives on the Guinea-pig Ileum. Japan J. Physiol., 11: 476-487.

TAKAHASHI, H. (1961c). Effects of Derivatives of γ-Aminobutyric Acid and Guanidino Acids on the Heart and Blood Vessels. Nippon Seirigaku Zasshi, 23: 417-25; Chem. Abstr., 55: 23837f (1961).

TAKAHASHI, H. and KOHIGA, M. (1962a). γ-Aminobutyric Acid Derivatives. Japan Patent 17,960; Chem. Abstr., 59: 11269c (1963).

TAKAHASHI, H. and KOHIGA, M. (1962b). γ-Aminobutyric Acid Derivatives. Japan Patent 17,961; Chem. Abstr., 59: 11269d (1963).

TENNANT, G. (1964). Heterocyclic N-oxides. Part III. Extensions of a Quinoxaline N-oxide Synthesis. J. Chem. Soc., 2666-2673.

THOMPSON, W.E., WARREN, R.J. EISDORFER, I.B. and ZAREMBO, J.E. (1965) Identification of Primary, Secondary and Tertiary Pharmaceutical Amines By the Infrared Spectra of Their Salts. J. Pharm. Sci., 54: 1819-1821.

TOWILL, G.A. (1971). Studies on Potential Hypotensive Agents. M.Sc. Thesis, Edmonton.

TURIN, M., FAURAN, C., RAYNAUD, G.M. and THOMAS, J. (1971). Benzhydrylpiperazine Derivatives. Ger. Offen. 2,111,776; Chem. Abstr., 76: 14576y (1972.

VOGEL, A.I. Practical Organic Chemistry, 3rd, Ed., Longmans, 1956, pp. 866,1067.

WEAST, R.C., Handbook of Chemistry and Physics, Chemical Rubber Company, 1970-1971.

WILLSTATTER, R. and PFANNENSTIEL, A. (1904). Ueber o-Chino, Ber , 37: 4744-4746.

YUNG, D.K., CHATTEN, L.G. and MACLEOD, D.P. (1968). Potential Antiarrhythmic Agents. I. Synthesis and Pharmacological Evaluation of Some Piperazine and Ethylenediamine Analogs of Procaine Amide. J. Pharm. Sci., 57: 2073-2080.

ZAUGG, H.E., DUNNIGAN, D.A. MICHAELS, R.J., SWETT, L.R., WANG T.S., SOMMERS, A.H. and DENET, R.W. (1960). Specific Solvent Effects in the Alkylation of Enolate Anions. III. Preparative Alkylations in Dimethylformamide. J. Org. Chem., 26: 644-651.

ZINCKE, T. and SCHWARZ, P. (1899). Ueber o-Dinitrosoverbindungen der Benzolreihe, Ann., 307: 28-49.