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TITLE OF THESIS....The Synthesis, Properties, and Pharmacological evaluation of some new hypotensive agents.

UNIVERSITY.........of Alberta..................

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

YEAR THIS DEGREE GRANTED........1969......................

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DATED..Sept. 17,........1969

NL-91 (10-68)

THE UNIVERSITY OF ALBERTA

THE SYNTHESIS, PROPERTIES, AND PHARMACOLOGICAL EVALUATION OF SOME NEW HYPOTENSIVE AGENTS

by

KAMAL KISHORE MIDHA

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

FACULTY OF PHARMACY
AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA
(FALL), (1969)

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled: "THE SYNTHESIS, PROPERTIES, AND PHARMACOLOGICAL EVALUATION OF SOME NEW HYPOTENSIVE AGENTS" submitted by KAMAL KISHORE MIDHA in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Edward E. Smissman
External Examiner

R T Coutts
Supervisor

A F Casy

L. G. Chatten

E J Triggs

Date SEP 9 1969

TO MY PARENTS

ABSTRACT

Various N-substituted-3-aminopropionates and related amino-hydroxamic acids have been prepared and screened for their effects on arterial blood pressure and heart rate of anaesthetized cats. The 3-amino-esters were either prepared by the interaction of an amine with a 3- or 4-halo-ester, or by the addition of amines to α, β-unsaturated esters. The N-substituted-3-amino-hydroxamic acids were prepared by reacting the appropriate ester with hydroxylamine hydrochloride in presence of potassium hydroxide, or by interaction of hydroxylamine hydrochloride and an amino-ester. PMR spectra of acyclic hydroxamic acids were recorded and an attempt has been made to identify the proton signals of the hydroxamate group in N-substituted-3-amino-hydroxamic acids.

During the course of preparing 3-halo-esters an interesting transesterification reaction was observed. This reaction has application in natural-product chemistry. The components of high molecular weight fatty ester mixtures can be identified.

All the 3-amino-esters and 3-amino-hydroxamic acids screened, produced a change in arterial blood pressure. Most of the esters and hydroxamic acids produced a fall in blood pressure and bradycardia, but there were some which caused a rise in blood pressure with or without tachycardia. It was noticed that the change in blood pressure caused by

by the esters was generally transient as compared to hydroxamic acids most of which produced a potent and prolonged fall in blood pressure. Structure-activity relationships have been studied, and an attempt has been made to design a compound which would produce a potent and prolonged fall in blood pressure. The activity of one of the hydroxamic acid namely 2-methyl-3-(4-phenylpiperidino)propiono-hydroxamic acid (K76) has been investigated in detail in an attempt to establish a possible mechanism of action on blood pressure. This fall in blood pressure appears to be the result of ganglion blockade. Evidence is presented in support of this. The action of K76 has been compared with that of pentolinium tartrate (Ansolysen), a known ganglion blocker. It was found to be as potent as Ansolysen in intensity and duration of fall in blood pressure, but unlike Ansolysen and other ganglion blockers K76 was almost ineffective in blocking the bradycardia produced by stimulation of cut right end of the vagus. This suggested that the hypotensive action of K76 was predominantly by blockade of sympathetic ganglia.

N-substituted-3-aminopropionic acids possessed no hypotensive properties.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the able supervision and guidance given by Dr. R.T. Coutts throughout the course of present investigation.

He thanks Dr. K. Prasad for his invaluable assistance in the pharmacological screening and testing of compounds synthesized during the course of the study. Finally, he thanks his friends Dr. E.J. Triggs and Dr. J.W. Hubbard for many useful discussions and suggestions.

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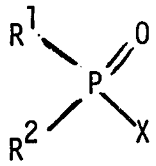
PREFACE

During the course of the research study reported in this thesis, certain interesting observations were made which altered the direction of the study. The introduction briefly summarizes the original intention of the research, describes why a change in emphasis occurred and summarizes the findings reported in the literature in the ultimate area of study.

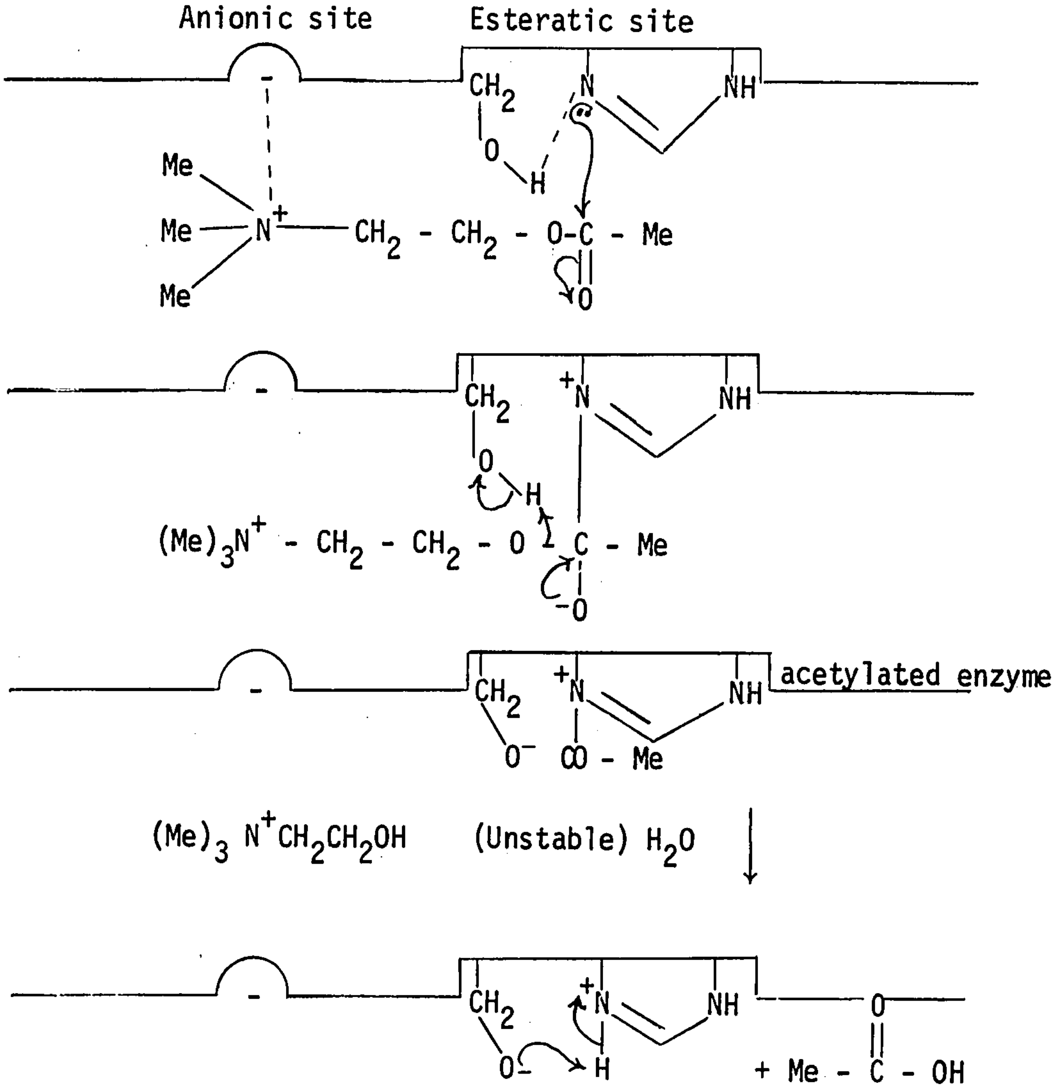
INTRODUCTION

Enzymes such as acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and *iso*propylcholinesterase are inhibited by organophosphorous compounds, especially the phosphofluoridates; e.g., TEPP (tetraethyl pyrophosphate), DFP (di-*iso*propylphosphorofluoridate), DDP (di-*n*-propyl 2, 2-dichlorovinyl phosphate), TABUN (ethyl-N, N-dimethyl phosphoramidocyanidate), and SARIN (*iso*propyl methylphosphonofluoridate) which possess the general formula (1) in which R^1 is an alkoxy group, R^2 is an alkoxy or alkyl or alkylamido group, and X is a halogen or a cyanide or a phenoxy or a disubstituted phosphoryloxy group. These are *per se* phosphorylating agents of AChE. A second group of organophosphates consist of compounds which are only weak anticholinesterase agents but are converted into highly potent phosphorylating agents *in vivo*. To this group belong the majority of organophosphate insecticides used at present; e.g., 0,0-diethyl o-(4-nitrophenyl) - phosphorothiate (Parathion) which is converted to deadly poisonous paraoxon *in vivo*.



(1)

The active centre of AChE contains two subsites which are generally called the anionic and esteratic sites (Bergmann and Shimoni, 1953; Wilson and Bergmann, 1950; Wilson, 1952; Wilson, 1951; Bergmann *et al*, 1950; Wilson, 1955). Hydrolysis of acetylcholine by AChE has been represented diagramatically (Beckett, 1961):

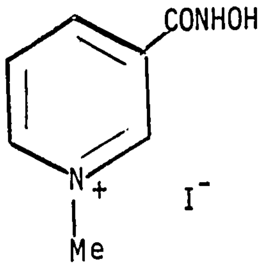


Formation of the acetylated enzyme is considered to be the rate determining step in the sequence of reactions. The acetylated enzyme is extremely unstable and has a half life of a fraction of a millisecond.

Acetylcholinesterase also reacts with organophosphorous compounds to give phosphorylated acetylcholinesterase which unlike acetylated AChE is extremely stable. The hydrolysis with water proceeds at very slow rate and for this reason organophosphates are inhibitors and not substrates of AChE.

Wilson in 1951 reported that choline and hydroxylamine reactivated diethylphosphorylated AChE considerably faster than water alone. Information gained in these studies led to the discovery of potent reactivators including hydroxamic acids and oximes. This is of considerable practical importance because the widespread use of organophosphate insecticides frequently leads to accidental, homicidal, or suicidal poisoning of man in some countries (Namba, 1958; Toivonen et al, 1959) and an effective treatment of organophosphate poisoning is urgently needed.

The most potent reactivators of the phosphorylated AChE are oximes; hydroxamic acids are less active. The best reactivators amongst the hydroxamic acids are nicotinohydroxamic acid methiodide (2), picolinohydroxamic acid (3) and pyrimidine-2-hydroxamic acid (4) which reactivate in the manner shown.



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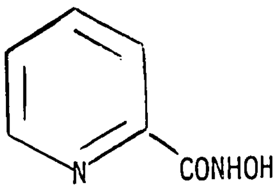

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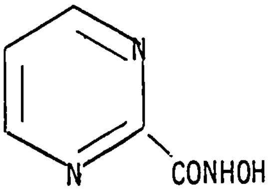

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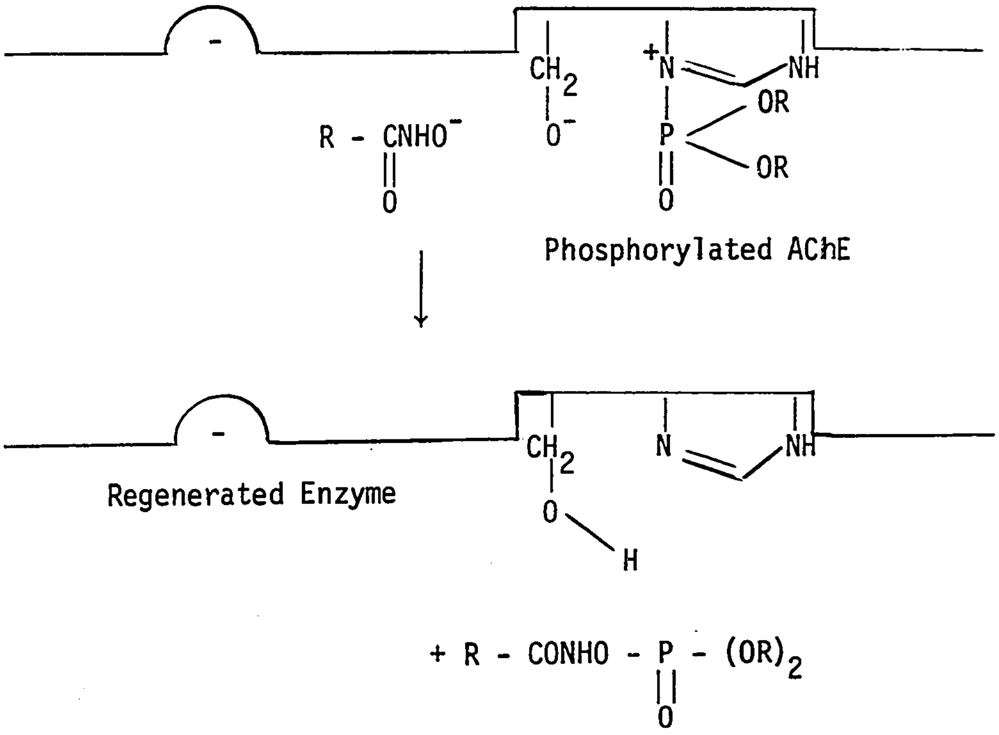
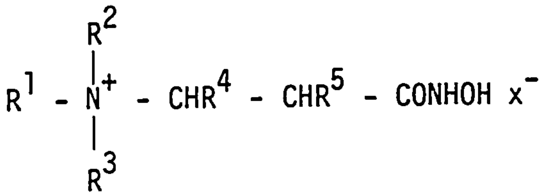
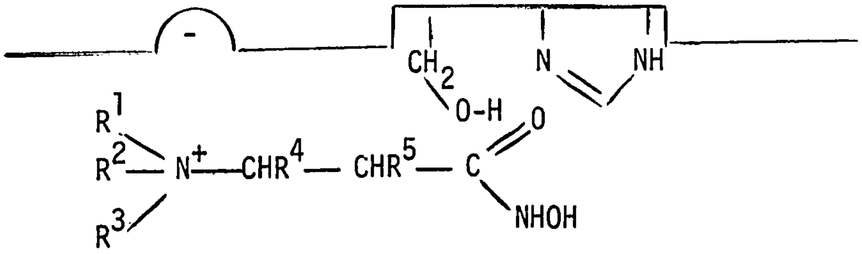


The present study was undertaken in an attempt to prepare hydroxamic acids which would act as competitors and therefore prevent phosphorylation of the enzyme, or else would be capable of reactivating the phosphorylated enzyme. Structures of type (5) were envisaged initially in which the various substituents were either alkyl groups or

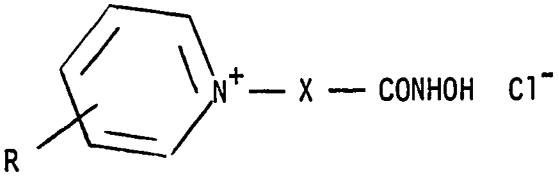

(5)

$$R^1 - \overset{\overset{\displaystyle R^2}{|}}{\underset{\underset{\displaystyle R^3}{|}}{N^+}} - CHR^4 - CHR^5 - CONHOH\ x^- \qquad (5)$$

hydrogen atoms. It was felt that these compounds could attach themselves to the enzyme surface at the anionic site and be so orientated that the hydroxamate group would be spacially near the esteratic site:



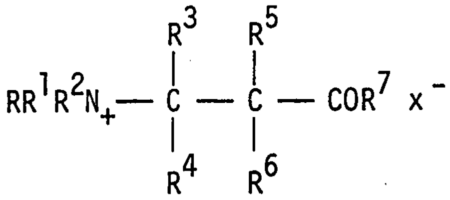
Such compounds would also be structurally similar to nicotinohydroxamic acid methiodide (2) and to other known quaternary hydroxamic acids derived from pyridine of structure (6) which were shown to be active in preventing and reversing some of the physiological effects of cholinesterase inhibition (Coe, 1959).



(6)

Numerous compounds of structure (5), therefore, were prepared. When a preliminary pharmacological screening of these hydroxamic acids and the ester precursors revealed that some were potent hypotensive agents and when a literature search showed that no hypotensive hydroxamic acids were known, the course of the proposed research was changed appreciably. Evaluation of the effects of the hydroxamic acids on inhibited acetylcholinesterase was postponed. The area of interest was expanded to include hydroxamic acids, esters, amides and ketones of general structure (7) in which the substituents R^2, R^3, R^4, R^5 and R^6 were hydrogen atoms or alkyl groups, R and R^1

were alkyl groups or a ring system and X was a halogen atom. A literature survey of the methods of preparation and pharmacological evalu-



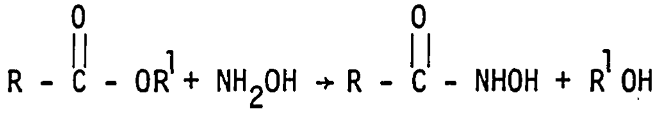
$$RR^1R^2\underset{+}{N} - \overset{R^3}{\underset{R^4}{\overset{|}{\underset{|}{C}}}} - \overset{R^5}{\underset{R^6}{\overset{|}{\underset{|}{C}}}} - COR^7\ x^- \qquad (7)$$

ation of these compounds (7) was carried out.

Acyclic 3- and 4-amino-hydroxamic acids and related compounds

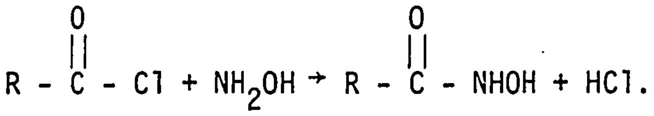
The chemistry and biological properties of numerous cyclic and acyclic hydroxamic acids have been reported in the literature and reviewed (Coutts, 1967). The most common methods of preparation are by the action of hydroxylamine on an ester, an acid chloride or an anhydride; e.g.,

(a)



$$R - \overset{O}{\overset{\|}{C}} - OR^1 + NH_2OH \rightarrow R - \overset{O}{\overset{\|}{C}} - NHOH + R^1OH$$

(b)

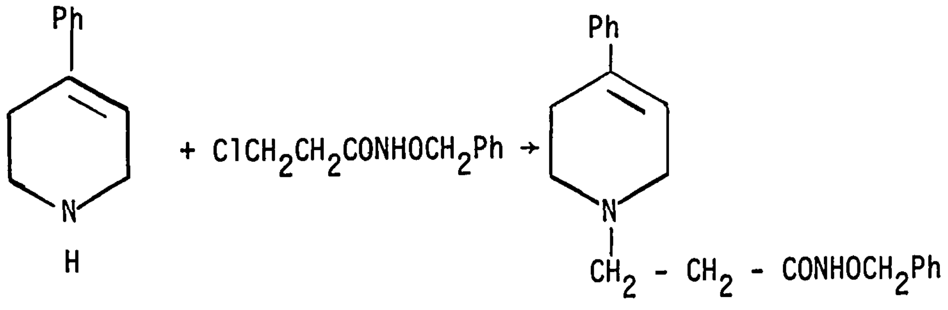


$$R - \overset{O}{\overset{\|}{C}} - Cl + NH_2OH \rightarrow R - \overset{O}{\overset{\|}{C}} - NHOH + HCl.$$

Sulfonyl chlorides are employed similarly in the preparation of sulfonhydroxamic acids; e.g.,

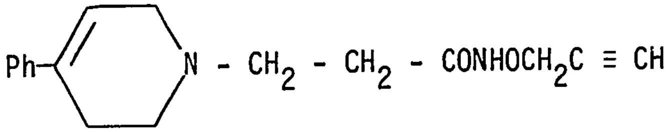
$$C_6H_5SO_2Cl + NH_2OH \rightarrow C_6H_5SO_2NHOH + HCl \ .$$

Although sufficient literature references are available which describe the synthesis of 3- or 4-amino-esters, very little work has been reported on the synthesis and pharmacology of the related amino-hydroxamic acids. Matveev et al (1964) synthesised nineteen aliphatic amino-hydroxamic acids for polarographic reduction studies but did not mention any pharmacology of these compounds. Hypertension and peripheral vascular disease have been treated (Biel, 1965), with aralkoxyamides of 4-phenyl -1,2,5,6-(tetrahydropyridino) alkanoic acid (8). This compound (8), prepared as illustrated, and related compounds produce a potent and prolonged blood pressure fall. Their mechanism of action does not involve blockage of peripheral and autonomic ganglia, thus making them useful



(8)

antihypertensive agents with minimum of side effects. The corresponding N-alkynyl and N-alkynyloxy derivatives (9) prepared in a similar manner show analgesic activity (Biel and Hopps, 1965).



(9)

The compounds just described were the only ones reported in the literature which were closely related to the hydroxamic acids prepared in the present study.

3- and 4-Amino-esters

Numerous references are available which describe the synthesis and pharmacological activity of these esters. (Howton, 1945; Vystrčil and Hudeček, 1950; Phillips, 1950; Dickermann and Besozzi, 1954; Nikitskaya and Rubtsov, 1956; Nazarov and Kazaryan, 1957; Gearien and Binkley, 1958; Matkovics et al, 1961; Nazarov and Sorokin, 1960; Harvieu et al, 1963; Humphreys et al, 1964).

It is convenient to discuss initially the synthesis of these compounds and to postpone comments on their pharmacology until later. The most common of these methods are given now.

(i) The first of these involves the addition of ammonia and primary, secondary and cyclic amines to α, β-unsaturated esters (Flurscheim, 1903; Morsch, 1932; Morsch, 1933; Adamson, 1950; Bieber, 1954). The large volume of work done in this area is summarized conveniently by means of relevant equations:

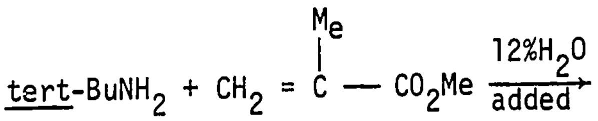
$$RNH_2 + R^1 - CH = CH - CO_2R^2 \rightarrow RNH - CHR^1 - CH_2\ CO_2R^2\ ,$$

where R = H or an alkyl group with 1 to 8 carbon atoms, R^1 = H, Me, Et, <u>n</u>Pr or phenyl and R^2 = alkyl, and

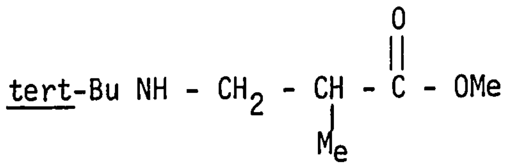
$$RRNH + CH_2 = CR^1 - CO_2R^2 \rightarrow RRN - CH_2 - CHR^1 - CO_2R^2\ ,$$

where R = Me, Et, <u>n</u>Pr and other alkyl groups, or substituted piperidine, piperazine, morpholine, pyrrolidine and ring systems, R^1 = H, Me, Et, or <u>n</u>Pr and R^2 = various alkyl groups.

It has been shown that ease of addition of amines to methylmethacrylate is in the order piperidine $>$ diethylamine $>$ aniline $>$ phenyl ethylamine (Bieber, 1954). According to Hughes, (1960) simple amines containing only two to three carbon atoms reacted with nitriles and lower esters of α, β-unsaturated acids such as acrylic, methacrylic and crotonic acids in the absence of a catalyst but higher amines and amines containing branched alkyl groups such as <u>tert</u>-butyl and <u>tert</u>-octyl did not react even in the presence of basic or acidic catalysts. They reacted, however, in the presence of water; e.g.,



$$\underline{tert}\text{-}BuNH_2 + CH_2 = \overset{\overset{Me}{|}}{C} — CO_2Me \xrightarrow[\text{added}]{12\%H_2O}$$



$$\textit{tert}\text{-Bu NH} - CH_2 - \underset{\displaystyle Me}{CH} - \overset{\displaystyle O}{\overset{\|}{C}} - OMe$$

Action of ammonia and amines upon esters of unsaturated acids has been studied in detail also by Phillippi and Galter (1929). According to them, reaction of methylamine with ethylcrotonate gave small quantity of the ethyl 3-methylamino butyrate but the main product was 3-methylaminobutyric methylamide. With piperidine the only product obtained was ethyl 3-piperidinobutyrate. Ethyl 2-ethylacrylate did not react with liquid ammonia or with methylamine. The interaction of ethyl 3, 3-dimethylacrylate and ammonia gave about 60% of the addition product but no amide; whereas when methylamine was the base used the ester, ethyl 3-methylamino*iso*-valerate was not the main product of the reaction. The main product was the corresponding methylamide. Ethyl allylacetate scarcely reacted with ammonia. Ethyl acrylate and piperidine reacted to give an 87% yield of ethyl 3-piperidinopropionate. The interaction of methyl hydrosorbate and liquid ammonia gave only the amide. Methyl sorbate and ethyl phenyl *iso*crotonate reacted similarly.

(ii) Addition of amines to α, β-unsaturated nitriles followed by hydrolysis and esterification, or alcoholysis of the intermediate nitriles (Whitemore *et al*, 1944; Takagi *et al*, 1952; Tsunetsugu *et al*, 1962; Zilka *et al*, 1958).

This method has been employed for the synthesis of quite a few aminoesters such as piperidino-, diethylamino- and di-*n*-butylamino-propionic esters. This can be represented by the following sequence:

$$CH_2 = CHCN + HNR_2 \rightarrow R_2NCH_2CH_2CN$$

$$R_2NCH_2CH_2CN + C_2H_5OH + H_2O \xrightarrow{H_2SO_4} R_2NCH_2CH_2CO_2C_2H_5 + NH_3 \;.$$

(iii) Condensation of acrylic esters with formamides (Hromatka and Eiles, 1948).

Secondary formamides such as N-methyl formamide, N-phenyl formamide, N-benzyl formamide react with acrylic esters and give an intermediate which hydrolyses to the corresponding amino-ester; e.g.,

$$HCONHR + CH_2 = CH - CO_2R^1$$

$$\longrightarrow HCONR - CH_2 - CH_2 - CO_2R^1$$

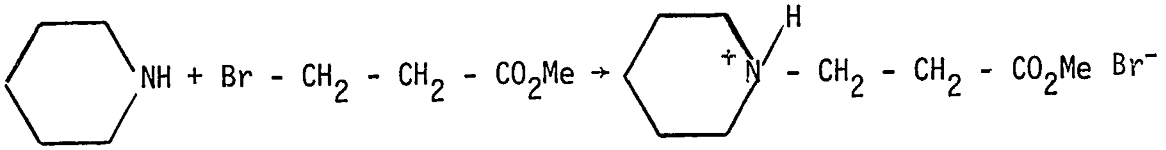
$$\Big\downarrow \text{HCl/MeOH}$$

$$RNH - CH_2 - CH_2 - CO_2R^1$$

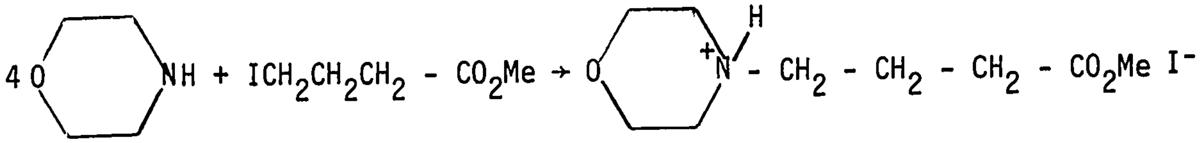
where R = Me, Et, phenyl, benzyl, or cyclohexyl and R^1 = Me, Et, or other alkyl groups.

(iv) Reaction of amines with halo-acids or -esters (Fuson et al, 1929; Drake and McElvain, 1934; Weisel et al, 1945; Takahashi and Kohiga, 1962; David et al, 1965).

A very common and widely used method for the synthesis of 3- or 4-amino-acids or -esters is through the reaction of a cyclic or acyclic amine with a 2-, or 3-, or 4- halo-ester, -acid or -nitrile. The preparation of methyl 3-piperidinopropionate (as a salt) is typical:



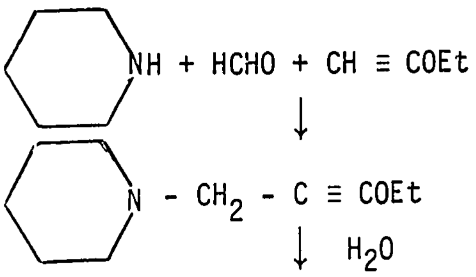
This method has most commonly been employed for the synthesis of 4- mono- or 4-di-alkylamino-acids and -esters. (Asano and Tomita, 1948; Treibs *et al*, 1956; Solovév *et al*, 1961). Cruickshank *et al* (1961) extended this to the preparation of 4-piperidino-, 4-morpholino-, 4-dibenzylamino-, and 4-di*iso*propylamino-butyrates by reacting four equivalents of the appropriate amine with an ester in benzene,



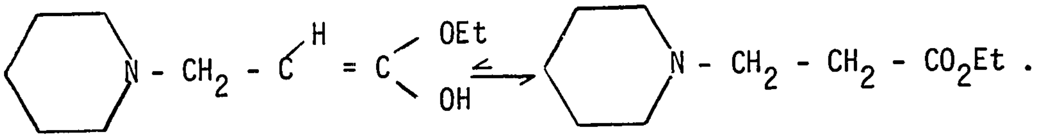
The corresponding acids were prepared by the acid or alkaline hydrolysis of these esters.

(v) Reaction of secondary amines with formaldehyde and acetylenic ether.

Arens *et al* (1956) in their attempt to prepare Mannich bases, obtained 3-dialkylamino-esters through the reaction of formaldehyde, dialkylamine and acetylenic ethers in an aqueous medium. A typical example is given:



(10)



Ethyl 3-piperidinopropionate may be considered as the hydration product of intermediate (10).

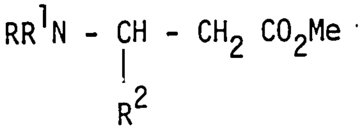
(vi) Oxidation of amino-alcohols

Olomucki and Marszak (1958) prepared 4-dialkylamino-acids and esters by the chromic acid oxidation of the appropriate 4-dialkylaminoalcohol; e.g.,

$$\begin{tikzcd} Me_2NCH_2CH_2CH_2CH_2OH \arrow[r, "CrO_3/H_2SO_4"] & Me_2NCH_2CH_2CH_2 - CO_2H \arrow[d, "ROH/H^+"] \\ & Me_2NCH_2CH_2CH_2 - CO_2R \end{tikzcd}$$

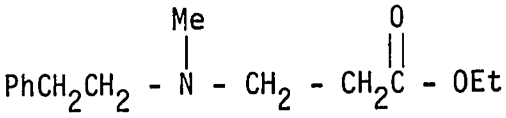
Aliphatic 3-, and 4-amino-esters and related compounds have become an important class of compounds responsible for a large variety of biological activities. Their importance in medicinal chemistry can be seen from the different pharmacological properties for which they have been screened and found useful. Some of the important uses are discussed below.

Methyl 3-substituted-butyrate esters and related compounds with general formula (11), where R is methyl, R^1 is methyl or ethyl and R^2 is methyl or propyl have demonstrated strong analgesic properties (Adamson, 1954).



$$RR^1N - \underset{\displaystyle R^2}{\underset{|}{CH}} - CH_2\,CO_2Me \qquad (11)$$

Ethyl 3-phenethyl methylaminopropionate (12) and similar compounds have been screened for oxytocic activity (Baltzly *et al*, 1949).


(12)

$$PhCH_2CH_2 - \overset{\overset{\displaystyle Me}{|}}{N} - CH_2 - CH_2\overset{\overset{\displaystyle O}{||}}{C} - OEt$$

Amino-acid esters with general formula (13) have been synthesised and screened for both muscarinic and nicotinic properties (Barrass *et al*, 1968; Brimblecome *et al*, 1968). Matkovics *et al* (1961) and Pórszász *et al* (1961) studied numerous esters possessing the same general formula (13) and found that esters containing lower aliphatic substituents (i.e. when R is methyl or ethyl) had nicotine-like action while those with higher molecular weight or with aromatic substituents (i.e. when R is phenyl or benzyl) showed antinicotinic action. The quaternary salts of these esters were potent ganglion blockers. Esters (14) containing two quaternary

$R^1(CH_2)_n - CO_2R$ $\quad$ $n = 1$ to 4 $\quad$ (13)

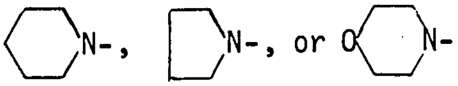
R = Me or Et

$R^1 = Me_2N$, or

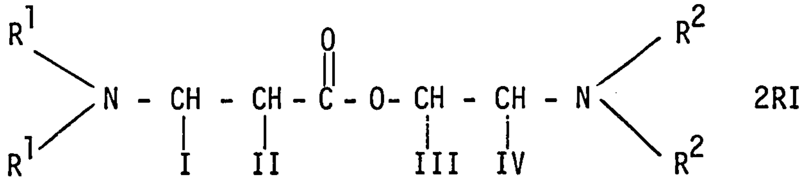
$R^1(CH_2)_nCO_2R$ $\quad$ $n = 1$ to 2 $\quad$ (13)

R = Me, Et, Bu or $PhCH_2$

R^1 =



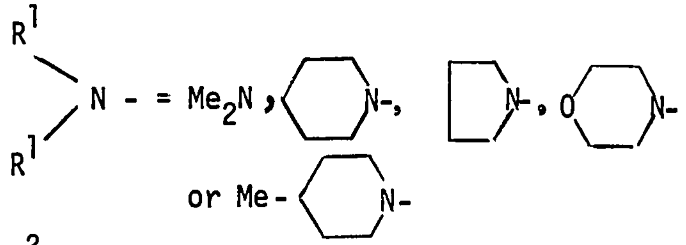
nitrogen atoms - a trialkyl nitrogen atom on the acid moiety and a heterocyclic nitrogen atom on the alcohol portion have been patented as useful hypotensive agents (Brit. Patent, 1957) as a result of ganglion blockage (Halverstadt, et al, 1959).

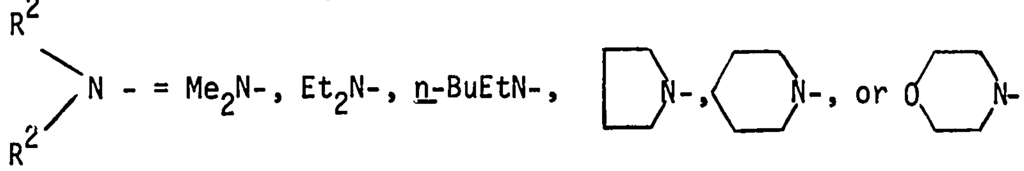


(14)

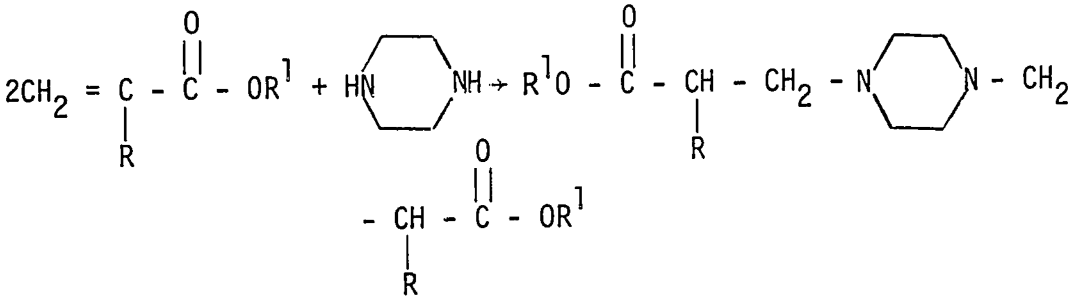
I to IV = H or Me or Et

R = Me or Et





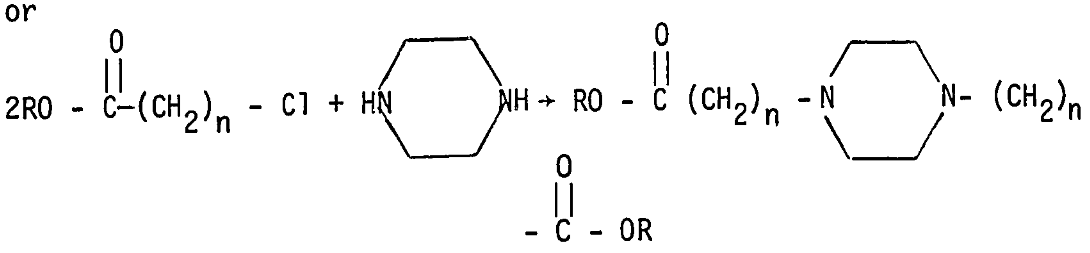
Diesters containing the piperazine ring system have been synthesised by the addition of one mole of piperazine to two moles of the acrylate esters or by reacting piperazine with 2-, or 3-, or 4- halo-esters (Cantatore, 1964); e.g.,



R = H

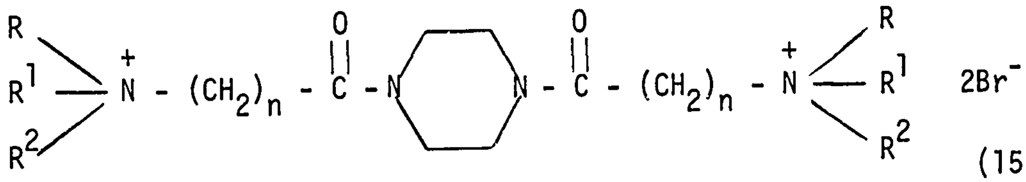
R^1 = Me, Et or any other alkyl group

or



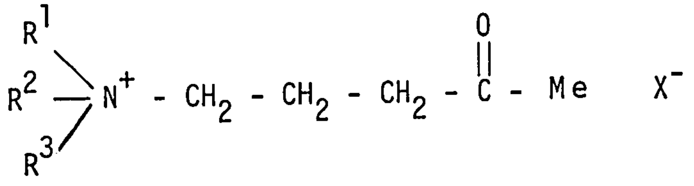
These esters have found use as intermediates in the synthesis of dyeable polypropylene-polyamide fibres (Neth. Patent, 1965), but no pharmacology of these compounds has been reported.

Quaternary ammonium salts of bis (dialkyl-aminoacyl) piperazines (15) show curare-like effects in rabbits (Hazard _et al_, 1964).



(15)

4-Amino-butyric acids and derivatives, especially esters, have been synthesised by various workers (Decombe, 1932; Breckpot, 1923; Graf, 1957). Esters of this type have been screened for a variety of biological actions. Esters (16a) and (16b) possess blood pressure depressant properties (Takahashi, <u>et al</u>, 1962); the related ester (17) displayed

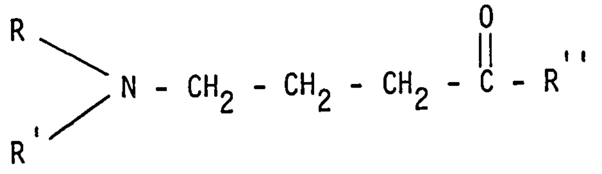


(16)

16a, $R^1 = Me$, $R^2 = H$, $R^3 = H$ and $X = Cl$

16b, $R^1 = Me$, $R^2 = Me$, $R^3 = Me$ and $X = I^-$

antistimulant properties on guinea pig ileum (Takahashi, 1961).



(17)

$R = H$ or Me

$R' = Me$ or Et or Ph and quarts

$R'' = OH, OMe, O\underline{n}Bu\ O\text{-}PhCH_2$.

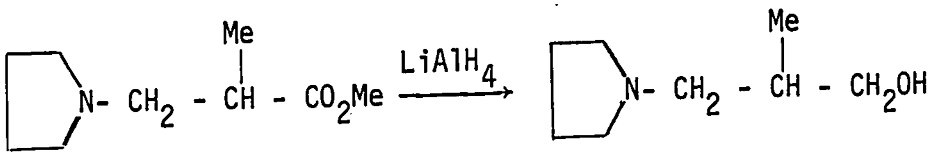
4- Amino-butyric acid (17, when $R = R^1 = H$, $R'' = OH$) inhibited the contraction of toad heart while its methylester (17a) (17 when $R = R^1 = H$, $R'' = OMe$) and N -methyl methyl ester (17b) (17, $R = H$, $R^1 = Me$ and $R'' = OMe$) in low concentrations increased the amplitude of the contraction. Esters (17a), and (17b), the N, N-dimethyl ester (17c) (17, $R = R^1 = R'' = Me$) and the quaternary methyl iodide (17c.MeI) showed a marked dilating action on rabbit ear blood vessels whereas 4-amino-butyric acid was devoid of this action (Takahashi, 1961).

The cholinergic-adrenergic dual action of some 4-amino-butyric acid methyl esters (17a) has been studied by Hashimoto et al (1963) on a preparation of toad-sinus-atrium. In this study it was found that 4-amino-(17a), 4-methylamino-(17b), and 4-dimethylamino-esters (17c) had positive inotropic effects;whereas methyl 4-trimethylamino-esters (17d) showed negative inotropic effects (Shiraishi et al, 1963). The amino ester (17d) in another study (Kumei, 1961), was shown to have highest tonic activity when tested on the isolated ileum of guinea pigs, cats, rats and rabbits, followed by (17c), (17b), and (17a) in that order. Farquharson et al (1961) determined the effects of 4-amino-butyric acid and some N-substituted amide and ester derivatives of this acid on the potentials arising in response to constant stretch of the slowly adapting stretch-receptor of Astacus fluviatilis to confirm its possible role as an inhibitory transmitter substance or regulator of neuronal activity in the central nervous system. Results indicated that 4-substitution by dimethylamine, diethylamine, acetamide, benzamide, piperidine, or morpholine residues increased the frequency of impulse, as did the two butyramides tested. Simultaneous substitution at both terminal groups of 4-amino-butyric acid had no noticeable effects on the impulse frequency.

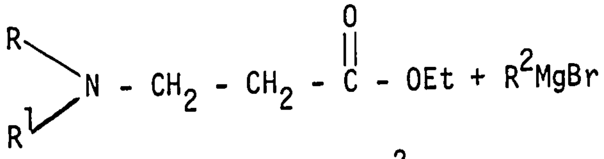
In another study, thirty derivatives or analogues of 4-amino-butyric acid were screened pharmacologically against the effects of Megimide ®, Leptazol ® and strychnine; which act in different ways on the central nervous system. Six of these compounds, when administered systemically, showed slight protection against chemically induced convulsions in mice (Lightowler et al, 1963).

Numerous references are available which describe the value of 3-amino-esters as intermediates, such as in the synthesis of side chains for incorporation into antimalarial compounds (Asano and Tomita, 1948; Clemo et al, 1951); in preparation of photographic color complexes (Kumura et al, 1965; Brit. Patent, 1966); in pantothenic acid studies (Lindsay and Cheldelin, 1950); in 1-substituted thymines (Smith and Binkley, 1959), and in the synthesis of compounds related to methadols (Perrine, 1953).

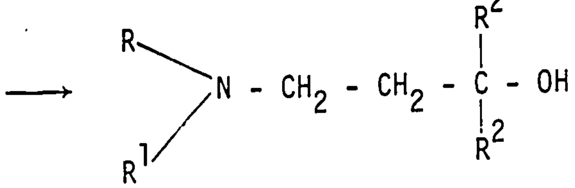
Seventeen new pyrrolidylalkanols have been prepared by the reduction of 2- or 3-pyrrolidylpropionates with lithium aluminum hydride (Moffet, 1949); e.g.,



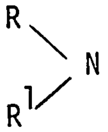
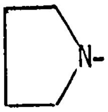
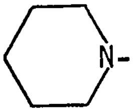
Adamson (1949) prepared amino-carbinols, which are used as antispasmodics, by reacting the appropriate substituted 3-monoalkyl- or -dialkylamino-propionates with phenyl magnesium bromide. The hydrochloride salts of these



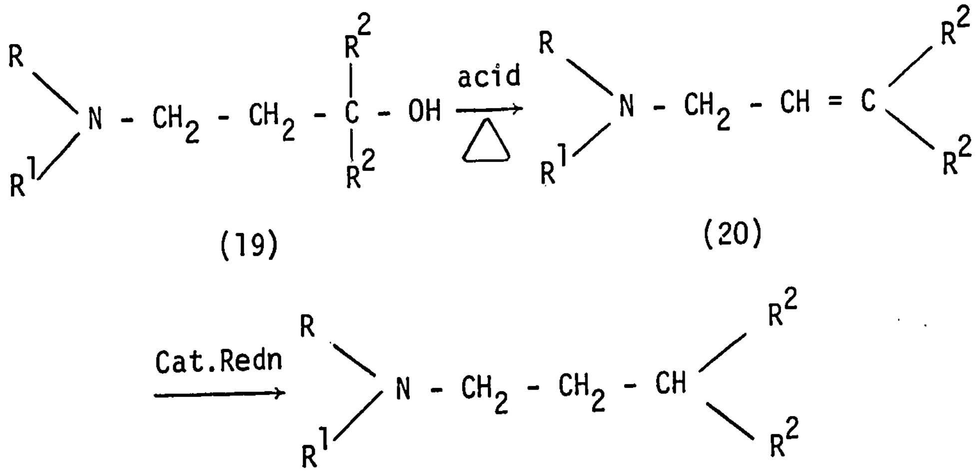
(18)



(19)

where  = Me_2N, Et_2N,  or  and other heterocyclic rings and, R^2 = phenyl or cyclohexyl.

amino-carbinols (19) readily dehydrated in acid solutions to give the corresponding allylamines (20) which then were catalytically reduced to give N-substituted-3-amino-1, 1-diphenylpropanes (21) which were also



(19)

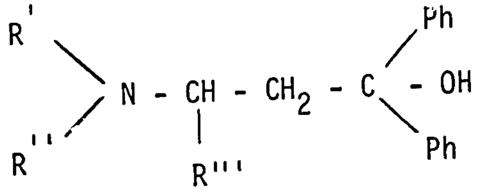
(20)

(21)

R^2 = Ph

tested for antispasmodic activity. The quaternary iodides of the intermediates 3-dialkylamino esters (18), their carbinols (19) and corresponding allylamines (20) have been patented as ganglion blockers (Adamson and Billinghurst, 1958.)

Takagi et al (1952) tested dimethylamino carbinols (22a) and their intermediate esters for antispasmodic activity. Although they showed weak antispasmodic action, some had a strong anticholinergic action. 3-Amino-propionates have also found use as intermediates in

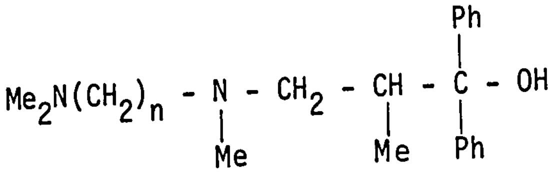


(22)

(22a), when $R' = R'' = R''' = Me$

(22b), when $R''' = H$; $R' = Me$; $R'' = Me_2N(CH_2)_n$

the synthesis of cholinolytic agents (22b) (Kuznetsov _et_ _al_, 1965). Barron _et_ _al_ (1965), prepared compounds related to (22b) and on testing these found that slight alteration in the structure of (22b) to (23) gave compounds which showed anticonvulsant and diuretic activity in test animals, and antidepressant activity (Spickett _et_ _al_, 1966; Ganellin and



(23)

$$Me_2N(CH_2)_n - \underset{Me}{N} - CH_2 - \underset{Me}{CH} - \overset{Ph}{\underset{Ph}{C}} - OH$$

Spickett, 1965; Barron _et_ _al_, 1965).

Compounds with the general formula (24), where R is Ph, R^1 is Ph or cyclohexyl and NB represents lower dialkylamino-, morpholino-,

$$RR^1C(OH)CH_2CH_2NB \qquad (24)$$

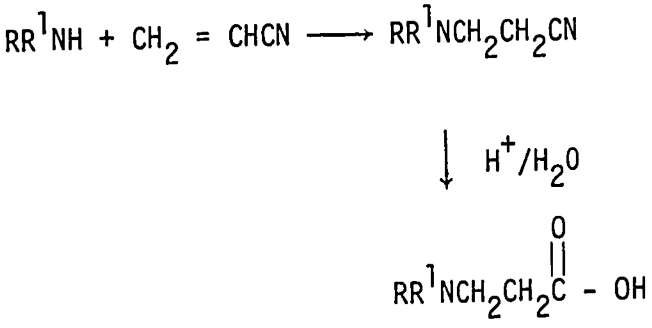
piperidino-, or pyrrolidino- groups have been patented as bronchodilators (Adamson, 1954).

3- and 4-Amino-acids

The importance of 3- and 4-amino-acids and related compounds in the field of medicinal chemistry is being increasingly felt as more and more data on various pharmacological actions are made available. 4-Aminobutyric acid (GABA), and its related compounds have acquired a unique position in medicinal research due to the various interesting pharmacological actions of these compounds, some of which have been discussed earlier. The biochemical importance of 3-amino-acids will be discussed after the general comments on the preparation of these acids.

3- and 4-Amino-acids have been prepared by various well-known methods (Gresham _et al_, 1951; Skita _et al_, 1927; Michaelis and Schubert, 1936; Lukeš _et al_, 1960; Mikhlina and Rubtsov, 1962). Those most commonly employed are:

(i) the hydrolysis of amino-nitriles which can be prepared by the addition of appropriate amines to α, β-unsaturated nitriles; e.g.,



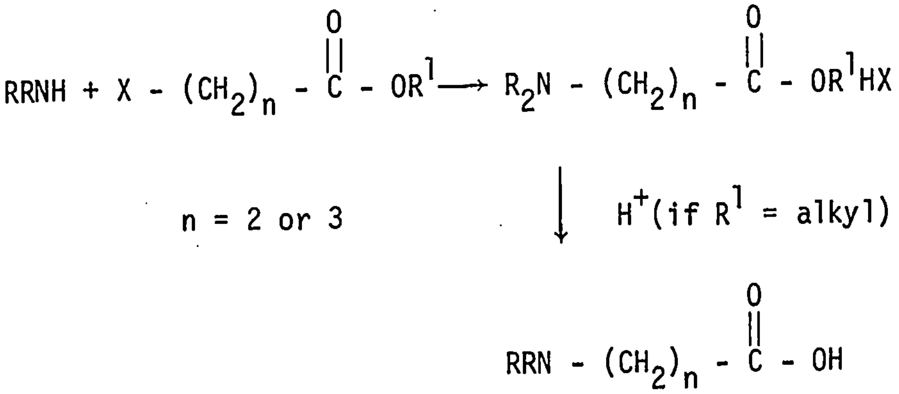
$$RR^1NH + CH_2 = CHCN \longrightarrow RR^1NCH_2CH_2CN$$

$$\downarrow H^+/H_2O$$

$$RR^1NCH_2CH_2\overset{\overset{O}{\|}}{C} - OH$$

(Takagi _et al_, 1952; Lindsay and Cheldelin, 1950).

(ii) by the reaction of an amine with a 3- or 4-halo-acid (Pollack, 1943) or ester, followed in the latter instance by hydrolysis of the 3- or 4-amino-ester:



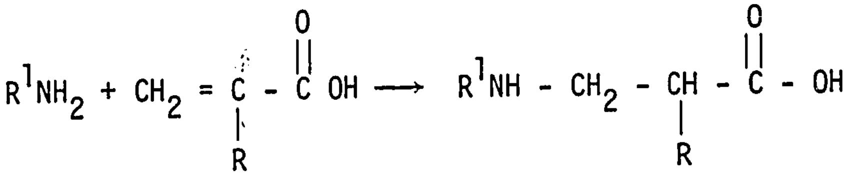
$$\mathrm{RRNH} + \mathrm{X} - (\mathrm{CH_2})_n - \overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}} - \mathrm{OR^1} \longrightarrow \mathrm{R_2N} - (\mathrm{CH_2})_n - \overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}} - \mathrm{OR^1HX}$$

$$n = 2 \text{ or } 3 \qquad \Big\downarrow \; \mathrm{H^+}(\text{if } \mathrm{R^1} = \text{alkyl})$$

$$\mathrm{RRN} - (\mathrm{CH_2})_n - \overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}} - \mathrm{OH}$$

(Cruickshank et al, 1961).

(iii) Addition of a primary or secondary alkylamine to an α, β-unsaturated acid:



$$\mathrm{R^1NH_2} + \mathrm{CH_2} = \underset{\underset{\mathrm{R}}{|}}{\mathrm{C}} - \overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}\,\mathrm{OH} \longrightarrow \mathrm{R^1NH} - \mathrm{CH_2} - \underset{\underset{\mathrm{R}}{|}}{\mathrm{CH}} - \overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}} - \mathrm{OH} \qquad (25)$$

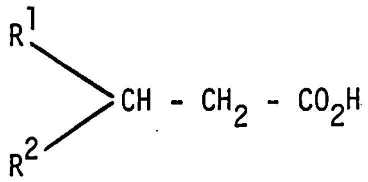
The reaction of amines with methacrylic acid [(25), where R is methyl] in various solvents gave various products, depending on the amine and the reaction solvent. The reaction of benzylamine, isobutylamine and n-hexylamine with methacrylic acid in pyridine gave N-alkyl- 2-methyl-3-amino-propionic acids (Zilka et al, 1961).

The importance of 2-methyl-3-amino-propionic acids can be seen from the research (Fruton and Simmonds, 1958) in the field of metabolic breakdown of pyrimidines, in which 2-methyl-3-amino-propionic acid is implicated. In 1951 L-2-methyl-3-amino-propionic acid was isolated for the first time (Crumpler et al, 1951) from human urine. It was found to accumulate in the urine of certain people and its presence was thought to be a matter of genetic peculiarity. Subsequent research (Fruton and Simmonds, 1958) has shown that introducing thymine to rats caused the appearance of 2-methyl-3-amino-propionic acid in their urine, while uracil caused the appearance of 3-amino-propionic acid (β-alanine). From this it may be supposed that the formation of these 3-amino-acids in the body is connected with metabolic degradation of nucleic acids.

Various 3- and 4-substituted amino-acids have also been used as intermediates in the synthesis of numerous substituted quinolizidines, indolizidine and pyrrolizidine derivatives (Murakoshi, 1958).

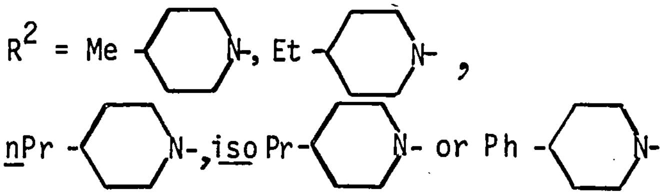
The N-nitroso-N-alkyl-3-amino-acids which have been reported to act as plant growth regulators employ N-alkyl-3-amino-acids as intermediates in their synthesis (Gerjovich and Harrison, 1966).

Both 3-piperidino-propionic and -butyric acids (26) have recently been screened for analgesic, antipyretic and antiphlogistic activity (Muehle <u>et</u> <u>al</u>, 1968).



(26)

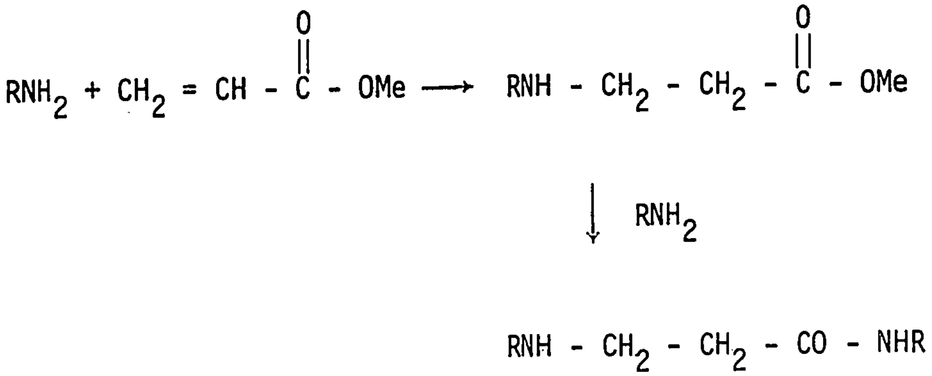
R^1 = H or Me



3- and 4-Amino-amides

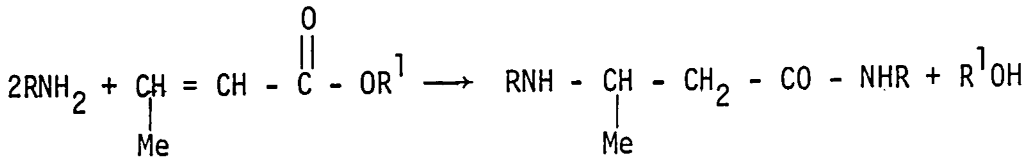
The preceding discussion on 3- and 4-amino-esters and amino-hydroxamic acids was detailed due to the fact that the emphasis of the research in the present study, was placed on the synthesis and pharmacological evaluation of these compounds. However, the related 3- and 4-amino-amides and 3- and 4-amino-ketones also possess interesting pharmacological properties. Selected references to the synthesis and pharmacology of these amides are now discussed. It should be emphasized that the literature survey of the 3- and 4-amino-amides and corresponding amino-ketones is not intended to be an exhaustive one.

(i) Primary and secondary N-alkyl-3-aminopropionamides and -3-amino-butyramides are synthesised by the reaction between two moles of the appropriate amine and one mole of an α, β-unsaturated ester:



$$\begin{array}{c} RNH_2 + CH_2 = CH - \overset{\overset{\displaystyle O}{\|}}{C} - OMe \longrightarrow RNH - CH_2 - CH_2 - \overset{\overset{\displaystyle O}{\|}}{C} - OMe \\ \downarrow RNH_2 \\ RNH - CH_2 - CH_2 - CO - NHR \end{array}$$

R = Me, Et, nPr etc.

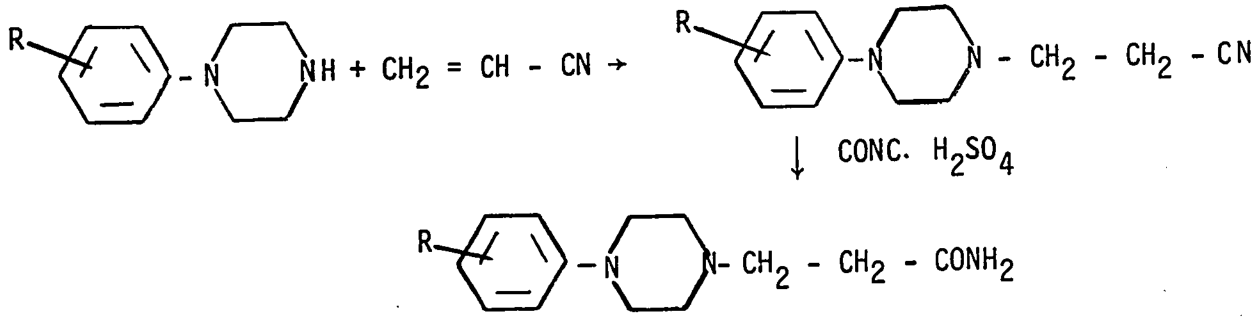


$$2RNH_2 + \underset{\underset{\displaystyle Me}{|}}{CH} = CH - \overset{\overset{\displaystyle O}{\|}}{C} - OR^1 \longrightarrow RNH - \underset{\underset{\displaystyle Me}{|}}{CH} - CH_2 - CO - NHR + R^1OH$$

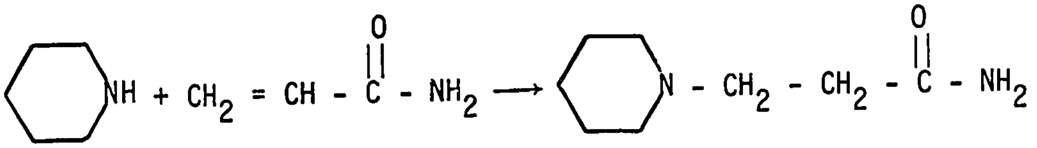
R = Me or Et

An increase in the temperature or a prolongation of the reaction time favours the formation of the amide (Morsch, 1932; David et al, 1965).

(ii) Alternately, substituted 3-amino-amides are the products of the addition of amine to acrylonitrile (only acrylonitrile and not methacrylonitrile or butacrylonitrile), followed by the hydrolysis of 3-amino-nitrile in strong acidic conditions; e.g.,(Pollard et al, 1953).

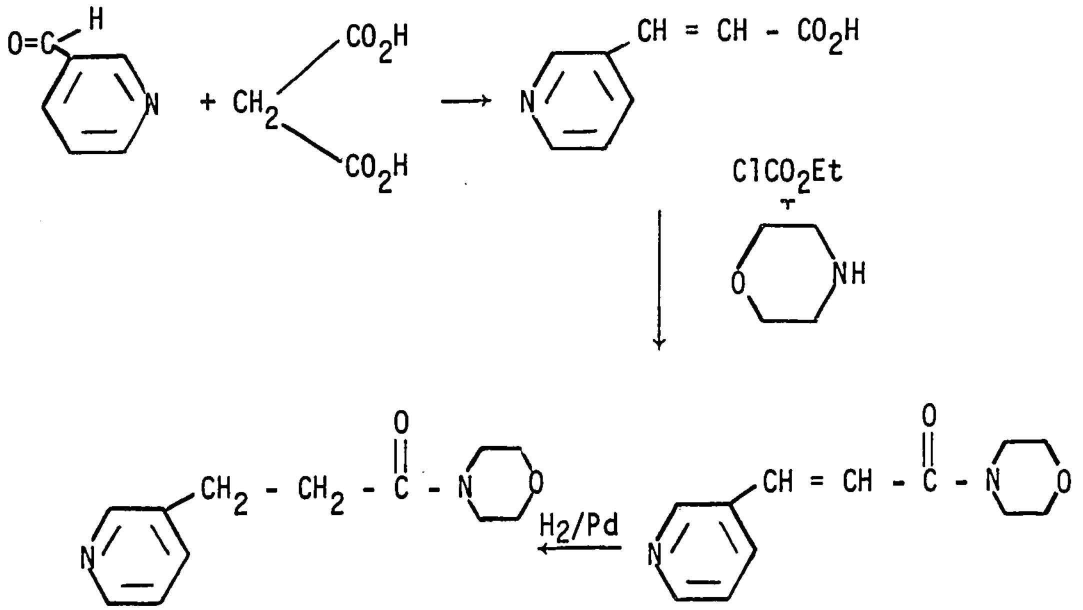


(iii) N-substituted-3-amino-amides can also be prepared by the addition of an amine across the double bond in acrylamide or its homologs; e.g.,



The influence of some solvents and catalytic mixtures on the rate of addition of amino compounds such as piperidine (A), hexamethylenimine (B), benzylamine (C), n-butylamine (D), hydrazine hydrate (E), 3,5,5,-trimethyl pyrazoline (F), and 3,5-dimethylpyrazole (G) to acrylamide has been the subject of a recent study, (Suminov, 1967), which concluded that amines A, D and E were most active while F and G reacted only very slowly at elevated temperatures and in the presence of water. Addition of amines to acrylamide took place most easily in water. In alcohol the reaction rate was slower and in aprotic solvents the reaction rate further decreased. The presence of proton donors such as ammonium chloride and boric acid in the reaction mixture increased the rate of the addition of (F) to acrylamide. The addition of sodium fluoride, potassium fluoride, sodium acetate and potassium acetate did not affect the reaction rate.

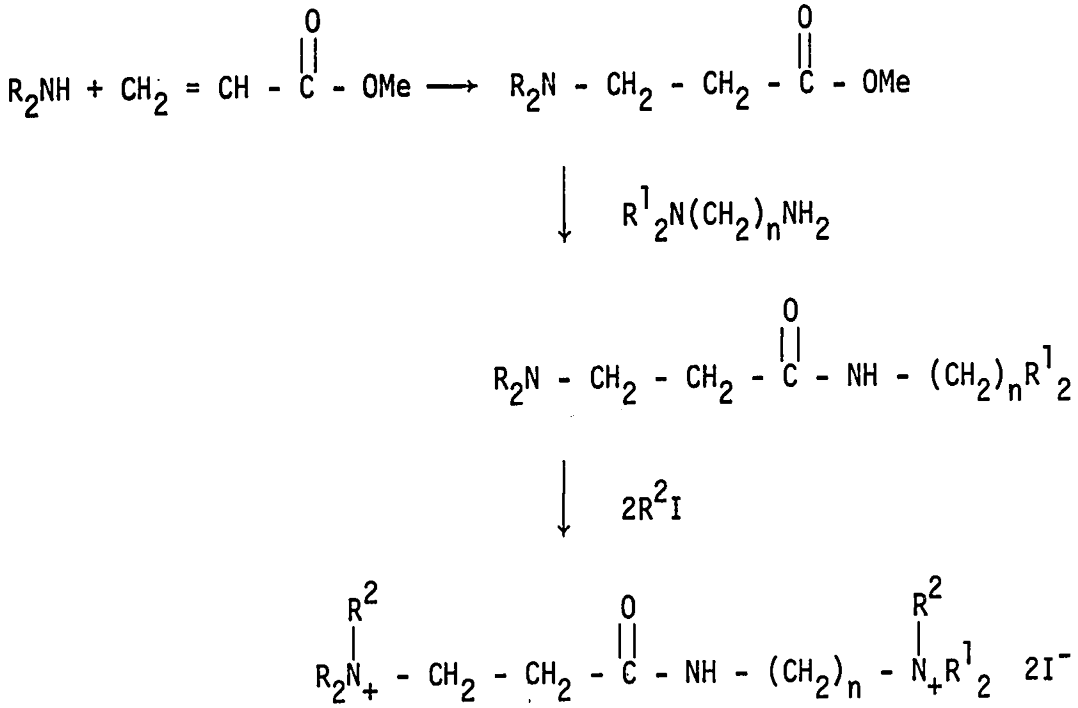
Series of pyridino-carboxamides and piperidino-carboxamides have been prepared and tested for hypotensive effects in anaesthetized dogs. One interesting amide preparation, in which the carboxamido-group is separated from the piperidine nucleus by an ethylene chain, follows the sequence:



(28) (27)

The unsaturated compound (27) showed slight hypotensive effect but its reduction product (28) was devoid of this effect (Sam _et_ _al_, 1959).

Di-quaternary ammonium salts have been prepared and evaluated as ganglion blockers in animals. A typical route of synthesis is as follows:



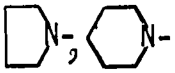
$$R_2NH + CH_2 = CH - \overset{O}{\overset{||}{C}} - OMe \longrightarrow R_2N - CH_2 - CH_2 - \overset{O}{\overset{||}{C}} - OMe$$

$$\downarrow R^1{}_2N(CH_2)_nNH_2$$

$$R_2N - CH_2 - CH_2 - \overset{O}{\overset{||}{C}} - NH - (CH_2)_nR^1{}_2$$

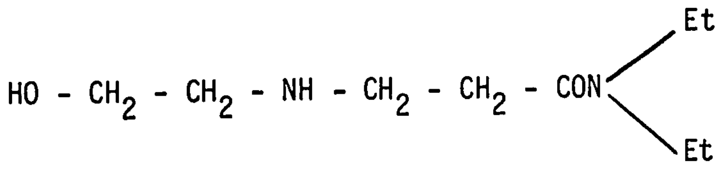
$$\downarrow 2R^2I$$

$$R_2\overset{R^2}{\overset{|}{N_+}} - CH_2 - CH_2 - \overset{O}{\overset{||}{C}} - NH - (CH_2)_n - \overset{R^2}{\overset{|}{N_+}}R^1{}_2 \quad 2I^-$$

where R_2N-= Et_2N-, _n_Pr_2N-, _n_Bu_2N-, 

(Surrey _et_ _al_, 1963).

Propionamides have also been screened for oxytocic activity by Rosen _et_ _al_ (1956), but of the ten substituted propionamides tested only N, N-diethyl-3-(2'-hydroxyethylamino)- propionamide (29) had oxytocic activity.

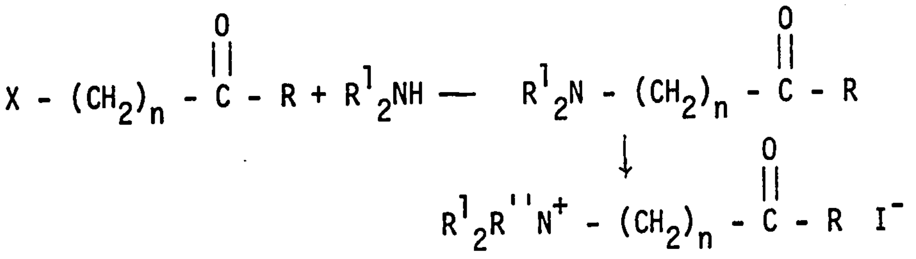


(29)

3- and 4-Amino-ketones

Numerous references are available in the literature which describe the synthesis and pharmacological uses of 3- and 4-amino-ketones. Selected interesting references only are discussed here because no ketones were prepared and tested in the present study.

3- and 4-Amino-ketones have been prepared from 3- and 4-halo-ketones and evaluated for their muscarinic and nicotinic activity



(30)

$$\mathrm{X-(CH_2)_n-\overset{O}{\overset{\|}{C}}-R + R^1{}_2NH \longrightarrow R^1{}_2N-(CH_2)_n-\overset{O}{\overset{\|}{C}}-R}$$

$$\downarrow$$

$$\mathrm{R^1{}_2R''N^+-(CH_2)_n-\overset{O}{\overset{\|}{C}}-R\ \ I^-}$$

R^1 = Me

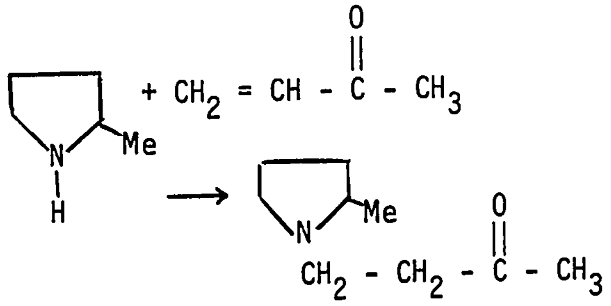
R'' = Me

R = Me or Et

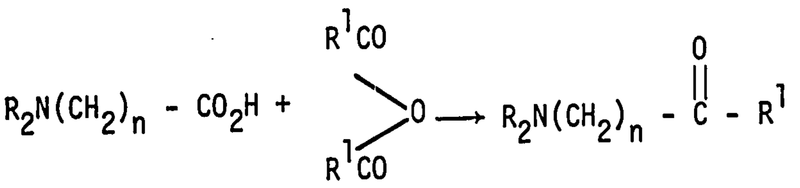
n = 2 or 3

on guinea pig ileum (Ing et al, 1952), rabbit ileum (Edward et al, 1951), and rabbit jejunum (Lands and Cavillito, 1954). In general, these ketones are weaker in cholinergic activity than their corresponding hydrocarbon analogs. They are predominantly nicotinic in action (Friedman, 1967), but also possess some muscarinic activity. Keto-amylamines (30, n = 3) are more active than keto-butylamines (30, n = 2).

3- Amino-ketones have also been prepared by the reaction between an α, β-unsaturated ketone and an amine by Tsunetsugu et al (1962) in their study on the synthesis of derivatives of 2- methyl-pyrroline and 2-methyl-pyrrolidine; i.e.,

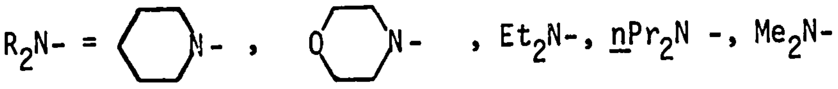


Cruickshank et al (1964) have patented the synthesis of dialkylaminoketones by the reaction between dialkylaminoacids and carboxylic acid anhydrides. Only ketones are obtained when both alkyl groups in the dialkylamino acids are straight-chained. If only one R group in (31) is unbranched then both ketones (32) and lactams (33) are formed and if neither



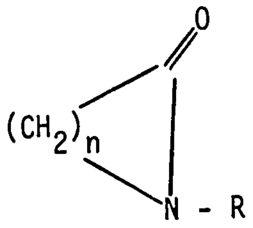
$$R_2N(CH_2)_n - CO_2H + (R^1CO)_2O \longrightarrow R_2N(CH_2)_n - \overset{O}{\overset{\|}{C}} - R^1$$

(31) (32)



R^1 = Me or Et , n = 3 or 4.

R group in (31) is of the normal series then lactams (33) only are formed. No pharmacology has been reported in this study.



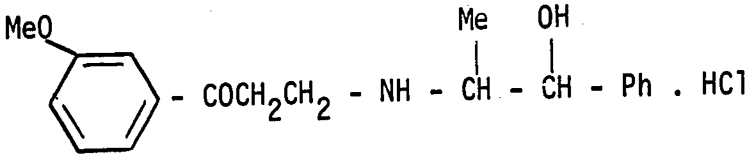
R = Me, n = 3

R = isoPr , n = 3

R = CH_2Ph, n = 3

(33)

A new cardioactive 3-amino-ketone (34) of the phenethylamine series has been described recently (Thiele et al, 1968). This compound differs in stability and rigidity of linkage from other 3-amino-ketones examined. Correlations between structure and pharmacological activity have been discussed. Maximum activity was observed only if the molecule



(34)

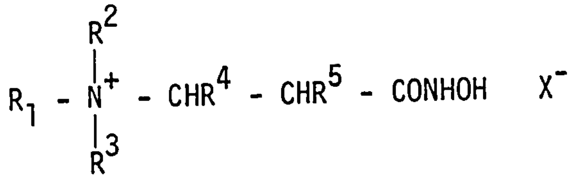
contained a hydroxyl group in the α-position of the phenethylamine portion and a carbonyl group conjugated with a phenyl nucleus, which had to be meta substituted with a methoxy-, hydroxy- or amino-group. For chemical and physiological reasons, compound (34) cannot be placed among the existing categories of the positive inotropic compounds, nor among the classic sympathomimetics, nor the cardioactive steroids. This compound should be regarded as a representative of a new class of cardioactive pharmaceuticals with a positive inotropic action.

CHEMISTRY

DISCUSSION

Preparation of 3- and 4-amino-esters

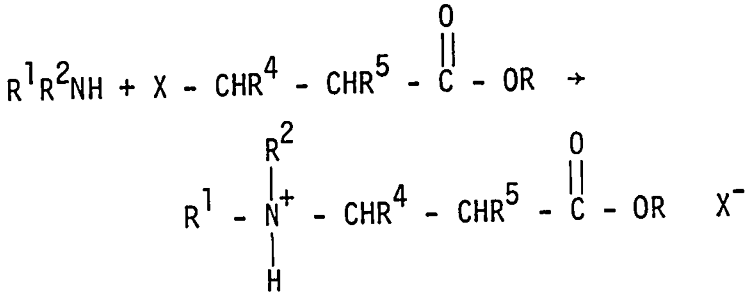
Acyclic hydroxamic acids are readily prepared by the action of hydroxylamine on esters. In order to prepare compounds with general formula (5), therefore, various 3- and 4-amino-esters were required.



$$R_1 - \overset{R^2}{\underset{R^3}{N^+}} - CHR^4 - CHR^5 - CONHOH \quad X^- \qquad (5)$$

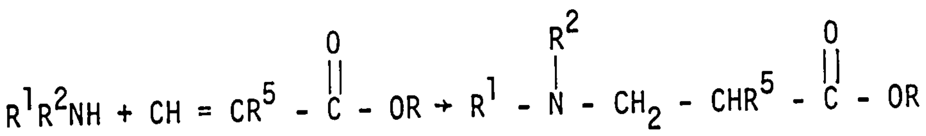
Two literature methods have been frequently employed for the preparation of such esters. They are:

(i) through the interaction of an amine with a 3- or 4-halo-ester, (Drake and McElvain, 1934; Cruickshank *et al*, 1961); e.g.,



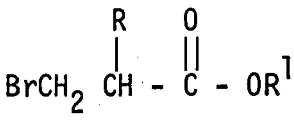
$$R^1R^2NH + X - CHR^4 - CHR^5 - \overset{O}{\overset{\|}{C}} - OR \rightarrow R^1 - \overset{R^2}{\underset{H}{N^+}} - CHR^4 - CHR^5 - \overset{O}{\overset{\|}{C}} - OR \quad X^-$$

and (ii) by the addition of amines to α, β-unsaturated esters (Adamson, 1950), i.e.



$$R^1R^2NH + CH = CR^5 - \overset{O}{\overset{\|}{C}} - OR \rightarrow R^1 - \overset{R^2}{N} - CH_2 - CHR^5 - \overset{O}{\overset{\|}{C}} - OR$$

To use the method (i) for the preparation of 3-amino-esters, 3-halo-esters such as methyl 3-bromopropionate (35a) and methyl 2-methyl-3-bromopropionate (35b) were needed. For the preparation (35b), three



$$BrCH_2\,\overset{\displaystyle R}{\overset{|}{C}H} - \overset{\displaystyle O}{\overset{||}{C}} - OR^1 \qquad (35)$$

$R = H;\ R^1 = Me$ (35a)

$R = Me;\ R^1 = Me$ (35b)

$R = Me;\ R^1 = Et$ (35c)

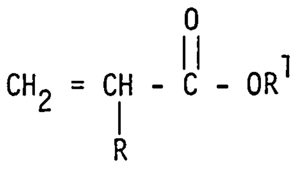
literature methods were employed.

(a) Dry hydrogen bromide was passed through a solution of methyl acrylate in diethylether, (Mozingo and Patterson, 1940).

(b) Dry hydrogen bromide was passed through a solution of methyl acrylate or methyl methacrylate in glacial acetic acid containing a trace of iodine, (Clemo and Melrose, 1942).

(c) Hydrobromic acid (48%) and methyl methyacrylate were reacted in carbon tetrachloride which contained trace quantities of benzoyl perioxide and hydroquinone, (Price and Coyner, 1940).

The hydrobromination of methyl acrylate (36a) in ether is said



$$CH_2 = \underset{\displaystyle R}{\underset{|}{C}H} - \overset{\displaystyle O}{\overset{||}{C}} - OR^1 \qquad (36)$$

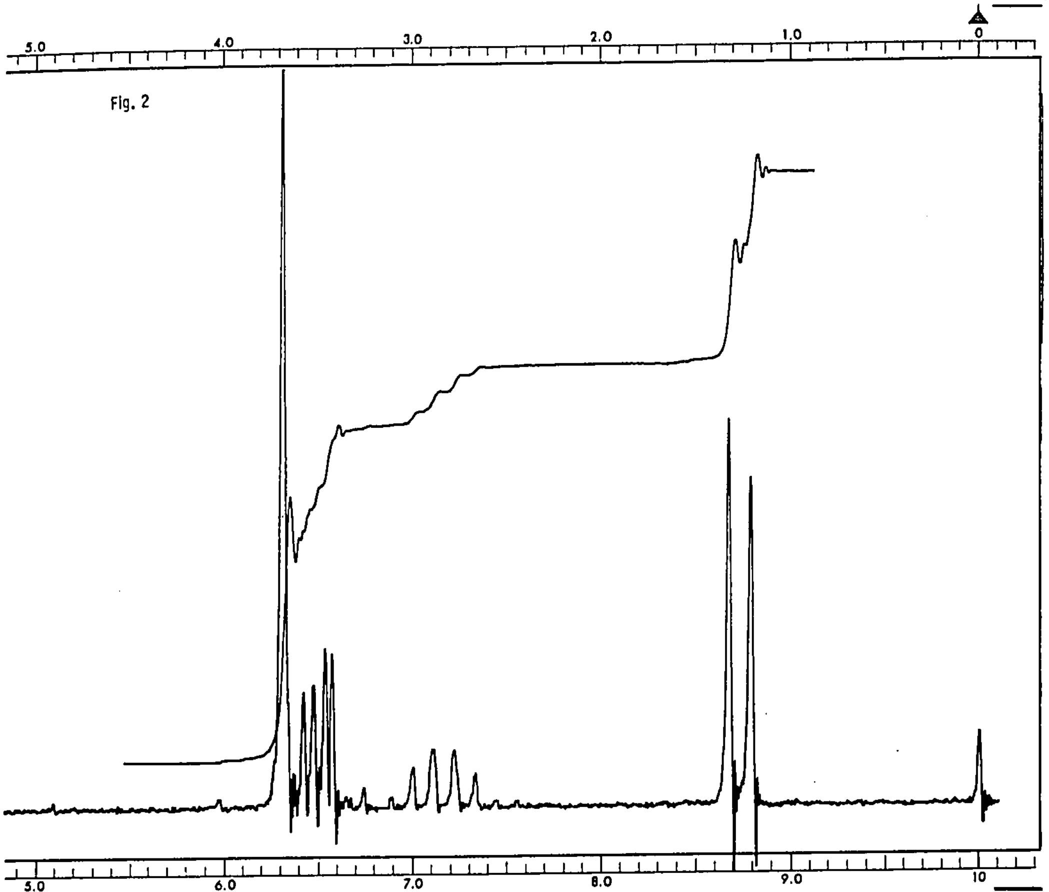
$R = H;\ R^1 = Me$ (36a)

$R = Me;\ R^1 = Me$ (36b)

$R = Me;\ R^1 = Et$ (36c)

to give methyl 3-bromopropionate (35a) exclusively, and in good yield (Mozingo and Patterson, 1940). When, however, the same conditions were employed in the present study to hydrobrominate methyl methacrylate (36b), a single product was not obtained. A gas-chromatogram of the reaction mixture showed that there were two major components present in approximately equal quantities. Fraction 1, which formed 46.4% of the mixture, had a retention time of 1.20 minutes and fraction 2, was present in 53.6% yield and had a retention time of 1.65 minutes 'on SE30 (10%)'. The mixture was separated by fractional distillation using a spinning band column. The more volatile fraction 1, had b.p. 69-70°/18 mm and less volatile fraction 2, had b.p. 78.5-79°/18 mm. A proton magnetic resonance (PMR) study (Fig. 1 and 2) assisted in the identification of the two components as methyl 2-methyl-3-bromopropionate (35b) and the corresponding ethyl ester (35c). The 3-proton singlet at 222 Hz in Fig.1 and 2 is due to the protons of the $-OCH_3$ group; the 3-proton triplet centered at 77Hz with J, 7Hz (overlapped by a 2-proton doublet of the 2-methyl group) and the 2-proton quartet centered at 247 Hz in Fig. 1 are ascribed to the methyl and methylene protons, respectively, of the $-OCH_2CH_3$ group.

Fig. 2 PMR spectrum of methyl 2-methyl-3-bromo-propionate in CCl_4



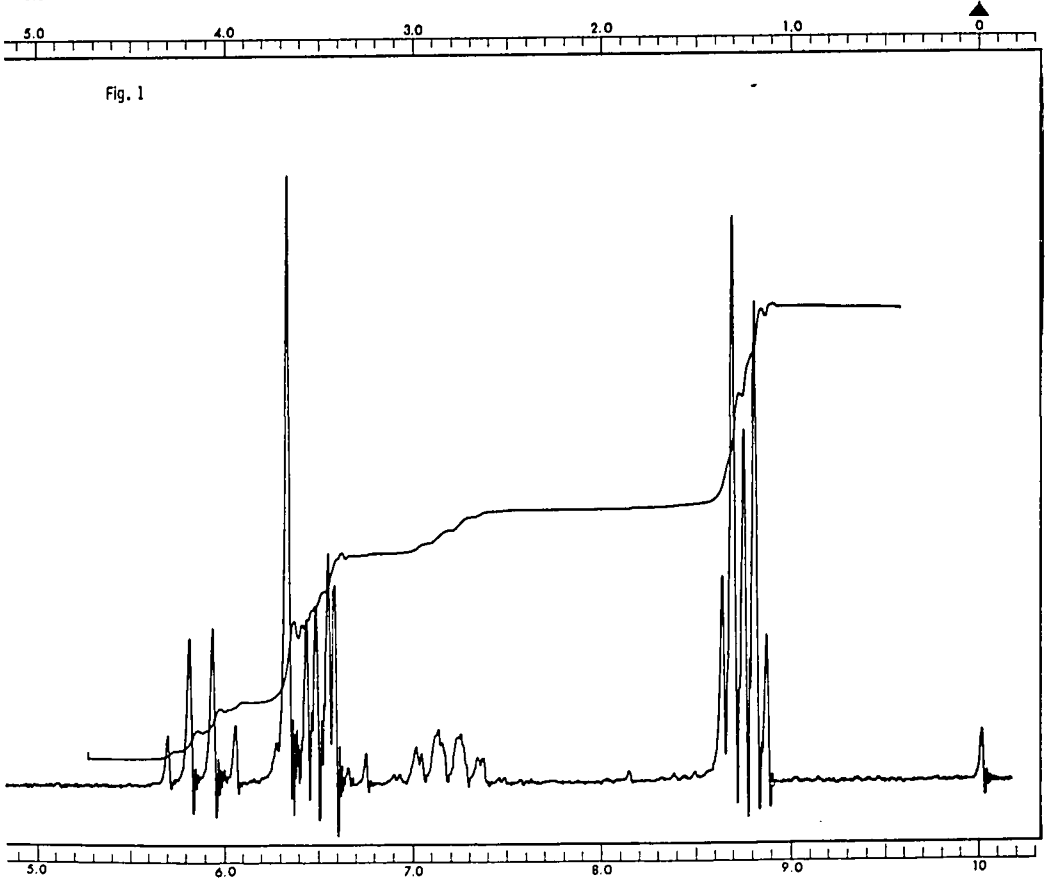


Fig. 1 PMR spectrum of the reaction mixture of methyl methacrylate after treatment with hydrogen bromide in diethyl ether in CCl_4

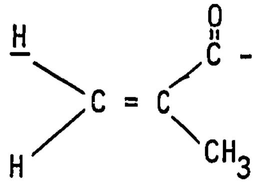
Elemental analysis, and comparisons of the PMR and IR spectra of both products with authentic samples of (35b) and ethyl 2-methyl-3-bromopropionate (35c) confirmed that hydrobromination of methyl methacrylate (36b) in diethyl ether gives both the methyl and ethyl esters of 2-methyl-3-bromopropionic acid.

The original reaction of methyl methacrylate with hydrogen bromide was then performed for a longer time. The yield of the ethyl ester (35c) improved at the expense of the methyl ester (35b), although complete transesterification was not observed. When the hydrobromination reaction was repeated using ethyl methacrylate (36c), a very good yield (75.3%) of (35c) was obtained and it was identical (b.p., IR, GLC, and PMR) with the ethyl ester (35c) isolated from the methyl methacrylate reaction.

These results suggested that the claim by Mozingo and Patterson (1940) that methyl 3-bromopropionate was obtained in 84% yield from methyl acrylate (36a), by reacting the latter (36a) with hydrogen bromide in ether was erroneous. This reaction therefore, was performed using the conditions reported, and, as expected, a mixture of the ethyl and methyl esters of 3-bromo-propionic acid was obtained. The ethyl ester in fact was the major product.

Higher esters can also be converted with ease to ethyl esters by this method and for this reason, this transesterification reaction has been applied to the identification of the components of mixtures of fatty esters. This application is discussed later (see appendix).

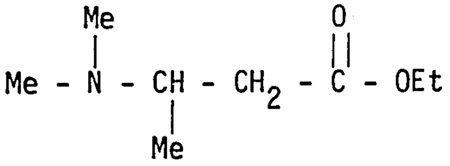
By repeatedly employing the conditions used by Clemo and Melrose (1942) i.e., by passing dry hydrogen bromide through a solution of methyl methacrylate (36b) in glacial acetic acid containing a trace of iodine, a mixture was obtained. The PMR spectrum in carbon tetrachloride showed it to consist of the required methyl 2-methyl-3-bromopropionate (35b) [a doublet at 80Hz, J, 6.5 Hz (i.e. $\mathrm{Br - CH_2 - \underset{\underset{C\underline{H}_3}{|}}{CH}-}$); a multiplet centered at 175 Hz, J, 6.8 Hz for $-\overset{|}{\mathrm{C\underline{H}}}-$] together with an appreciable quantity of the starting material methyl methacrylate (36b), [singlet at 117 Hz due to the methyl group attached to the double bond i.e. $\mathrm{CH_2 = \overset{\overset{C\underline{H}_3}{|}}{C} -}$; a quatret centered at 341 Hz for the illustrated methylene proton



; and a broad singlet at 374 Hz for the other proton of the methylene group attached to the double bond].

On using the conditions employed by Price and Coyner (1940), the desired product methyl 2-methyl-3-bromopropionate (35b) was obtained in 34% yield as compared to the 87% yield claimed by the authors.

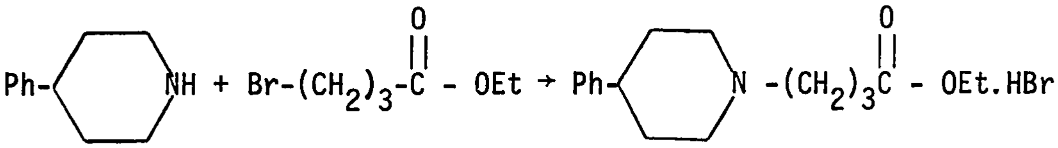
Some bromo-acids are available commercially. Esters were readily obtained from them using the Fischer and Speier (1895) esterification procedure. In this way, ethyl 3-bromobutyrate was prepared. This was reacted with dimethylamine and ethyl 3-dimethylamino-butyrate (37) was isolated in poor yield. Similarly, ethyl 4-(4-phenylpiperidino) butyrate (38) was obtained as its hydrobromide salt through the reaction



$$\mathrm{Me_2N{-}CH(Me){-}CH_2{-}C({=}O){-}OEt}$$

(37)

between 4-phenylpiperidine and a commercially available ester ethyl



(38)

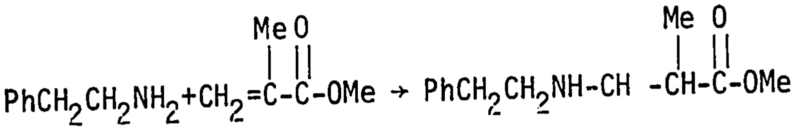
4-bromobutyrate.

The alternative method of preparing 3-amino-esters is by the addition of amines across the double bond of α, β-unsaturated ester (Adamson, 1950). This synthetic method and the bromination study just described were investigated simultaneously and when it was found that Adamson's method gave good yields of amino-esters in a one-step synthesis, the bromination studies were discontinued. Most of the esters prepared in this study therefore, were the products obtained by the addition of an amine to an α, β-unsaturated ester.

Ease of addition of amines to methyl methacrylate (36b) has been reported (Bieber, 1954) to be in the order piperidine > diethylamine > aniline. The same order of reaction was noted in this study. In fact, aniline failed to give any addition product with methyl methacrylate.

In general, the reaction between amines and methyl methacrylate (36b) takes place easily and the yields are good. All the esters prepared in the present study were isolated in yields varying from 40 to 90% of the theoretical quantity and were obtained without much difficulty.

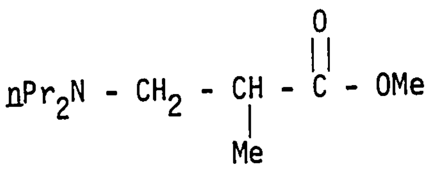
One exception was the addition of phenethylamine to methyl methacrylate (36b) to give methyl 2-methyl-3-phenethylamino- propionate (39). This reaction was slow and required a 12 months reaction period to give a



$$\mathrm{PhCH_2CH_2NH_2{+}CH_2{=}\overset{Me}{\overset{|}{C}}{-}\overset{O}{\overset{||}{C}}{-}OMe \rightarrow PhCH_2CH_2NH{-}CH\ {-}\overset{Me}{\overset{|}{C}}H{-}\overset{O}{\overset{||}{C}}{-}OMe} \qquad (39)$$

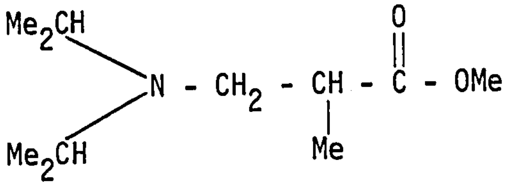
62% yield of the desired product.

It has been reported that simple amines containing 2 or 3 carbon atoms react with lower esters of α, β-unsaturated acids such as acrylic, methacrylic, and crotonic acids in the absence of a catalyst but higher amines and amines containing branched alkyl groups, such as *tert*-butyl and *tert*-octyl did not react even in the presence of basic or acidic catalysts (Hughes, 1960). In the present study, dialkylamines such as dimethylamine and diethylamine reacted readily with methyl methacrylate (36b) in the absence of catalysts to give good yields (i.e. 75% and 68% respectively of the corresponding esters). Di-*n*-propylamine and methyl methacrylate required heating under reflux for a longer period of time (5 days) to give 60% yield of *methyl 2-methyl-3-di-n-propylpropionate* (40). Attempts to react di-*iso*propylamine with



$$\underline{n}\mathrm{Pr_2N - CH_2 - \underset{Me}{\underset{|}{CH}} - \overset{O}{\overset{||}{C}} - OMe} \qquad (40)$$

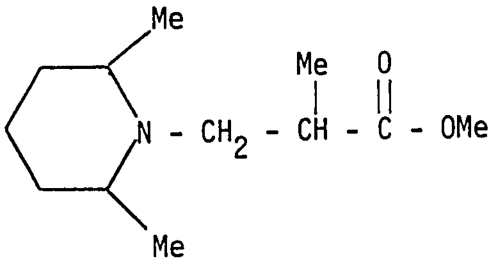
methylmethacrylate (36b) in methanol by boiling under reflux for 7 days gave only the starting materials. Even heating vigorously under reflux for 10 days in n-butanol was unsuccessful in giving the desired product. This result is in agreement with the results of Hughes (1960) but contrasts the findings of Suminov (1967), who reported that he obtained the desired ester i.e. methyl 2-methyl-3-di-isopropylamino-propionate (41), in 35.3% yield by boiling a mixture of di-isopropylamine and



(41)

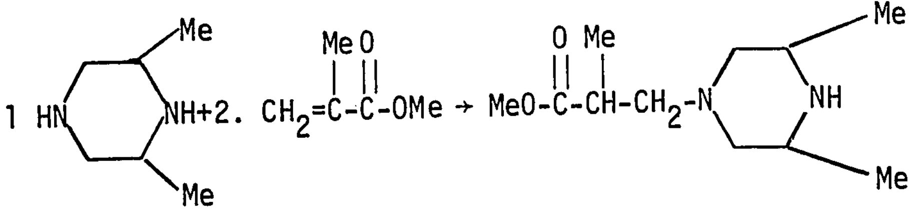
methyl methacrylate (36b) in methanol for 24 hours. This appears rather unlikely due to steric hinderance caused by the bulky isopropyl groups in the amine.

Suminov (1967) has also reported a successful preparation of methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (42) in 70% yield when methyl methacrylate (36b) was heated under reflux with



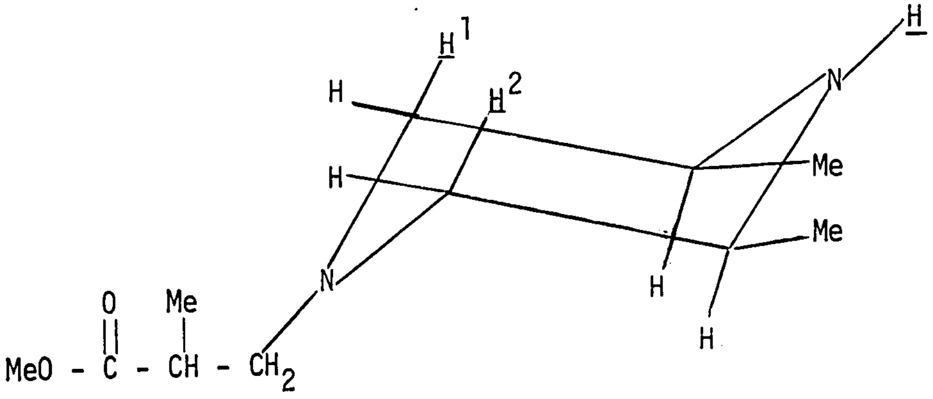
(42)

2,6-dimethyl piperidine in methanol for 24 hours. Attempts to repeat this reaction under the same conditions failed to give any amount of the desired product (42). Heating vigorously the same reactants in methanol did not give any of the desired product, methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (42) either. The only product isolated when 2-moles of methyl methacrylate (36b) and 1 mole of 2,6-dimethylpiperazine were heated under reflux in methanol, was *methyl 2-methyl-3-(2,6-dimethyl-4-piperazinyl)propionate* (43) together with starting material (methyl methacrylate). The structure of compound (43)



(43)

was confirmed by elemental analysis, IR and PMR spectra. The PMR spectrum in carbon tetrachloride showed a 3-proton triplet centered at 90 Hz, J, 9.8 Hz which on deuterium exchange changed to a 2-proton triplet, J, 9.8 Hz. This indicated that 3-proton triplet was attributable to the protons indicated in structure (44). The shape of the 2-proton triplet after deuterium exchange indicated that the centerline of the triplet in the spectrum of the non-deuterated compound had intensified due to N - H having the same chemical shift as that of H^1 and H^2. The formation of monoester (43) rather than a diester would suggest that the reported

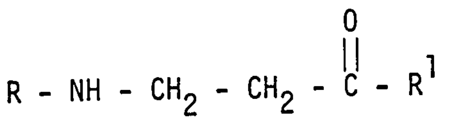


(44)

reaction of methyl methacrylate (36b) with 2,6-dimethylpiperidine by Suminov (1967) to give methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (42) was rather unlikely. Steric hinderance would also be expected to hamper the reaction.

Formation of amino-amides as side products in the preparation of amino-esters

The reaction between a primary amine and α, β-unsaturated ester is known to give 3-amino-esters, but an increase in temperature and more especially the prolongation of the reaction time favours the formation of N-alkyl-3-amino-propionamides (Morsch, 1932; David *et al*, 1965). During the synthesis of N-alkyl-3-amino-propionates, (45) where R = Me, Et, *n*Pr, *n*Bu, or *n*-Hexyl,



$$R - NH - CH_2 - CH_2 - \overset{\overset{O}{\|}}{C} - R^1 \qquad (45)$$

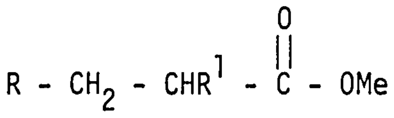
and R^1 = OMe, similar observationswere made. One attempt to prepare methyl 3-ethylamino-propionate (45, when R = Et, and R^1 = OMe), gave only 3-ethylamino-propionic ethylamide (45, when R = Et, and R^1 = - NHEt) when the reactants ethylamine and methyl acrylate (36a), were kept in methanol at room temperature for 10 days. A shorter reaction time (2 days at room temperature) yields the ester methyl 3-ethylamino-propionate. The PMR spectrum of 3-ethylamino-propionic ethylamide showed 1-proton singlet at 88 Hz for - $N\underline{H}$ - and 1-proton broad singlet at 498 Hz for the amide proton, $- \overset{\overset{O}{\|}}{C} - N\underline{H} -$. Both of these protons were exchanged in D_2O.

Secondary amides were also observed as being side products in the reaction between methylacrylate (36a) and primary amines such as methylamine, *n*-propylamine , *n*-butylamine and *n*-hexylamine especially when the reaction period was prolonged. Their presence was detected in the less volatile portion of the reaction mixture by means of their IR spectra, which showed in each case a strong carbonyl stretching absorption, characteristic of amides, in the region 1645-1690 cm^{-1}, but these amides were not isolated.

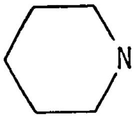
Formation of 3-amino-amides was not observed in the reaction between methyl methacrylate (36b) and secondary amines such as dimethylamine, diethylamine, and di-*n*-propyl-amines. Reaction between methyl methacrylate and cyclic amines such as piperidine, substituted piperidine, homopiperidine, 4-methylpiperazine, pyrrolidine, morpholine and 2,6-dimethylmorpholine also failed to produce amide by-products.

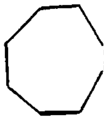
Hydrolysis of 3-amino-esters

When some of the esters prepared in this study i.e. methyl 2-methyl-3-piperidinopropionate (46a), *methyl 2-methyl-3-homopiperidinopropionate* (46b), methyl 3-ethyamino-propionate (46c), and *methyl 2-methyl-3-(3-methylpiperidino)propionate* (46d) were stored for a long period of time (6 to 8 weeks) at room temperature, white crystalline

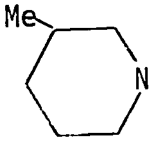


$$R - CH_2 - CHR^1 - \overset{\overset{\displaystyle O}{||}}{C} - OMe \qquad (46)$$

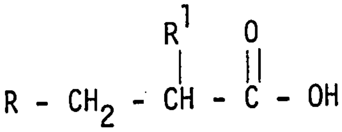
when R = N-; R^1 = Me (46a)

when R = N-; R^1 = Me (46b)

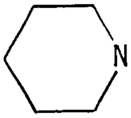
when R = EtNH-, R^1 = H (46c)

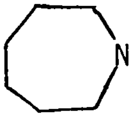
when R = N-, R^1 = Me (46d)

solids slowly deposited. There products were characterised as the corresponding 3-amino-acids (47a), (47b), (47c) and (47d) (Table I) respectively, by m.p., elemental analysis, IR and PMR spectra. The IR spectra of the

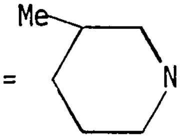


$$R - CH_2 - \overset{\overset{\displaystyle R^1}{|}}{CH} - \overset{\overset{\displaystyle O}{||}}{C} - OH \qquad (47)$$

when R = N-; R^1 = Me (47a)

when R = N-; R^1 = Me (47b)

when R = EtNH-; R^1 = H (47c)

when R = N-; R^1 = Me (47d)

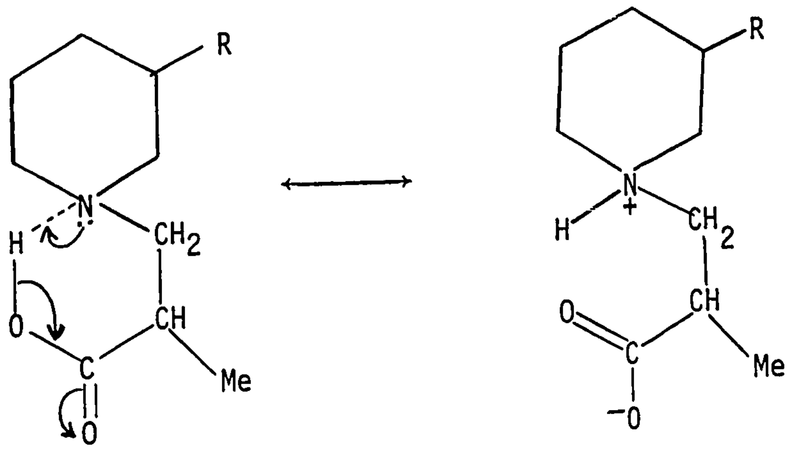
tertiary amino-acids (47a), (47b), and (47d) showed broad bands between 2120 and 2400 cm^{-1} for $\overset{+}{N}H$, and 3-ethylamino-propionic acid (47c) showed broad

TABLE I

Physical characteristics of N-substituted-3-amino-acids

Ester precursors	Crystallization solvent for (47)	M.P.	Identity
(46a)	benzene/hexane	109-110°	2-methyl-3-piperidino propionic acid (47a)
(46b)	benzene/hexane	100°	2-methyl-3-homopiperidino-propionic acid (47b)
(46c)	ethanol/acetone	188°	3-ethylamino-propionic acid (47c)
(46d)	ethanol/acetone	122-124°	2-methyl-3-(3-methyl-piperidino)propionic acid (47d)

bands between 2740 and 2660 cm^{-1} for $\overset{+}{N}H_2$; all these acids (47a), (47b), (47c), and (47d) showed bands near 1620 and 1450 cm^{-1} for COO^-. The PMR spectra (in $CDCl_3$) of 2-methyl-3-piperidinopropionic acid (47a) and 2-methyl-3-(3-methylpiperidino)propionic acid (47d) showed a deuterium-exchangable singlet between 843 and 887 Hz for $- \overset{O}{\overset{\|}{C}} - O - \underline{H}$ considerably further downfield than usually observed for non-intramolecularly hydrogen-bonded carboxylic acid protons. All this evidence of IR and PMR indicates that these acids might exist in the tautomeric forms (48) or (49). Confirmation of this could be established by further IR and PMR studies of other such acids. The formation of acids (47) was presumably



PMR (48)

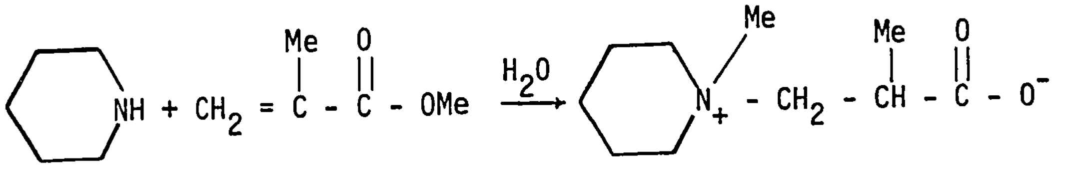
IR (49)

47(a) when R = H, and 47(d) when R = Me

the result of hydrolysis of the esters (46) by trace quantities of moisture present. This is in agreement with the findings of Bieber (1954) who also noticed the same phenomenon in the case of (46a).

This was an important observation as this readily made available the 3-amino-acids which were also subjected to preliminary screening for their effects on blood pressure and heart rate of anaesthetized cats.

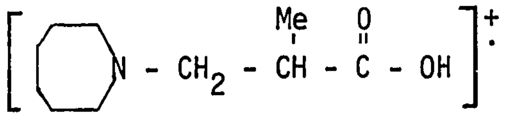
Bieber (1954) also obtained the same acid 2-methyl-3-piperidinopropionic acid (47a) by shaking the ester, methyl 2-methyl-3-piperidinopropionate (46a) with cold water. Vystrčil and Hudeček (1950) have studied the addition of amines to methyl methacrylate (36b) and the effect of water on the reaction product. According to this study, aliphatic amines such as diethylamine, piperidine, and morpholine added to methyl methacrylate (36b) to form N-substituted-3-amino-esters in nonaqueous medium, but in an aqueous medium, a shift of esterically-bound methyl group to the amino group occurred and resulted in the formation of N-methyl substituted betaines in very good yields. One of the betaines which they prepared by this method was (50), the product of the reaction between piperidine and methyl methacrylate in water. Betaine (50) was reported to melt at 106-108°.

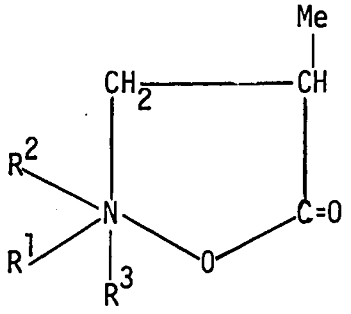


(50)

In the present study the ester methyl 2-methyl-3-piperidinopropionate (46a) was obtained by the reaction between piperidine and methyl methacrylate (36b) in methanol. This ester (46a) on long standing in moist air gave 2-methyl-3-piperidinopropionic acid (47a), characterised by IR (KBr disc; 2300-2120 cm^{-1} broad band for $\overset{+}{N}H$; 1610, 1540 cm^{-1} for

COO$^-$); elemental analysis and PMR spectrum ($\overset{O}{\overset{\|}{C}}$ - OC$\underline{H}_3$) signal absent; - $\overset{O}{\overset{\|}{C}}$ - O$\underline{H}$ sharp singlet at 843 Hz, disappeared in D_2O). The acid (47a) had a melting point of 109-110° (Table I) suspiciously close to that reported for the betaine (50). The formation of acid was further confirmed by the mass spectrum of (47b) which showed the molecular ion peak at m/e 185,

for  . Thus it is rather unlikely that Vystrčil and Hudeček (1950) had obtained the betaine (50) or, indeed any other betaines. They had characterised betaine (50) and other such betaines only by nitrogen analysis and m.p. data. Their postulate that the reaction had taken place through the intermediate (51), an impossible



(51)

structure, discredits their argument for betaine formation. Because of limited time, the formation of betaine (50) using the reported conditions of Vystrčil and Hudeček (1950) was not carried out.

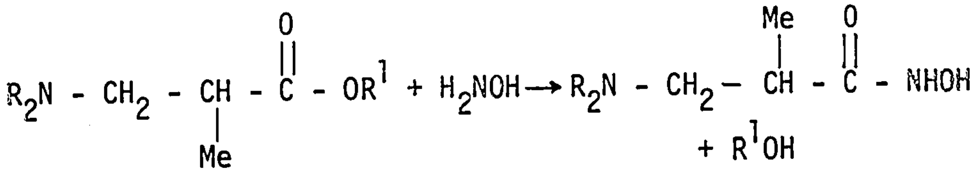
Preparation of hydrochloride salts

Hydrochlorides of the esters prepared in this study were obtained easily by passing dry hydrogen chloride gas through an ether solution of the appropriate amino-ester. They were recrystallized from methanol/ether and were found to be hygroscopic. The hydrochloride of methyl 3-methylamino-propionate, for example, liquified in air.

Preparation of 3-amino-hydroxamic acids

The 3-amino-hydroxamic acids were initially prepared by the interaction of hydroxylamine with the corresponding amino-ester. The hydroxylamine was obtained in situ by reacting hydroxylamine hydrochloride with potassium hydroxide in methanol. The potassium chloride, formed as a side product, was removed by filteration.

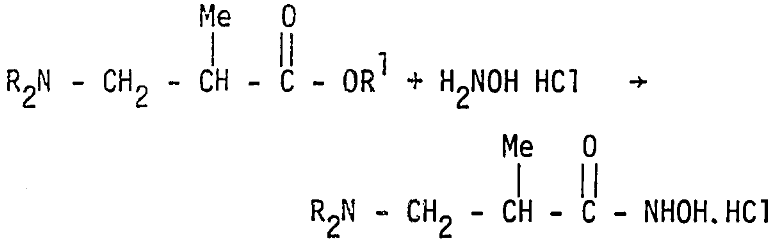
$$H_2NOH\ HCl + KOH \rightarrow \downarrow KCl + H_2NOH + H_2O$$



$$R_2N - CH_2 - \underset{\underset{Me}{|}}{CH} - \overset{\overset{O}{\|}}{C} - OR^1 + H_2NOH \longrightarrow R_2N - CH_2 - \overset{\overset{Me}{|}}{CH} - \overset{\overset{O}{\|}}{C} - NHOH + R^1OH$$

The hydroxamic acids, thus obtained were generally contaminated with varying amounts of potassium chloride and offered some difficulty in their purification. The hydroxamic acids were soluble only in methanol and ethanol in addition to water, and potassium chloride was also soluble in methanol and ethanol to a certain extent. These hydroxamic acids, therefore, had to be crystallized three or four times before they were obtained in pure state. An improvement in this method of preparing hydroxamic acids was achieved by using a large excess of hydroxylamine hydrochloride as compared to potassium chloride, so that an excess of free hydroxylamine as well as hydroxylamine hydrochloride were available for interaction with the amino-ester. The amino-hydroxamic acids were thus obtained directly as hydrochlorides which were contaminated only with trace amounts of potassium chloride and were crystallized more easily.

The other method employed for the synthesis of 3-amino-hydroxamic acids in the present study was by the reaction of hydroxylamine hydrochloride with the desired amino-ester. The 3-amino-hydroxamic acids prepared by this method were contaminated with certain amounts of 3-amino-ester hydrochlorides. This difficulty was solved by prolongation of the reaction time between the ester and hydroxylamine hydrochloride. The

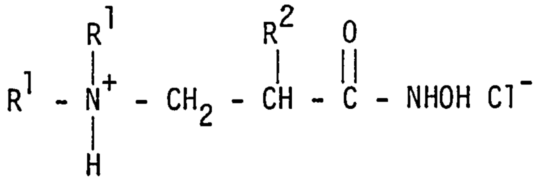


$$R_2N - CH_2 - \overset{\overset{Me}{|}}{CH} - \overset{\overset{O}{\|}}{C} - OR^1 + H_2NOH\ HCl \rightarrow R_2N - CH_2 - \overset{\overset{Me}{|}}{CH} - \overset{\overset{O}{\|}}{C} - NHOH.HCl$$

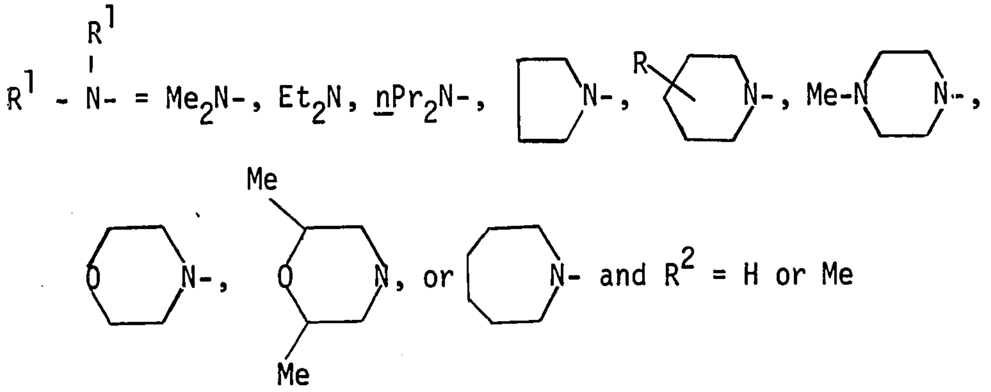
amino-hydroxamic acid hydrochlorides thus obtained in good yields, were generally found to be quite hygroscopic.

Each of the hydroxamic acid prepared in this study, gave a violet color with alcoholic ferric chloride and was characterised by elemental analysis, IR and PMR spectra. They all showed in their IR spectra a carbonyl absorption band attributable to the hydroxamic acid group between 1635-1675 cm^{-1}.

No PMR studies on the protons of the hydroxamate group of cyclic and acyclic hydroxamic acids have been reported. The present study has shown that the PMR spectra of acyclic hydroxamic acid salts of general structure (52) in DMSO or DMSO-d_6 have three characteristic 1-proton signals within the 500-700 Hz range as shown in Fig. 3.



$$R^1-\overset{\overset{R^1}{|}}{\underset{\underset{H}{|}}{N^+}}-CH_2-\overset{\overset{R^2}{|}}{CH}-\overset{\overset{O}{\|}}{C}-NHOH\ Cl^- \qquad (52)$$



R^1R^1N- = Me_2N-, Et_2N, $\underline{n}Pr_2N-$, ..., or ... and R^2 = H or Me

For the reasons which follow, it is concluded that the protons of the hydroxamate group, i.e. -CON$\underline{H}$O$\underline{H}$, come to resonance in DMSO-d_6 or DMSO (these hydroxamic acids are insoluble in solvents for PMR study other than DMSO-d_6, DMSO and D_2O, but in D_2O these protons are exchanged readily) within the region 521 to 635 Hz (Table II) and the signal which is located between 645 and 663 Hz is attributable to the $\overset{+}{N\underline{H}}$ proton of the salt. All the three protons exchange in D_2O. To confirm the latter assignment, PMR spectra of two base amino-hydroxamic acids were done in DMSO-d_6 and in both cases the signal between 645 to 663 Hz was found absent. The proton H^1, $(-\overset{O}{\overset{\|}{C}} - \overset{H^2}{\overset{|}{N}} - O - \underline{H}^1)$ was tentatively assigned to the signal between 521-540 Hz, as it is attached to electronegative oxygen only, and the proton H^2 was assigned to the signal in

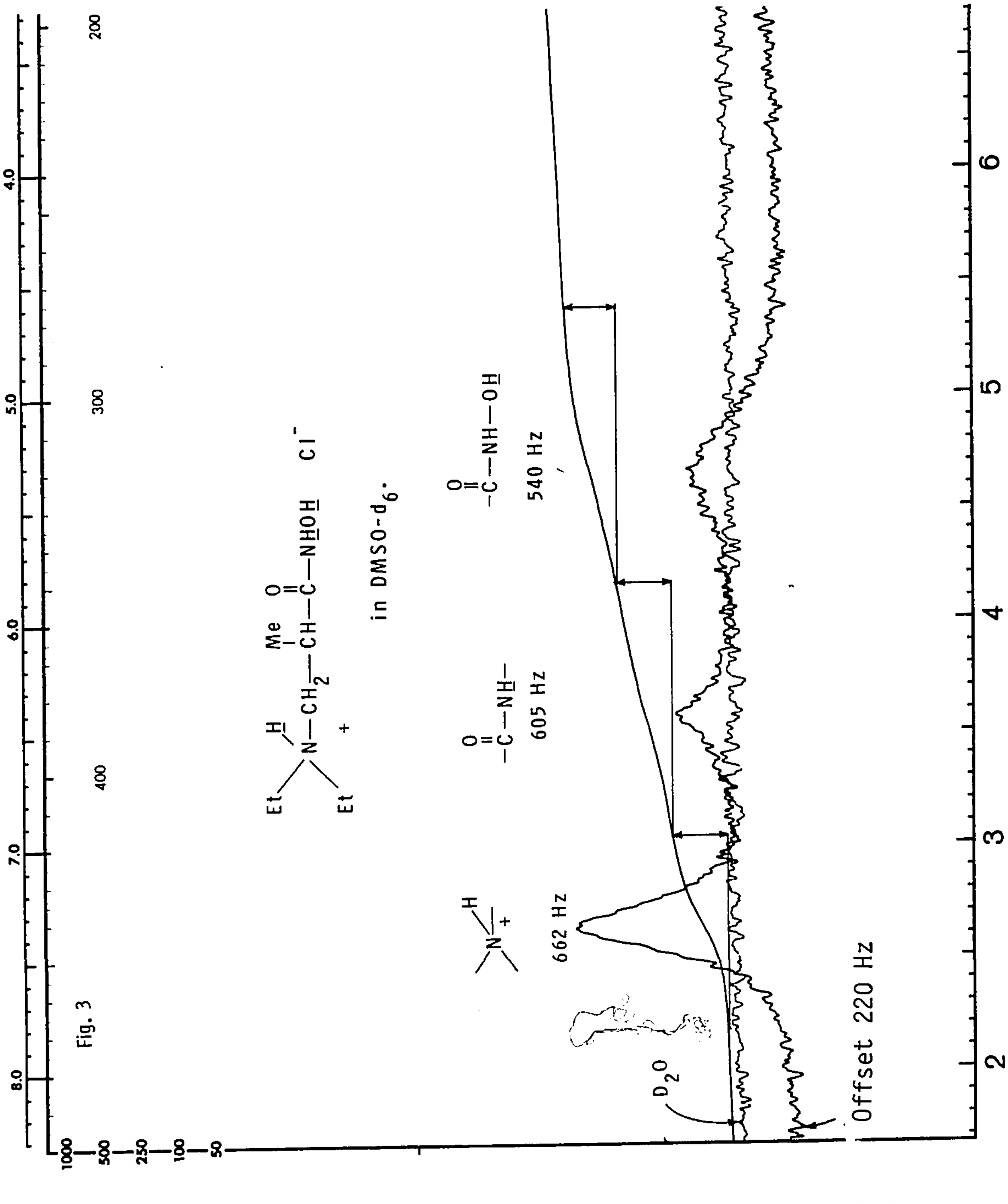
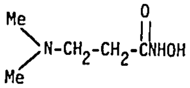
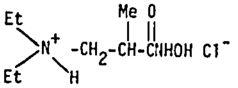
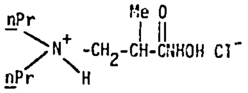
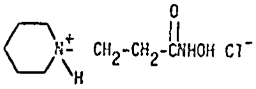
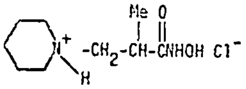
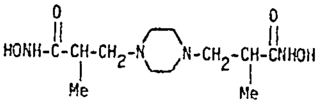
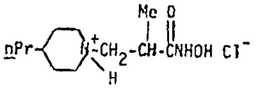
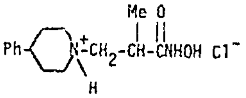
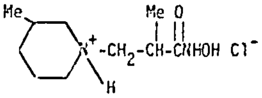
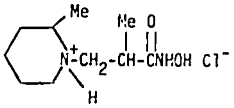
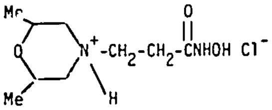
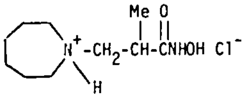
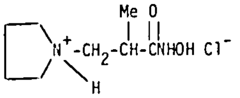


Fig. 3 PMR spectrum of hydroxamic acid protons of 2-methyl-3-diethylamino-propiono-hydroxamic acid hydrochloride

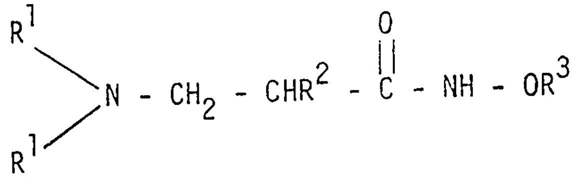
TABLE II

PMR characteristics of N-substituted-3-amino hydroxamic acid hydrochlorides

Compound	Solvent	Chemical Shift* -O-$\underline{H}$	 -N(H)-	 $>\overset{+}{N}<\underline{H}$
	DMSO	529 VB	603 VB	
	DMSO-d_6	527 B	622B	660 s
	DMSO-d_6	521 VB	625 VB	663 s
	DMSO-d_6	540 VB	610 VB	648 s
	DMSO-d_6	528 B	635 B	658 s
	DMSO-d_6	535 B	603 B	
	DMSO	535 VB	603 VB	655 B
	DMSO-d_6	533 VB	604 VB	655 B
	DMSO-d_6	534 VB	620 VB	653 B
	DMSO	535 VB	603 VB	660 B
	DMSO	540 VB	604 VB	645 B
	DMSO	533 VB	603 VB	656 B
	DMSO	570 VB	605 VB	653 B

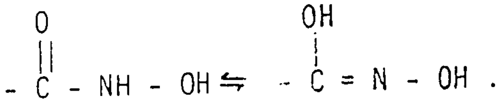
*In H_z from TMS. All signals are generally broad and are centered at the H_z value quoted. VB stands for very broad signal, B for broad signal and s for singlet.

the 603 to 635 Hz range as this proton is attached to nitrogen, which in turn is attached to an electron-withdrawing carbonyl group and to the oxygen atom of the hydroxy group. Presumably the H^2 proton would be more deshielded than H^1. These are only tentative assignments and a more detailed study is required to confirm the magnitudes of the chemical shifts of protons H^1 and H^2. A low temperature PMR study of these compounds and other hydroxamic acids (53) could prove useful,


(53)

where $R^1{}_2N$ and R^2 same as in (52) and R^3 is neither electron withdrawing nor electron donating,

in positively assigning the protons to the chemical shifts mentioned before, as the hydroxamate group can tautomerize as shown below:



$$-\overset{\overset{\displaystyle O}{\|}}{C} - NH - OH \leftrightharpoons -\overset{\overset{\displaystyle OH}{|}}{C} = N - OH \; .$$

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All the melting points and boiling points quoted are uncorrected. Infrared spectra were recorded on either a Perkin-Elmer Model 21 Infrared Spectrophotometer or a Beckman IR10 Spectrophotometer; and proton magnetic resonance (PMR) spectra were taken on a Varian A-60D Spectrophotometer. Mass spectra were recorded on an A.E.I. MS9 instrument equipped with a heated inlet system; the electron beam energy was 70 eV. For gas-liquid chromatography (GLC), an F and M Model 500 Gas Chromatograph was employed. The GLC determinations were carried out using SE-30 (10%) on Diatoport S contained in a stainless steel column (6 ft. x 1/4 in.), and percentages were calculated by measuring the areas under the curve using the triangulation method. Elemental analyses were done by the Department of Chemistry, the Microanalytical Laboratory, and the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. All the chemicals obtained from commercial sources were used without further purification unless otherwise specified.

Reaction of methyl methacrylate with hydrogen bromide

Dry hydrogen bromide gas was bubbled through a solution of methyl methacrylate (50g) in diethylether (200 ml) at 0^{c} for 30 minutes. There was a weight increase of 49g. The reaction mixture was kept at room temperature for 48 hours. Ether was removed under reduced pressure to give an oil which was dissolved in fresh diethylether (100 ml). The ethereal solution was washed with 10% sodium bicarbonate solution (3 x 25 ml) and water (2 x 25 ml) then dried over anhydrous sodium sulfate. [The combined aqueous washings were treated with excess of 10% hydrochloric acid and a solid which gave effervescence with sodium bicarbonate solution was obtained. This was recrystallized from absolute ethanol and had m.p. 210-215°, IR spectrum (KBr disc): 2610 (bonded OH), 1710 cm^{-1} ($C{=}O$). This was not identified]. The ether was removed and an oil (96.5g) was obtained which was distilled under reduced pressure. The major fraction which had b.p. 93-99°/43 mm was collected. The IR spectrum (film) showed no band near 1630 cm^{-1} (absence of C = C). A portion (4uL) was gas-chromatographed and the retention times of two major components present were obtained when

(a) the column temperature was 125°,

(b) the injection port temperature was 150°, and

(c) the helium flow rate was 60 ml/minute.

Fraction 1. (46.4%), had a retention time of 1.20 minutes;

Fraction 2. (53.6%), had a retention time of 1.65 minutes.

The two components were separated by fractional distillation using a spinning band column.

Fraction 1, b.p. 69-70°/18 mm was methyl 2-methyl-3-bromopropionate. Reported (Price and Coyner, 1940) b.p. 67°/17 mm.

Anal. Found: C, 32.81; H, 4.62; Br, 44.50. Calcd. for $C_5H_9O_2Br$: C, 33.27; H, 4.97; Br, 44.20.

Fraction 2, b.p. 78.5-79°/18 mm, was ethyl 2-methyl-3-bromopropionate. Reported (Rydon, 1936) b.p. 91-93°/19 mm.

Anal. Found: C, 37.15; H, 5.68. Calcd. for $C_6H_{11}O_2Br$: C, 36.92; H, 5.64.

Reaction of ethyl methacrylate with hydrogen bromide

The method employed was the same as that described for the methyl ester. The only product obtained ethyl 2-methyl-3-bromopropionate had b.p. 130-135°/30 mm and retention time (GLC same conditions as described for methyl ester) of 1.6 minutes.

Reaction of methyl acrylate with hydrogen bromide

The method employed was essentially that described for methyl methacrylate. In this case the two neutral products were methyl 3-bromopropionate (38.6%), retention time (GLC conditions, as in the methyl methacrylate reaction (p. 56) 0.9 minute; and ethyl 3-bromopropionate (61.4%) retention time, 1.4 minutes. Their b.p.'s IR spectra and GLC behaviour were identical to those of authentic samples.

General methods for the synthesis of Amino-esters

Each of the esters prepared in this series was characterised by means of microanalysis, PMR, and IR spectra. PMR spectra were taken in deuetrochloroform or carbontetrachloride. PMR data (chemical shifts as well as coupling constants) are quoted in Hertz units from TMS signal. IR spectra were recorded as films unless specified otherwise. A C = C stretching band near 1630 cm^{-1} (due to the acrylate starting material) was confirmed to be absent from each spectrum. Whenever a secondary amine was used as starting material with acrylate, absence of a band within the range of 3310 to 3500 cm^{-1} (due to secondary amine NH) was considered as a positive indication that the reaction had gone to completion. No attempts were made to separate the optical isomers or steroisomers.

Method A

The appropriate acrylate (0.25 mole) or methacrylate (0.25 mole) was dissolved in anhydrous methanol (50-100 ml) and to this was added the appropriate amine (0.25 mole) and 0.005% hydroquinone. The reaction mixture was then heated under reflux for 20 hours to 10 days. The solvent was removed, and the residue was dissolved in diethylether (100 ml) and extracted with 5% hydrochloric acid solution (3 x 25 ml). The aqueous extract was made basic with 10% ammonia solution and re-extracted with diethylether (3 x 50 ml). The combined ethereal extract was washed with water and dried over anhydrous sodium sulfate, after which the ether was removed. The oil which resulted was fractionally distilled using a fractionating column and the appropriate fraction was collected.

Method B (Adamson, 1949)

Methyl acrylate (0.25 mole) and the appropriate amine (0.25 mole) were dissolved in separate 40 ml. portions of anhydrous methanol. Both solutions were cooled to -5°C in an ice-salt mixture. The acrylate solution was added to the stirred amine solution, and the resulting mixture was kept in an ice-salt mixture for 1/2 hour to 4 hours; then allowed to stand at room temperature from 18 hours to 5 days. Methanol was removed under diminished pressure and the residual oil was distilled under reduced pressure.

Method C (Perrine, 1953)

To a stirred, cold solution of the appropriate secondary amine (0.25 mole in 25 ml of anhydrous methanol), was added an ice cold methanolic solution of methyl methacrylate (0.25 mole in 25 ml anhydrous methanol). The resulting mixture was stirred and kept cold for 30 minutes and then kept at 30° for 20 to 40 hours; finally, it was heated under reflux for 1 to 3 days. The methanol was removed under reduced pressure and the residual oil was distilled using a fractionating column under diminished pressure.

Preparation of hydrochloride salts

The hydrochloride salts of the amino-esters were prepared by passing dry hydrogen chloride through an ether solution of the appropriate ester. The hydrochlorides were recrystallized from methanol/diethyl ether unless mentioned otherwise. They were obtained as colorless solids and were characterized by their infrared spectra. All showed strong N^+-H stretching bands in the 2350-2710 cm^{-1} region (Thompson et al, 1965).

Methyl 3-methylamino-propionate

The title compound was prepared in 40% yield using general method B for the synthesis of amino-esters. The reaction mixture was kept at -5° for 4 hours and then at room temperature for 18 hours. The product had b.p. 70-72°/20 mm. Reported (Howton, 1945) b.p. 43.3-43.8/8mm.

Anal. Found: C, 51.48; H, 9.58. Calcd. for $C_5H_{11}NO_2$: C, 51.26; H, 9.47.

IR spectrum (film): 3340 (N-H), 1740 cm^{-1} (C = 0).

The hydrochloride salt liquified in air (Morsch, 1933).

Methyl 3-ethylamino-propionate

The title compound was prepared in 73% yield by general method B for the synthesis of amino-esters. The reaction mixture was kept at -5° for 1 hour and then at room temperature for 2 days. The title product had b.p. 28°/0.5 mm. Reported (David *et al*, 1965) b.p. 27°/0.3 mm.

Anal. Found: C, 54.72; H, 9.89; N, 10.84. Calcd. for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68.

IR spectrum (film): 3330 (N-H); 1740 cm^{-1} (C = 0).

The hydrochloride salt had m.p. 70-71°.

On long standing the title compound deposited a white crystalline substance which was collected and recrystallized from ethanol/acetone. The crystalline substance was characterised as 3-ethylamino-propionic acid, m.p. 188°. Reported (Gerjovich and Harrison, 1966) m.p. 174-176°.

Anal. Found: C, 50.97; H, 9.32 Calcd. for $C_5H_{11}NO_2$: C, 51.26; H, 9.46.

IR spectrum (KBr disc): 2740-2660 broad band; 2500-2420 broad band ($\overset{+}{N}H_2$); 1630, 1450 cm^{-1} (COO^-).

PMR spectrum (D_2O): 3-proton triplet centered at 78 Hz, J, 7Hz for $\underline{CH_3} - CH_2 -$; 2 - proton triplet split at top centered at 154 Hz, J, 6.6 Hz characterised for $- \underline{CH_2} - \overset{O}{\overset{\|}{C}}-$; 4 - proton multiplet due to overlapping of of a quatret J, 7Hz and a triplet J, 6.6Hz between 177 to 201 Hz for $- C\underline{H_2} - NH_2 - C\underline{H_2} -$.

3-Ethylamino-propionic ethylamide

The title compound was obtained in 46% yield during the course of an attempted preparation of methyl 3-ethylamino-propionate by the general method B for the synthesis of amino-esters, when the reaction mixture was kept at -5° for 1 hour and then at room temperature for 10 days. On fractional distillation, the first fraction was methyl acrylate and then the title compound was collected at 113-114°/2 mm.

Anal. Found: C, 54.85; H, 10.78; $C_6H_{14}N_2O$ requires: C, 55.35; H, 10.84.

IR spectrum (film): 3300 (N-H), 1645 cm^{-1} (C = O).

PMR spectrum (CCl_4): 3-proton triplet centered at 63Hz, J, 7Hz for $C\underline{H_3} - CH_2 - NH - CH_2$; 3-proton triplet centered at 66 Hz; J, 7Hz for $CONH - CH_2 - \underline{CH_3}$; 1-proton singlet at 88 Hz for $-N\underline{H}-$; 1-proton broad singlet at 498 Hz for $-CO - N\underline{H}-$. The singlet at 88 Hz and the singlet at 498 Hz exchanged in D_2O.

Methyl 3-n-propylamino-propionate

The title compound was prepared in 70% yield using general method B for the synthesis of amino-esters. The reaction mixture was kept at -5° for 1/2 hour and then at room temperature for 3 days. It was heated under reflux for 1/2 hour. The product had b.p. 86-88/16 mm. Reported (Brit. Patent, 1966) b.p. 57°/3 mm.

Anal. Found: C, 57.48; H, 9.94; N, 9.80 Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65.

IR spectrum (film): 3330 (N-H), 1740 cm^{-1}(C = 0).

The hydrochloride salt had m.p. 130-132°.

Methyl 3-n-butylamino-propionate

Using general method B for the synthesis of amino-esters, the title compound was obtained in 75% yield. The reaction mixture was kept at -5° for 1/2 hour and then at room temperature for 5 days, followed by heating under reflux for 1 hour. The title compound had b.p. 48-50°/0.5 mm. Reported (Kumura et al, 1965) b.p. 75°/5 mm.

Anal. Found: C, 60.22; H, 10.53. Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.76.

IR spectrum (film): 3330 (N-H), 1740 cm^{-1} (C = 0).

The hydrochloride salt of the title compound melted at 174-176°.

Methyl 3-n-hexylamino-propionate

When general method B for the synthesis of amino-esters was employed, the title compound was prepared in 62% yield. The reaction mixture was kept at -5° for 1/2 hour then at room temperature for 5 days. It was then boiled under reflux for 8 hours. The compound had b.p. 80-84/0.5 mm.

Anal. Found: C, 64.13; H, 11.50; N, 7.11. $C_{10}H_{21}NO_2$ requires: C, 64.13; H, 11.30; N, 7.48.

IR spectrum (film): 3320 (N-H), 1740 cm^{-1} (C = 0).

The m.p. of the hydrochloride was 190-192.5°.

Methyl 2-methyl-3-dimethylamino-propionate

The title compound was prepared in 75% yield by general method C for the synthesis of amino-esters. The reactants were kept at 30° for 20 hours and then heated under reflux for 1 day. The title compound had b.p. 66-68°/30 mm. Reported (Traynelis and Dadura, 1961) b.p. 70-71°/ 24 mm.

Anal. Found: C, 57.81; H, 10.33; N, 9.32. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65.

I.R. spectrum (film): 1740 cm^{-1} (C = 0).

The hydrochloride of the title compound had m.p. 123-125°. Reported (Perrine, 1953) m.p. 124-127.5°.

Ethyl 3-dimethylamino-propionate hydrochloride

The title ester was obtained from a commercial source and its hydrochloride salt m.p. 137-138° was prepared using the method previously described.

Methyl 2-methyl-3-diethylamino-propionate

Using general method C for the synthesis of amino-esters, the title compound was prepared in 68% yield. The reaction mixture was kept at 30° for 30 hours and then heated under reflux for 2 days. The product had b.p. 98-99°/35 mm. Reported (Bieber, 1954) b.p. 77-79/15 mm.

Anal. Found: C, 62.52; H, 10.61; N, 8.23. Calcd. for $C_9H_{19}NO_2$: C, 62.39; H, 11.05; N, 8.09.

IR spectrum (film): 1750 cm^{-1} (C = 0).

The hydrochloride of the title compound melted at 168-170°.

Methyl 2-methyl-3-(di-n-propylamino)propionate

The title compound was prepared in 60% yield by the general method C. The reaction mixture was allowed to stand at 30° for 40 hours and then heated under reflux for 5 days. The product had b.p. 58-60°/1 mm.

Anal. Found: C, 65.81; H, 11.74; $C_{11}H_{23}NO_2$ requires: C, 65.63; H, 11.52.

IR spectrum (film): 1740 cm^{-1} (C = 0).

Its hydrochloride melted at 175-176°.

Ethyl 3-dimethylamino-butyrate (Viscontini *et al*, 1950; Edwards *et al*, 1964)

To a solution of dimethylamine (0.1 mole) in benzene (40 ml) at 0° was added slowly with stirring a benzene solution of ethyl 3-bromobutyrate (0.05 mole in 20 ml benzene). After being kept at room temperature for 48 hours, the solution was flash-evaporated to remove the benzene and then treated with excess 5% sodium carbonate solution. The aqueous layer was extracted with diethyl ether (3 x 30 ml). The ether layer was dried over anhydrous sodium sulfate; the ether was removed under reduced pressure and the mixture was distilled to give the product (5.8 g, 33%); b.p. 74-76°/15 mm. Reported (Adamson, 1950) b.p. 178-180°.

Anal. Found: C, 60.24; H, 10.35; N, 8.56. Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.76; N, 8.80.

IR spectrum (film): 1740 cm^{-1} (C = 0).

Ethyl 4-(4-phenylpiperidino)butyrate hydrobromide

To a benzene (40 ml) solution containing 4-phenyl-piperdine (12g) was added slowly with constant stirring at room temperature a solution of methyl 4-bromobutyrate (15g) in benzene (40 ml). The mixture was kept at room temperature for 18 hours and then heated under reflux for 4 days. On removing the benzene under reduced pressure, a solid (21g, 77.7%) was collected, which was dissolved in anhydrous methanol (40 ml). To this a few drops of ether were added and after refrigeration for 24 hours the desired product was collected and recrystallized from acetone/ether; m.p. 131-133°.

Anal. Found: C, 57.49; H, 7.62; N, 3.28; $C_{17}H_{26}NO_2Br$ requires: C, 57.30; H, 7.36; N, 3.93.

IR spectrum (Nujol mull): 2700-2300 (N^+-H), 1725 cm^{-1} (C = 0).

Methyl 3-(2,6-dimethylmorpholine)propionate

The title compound was prepared in 89% yield using the general method A for the synthesis of amino-esters. The reaction mixture was boiled under reflux for 20 hours. The title compound had b.p. 76-78°/ 0.5 mm.

Anal. Found: C, 59.74; H, 9.59; N, 6.51; $C_{10}H_{19}NO_3$ requires: C, 59.68; H, 9.52; N, 6.96.

IR spectrum (film): 1745 cm^{-1} (C = 0).

The hydrochloride salt of the title compound melted at 231-233°.

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

Using general method A for the synthesis of amino-esters, the title compound was obtained in 65% yield. The reaction mixture was heated under reflux for 3 days. The title product had b.p. 42-44°/0.5 mm. Reported (Moffet, 1949) b.p. 86°/13 mm; (Barron *et al*, 1965) b.p. 53°/0.2 mm.

Anal. Found: C, 62.98; H, 9.66. Calcd. for $C_9H_{17}NO_2$: C, 63.16; H, 10.00.

IR spectrum (film): 1740 cm^{-1} (C = 0).

Its hydrochloride melted at 146-148°.

Methyl 2-methyl-3-(4-methyl-1-piperazinyl)propionate

The title compound was prepared in 90% yield by the general method A for the synthesis of amino-esters. The reaction mixture was heated under reflux for 7 days. The title product had b.p. 85-86°/3 mm. Reported (Barron et al, 1965) b.p. 60-62°/0.5 mm; (French Patent, 1958), b.p. 105-106°/11 mm.

Anal. Found: C, 59.52; H, 9.59. Calcd. for $C_{10}H_{22}N_2O_2$: C, 59.97; H, 10.07.

IR spectrum (film): 1745 cm^{-1} (C = 0).

Its hydrochloride had m.p. 208-210°.

Methyl 2-methyl-3-(3-methylpiperidino)propionate

When general method A for the synthesis of amino-esters was employed the title compound was prepared in 90% yield. The reactants were heated under reflux for 6 days. The title compound had the b.p. 58-60°/0.5 mm.

Anal. Found: C, 66.61; H, 10.71. $C_{11}H_{21}NO_2$ requires: C, 66.29; H, 10.62.

IR spectrum (film): 1740 cm^{-1} (C = 0).

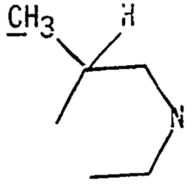
The hydrochloride salt melted at 129-130°.

On long standing, the title compound deposited a white crystalline solid which was recrystallized from absolute ethanol/acetone. This was characterised as <u>2-methyl-3-(3-methylpiperidino)propionic acid,</u> m.p. 122-124°.

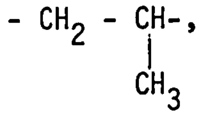
Anal. Found: C, 64.64; H, 10.16; $C_{10}H_{19}NC_2$ requires: C, 64.83; H, 10.34.

IR spectrum (KBr disc): 2260-2120 broad band (N^+-H); 1610, 1450 cm^{-1} (COO^-).

PMR spectrum (D_2O): 3-proton doublet centered at 57Hz, J, 6Hz

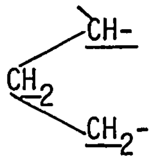


–; 3-proton doublet centered at 69Hz, J, 7Hz for

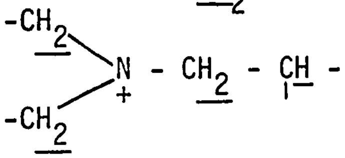


$-CH_2-\underset{\underset{CH_3}{|}}{CH}-$,

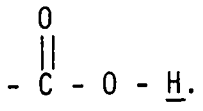
5-proton broad complex between 93 to 134 Hz for



, 7-proton broad complex between 135 to 230 Hz for



PMR spectrum ($CDCl_3$): 1-proton sharp singlet at 887Hz for



$-\overset{\overset{O}{\|}}{C}-O-\underline{H}$. This singlet disappeared on deuterium exchange.

Methyl 2-methyl-3-(2-methylpiperidino)propionate

The title compound was obtained in 80% yield using general method A for the synthesis of amino-esters. The reaction products were boiled under reflux for 10 days. The title compound had the b.p. 122-124°/ 25 mm.

Anal. Found: C, 65.81; H, 10.81. $C_{11}H_{21}NO_2$ requires: C, 66.29; H, 10.62.

IR spectrum (film): 1740 cm^{-1} (C = 0).

Repeated attempts to prepare the hydrochloride salt of the title compound were unsuccessful.

Ethyl 3-piperidinopropionate

General method A for the synthesis of amino-esters was employed for the title compound which was obtained in 74% yield. The reaction mixture was heated under reflux for 5 days. The product had b.p. 109-110°/15 mm. Reported (Matkovics et al, 1961) b.p. 102-103°/5 mm.

Anal. Found: C, 65.21; H, 9.84. Calcd. for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34.

IR spectrum (film): 1740 cm^{-1} (C = 0).

Its hydrochloride melted at 183-184°. Reported (Matkovics et al, 1961) m.p. 169°.

Methyl 2-methyl-3-piperidinopropionate

When general method A for the synthesis of amino-esters was employed, the title compound was prepared in 78% yield. The reaction mixture was heated under reflux for 5 days. The product had b.p. 107-108°/18 mm. Reported (Bieber, 1954) b.p. 102-103°/18 mm; (Spickett et al, 1966) gave b.p. 56°/0.5 mm.

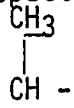
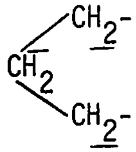
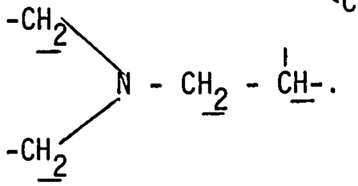
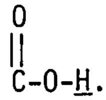
IR spectrum (film): 1740 cm^{-1} (C = 0).

M.P. of the hydrochloride was 157-158°.

On long standing, the title compound deposited a white crystalline solid which was collected and recrystallized from benzene/hexane. This was characterized as 2-methyl-3-piperdinopropionic acid, m.p. 109-110°. Reported (Bieber, 1954) m.p. 108°.

Anal. Found: C, 63.38; H, 9.91. Calcd. for $C_9H_{17}NO_2$: C, 63.16; H, 9.94.

IR spectrum (KBr disc): 2300-2120 broad band (N^+-H); 1610, 1450 cm^{-1} (COO^-).

PMR spectrum ($CDCl_3$): 3-proton doublet of a doublet centered at 67 Hz for  $-\overset{C\underline{H}_3}{\overset{|}{CH}}-$, 6-proton broad signal centered at 98 Hz for  , 7-proton complex multiplet centered at 162Hz for  1-proton sharp singlet at 843Hz for  $-\overset{O}{\overset{\|}{C}}-O-\underline{H}$. This singlet disappeared on deuterium exchange.

1, 4-Bis(2-methoxy carbonylpropyl)piperazine

The title compound was prepared in 60% yield by the general method A for the synthesis of amino-esters except that twice the amount of methyl methacrylate was used. The reaction mixture was heated under reflux for 7 days. The title compound had the b.p. 138-140°/2 mm.

Anal. Found: C, 58.49; H, 9.0; $C_{14}H_{26}N_2O_4$ requires: C, 58.72; H, 9.15.

IR spectrum (film): 1740 cm^{-1} (C = 0).

The hydrochloride of the title compound had m.p. 203-204.5°.

Methyl 2-methyl-3-(4-methylpiperidino)propionate

The title compound was prepared in 90% yield when general method A for the synthesis of amino-esters was applied. The reactants were heated under reflux for 7 days. The title product had b.p. 66-68°/ 2 mm.

Anal. Found: C, 66.19; H, 10.49; $C_{11}H_{21}NO_2$ requires: C, 66.29; H, 10.62.

IR spectrum (film): 1740 cm^{-1} (C = 0).

M.P. of the hydrochloride salt was 145-146°.

Methyl 2-methyl-3-(4-n-propylpiperidino) propionate

Using general method A for the synthesis of amino-esters, the title compound was prepared in 80% yield after heating under reflux for 7 days. The title compound had the b.p. 88-90°/2 mm.

Anal. Found: C, 68.43; H, 11.17. $C_{13}H_{25}NO_2$ requires: C, 68.68; H, 11.08.

IR spectrum (film): 1745 cm^{-1} (C = 0).

The hydrochloride salt of the title compound melted at 164-165°.

Methyl 2-methyl-3-(4-phenyl-piperidino)propionate

The title compound, b.p. 143-145°/2 mm was prepared in 85% yield employing general method A for the synthesis of amino-esters. The reaction mixture was heated under reflux for 7 days.

Anal. Found: C, 73.37; H, 8.50. $C_{11}H_{23}NO_2$ requires: C, 73.53; H, 8.87.

IR spectrum (film): 1740 cm^{-1} (C = 0).
Hydrochloride salt melted at 180-181°.

Methyl 2-methyl-3-morpholinopropionate

The title compound was prepared in 90% yield using general method A for the synthesis of amino-esters, and heating under reflux for 7 days. The desired product had the b.p. 120-122°/19 mm. Reported (Vystrčil and Hudeček, 1950) b.p. 112°/13 mm.

Anal. Found: C, 57.64; H, 9.16. Calcd. for $C_9H_{17}NO_3$: C, 57.73; H, 9.15.

IR spectrum (film): 1740 cm^{-1} (C = 0).
The hydrochloride salt of the title compound had m.p. 165-166°.

Methyl 2-methyl-3-(2,6-dimethylmorpholino)propionate

The title compound was prepared in 95% yield using the general method A for the synthesis of amino-esters. The reaction mixture was heated under reflux for 7 days. The desired product had the b.p. 76-78°/2 mm.

Anal. Found: C, 61.05; H, 10.03. $C_{11}H_{21}NO_3$ requires: C, 61.36; H, 9.83.

IR spectrum (film): 1740 cm^{-1} (C = 0).
Its hydrochloride had m.p. 148-149°.

Methyl 2-methyl-3-homopiperidinopropionate

When general method A for the synthesis of amino-esters was employed and the reactants were heated under reflux for 30 hours, the title compound was prepared in 80% yield. It had b.p. 76-78°/2 mm. Reported (Barron et al, 1965) b.p. 60°/0.05 mm.

Anal. Found: C, 66.42; H, 10.57. Calcd. for $C_{11}H_{21}NO_2$: C, 66.29; H, 10.62.

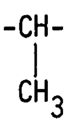
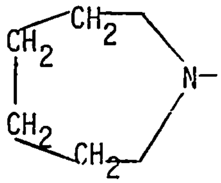
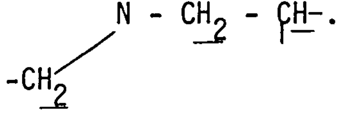
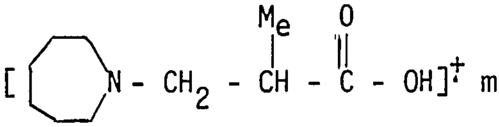
IR spectrum (film): 1740 cm^{-1} (C = 0).

M.P. of the hydrochloride was 133-134°.

The title compound, on long standing, deposited a white crystalline substance which was collected and recrystallized from benzene/hexane. This was characterised as 2-methyl-3-homopiperidinopropionic acid m.p. 100°.

Anal. Found: C, 61.69; H, 10.58. $C_{10}H_{19}NO_2 \cdot \frac{1}{2} H_2O$ requires: C, 61.85; H, 10.31.

IR spectrum (KBr disc): 2400-2120 broad band (N^+-H), 1620, 1450 cm^{-1} (COO^-).

PMR spectrum (D_2O): 3-proton doublet centered at 69Hz, J, 6.5Hz for  $-\underset{\underset{CH_3}{|}}{CH}-$; 8-proton singlet at 105Hz for ; 7-proton complex signal between 138-181Hz for  Mass spectrum: 185 (23.8%),  $[\,N - CH_2 - \overset{\overset{Me}{|}}{CH} - \overset{\overset{O}{\|}}{C} - OH]^{+\cdot}$ m/e (percent abundance relative to peak at m/e 41 = 100).

Methyl 3-benzylamino-propionate

Using general method A for the synthesis of amino-esters the title compound was prepared in 62% yield, when the reactants were heated under gentle reflux for 2 days. The title compound had the b.p. 100-104°/1 mm. Reported (Southwick and Crouch, 1953) b.p. 145-147°/7mm.

Anal. Found: C, 68.26; H, 7.82; N, 7.18. Calcd. for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25.

IR spectrum (film): 3340 (N-H), 1740 cm^{-1} (C = O). The hydrochloride salt of the title compound had m.p. 158-159°. Reported (Southwick and Crouch, 1953) m.p. 164-165°.

Methyl 2-methyl-3-phenethylamino-propionate

Methyl methacrylate (316g) was mixed with phenethylamine (246g) in absolute methanol (150 ml). This solution was allowed to stand at room temperature for 13 months. The methanol was flash-evaporated and the oil was taken up in diethyl ether (150 ml), which was extracted with 5% hydrochloric acid solution (3 x 50 ml). The acidic extract was treated with an excess of 20% sodium carbonate solution and then re-extracted with ether (3 x 100 ml). The ether was removed under reduced pressure and the product was distilled to give the desired compound (300g, 65%); b.p. 124-126°/1 mm. Reported (Kirk, 1958) b.p. 116-118°/0.6 mm.

Anal. Found: C, 70.77; H, 8.54. Calcd. for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65.

IR spectrum (film): 3330 (N-H), 1740 cm^{-1} (C = O). M.P. of the hydrochloride was 116-118°.

Attempted synthesis of methyl 2-methyl-3-di*iso*propyl-amino-propionate

General method C for the synthesis of amino-esters was employed. The reaction mixture was vigorously boiled under reflux for 7 days, but only the starting materials were recovered. Using *n*-butanol instead of methanol in general method C and boiling under reflux for 10 days failed to give the title compound; again the starting materials were obtained.

Attempted synthesis of methyl 2-methyl-3-(2,6 dimethyl-piperidino)propionate

General method A for the synthesis of amino-esters was employed for the preparation of the title compound. Boiling the reaction mixture vigorously under reflux for 16 days failed to give the desired product. In all attempts, the starting materials were recovered unreacted.

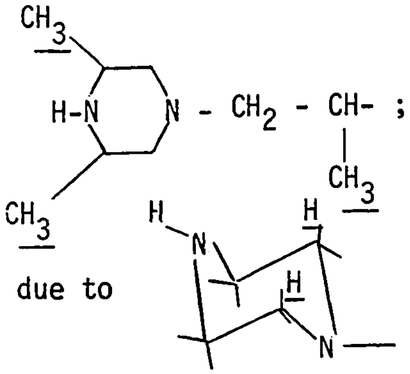
Attempted synthesis of 1,4-bis(2-methoxy-carbonyl-propyl)2,6-dimethyl-piperazine

2,6-Dimethyl-piperazine (14.25g) was dissolved in anhydrous methanol (50 ml) and to this methylmethacrylate (25g) was added. This was kept at room temperature for 24 hours and then boiled under reflux for 60 hours. An IR spectrum of a small portion from which the solvent had been removed, showed a band of medium intensity at 1630 cm^{-1} for C = C stretching., The reaction mixture was heated under reflux for a further two weeks. The methanol was removed *in vacuo*, and the residual oil was distilled under pressure. The first fraction collected was methyl methacrylate, the other fraction was characterised as *methyl 2-methyl-3-(2,6-dimethyl-4-piperazinyl)propionate* b.p. 88-90°/4 mm.

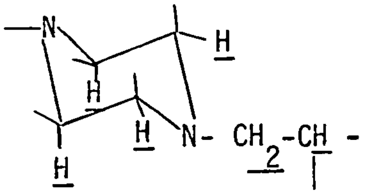
Anal. Found: C, 61.12; H, 9.85. $C_{11}H_{22}N_2O_2$ requires: C, 61.64; H, 10.35.

IR spectrum (film): 3320 (N-H); 1740 (C = O); 1630 cm^{-1} (C = C) absent.

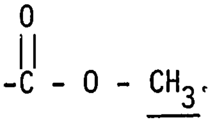
PMR spectrum (carbon tetrachloride): 9-proton signal centered at 60Hz (a triplet formed by the overlapping of two doublets) for



3-proton triplet centered at 90Hz, J, 9.8Hz, due to on adding D_2O changed to 2-proton triplet centered at 90Hz, J, 9.8Hz; 7-proton complex signal between 127-182Hz for



3-proton signlet at 211 Hz for



$-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{O}-\underline{\text{CH}_3}$.

General methods for the synthesis of amino-hydroxamic acid hydrochlorides

Each of the hydroxamic acids prepared in this series gave a violet color with alcoholic ferric chloride and was characterised by its PMR and IR spectra and by elemental analysis. PMR spectra were taken in DMSO-d_6, DMSO, or D_2O. Chemical shifts as well as coupling constants are quoted in Hz units. IR spectra were recorded as KBr discs or Nujol mulls. All hydrochlorides showed strong N^+-H stretching bands in the 2340-2760 cm^{-1} region (Thompson et al, 1965).

Method I

A constantly stirred solution of hydroxylamine hydrochloride (0.02 mole) in anhydrous methanol (50 ml) was cooled to 0° and to this was added dropwise a methanolic solution of the appropriate amino-ester (0.02 mole in 20 ml MeOH). Stirring was continued at room temperature for a further 10 hours to 3 days and then at 50° with constant stirring for another 3 days. Methanol was removed *in vacuo* and the resulting semi-solid was redissolved in the minimum of dry methanol. The solution was cooled to 0° and dry acetone was added until it turned turbid. The products separated on standing. The hydroxamic acid hydrochlorides were crystallized from methanol/acetone or ethanol/acetone.

Method II

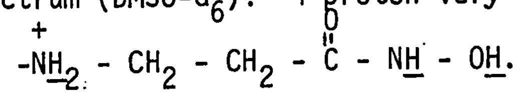
To a constantly stirred solution of hydroxylamine hydrochloride (0.18 mole in 50 ml of anhydrous MeOH) at 0° was added a cold methanolic solution of potassium hydroxide (0.12 mole in 40 ml of MeOH), and after 3 minutes the precipitated potassium chloride was filtered out. To the filtrate was added a methanolic solution of the appropriate ester (0.03 mole in 20 ml of MeOH). The temperature of the stirred solution was maintained at 0° to -5° for an hour and then allowed to come to room temperature. Stirring was continued for 18 hours to 48 hours. Methanol was removed under reduced pressure and to the remaining viscous oil, sufficient acetone was added to give a turbidity. On refrigeration a solid formed. It was recrystallized at least three times from absolute ethanol to remove any potassium chloride.

3-Methylamino-propiono-hydroxamic acid hydrochloride

The title compound was prepared in 65% yield using general method I for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred at 0° for 60 hours then raised to 50°. The title product had m.p. 114-115°.

Anal. Found: C, 31.1; H, 7.19; N, 17.91. $C_4H_{11}N_2O_2Cl$ requires: C, 31.07; H, 7.17; N, 18.12.

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

PMR spectrum (DMSO-d_6): 4-proton very broad peak between 510 to 605 H_z for  $-\overset{+}{N}\underline{H}_2 - CH_2 - CH_2 - \overset{\overset{O}{\|}}{C} - N\underline{H} - O\underline{H}$.

3-Ethylamino-propiono-hydroxamic acid hydrochloride

By employing general method II for the synthesis of amino-hydroxamic acids the title compound m.p. 44-45° was obtained in 64% yield. The reaction mixture was allowed to stir for 40 hours.

Anal. Found: C, 32.22; H, 7.99; N, 15.36. $C_5H_{13}N_2O_2Cl \cdot H_2O$ requires: C, 32.17; H, 8.10; N, 15.01.

IR spectrum (KBr disc): 1660 cm^{-1} (C = O).

3-n-Propylamino-propiono-hydroxamic acid hydrochloride

Using general method I for the synthesis of amino-hydroxamic acids, the title compound was prepared in 72% yield. The reactants were stirred for 48 hours at 0° then at 50°. The title compound had m.p. 78°.

Anal. Found: C, 39.36; H, 8.62; N, 15.34. $C_6H_{15}N_2O_2Cl$ requires: C, 39.45; H, 8.27; N, 15.34.

IR spectrum (Nujol mull): 1650 cm^{-1} (C = O).

3-n-Butylamino-propiono-hydroxamic acid hydrochloride

The title compound was prepared in 61% yield, when general method II for the synthesis of amino-hydroxamic acids was employed. The reaction mixture was stirred for 48 hours and the product had m.p. 108°.

Anal. Found: C, 40.49; H, 8.68; N, 13.95. $C_7H_{17}N_2O_2Cl\frac{1}{2}H_2O$ requires: C, 40.87; H, 8.81; N, 13.62.

IR spectrum (Nujol mull): 1650 cm^{-1} (C = 0).

3-n-Hexylamino-propiono-hydroxamic acid hydrochloride

The title compound was prepared in 79% yield by general method II for the synthesis of amino-hydroxamic acids. The reactants were stirred for 40 hours and the product had m.p. 134-135°.

Anal. Found: C, 47.72; H, 9.49; N, 12.72. $C_9H_{21}N_2O_2Cl$ requires: C, 48.10; H, 9.42; N, 12.47.

IR spectrum (Nujol mull): 1655 cm^{-1} (C = 0).

PMR spectrum (DMSO-d_6): 4-proton very broad signal between 472 to 615 Hz for $-\overset{+}{N}\underline{H}_2-CH_2-CH_2-\overset{\overset{\displaystyle O}{\|}}{C}-N\underline{H}-O\underline{H}$.

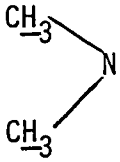
3-Dimethylamino-propiono-hydroxamic acid hydrochloride

The title compound was obtained in 71% yield, when general method I for the synthesis of amino-hydroxamic acids was applied. The reaction mixture was stirred for 10 hours at 0° then at 50°. The product had m.p. 86-88°. Reported (Matveev <u>et al</u>, 1964) m.p. 90-91°.

Anal. Found: C, 35.59; H, 7.94; N, 16.77. Calcd. for $C_5H_{13}N_2O_2Cl$: C, 35.61; H, 7.77; N, 16.62.

IR spectrum (KBr disc): 1665 cm^{-1} (C = 0).

PMR spectrum (D_2O): 2-proton triplet centered at 168 Hz, J, 7Hz for $- CH_2 - C\underline{H}_2 - \overset{O}{\overset{\|}{C}}-$' 6-proton singlet at 180 Hz for



$\underline{CH}_3$ N $\underline{CH}_3$; 2-proton triplet centered at 213 Hz, J, 7Hz for $- C\underline{H}_2 - CH_2 - \overset{O}{\overset{\|}{C}}-$.

PMR spectrum of 3-dimethylamino-propiono-hydroxamic acid (DMSO): Very broad signal centered at 529Hz for $- \overset{O}{\overset{\|}{C}} - N - H - O - \underline{H}$; very broad signal centered at 603 Hz for $- \overset{O}{\overset{\|}{C}} - N - \underline{H} - OH$.

2-Methyl-3-dimethylamino-propiono-hydroxamic acid hydrochloride

Using general method I for the synthesis of amino-hydroxamic acids the title compound, m.p. 140-142° was obtained in 77% yield. The reaction mixture was stirred for 10 hours at room temperature.

Anal. Found: C, 39.29; H, 7.91; N, 15.32. $C_6H_{15}N_2O_2Cl$ requires: C, 39.45; H, 8.27; N, 15.34.

IR spectrum (KBr disc): 1665 cm^{-1} (C = 0).

Attempted synthesis of 3-dimethylamino-butyrohydroxamic acid hydrochloride

General method I for the synthesis of amino-hydroxamic acids was employed for the preparation of the title compound. The reaction mixture was stirred for 10 hours at room temperature. The title product was obtained as a thick viscous oil. Repeated attempts to obtain it in crystalline form were unsuccessful.

IR spectrum (film): 1640 cm^{-1} (C = 0).

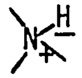
It formed a reineckate salt m.p. 133-34°.

2-Methyl-3-diethylamino-propiono-hydroxamic acid hydrochloride

The title compound was prepared in 73% yield using general method I for the synthesis of amino-hydroxamic acids. The reactants were stirred for 10 hours at room temperature before being kept at 50°. The title product had m.p. 118-119°.

Anal Found: C, 45.82; H, 9.28; N, 13.09. $C_8H_{19}N_2O_2Cl$ requires: C, 45.59; H, 9.08; N, 13.29.

IR spectrum (KBr disc): 1660 cm^{-1} (C = 0).

PMR spectrum (DMSO-d_6): Broad singlet at 527Hz for $-\overset{O}{\overset{\|}{C}}-NH-O\underline{H}$, broad singlet at 622 Hz for $-\overset{O}{\overset{\|}{C}}-N\underline{H}-0-$, sharp singlet at 660 Hz for  . Combined signal integrated for 3-protons which were exchanged on addition of D_2O.

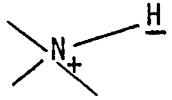
2-Methyl-3-di-n-propylamino-propiono-hydroxamic acid hydrochloride

By applying general method I for the synthesis of amino-hydroxamic acids, the title compound was prepared in 62% yield. The reaction mixture was stirred at room temperature for 10 hours before keeping it at 50°. The title compound melted at 152-153°.

Anal. Found: C, 50.60; H, 9.93; N, 11.30. $C_{10}H_{23}N_2O_2Cl$ requires: C, 50.30; H, 9.71; N, 11.74.

IR spectrum (KBr disc): 1655 cm^{-1} (C = 0).

PMR spectrum (DMSO-d_6): Broad signlet at 521 Hz for $-NH-0-\underline{H}$;

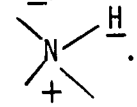
broad singlet at 625 Hz for - NH_ - 0 -; sharp singlet at 663 Hz for  . Combined signal integrated for 3-protons.

2-Methyl-3-(1-pyrrolidinyl)propiono-hydroxamic acid hydrochloride

The title compound was obtained in 72% yield when general method II for the synthesis of amino-hydroxamic acids was used. The reactants were stirred for 24 hours. The product had m.p. 168-169°.

Anal. Found: C, 45.70; H, 8.25; N, 13.04. $C_8H_{17}N_2O_2Cl$ requires: C, 46.03; H, 8.21; N, 13.42.

IR spectrum (KBr disc): 1660 cm^{-1} (C = 0).

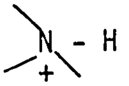
PMR spectrum (DMSO): Broad singlet at 570 Hz for - NH - 0$\underline{H}$; broad singlet at 605 Hz for - N - $\underline{H}$ - 0 -; singlet at 653 Hz for .

2-Methyl-3-(3-methyl-piperdino)propiono-hydroxamic acid hydrochloride

Using general method II for the synthesis of amino-hydroxamic acids, the title compound m.p. 191-193° was prepared in 86% yield when the reaction mixture was stirred for 18 hours.

Anal. Found: C, 50.23; H, 8.93; N, 11.81. $C_{10}H_{21}N_2O_2Cl$ requires: C, 50.73; H, 8.94; N, 11.84.

IR spectrum (KBr disc): 1660 cm^{-1} (C = 0).

PMR spectrum ($DMSO-d_6$): Broad singlet at 534 Hz for - NH - 0 - $\underline{H}$; broad singlet at 620 Hz for - N$\underline{H}$ - 0 -; singlet at 653 Hz for  . Combined signal integrated for 3-protons.

2-Methyl-3-(2-methyl-piperdino)propiono-hydroxamic acid hydrochloride

The title compound was obtained in 69% yield by employing general method II for the synthesis of amino-hydroxamic acids. The reactants were stirred for 48 hours. The product had m.p. 170-172°.

Anal. Found: C, 50.45; H, 9.31; N, 11.63. $C_{10}H_{21}N_2O_2Cl$ requires: C, 50.73; H, 8.94; N, 11.84.

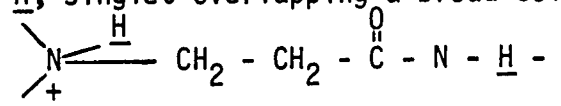
IR spectrum (Nujol mull): 1645 cm^{-1} (C = O).

2-Methyl-3-(1-methyl-4-piperazinyl)propiono hydroxamic acid monohydrochloride

The title compound was prepared in 69% yield using general method I for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for 24 hours at 0° before keeping it at 50°. The product had m.p. 177-178°.

Anal. Found: C, 45.49; H, 8.78; N, 17.60. $C_9H_{20}N_3O_2Cl$ requires: C, 45.47; H, 8.48; N, 17.67.

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

PMR spectrum (DMSO-d_6): Very broad singlet centered at 580 Hz for - NH - O - $\underline{H}$, singlet overlapping a broad collapsed multiplet centered at 632 Hz for  $>\overset{\underline{H}}{\overset{+}{N}}—CH_2 - CH_2 - \overset{O}{\overset{\|}{C}} - N - \underline{H} -$. Combined signal integrated for 3-protons.

The di-picrate of title compound had m.p. 209-211°.

Anal. Found: C, 38.22; H, 3.82; N, 19.12. $C_{21}H_{25}N_9O_{16}$ requires: C, 38.24; H, 3.82; N, 19.12.

2-Methyl-3-(phenethylamino)propiono-hydroxamic acid hydrochloride

The title compound was prepared in 75% yield, when general method II for the synthesis of amino-hydroxamic acids was used and the reactants were stirred for 48 hours. The title compound had m.p. 126-127°.

Anal. Found: C, 55.77; H, 7.69; N, 10.94. $C_{12}H_{19}N_2O_2Cl$ requires: C, 55.70; H, 7.40; N, 10.83.

IR spectrum (Nujol mull): 1640 cm^{-1} (C = 0).

The picrate of the title compound melted at 139-141°.

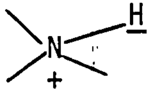
Anal. Found: C, 47.70; H, 4.53; N, 15.84. $C_{18}H_{21}N_5O_9$ requires: C, 47.89; H, 4.69; N, 15.52.

3-(2,6-Dimethylmorpholino)propiono-hydroxamic acid hydrochloride

Using general method II for the synthesis of amino-hydroxamic acids, the title compound was obtained in 48% yield. The reaction mixture was stirred for 20 hours. The product obtained had m.p. 170-171°.

Anal. Found: C, 45.56; H, 8.24; N, 11.50. $C_9H_{19}N_2O_3Cl$ requires: C, 45.28; H, 8.02; N, 11.74.

IR spectrum (Nujol mull): 1660 cm^{-1} (C = 0).

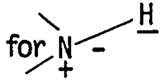
PMR spectrum (DMSO): Very broad singlet centered at 540 Hz for - NH - 0 - H; very broad singlet centered at 604 Hz for - NH - 0 -; broad singlet at 645 Hz for  .

3-Piperidino-propiono-hydroxamic acid hydrochloride

The title compound was obtained in 70% yield by employing general method I for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for 18 hours at room temperature. The title product had m.p. 162-163.5°.

Anal. Found: C, 46.19; H, 8.26; N, 13.81; $C_8H_{17}N_2O_2Cl$ requires: C, 46.03; H, 8.21; N, 13.42.

IR spectrum (Nujol mull): 1630, 1675 cm^{-1} (C = 0).

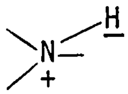
PMR spectrum (DMSO-d_6): Very broad signal between 510 to 630 Hz for  $-\overset{O}{\overset{\|}{C}}-N\underline{H}-O\underline{H}$, singlet at 648 Hz for  $>\overset{+}{N}-\underline{H}$. Combined signal integrated for 3-protons.

2-Methyl-3-piperidino-propiono-hydroxamic acid hydrochloride

The title compound was synthesised in 68% yield using general method I for the synthesis of amino-hydroxamic acids. The reactants were stirred for 48 hours at 0° then kept at 50°. The compound thus synthesised had m.p. 166-167°.

Anal. Found: C, 48.38; H, 8.59; N, 12.68. $C_9H_{19}N_2O_2Cl$ requires: C, 48.53; H, 8.60; N, 12.58.

IR spectrum (Nujol mull): 1620, 1655 cm^{-1} (C = 0).

PMR spectrum (DMSO-d_6): Broad signal centered at 528 Hz for $- NH - O - \underline{H}$, broad signal centered at 635 Hz for $- N\underline{H} - O -$, singlet at 658 Hz for  $>\overset{+}{N}-\underline{H}$. The combined signal integrated for 3-protons and exchanged in D_2O.

Piperazine-1,4-bis-(2-methyl-3-propiono-hydroxamic acid)dihydrochloride

When general method I for the synthesis of amino-hydroxamic acids was employed, the title compound was obtained in 70% yield. The reaction mixture was stirred for 3 days at room temperature before being kept at 50°. The title compound had m.p. 205-207°.

Anal. Found: C, 39.66; H, 7.51; N, 15.18. $C_{12}H_{26}N_4O_4Cl_2$ requires: C, 39.89; H, 7.25; N, 15.51.

IR spectrum (Nujol mull): 1690, 1655 cm^{-1} (C = O).

PMR spectrum of the title compound as base (DMSO-d_6): broad singlet centered at 535 Hz for - O - H; broad singlet centered at 603 Hz for - NH - O - H. Combined signal integrated for 4-protons.

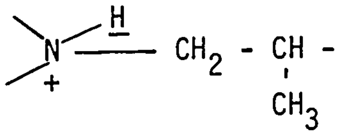
2-Methyl-3-(4-methylpiperidino)propiono-hydroxamic acid hydrochloride

Using general method I for the synthesis of amino-hydroxamic acids, the title compound was prepared in 80% yield. The reaction mixture was stirred for 24 hours before keeping it at 50°. The title compound had m.p. 147-148°.

Anal. Found: C, 50.61; H, 8.85; N, 11.56. $C_{10}H_{21}N_2O_2Cl$ requires: C, 50.73; H, 8.94; N, 11.84.

IR spectrum (KBr disc): 1660 cm^{-1} (C = O).

PMR spectrum (DMSO-d_6): Very broad signal centered at 515 Hz for - O - H; very broad signal centered at 630 Hz for



$>\overset{+}{N}(\underline{H})-CH_2-\underset{CH_3}{CH}-$

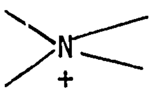
CO - NH -. The combined signal integrated for 3-protons.

2-Methyl-3-(4-n-propylpiperidino)propiono-hydroxamic acid hydrochloride

The title compound was obtained in 73% yield by employing general method I for the synthesis of amino-hydroxamic acids. The reactants were stirred for 48 hours at room temperature. The title product thus obtained had m.p. 171.5-173°.

Anal. Found: C, 54.33; H, 9.32; N, 10.55. $C_{12}H_{25}N_2O_2Cl$ requires: C, 54.43; H, 9.52; N, 10.58.

IR spectrum (KBr disc): 1628, 1665 cm^{-1} (C = O).

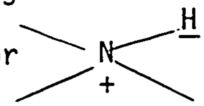
PMR spectrum (DMSO): Very broad signal centered at 535 Hz for - NH - O$\underline{H}$; very broad signal centered at 603 Hz for - $\overset{O}{\overset{\|}{C}}$ - N$\underline{H}$ - O -; broad singlet at 655 Hz for  ; all exchanged in D_2O.

2-Methyl-3-(4-phenyl-piperidino)propiono-hydroxamic acid hydrochloride

General method I for the synthesis of amino-hydroxamic acids was employed for the preparation of the title compound. The reaction mixture was stirred for 48 hours at room temperature prior to keeping at 50°. The title compound was obtained in 78% yield and had m.p. 199.5-200.5°.

Anal. Found: C, 60.17; H, 7.79; N, 9.27. $C_{15}H_{23}N_2O_2Cl$ requires: C, 60.29; H, 7.76; N, 9.38.

IR spectrum (KBr disc): 1605, 1655 cm^{-1} (C = O).

PMR spectrum (DMSO-d_6): very broad signal centered at 533 Hz, for - O - $\underline{H}$; very broad signal centered at 604 Hz, for $\overset{O}{\overset{\|}{C}}$ - N$\underline{H}$ - O - H; broad singlet at 655 Hz for  .

2-Methyl-3-morpholinopropiono-hydroxamic acid hydrochloride

The title compound was prepared in 71% yield using general method I for the synthesis of amino-hydroxamic acids. The reaction mixture was stirred for 24 hours at room temperature before keeping it at 50°. The title compound had m.p. 156-157°.

Anal. Found: C, 42.62; H, 7.56; N, 12.49. $C_8H_{17}N_2O_3Cl$ requires: C, 42.75; H, 7.62; N, 12.47.

IR spectrum (Nujol mull): 1620, 1655 cm^{-1} (C = O).

2-Methyl-3-(2,6-dimethylmorpholino)propiono-hydroxamic acid hydrochloride

The title compound was obtained in 73% yield when general method I for the synthesis of amino-hydroxamic acids was employed. The reactants were stirred for 3 days at room temperature. The product had m.p. 196-197°.

Anal. Found: C, 47.80; H, 8.40; N, 11.16. $C_{10}H_{21}N_2O_3Cl$ requires: C, 47.52; H, 8.38; N, 11.09.

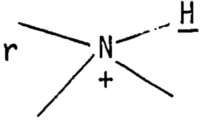
IR spectrum (Nujol mull): 1620, 1655 cm^{-1} (C = O).

2-Methyl-3-homopiperidino-hydroxamic acid hydrochloride

Using general method I for the synthesis of amino-hydroxamic acids, the title compound, m.p. 193.5-194° was obtained in 76% yield. The reaction mixture was kept stirred for 18 hours at room temperature.

Anal. Found: C, 50.49; H, 9.21; N, 11.62. $C_{10}H_{21}N_2O_2Cl$ requires: C, 50.73; H, 8.94; N, 11.84.

IR spectrum (Nujol mull): 1620, 1660 cm^{-1} (C = O).

PMR spectrum (DMSO): very broad singlet centered at 533 Hz for - O - $\underline{H}$, very broad singlet centered at 603 Hz for - $\overset{O}{\overset{\|}{C}}$ - N - $\underline{H}$ - OH; broad singlet at 656 Hz for  .

3-Benzylamino-propiono-hydroxamic acid hydrochloride

General method I for the synthesis of amino-hydroxamic acids, was employed for the preparation of the title compound. The reaction mixture was stirred for 10 hours at room temperature. This gave the title product in 79% yield, m.p. 152-153°.

Anal. Found: C, 52.52; H, 6.70; N, 12.13. $C_{10}H_{15}N_2O_2Cl$ requires: C, 52.06; H, 6.55; N, 12.15.

IR spectrum (Nujol mull): 1640, 1670 cm^{-1} (C = O).

PHARMACOLOGY

METHODS

1. Blood pressure and heart rate in anaesthetized cats

Experiments were conducted on cats (2 to 4 kg) of both sexes anaesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg). The operative procedures were the same as used by Malhotra et al (1961). The legs of the cat were tied to the operating table with the cat lying in a supine position, and its head extended. The skin and fascia were cut out from the sternum to the jaw. The trachea was exposed by dividing the overlying muscle in the midline. An incision was made in the trachea. A tracheal cannula was inserted into the trachea and was tied in place to keep the airway patent. The carotid arteries were then exposed and were separated from the accompanying vagosympathetic trunks. One of the common carotid arteries was tied as near to the head as possible. A bull dog clip was then placed on the artery 3 to 5 cm nearer the heart and a thread was passed around the artery. A small nick was made in the artery, and the arterial cannula connected to the Statham Physiological Transducer, was inserted into the common carotid artery and tied in place. The arterial blood pressure (B.P.) was recorded from the common carotid artery using the Statham Pressure Transducer.

One of the femoral veins was exposed and a venous cannula connected to a burette was inserted into the vein for infusion of drugs into the system.

The carotid baroreceptor reflex was tested by occluding both the common carotid arteries for 30 seconds, the femoral arterial blood pressure having been recorded.

The standard limb lead II was used for taking electrocardiograms (E.C.G.) of the anaesthetized cat to record only the changes in the heart rate.

Blood pressure and heart rate were recorded simultaneously on the physiograph.

2. Nictitating membrane preparation

Cats of either sex weighing 2 to 4 kg were anaesthetized as above. In each case, the trachea was cannulated. The left cervical sympathetic trunk was dissected free from the vagus nerve and cut at a point 2 to 2.5 cm caudal to the superior cervical ganglion. The superior cervical ganglion and the post ganglionic fibres were also exposed. For electrical stimulation of pre- and post-ganglionic fibres a pair of platinum electrodes was placed under the fibres. Rectangular pulses of supramaximal voltage at a frequency of 1/second and 1 millisecond duration were applied with the Grass Stimulator.

The left common carotid artery was exposed to the branch point of the internal and external carotid arteries. The left external carotid artery above the branch point was also exposed. The drug was administered slowly to the ganglion through the common carotid artery, the external carotid artery being occluded during the injection period thus allowing the drug to act directly on the superior cervical ganglion.

In a few experiments the drug was applied directly to the ganglion with a cotton swab. The head of the cat was fixed in a rigid position. By means of a fine needle, a silk thread was passed through the centre of the edge of the left nictitating membrane and was tied. The other end of the silk thread was taken outward and forward so as to be at an angle of 30° from the axis of the cat to a force displacement transducer. Changes in the tension of the nictitating membrane were recorded through the force displacement transducer on an Offner Dynograph with an applied tension of 4 to 6 g. The preganglionic sympathetic chain was stimulated to maintain steady contraction of the nictitating membrane. The drug was then applied to the ganglion either through infusion or direct swab and the stimulation of the pre- and post-ganglionic fibres was repeated.

3. Vagal ganglion preparation

Cats of either sex weighing 2 to 4 kg were anaesthetized and the B.P. and the E.C.G. were recorded as described earlier in the method section. The vagosympathetic trunk on the right side was exposed and the vagus nerve separated from the sympathetic trunk. The vagus nerve was then cut and the cardiac end of the cut right vagus was stimulated electrically for 5 to 15 seconds and the effects were recorded in lead II of the E.C.G. The electrical stimulation was applied with the help of the Grass Stimulator giving rectangular pulses of 5 to 10 volts, at 60/second frequency and of 0.5 millisecond duration. The cardiac end of the vagus was stimulated before and after the administration of drug and the effects on the E.C.G. and B.P. were observed.

4. Isolated Guinea-pig Ileum

Experiments were carried out with pieces of ileum (1.5 to 2.0 cm long) removed from the animal after it was stunned by a sharp blow on the head. The tissue was suspended in a jacketed 15 ml organ bath containing modified Kreb's-Ringer solutions of the following compositions in milliequivalents per litre: Na, 138.5; K, 4.6; Ca, 4.9; Mg, 2.3; HCO_3, 21.91; PO_4, 3.48; Cl, 125; and glucose 5 mM. The solution was equilibrated with 95% oxygen and 5% carbon dioxide and the temperature was maintained at 37°. The contractions were recorded at a resting tension of 2 to 3 g by means of the Statham force displacement transducer on an Offner Dynograph. The tissue was equilibrated in oxygenated modified Krebs-Ringer solution for 30 minutes before the effects of drugs were investigated.

All the solutions of the drugs (hydroxamic acid hydrochlorides and ester hydrochlorides) were made in purified water (distilled twice).

RESULTS

Effects of some of the compounds on general behavior of rats and cats

Some of the compounds were administered either intraperitoneally or intravenously to rats in the various doses range as shown in Table III, and the effects on the general behavior were noted. These compounds produced an increased spontantaneous activity and an increased response to external stimuli. The external stimuli sometimes produced clonic type of convulsions in the drug-treated excited rats. The stimulation was always followed by depression. These effects lasted for 2 to 3 hours. The degree of abnormal behavior was varied, and dependent upon the substituents in the piperidine ring. Thus compound 3-piperidinopropiono-hydroxamic acid hydrochloride (K71) did not produce any apparent change in the general behavior of rats while compound 2-methyl 3-(4-phenylpiperidino-)propiono-hydroxamic acid hydrochloride (K76) had marked effects on general behavior. None of the rats died even with large doses of the drug when administered intraperitoneally. However, the intravenous administration of compound 2-methyl-3-(4-n-propylpiperidino)propiono-hydroxamic acid hydrochloride (K75) in 50 mg/kg dose had lethal effect in one out of 6 rats.

The compounds K75 and K76 were administered subcutaneously (30 mg/kg) and intravenously (15 mg/kg) each dose in 5 conscious cats and the effects were noted for two hours. Intravenous administration of K75 and K76 produced an immediate effect on the nictitating membrane. The whole eye was covered with nictitating membrane indicating the relaxation of the membrane. There was a decrease in the heart rate. There was a varied and mild effect on the size of the pupil. Subcutaneous administration of drug produced effects similar to the intra-

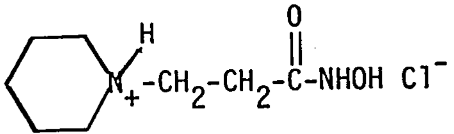
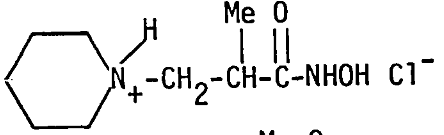
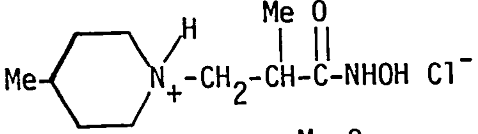
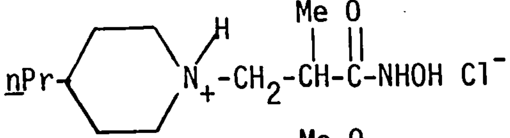
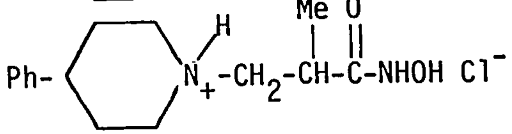
Pharm. Code No.	Compound	No. of rats which survived	Route of administeration	Dose mg/kg
K71		6 out of 6	I.P.	400
K72		12 out of 12	I.P.	100-300
K74		12 out of 12	I.P.	100-300
K75		11 out of 12 5 out of 6	I.P. I.V.	100-300 50
K76		12 out of 12	I.P.	100-300

TABLE III

Effects of 3-piperidino-propiono-hydroxamic acid hydrochlorides on survivability of rats venous administration except that the onset of the effects was slow and the effects were not well marked.

Effects of 3-monoalkylamino-hydroxamic acid hydrochlorides and ester hydrochlorides on the blood pressure and heart rate of anaesthetized cats

The effects of intravenous administration of various 3-monoalkylamino-hydroxamic acids and the corresponding methyl esters were investigated on the blood pressure and heart rate of anaesthetized cats. The results of this preliminary study are summarised in Table IV. All the compounds produced a fall in the blood pressure to a varying degree except K66 and K87 which caused a rise in blood pressure. All the compounds in this study produced either a decrease or no change in the heart rate except K87 which caused an increase in the heart rate.

Effects of various 3-dialkylamino-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on the blood pressure and heart rate of anaesthetized cats

A preliminary study with intravenous administration of five 3-dialkylamino-hydroxamic acid hydrochlorides and corresponding ester hydrochlorides on the blood pressure and heart rate, showed that all of them produced a fall in the blood pressure except the ester K22 which caused a rise in blood pressure. All of the compounds studied in this series produced a decrease in the heart rate although to a varying degree. The results are shown in Table V.

TABLE IV

Effects of 3-monoalkylamino-hydroxamic acid hydrochlorides and corresponding ester hydrochlorides on arterial blood pressure and heart rate of anesthetized cats.[a] The values are expressed as percent change from the control (before treatment).

		Hydroxamic Acids						Esters			
Pharm. Code No.[b]	Compound	Dose mg/kg	Percent Change in B.P.	Duration (min.)	Percent change in heart rate	Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate
K87	$\mathrm{Me\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-}$	25	+33	10	+2.1	K63	$\mathrm{Me\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	25	-62	5	- 4.6
K96	$\mathrm{Et\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-}$	25	-53	5	-6.3	K70	$\mathrm{Et\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	25	-48	10	0.0
K90	$\mathrm{\underline{n}Pr\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-}$	25	-30	15	-4.7	K66	$\mathrm{\underline{n}Pr\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	25	+27	50	- 6.59
K88	$\mathrm{\underline{n}Bu\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-}$	25	-39	15	-2.0	K64	$\mathrm{\underline{n}Bu\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	25	-13	10	0.0
K98	$\mathrm{\underline{n}Hex\overset{+}{N}H_2CH_2CH_2CONHOH\ Cl^-}$	25	-40	130	-9.8	K89	$\mathrm{\underline{n}Hex\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	25	-87	80	-14.8
						K89	$\mathrm{nHex\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	10	-54	10	-11.9
						K89	$\mathrm{\underline{n}Hex\overset{+}{N}H_2CH_2CH_2CO_2Me\ Cl^-}$	5	-42	8	

[a] Averagesof at least two results are given.

[b] These identification numbers are used in pharmacology result and discussion sections.

(+) denotes increase and (-) denotes decrease in heart rate and blood pressure.

TABLE V

Effects of 3-dialkylamino-hydroxamic acid hydrochloridesand corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.[a] The values are expressed as percent change from the control (before treatment).

Hydroxamic Acids						Esters					
Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate	Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate
K16	$\mathrm{Me_2\overset{+}{N}HCH_2CH_2CONHOH\ Cl^-}$	10 25	-11 -56	2.5 2.5	- 4.2	K15	$\mathrm{Me_2\overset{+}{N}HCH_2CH_2CO_2Et\ Cl^-}$	25	-38	5	-10.6
K23	$\mathrm{Me_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCONHOH\ Cl^-}$	10 25	-52 -58	20 30	- 6.0	K21	$\mathrm{Me_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCO_2Me\ Cl^-}$	25 10 5	-67 -72 -20	115 90 14	-25.7 -12.4
K50	$\mathrm{Me_2\overset{+}{N}H\underset{\substack{\mid \\ Me}}{C}HCH_2CONHOH\ Cl^-}$	25	-39	2.5	- 7.9	K22	$\mathrm{Me_2\overset{+}{N}H\underset{\substack{\mid \\ Me}}{C}HCH_2CO_2Me\ Cl^-}$	25	+54	5	-21.1
K34	$\mathrm{Et_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCONHOH\ Cl^-}$	25	-50	15	- 6.0	K33	$\mathrm{Et_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCO_2Me\ Cl^-}$	25	-39	5	-29.4
K40	$\mathrm{\underline{n}Pr_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCONHOH\ Cl^-}$	25	-41	20	-12.1	K31	$\mathrm{\underline{n}Pr_2\overset{+}{N}HCH_2\underset{\substack{\mid \\ Me}}{C}HCO_2Me\ Cl^-}$	25	-28	5	-21.6

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[a] The figure shown in each case is the average of two results.

[b] These identification numbers are used in the pharmacology results and discussion.

(+) denotes increase and (-) denotes decrease in blood pressure and heart rate.

Effects of various ring substituents in 3-position of propiono-hydroxamic acid hydrochlorides and the corresponding esters on the blood pressure and heart rate of anaesthetized cats

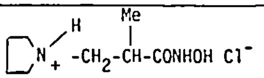
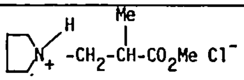
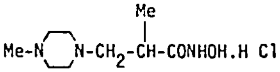
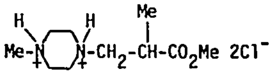
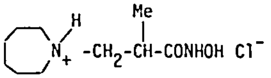
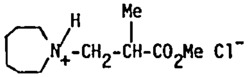
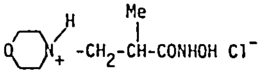
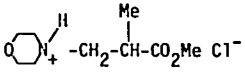
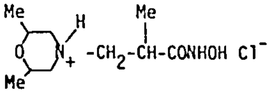
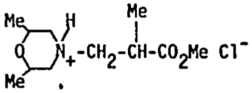
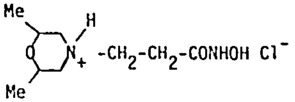
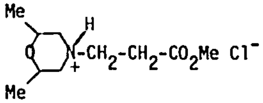
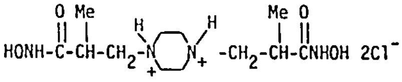
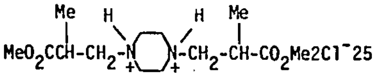
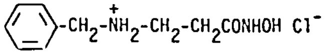
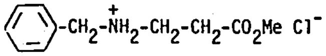
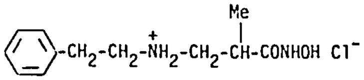
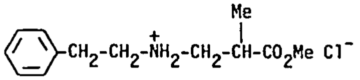
Preliminary investigations of intravenous administration of nine 3-substituted propiono-hydroxamic acid hydrochlorides and nine corresponding ester hydrochlorides on the blood pressure and heart rate of anaesthetized cats were made. All of them produced a fall in the blood pressure and decrease in heart rate. The results are summarised in Table VI.

Effects of various alkyl and aryl substituents in the piperidine ring of 3-(substituted-piperidino)propiono-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on the blood pressure and heart rate of anaesthetized cats

Preliminary studies of the intravenous administration of various 3-(substituted-piperidino)propiono-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on the blood pressure and heart rate showed that all of them caused a fall in the blood pressure and a decrease in the heartrate except the compound K52 which produced a rise in blood pressure and an increase in heart rate. The results are given in Table VII.

TABLE VI

Effects of various amino-substituents in 3-position of propiono-hydroxamic acid hydrochlorides and corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.[a] The values are expressed as percent change from the control (before treatment).

	Hydroxamic Acids						Esters				
Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate	Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate
K92		25	-38	20	-11.1	K83		25	-48	15	- 6.2
K93		25	-43	10	- 9.2	K82		25	-43	5	- 4.1
K81		25	-33	10	- 5.1	K67		25	-21	5	- 8.9
K77		25	-42	5	- 2.0	K61		25	-41	5	- 1.9
K78		25	-22	10	- 4.3	K62		25	-44	2.5	- 4.0
K104		25	-45	5	-2.1	K102		25	-13	2.5	- 2.0
K 73		25	-26	30	- 4.2	K53		25	-43	5	- 2.1
K91		25	-35	70	- 3.4	K65		25	-35	25	-12.5
K97		25	-50	100		K86		10	-35	30	-12.8
		10	-39	81	- 2.5			5	-18	8	
		5	-17	67	- 2.2						

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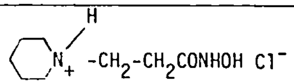
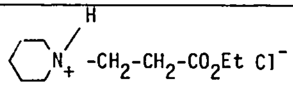
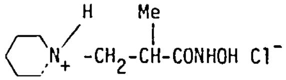
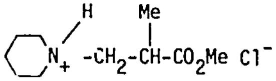
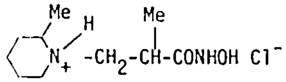
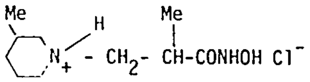
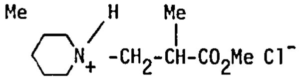
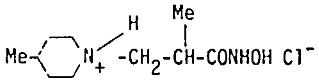
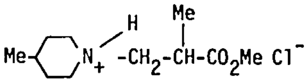
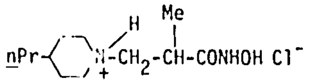
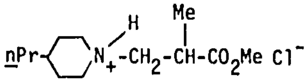
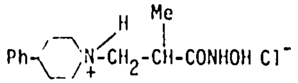
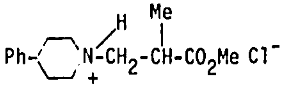
[a] Averagesof at least two results are given.

[b] These identification numbers are used in the pharmacology results and discussion.

(-) denotes fall in blood pressure and decrease in heart rate

TABLE VII

Effects of various 3-(substituted piperidino)propiono-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.[a] The values are expressed as percent change from the control (before treatment).

Hydroxamic Acids						Esters					
Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (Min.)	Percent change in heart rate	Pharm. Code No.[b]	Compound	Dose mg/kg	Percent change in B.P.	Duration (min.)	Percent change in heart rate
K71		25	-36	5	0.0	K51		25	-42	5	- 6.3
K72		25	-48	10	- 3.4	K52		25	+30	5	+ 5.1
K95		25	-33	5	- 5.5						
K94		25	-39	5	- 2.6	K80		25	-43	5	-11.3
K74		25	-56	10	- 3.8	K54		25	-38	5	- 8.2
K75		25	-45	120	-13.1	K55		25	-35	5	- 7.1
		10	-34	93	- 5.9						
		5	-31	68	- 3.0						
K76		25	-81	160	-16.7	K56		25	-44	30	- 9.2
		15	-65	148	- 9.8			10	-35	20	- 6.5
		10	-64	123	-14.8						
		5	-57	90	-25.2						
		2.5	-53	70	- 4.9						

[a] Averages of at least two results are given.

[b] These identification numbers are used in the pharmacology results and discussion.

(+) denotes increase and (-) decrease in heart rate and blood pressure

Effects of Ethyl 4-(4-phenyl-piperidino)butyrate hydrobromide on the blood pressure and heart rate of anaesthetized cats

A preliminary study with intravenous administration of 5 mg/kg of ethyl 4-(4-phenyl-piperidino)butyrate hydrobromide on the blood pressure and heart rate showed that the compound produced a 33% fall in blood pressure and 8.8% decrease in heart rate. The blood pressure came back to normal after 53 minutes.

Effects of 3-substituted amino-acids on the blood pressure and heart rate of anaesthetized cats

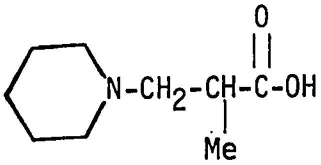
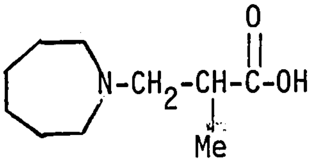
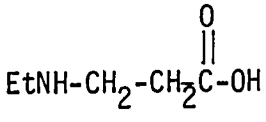
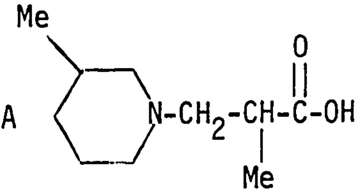
Aqueous solutions of 3-substituted-amino-acids were administered intravenously to observe their effects on the blood pressure and heart rate of anaesthetized cats. The results are summarized in Table VIII. None of the acids tested were found to have any effect on blood pressure or heart rate.

Detailed pharmacological investigation of 2-methyl-3-(4-phenylpiperidino)-propiono-hydroxamic acid hydrochloride (K76) as to establish its mechanism of fall in blood pressure

One of the compounds, namely 2-methyl-3-(4-phenylpiperidino) propiono-hydroxamic acid hydrochloride (K76) was selected for detailed investigation of the mechanism of fall in the blood pressure.

TABLE VIII

The effects of intravenous administration of 3-substituted–amino-acids on the blood pressure and heart rate of anaesthetized cats. The values are expressed as percent decrease from the control (before treatment). Two experiments on each compound were performed.

Pharm. Code No.	Compound	Dose mg/kg	Effect on B.P.	Effect on the heart rate
K52A		25	Nil	Nil
K67A		25	Nil	Nil
K70A	 $\mathrm{EtNH{-}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}OH}$	25	Nil	Nil
K80A		25	Nil	Nil

Effect of 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride (K76) on the blood pressure of anaesthetized cats

Fifteen experiments were carried out in which various doses (1.25, 2.5, 5.0, 10.0, 15.0, and 25 mg/kg) of K76 were administered intravenously into anaesthetized cats. Typical responses after the administration of 15 mg/kg of the drug are shown in Fig. 4 . The drug produced a maximum fall in the blood pressure immediately after injection and there was a lapse of 2 to 3 hours before the blood pressure reached the control level. This fall in the blood pressure produced by K76 was dose dependent (Table IX).

Effect of K76 on heart rate

Intravenous administration of various doses (2.5, 5.0, 10.0, 15.0, 25.0 mg/kg) of K76 into cats initially produced a decrease in the heart rate associated with a fall in the blood pressure. Later on the heart rate not only returned to control level but there was a tendency towards an increase over the control value (Fig. 4). This decrease in the heart rate was variable and not dose dependent (Table X).

Effect of K76 on the blood pressure in atropinized cats

To determine if this fall in the blood pressure produced by K76 was due to acetylcholine-like effects, six experiments were carried out in which the effects of intravenous administration of K76 (5 mg/kg) on the blood pressure of atropine treated (4 mg/kg) cats were investigated. It was observed that although atropine was able to block the effects of

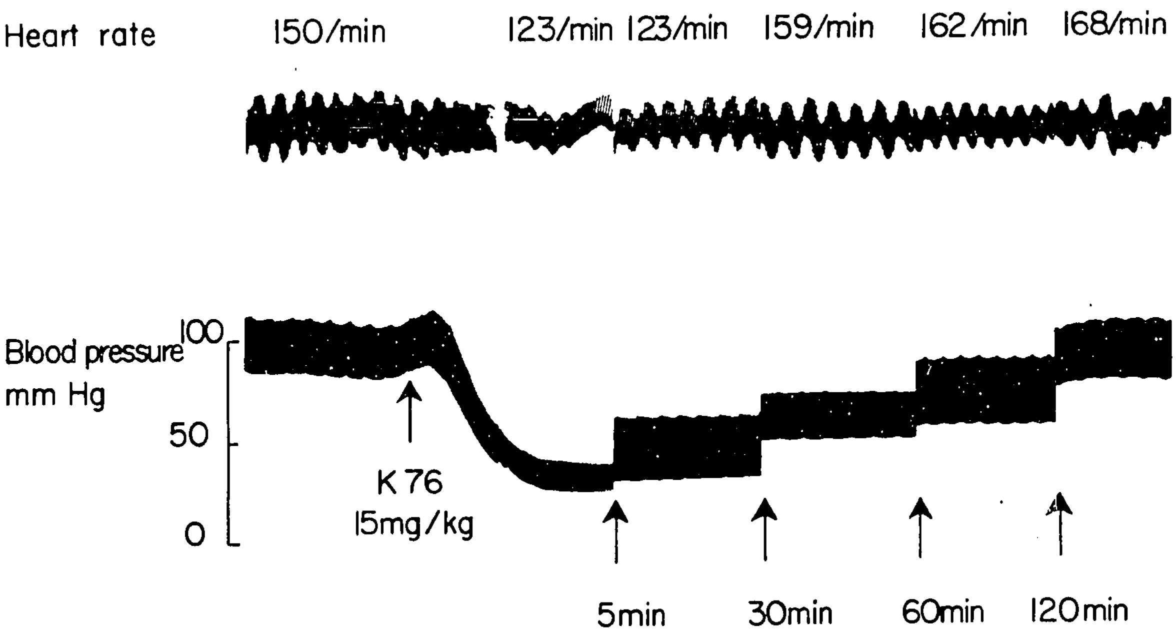


Fig. 4 Effects of intravenous administration of K76 15 mg/kg on the blood pressure and heart rate of anaesthetized cat. Note the prolonged fall in B.P. and initial bradycardia followed by tachycardia.

TABLE IX

The effects of various doses of K76 on the blood pressure of anesthetized cats. The values are expressed as percent fall from the control (before treatment) taken as 100%.

Dose mg/kg, I.V.	No. of expts.	Percent fall in B.P.	Mean ± S.E.
1.25	1	16.7	--
2.5	4	52 43 57 59	53 ± 3.57
5	3	43 64 65	57 ± 7.17
10	3	65 74 52	64 ± 6.39
15	2	59 70	65 ± 5.50
25	2	89 72	81 ± 8.50

TABLE X

The effects of I.V. administration of various doses of K76 on the heart rate of anesthetized cats. The values are expressed as percent decrease from the control (before treatment) taken as 100%. Note the varied effects on the heart rate.

Dose mg/kg, I.V.	No. of expts.	Percent decrease in heart rate	Mean ± S.E.
2.5	4	0.0 7.2 6.0 1.6	4.9 ± 1.72
5	2	18.5 32.0	25.2 ± 6.78
10	2	11.5 18.0	14.8 ± 3.18
15	2	9.8 9.7	9.8 ± 0.05
25	2	11.5 21	16.7 ± 4.75

acetylcholine (1 μg/kg) completely, it was unable to block the effects of K76 on the blood pressure. These effects were observed in all six experiments. Figure 5 is representative of the observed effects in a single experiment.

Effect of epinephrine on the blood pressure of K76-treated cats

Six experiments were carried out in which the effect of epinephrine (1 μg/kg) on the blood pressure of K76-treated (5 mg/kg) cats were investigated to find out whether the fall in the blood pressure produced by K76 was sympatholytic in nature. The effect of epinephrine on blood pressure was not blocked by K76. On the other hand epinephrine-induced rise in the blood pressure was potentiated in the K76 treated cats. Figure 6 is representative of the effects which were observed in all six experiments.

Interaction between the effects of K76 and nicotine on blood pressure

Three experiments were conducted to find out whether the fall in the blood pressure produced by K76 was due to a ganglion blocking property. Nicotine in the dose of 10 μg/kg was administered intravenously in 3 cats to produce a rise in the blood pressure. The cats were injected with K76 (5 mg/kg) and while its effects were being observed, nicotine (10 μg/kg) was administered intravenously. It was observed in all the three experiments that the effect of nicotine was completely blocked in the K76-treated cats. A representative of the results with such treatment is shown in Fig. 7.

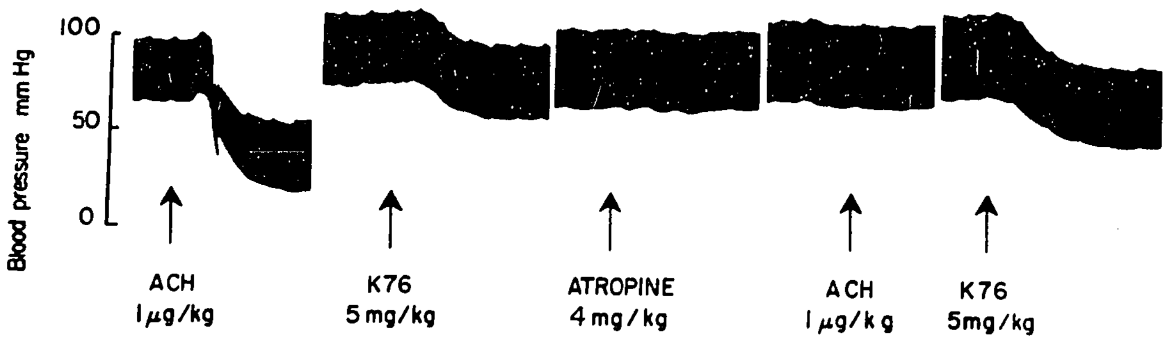


Fig. 5 Effects of K76 on the B.P. in atropine-treated cats. Note that acetylcholine (1µg/kg) and K76 (5 mg/kg) produced fall in the B.P. before atropine treatments. Note also that after atropine, the effect of acetylcholine (1 µg/kg) was completely blocked while that of K76 still persisted.

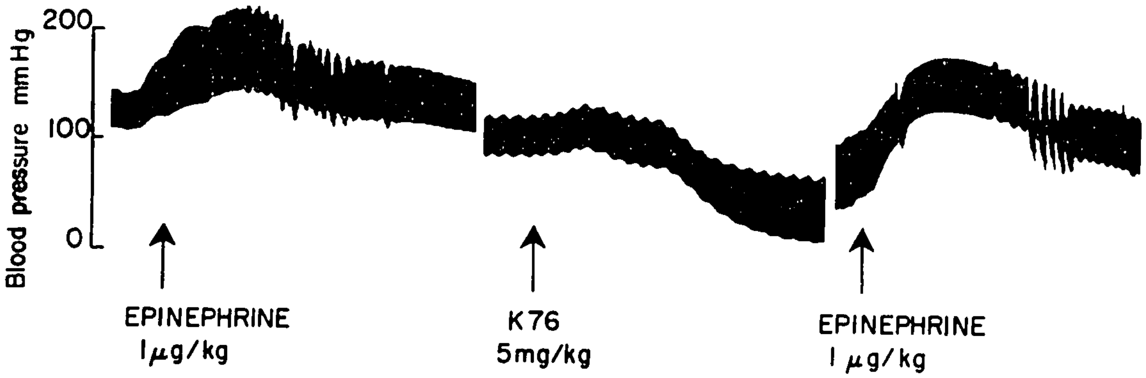


Fig. 6 Effects of epinephrine on the B.P. of K76-treated cats. Epinephrine was administered 15 minutes after K76 administration. Note that the epinephrine-induced rise in B.P. was not blocked in the presence of K76. On the other hand there was a potentiation of the effect of epinephrine on the B.P.

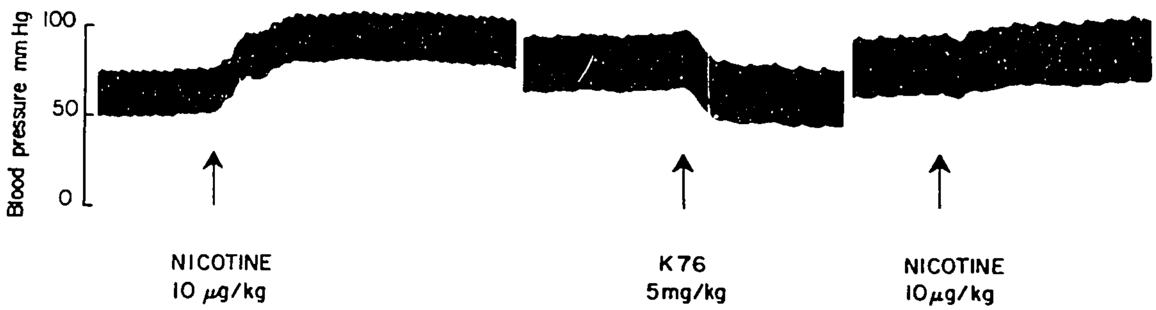


Fig. 7 Effects of nicotine on the B.P. of K76-treated cats. Nicotine (10 μg/kg) was administered I.V. to the cat before and after K76 administration. Note that the pressor response of nicotine was completely blocked in the presence of K76 when it was administered 15 minutes after K76.

Effect of K76 on the acetylcholine-induced contraction in the isolated guinea-pig ileum

Four experiments were carried out in which the response of guinea-pig ileum to acetylcholine was tested in the absence and in the presence of K76. The results are shown in Fig. 8. It was observed that K76 was completely ineffective in antagonizing the acetylcholine-produced contraction of the ileum.

The results of the experiments carried out in the preceding sections indicated that K76 might be producing a fall in the blood pressure through blockade of the sympathetic ganglia. To ascertain if this was the case the following sets of experiments were carried out.

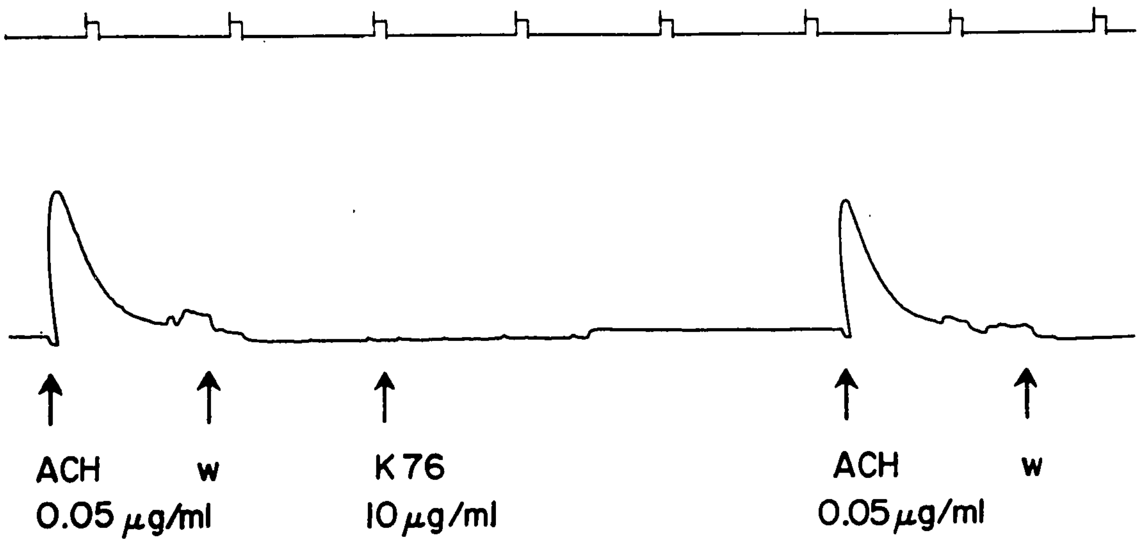


Fig. 8 Effects of K76 on the acetylcholine-induced contraction of a piece of guinea pig ileum.
Upper tracing - indicates the time mark every 2.5 minutes.
Lower tracing - contraction of guinea pig ileum.
W - wash.

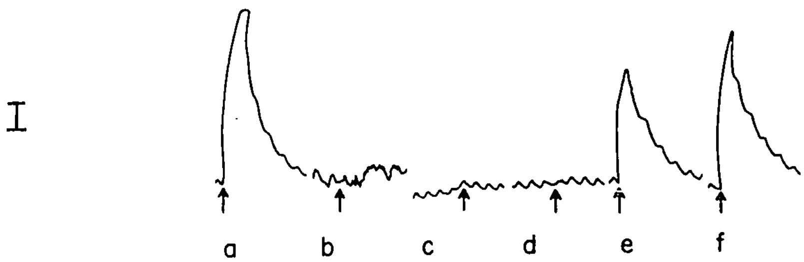
Effect of K76 on the superior cervical ganglia

Six experiments were carried out in which the pre- and post-ganglionic fibres were stimulated to produce a steady contraction in the nictitating membrane. The drug (K76) in the total dose of 0.25 or 1.0 mg was then injected into the common carotid artery while occluding the external carotid artery. The preganglionic fibres were stimulated and the effects on the contraction of the nictitating membrane were observed. It was noted that in four cats the intra-arterial injection of 0.25 or 1.0 mg of K76 completely inhibited the contraction of the nictitating membrane when the cut cranial end of the ipsilateral sympathetic trunk (preganglionic) fibres was electrically stimulated. The nictitating membrane, however, responded to the stimulation of the post-ganglionic fibres (Fig. 9I). It was also observed that the time for complete inhibition varied between 3 to 9 minutes and lasted for 30 to 50 minutes (Table XI). The same results were obtained in two cats when 25 mg/kg of K76 was injected intravenously (Table XII). In three cats, where the drug in 5% solution was directly applied to the superior cervical ganglion, the results were the same (Fig. 9II, Table XIII). The drug in 0.5% solution when applied locally to the superior cervical ganglion in three cats, however, produced only a 25% inhibition of the contraction produced by preganglionic stimulation.

Effect on vagal ganglion

In nine cats, intravenous administration of 2.5 to 25 mg/kg of K76 blocked the cardio-decelerator effect of the electrical stimulation

K 76 injected into the common carotid artery



K 76 5 % solution by direct swab to superior cervical ganglion

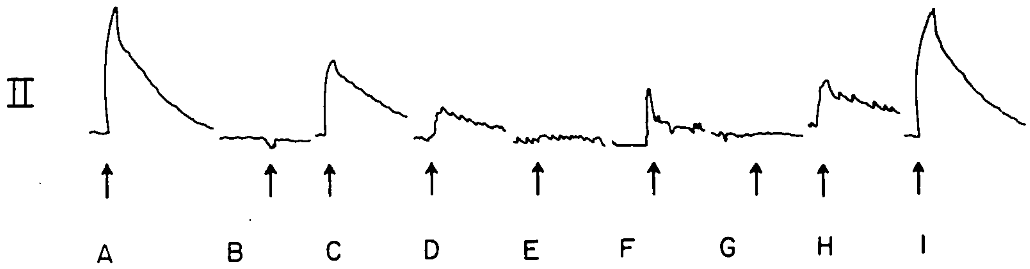


Fig. 9 Effects of K76 on the superior cervical ganglion of anaesthetized cat.

I. The effects of K76 (1mg) injected via the common carotid artery to the superior cervical ganglion, on the contraction of the nictiating membrane produced by the stimulation of the pre- and post-ganglionic fibres. a, contraction of the nictitating membrane to preganglionic stimulation; b, intra-arterial administration of K76 (1 mg); c and d, preganglionic stimulation; e and f, indicate post ganglionic stimulation. Note that K76 completely blocked the contraction of the nictitating membrane produced by preganglionic stimulation. Note also that postganglionic stimulation could still produce contraction of the nictitating membrane.

II. The effects of local application of K76 to the superior cervical ganglion. A,C,D,E,G, and I - responses of the nictitating membrane to stimulation of the preganglionic fibres; B, local application of a 5% solution of K76 on the superior cervical ganglion; F and H, responses of the nictitating membrane to postganglionic stimulation; I, response of the nictitating membrane to stimulation of preganglionic fibres after 5 - 6 washings of the superior cervical ganglion for 10 minutes with normal saline.

Note that the contraction of the nictitating membrane to preganglionic stimulation was completely blocked by K76 while post ganglionic stimulation could still produce contraction of the nictitating membrane. Note also that repeated washings of the superior cervical ganglion restored the responses of the nictitating membrane to preganglionic stimulation.

TABLE XI

The effect of intra-arterial administration of K76 on the responses of the nictitating membrane of anesthetized cats to stimulation of preganglionic fibres. The values are expressed as percent inhibition of the control (before treatment) contraction of the nictitating membrane produced by preganglionic stimulation.

Total Dose mg	No. of expts.	Percent inhibition	Time (mins.) to max. inhibition	Time for restoration of response to preganglion stimulation	Mean ± S.E.
0.25	2	100	5	30	34 ± 4.40
		100	3	38	
1.0	2	100	3	51	48.5 ± 2.50
		100	9	46	

TABLE XII

The effect of intravenous administration of K76 on the responses of the nictitating membrane of anesthetized cats to stimulation of preganglionic fibres. The values are expressed as percent inhibition of the control (before treatment) contraction of the nictiating membrane produced by preganglionic stimulation

Dose mg/kg	No. of expts.	Percent inhibition	Time (mins.) to max. inhibition	Time for restoration of response to preganglion stimulation	Mean ± S.E.
25	1	100	6	85	82.5 ± 2.5
25	1	100	9	80	

TABLE XIII

The effect of direct application of K76 to the superior cervical ganglion of anesthetized cat on the responses of the nictitating membrane to stimulation of preganglionic fibres. The values are expressed as percent inhibition of the control (before treatment) contraction of the nictitating membrane produced by preganglionic stimulation.

Concentration of K76	No. of expts.	Mean time (mins.) to Max. inhibition ± S.E.
5%	3	7 ± 0.06
10%	1	6

of the cardiac cut end of the right vagus to a varying degree (Table XIV).

Effect of K76 on the pressor responses to carotid arteries occlusion

In four cats, the intravenous administration of K76 in the dose of 5 to 25 mg/kg blocked markedly and sometimes completely the pressor responses to the occlusion of both common carotid arteries (Fig. 10).

Effect on the isolated guinea-pig ileum

Nineteen experiments were carried out in which guinea-pig ileum was exposed to various concentrations (1,5,10,20,30,40 μg/ml) of K76. It was observed that in none of the concentrations the compound was effective in producing either contraction or relaxation, thus excluding any parasympathomimetic or sympathomimetic properties of the compound (Table XV).

Effect of K76 on the nicotine-induced contractions in isolated guinea-pig ileum

K76 in the concentration of 5,10,20 and 30 μg/ml blocked the contraction response of the ileum to nicotine (1 μg/ml) to a varying degree (Table XVI). Although K76 was effective in blocking the effect of nicotine, it was ineffective in blocking acetylcholine-induced contraction of isolated guinea-pig ileum (Fig. 11).

TABLE XIV

The effect of various doses of K76 on the cardiac decelerator effect of stimulation of the cardiac end of the cut right vagus in anesthetized cat.

Dose mg/kg, I.V.	No. of expts.	Percent decrease in the heart rate Before drug	After drug	Percent ganglion block	Mean ± S.E.
2.5	3	56	50	10.7	
		35	24	31.4	14 ± 9.20
		35	35	0.0	
5	2	41	32	21.9	26.5 ± 4.55
		49	34	31	
10	1	54	13	75	
15	1	54	14	64	
25	2	32	8	75	87.5 ± 13.00
		20	0	100	

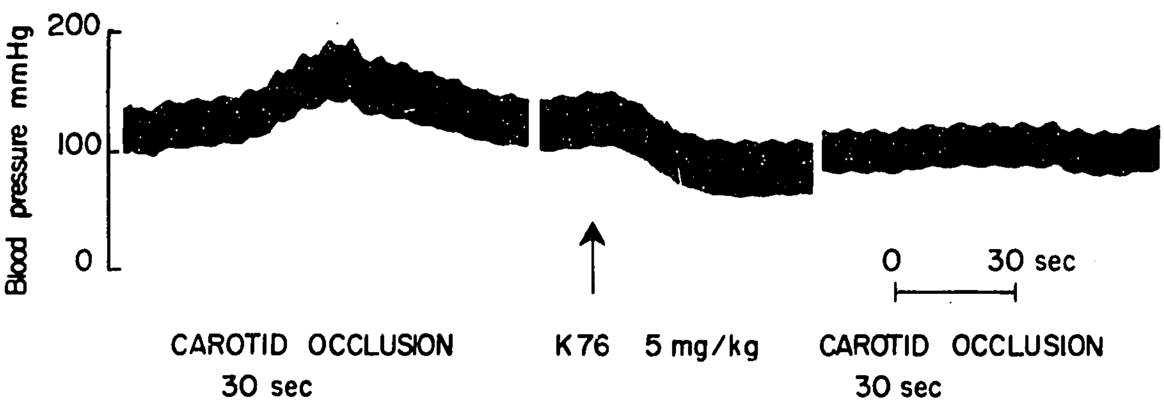


Fig. 10 Effects of K76 on the pressor responses to carotid occlusion in the anaesthetized cats. The carotid arteries were occluded for 30 seconds before and after K76 treatment. Note that the rise in B.P. due to carotid occlusion was completely blocked in the presence of K76.

TABLE XV

The effects of various concentrations of K76 on the isolated guinea-pig ileum

Concentration µg/ml	No. of expts.	Response (contraction or relaxation)
1	2	Nil
5	3	Nil
10	4	Nil
20	5	Nil
30	2	Nil
40	3	Nil

TABLE XVI

The effect of various concentrations of K76 on the nicotine-induced contraction in isolated guinea-pig ileum

Concentration of K76 μg/ml	Percent reduction in response to 1.0 μg/ml nicotine	Mean reduction in contraction ± S.E.
5	55 57 49	53 ± 2.40
10	76 85 80	80 ± 2.60
20	83 84 73	80 ± 3.51
30	100 100	100 ± 0

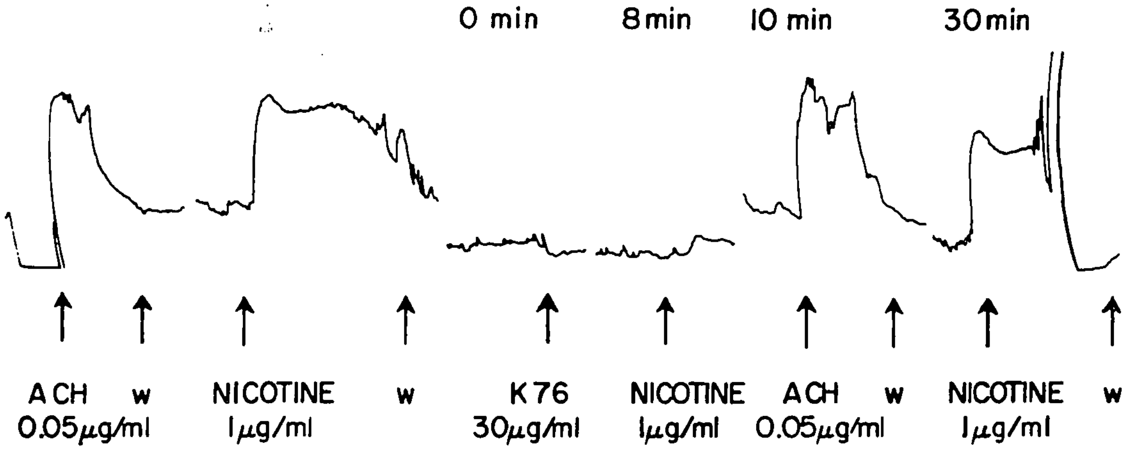
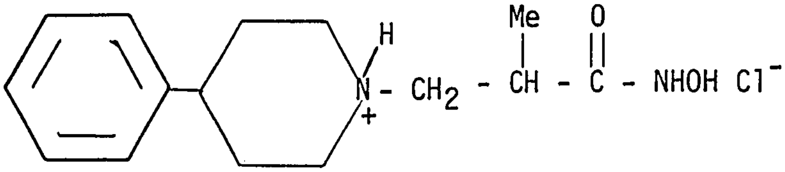


Fig. 11 The effects of K76 on the nicotine-induced contraction of isolated guinea pig ileum. Arrows indicate the start of exposure of the tissue to the drugs or washings with modified Kreb's-Ringer solution. Note that K76 completely blocked the effects of nicotine while the effects of acetylcholine could still be elucidated.



(K76)

These results confirmed the ganglion-blocking properties of K76. It was therefore decided to compare its potency in relation to a known ganglion blocking agent, pentolinium tartrate ("Ansolysen").

Comparison of the effects of K76 and Ansolysen on the blood pressure of anaesthetized cats

Experiments were carried out in which the equimolar doses of K76 (2.5 mg/kg) or Ansolysen (5 mg/kg) were administered intravenously, each into three anaesthetized cats and their effects on blood pressure were observed. The results of such experiments are given in Table XVII It was observed that K76 was 1.05 times as potent as Ansolysen in producing a fall in blood pressure and both effects persisted for nearly the same length of time.

Comparison of the effects of K76 and Ansolysen on the heart rate in anaesthetized cats

Equimolar doses of K76 (2.5 mg/kg) and Ansolysen (5 mg/kg) were administered each into three anaesthetized cats and their effects on the heart rate were observed. The results are summarized in Table XVIII.

TABLE XVII

Comparison of the effects of K76 and Ansolysen in equimolar doses on the B.P. of anaesthetized cats. The values are expressed as percent fall from the control (before treatment)

Drugs and Doses, I.V.	No. of expts.	Percent fall in B.P.	Mean percent fall in B.P. ± S.E.
K76 2.5 mg/kg	3	52 43 59	51.3 ± 4.63
Ansolysen 5.0 mg/kg	3	44 48 54	48.6 ± 2.91

TABLE XVIII

Comparison of the effects of K76 and Ansolysen on the heart rate of anaesthetized cats. The results are expressed as a percent of the control (before treatment).

Drugs and doses, I.V.	No. of expts.	Percent decrease in heart rate	Mean percent decrease in heart rate ± S.E.
K76 2.5 mg/kg	3	0 7.2 1.6	2.93 ± 2.18
Ansolysen 5 mg/kg	3	33.3 38 38	36.4 ± 1.57

The results showed that K76 was 0.083 as potent as Ansolysen in producing bradycardia in the anaesthetized cat.

Comparison of the effects of K76 and Ansolysen on the vagal ganglion

Ansolysen (5 mg/kg) or K76 (2.5 mg/kg) were administered intravenously each into three anaesthetized cats and the effects of stimulation of the cardiac end of the cut right vagus on the heart rate before and after such treatments were investigated. The results of such treatments are shown in Table XIX . It was observed that K76 was almost ineffective in blocking the bradycardia produced by vagal stimulation whereas Ansolysen in equimolar dose blocked the effect of vagal stimulation almost completely.

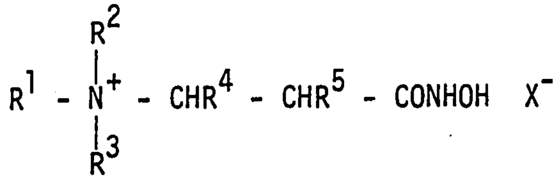
TABLE XIX

Comparison of the ability of K76 and Ansolysen to block the vagal ganglion in anaesthetized cats.

Drugs and doses, I.V.	No. of expts.	Percent decrease in heart rate caused by stimulation of vagus before drug		Percent decrease in heart rate caused by stimulation of vagus after drug	
		Decrease	Mean decrease ± S.E.	Decrease	Mean decrease + S.E.
K76 2.5 mg/kg	3	56 35 35	42 ± 7	50 24 35	36.3 ± 7.53
Ansolysen 5 mg/kg	3	56 21 36	38 ± 10.13	9.7 3 0	4.3 ± 2.85

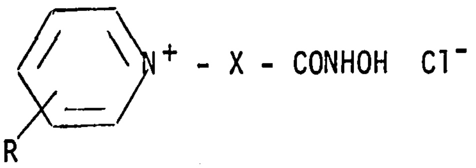
DISCUSSION

Long acting cholinesterase inhibitors, i.e. organophosphorus compounds, react with the enzyme cholinesterase to give phosphorylated cholinesterase which is extremely stable and is not easily hydrolysed by water. The findings by Wilson (1951) that choline and hydroxylamine reactivate phosphorylated cholinesterase led to the present knowledge of the reactivating properties of oximes and hydroxamic acids. Nicotinohydroxamic acid methiodide, picolinohydroxamic acid, and pyrimidine-2-hydroxamic acid are the best reactivators of phosphorylated cholinesterase in the hydroxamic acid series. The present study was undertaken to prepare hydroxamic acids of general structure (5) which would either reactivate the phosphorylated enzyme or compete with organophosphorous compounds for the enzyme surface. The hydroxamic acids (5) were also structurally similar to other known quaternary hydroxamic acids (6), synthesized by Coe (1959), which were known to be active in preventing and reversing some of the physiological effects of inhibited cholinesterase.



$$R^1 - \overset{\overset{\large R^2}{|}}{\underset{\underset{\large R^3}{|}}{N^+}} - CHR^4 - CHR^5 - CONHOH \quad X^-$$

(5)



(6)

A preliminary pharmacological study on some of these compounds using unanesthetized rats and cats demonstrated that they might have some effects on the autonomic nervous system.

The compounds (K71, K72, K74, K75, K76) in the doses shown in Table III initially produced an increased spontaneous activity, an increased response to external stimuli followed by a decrease in the spontaneous activity and response to external stimuli. Some of the animals developed clonic type of convulsions. Bradycardia and relaxation of the nictating membrane in cats were observed with smaller doses (15 to 30 mg/kg) of K75 and K76 administered either subcutaneously or intravenously. The neurological signs observed with these compounds in higher doses might be toxic effects. On the basis of these preliminary results the effects 3-amino-hydroxamic acids, and 3-amino-propionates on the cardiovascular system of anaesthetized cats were investigated.

Effects of intravenous administeration of 3-monoalkylamino-hydroxamic acid hydrochlorides and corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.

With one exception, all the hydroxamic acids caused a fall in the blood pressure (B.P.) and a decrease in the heart rate. The exception was 3-methylamino-propiono-hydroxamic acid hydrochloride (K87) which produced a rise in blood pressure and tachycardia (Table IV).

It is known that methyl 4-methylamino-butyrate and other related compounds possess hypotensive properties (Takahashi *et al*, 1962). Hashimoto *et al* (1963) reported that methyl 4-methylamino-butyrate and methyl 4-amino-butyrate produced a positive inotropic effect whereas methyl 4-trimethylamino-butyrate produced a negative inotropic effect.

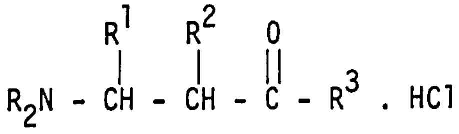
No such work, however, has been reported on 3-amino-esters.

The duration of hypotensive effect increased with an increase in alkyl chain length of the amine. Thus 3-n-hexylaminopropionohydroxamic acid hydrochloride (K98) produced a 40% fall in the blood pressure lasting for more than 2 hours as compared to 3-ethylaminopropiono-hydroxamic acid hydrochloride (K96) which showed a 53% fall for only 5 minutes (Table IV). The corresponding ester hydrochlorides showed the same effect. The intensity as well as duration of fall in the blood pressure increased with an increase in the alkyl chain length of the amine portion. Thus methyl 3-methylamino-propionate hydrochloride (K63) produced a 62% fall in the blood pressure for 5 minutes, whereas, methyl 3-n-hexylamino-propionate hydrochloride (K89) produced 87% fall in the blood pressure for a period of 80 minutes (Table IV). However, the intensity of hypotensive effect in 3-monoalkylamino-hydroxamic acids as well as the corresponding esters was not increased when the alkyl chain of the amine portion of these compounds was lengthened; a variable effect was observed (Table IV). All the methyl 3-monoalkylamino-propionates investigated, produced various degree of fall in the blood pressure with or without bradycardia except methyl 3-n-propylamino-propionate hydrochloride (K66) which produced a 27% rise in the blood pressure associated with a decrease in the heart rate (Table IV). It appears that an increase in alkyl chain length in the amine portion of 3-monoamino-ester hydrochlorides and corresponding hydroxamic acid hydrochlorides increased the duration of hypotensive effect.

At present it can not be explained why the hydroxamic acid (K87) produced a rise in the blood pressure and an increase in the heart rate, whereas the corresponding methylester (K63) caused a fall in the blood pressure and a decrease in the heart rate. Similarly it is difficult to explain why methyl 3-*n*-propylamino-propionate hydrochloride (K66) produced a rise in the blood pressure and a decrease in heart rate; whereas the corresponding hydroxamic acid (K90) caused a fall in the blood pressure and a decrease in heart rate.

Effects of various 3-dialkylamino-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on the arterial blood pressure and heart rate of anaesthetized cats.

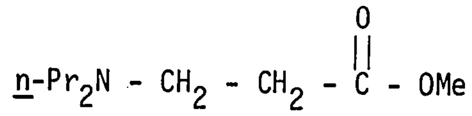
The compounds with general structure (54) where R_2N- is dimethylamino-, diethylamino-, or di-*n*- propylamino-, R^1 and R^2 are either methyl



$$\mathrm{R_2N - \overset{\overset{R^1}{|}}{CH} - \overset{\overset{R^2}{|}}{CH} - \overset{\overset{O}{\|}}{C} - R^3 \,.\, HCl} \tag{54}$$

groups or hydrogen atoms, and R^3 is - NHOH, are not known in literature except for 3-dimethylamino-propiono-hydroxamic acid hydrochloride (K16). This hydroxamic acid (K16) has been prepared by Matveev *et al* (1964) for polarographic reduction studies but its pharmacology has not been reported. Except for methyl 2-methyl-3-di-n-propylamino-propionate (K31), the corresponding esters (54, where R^3 is methoxy or ethoxy) are known (Traynelis and Dadura, 1961; Bieber, 1954; Edwards *et al*, 1964, and

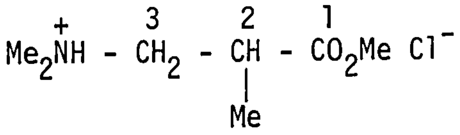
Viscontini et al, 1950). The compound methyl 3-di-n-propylamino-propionate (55) which is closely related to K31 has been screened for



$$\underline{n}\text{-}Pr_2N - CH_2 - CH_2 - \overset{\overset{O}{||}}{C} - OMe \qquad (55)$$

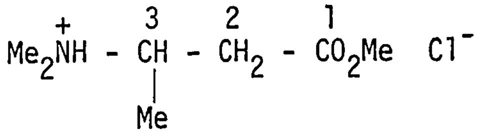
oxytocic activity and has been found devoid of any significant oxytocic activity (Phillips, 1950).

Methyl 3-dimethylamino-propionate hydrochloride (K16) is known to possess weak muscarinic activity (Barrass et al, 1968). Its quaternary iodide and the quaternary iodide of ethyl 3-di-methylamino-propionate have been reported to possess ganglion stimulating properties when studied on cats' blood pressure and superior cervical ganglion (Brimblecombe et al, 1968). In this study they reported that these esters produced effects which were blocked by hexamethonium and reduced by atropine and thus it was suggested that these esters produced their effects by reacting with post-synaptic receptors in the ganglia. In the present study the corresponding ethyl ester, namely ethyl 3-dimethylamino-propionate (K15) caused a 38% fall in the blood pressure and 10.6% decrease in the heart rate of the cat. The related ester, methyl 2-methyl-3-dimethylamino-propionate (K21), produced a profound fall in the blood pressure lasting for 90 to 115 minutes (Table V). This prolonged and marked fall in the blood pressure was accompanied by muscle spasm or muscle twitch, lachrymation, salivation, urination and defacation, suggestive of a strong parasympathomimetic nature of the compound. It is interesting that a



$$\overset{+}{Me_2NH} - \overset{3}{CH_2} - \overset{2}{\underset{\underset{Me}{|}}{CH}} - \overset{1}{CO_2Me}\ Cl^-$$

K21



$$\overset{+}{Me_2NH} - \overset{3}{\underset{\underset{Me}{|}}{CH}} - \overset{2}{CH_2} - \overset{1}{CO_2Me}\ Cl^-$$

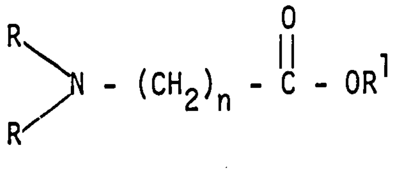
K22

change in the position of methyl group from carbon 2 in methyl 2-methyl-3-dimethylamino-propionate (K21) to carbon 3 in methyl 3-dimethylamino-butyrate (K22) produced a compound which caused a marked rise in the blood pressure (54%) (Fig. 12), and a decrease in the heart rate. The decrease in the heart rate produced by K22 might be reflex in nature secondary to a rise in the blood pressure.

These preliminary results suggested that a more detailed study of the effect of branching on simple 3-dialkylamino-esters should be carried out.

Effects of various substituted 3-amino-propiono-hydroxamic acid hydrochlorides and corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.

Amino-acid esters with general formula (56) where n is 1 to



(56)

4, R^1 is methyl or ethyl and R_2N- is dimethylamino- or pyrrolidino, have been shown to possess both muscarinic and nicotinic properties

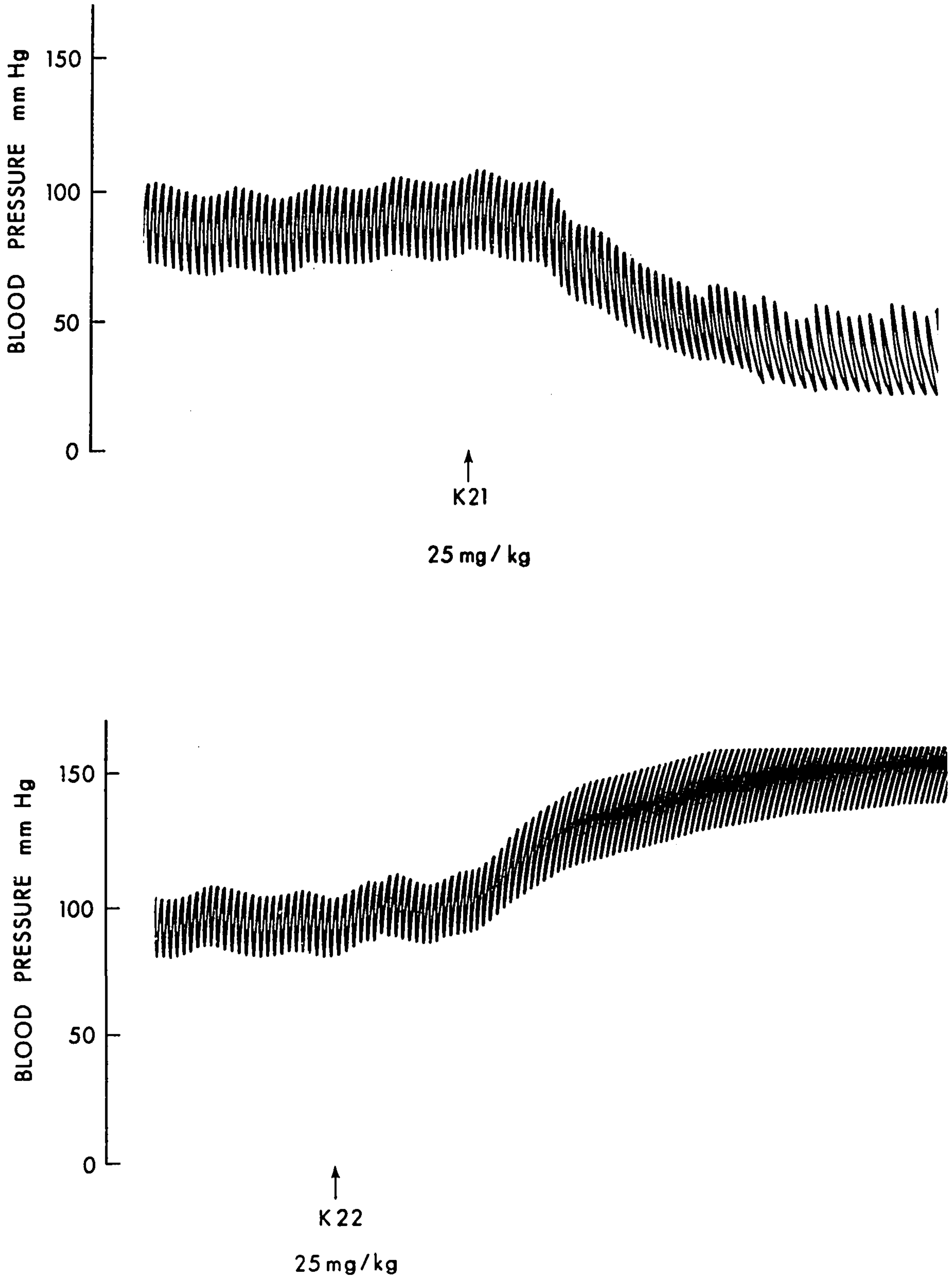
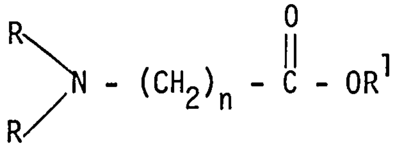


Fig. 12 Effects of intravenous administeration of K21 and K22 on the arterial blood pressure of anaesthetized cat.

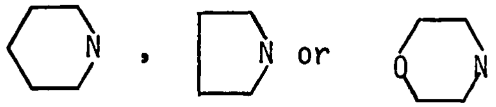
(Barrass et al, 1968). Various esters with the same general formula (56) with substituents as shown below were studied by Matkovics et al, (1961), and Porszasz et al (1961). These investigations demonstrated



(56)

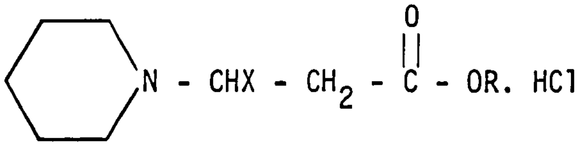
where n = 1 or 2;

R^1 = Me, Et, n-Bu, Ph or $PhCH_2$, and

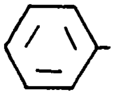
R_2N- = 

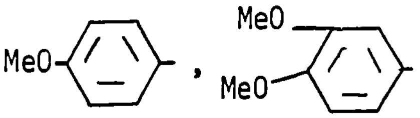
that the esters with lower aliphatic substituents (when R^1 is methyl or ethyl) had nicotinic action while those with higher aliphatic or aromatic substituents (when R^1 is phenyl or benzyl) showed antinicotinic action. The quaternary salts of these compounds were ganglion-blockers.

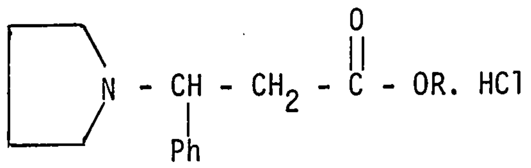
In another study, the compounds with general structures (57), (58), and (59) were screened for antispasmodic activity (Pacheco et al, 1962).



(57)

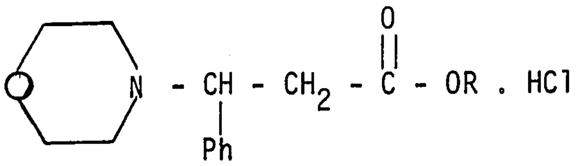
R = H, Me, Et, n-Pr, iso-Pr, n-Bu, iso-Bu, or sec-Bu, and X = 

 and others.



(58)

R = H, Me, Et, n-Pr, n-Bu, iso-Bu, sec-Bu and others.



(59)

R = Me, Et, n-Pr, n-Bu, iso-Bu, sec-Bu, and others.

Out of these 57a (57, X = Ph and R = n-Bu), and 58a (58, R = n-Bu) showed high antispasmodic activity and very low toxicity.

In the present study, an investigation to reveal the best amino-groups for substitution at the 3-position of hydroxamic acids and related esters, showed that all the nine 3-substituted amino-hydroxamic acid hydrochlorides and the corresponding esters prepared, produced a fall in the blood pressure and a decrease in the heart rate (Table VI). In most cases the hydroxamic acids were found to be more potent than the corresponding esters as far as the magnitude and duration of fall in the blood pressure was concerned. 2-methyl-3-phenethylamino-propiono-hydroxamic acid hydrochloride (K97) was the most potent compound followed by 3-benzylamino-propiono-hydroxamic acid hydrochloride (K91) in this series. These findings suggested that substitution by phenethylamino (as in K97) or benzylamino-group (as in K91) at 3-position gives the most active compounds capable of producing a fall in the blood pressure. All the esters investigated in this series with the exception of methyl 2-methyl 3-phenethylamino-propionate (K86) and methyl 3-benzylamino-propionate (K65), produced only a transient fall in blood pressure. These results indicated that an increase in the lypophilic nature of the compounds (such as K97, K86, K91 and K65) leads to increase in hypotensive properties of the compounds.

Effects of various 3-(substituted piperidino)propiono-hydroxamic acid hydrochlorides and the corresponding ester hydrochlorides on arterial blood pressure and heart rate of anaesthetized cats.

In our search to find a suitable amino-group for substitution at the 3-position of these esters and hydroxamic acids, propionic acid esters and propiono-hydroxamic acids with a piperidine ring, either unsubstituted or substituted with aryl and alkyl substituents at different positions, were synthesised and their effects on blood pressure and heart rate of cats were observed. All the 3-piperidinopropiono-hydroxamic acids produced a fall in blood pressure and a decrease in heart rate except K71 which had no effect on heart rate (Table VII). In studying this series, it was observed that for a marked and prolonged fall in the blood pressure the aryl or alkyl substituents should be at position 4 and not at 2 or 3 of the piperidine ring. Thus 2-methyl-(4-methyl piperidino)propiono-hydroxamic acid hydrochloride (K74) produced a 56% fall in blood pressure for a period of 10 minutes as compared to 2-methyl-(2-methylpiperidino)propiono-hydroxamic acid hydrochloride (K95) which caused a 33% fall in blood pressure for 5 minutes and *2-methyl-(3-methylpiperidino)propiono-hydroxamic acid hydrochloride* (K94) which produced a 39% fall in blood pressure for 5 minutes. It seems (Table VII) that substitution of methyl group on carbon 2 of the propionic acid-chain increases the extent and duration of fall in blood pressure. This is evident from the fact that 2-methyl-3-piperidinopropiono-hydroxamic acid hydrochloride (K72) produced a 48% fall in blood pressure for 10 minutes as compared to the unbranched hydroxamic acid namely 3-propiono-hydroxamic acid hydrochloride (K71) which showed a 36% fall in blood pressure for a period of 5 minutes.

The nature of the substituent at the 4-position of the piperidine ring of these piperidino-hydroxamic acids also affected hypotensive potency of these compounds. When the methyl group at 4-position of piperidine ring in *2-methyl-3-(4-methylpiperidino)propiono-hydroxamic acid hydrochloride* (K74) was changed to n-propyl group as in *2-methyl-3-(4-n-propylpiperidino)propiono-hydroxamic hydrochloride* (K75), the duration of fall in the blood pressure increased from 10 minutes to 120 minutes. Substutition of a phenyl group at 4-position of the piperidine ring gave the compound *2-methyl-3-(4-phenylpiperidino)propiono-hydroxamic acid hydrochloride*(K76) which produced a fall in the blood pressure (81%) for a period of more than 2 hours. This was the most potent hypotensive compound in this series. This is not unexpected as 4-phenylpiperidine ring system has been used in many other pharmacologically active compounds, such as neurotropic and psychotropic substances (Pelz and Protiva, 1967), antihypertensive agents (Biel, 1965) and analgesics (Biel and Hopps, 1965).

All the corresponding esters (Table VII) tested showed a fall in the blood pressure and decrease in the heart rate except methyl 2-methyl-3-piperidinopropionate (K52) which instead produced a rise in the blood pressure and increase in the heart rate. These esters were less potent than the corresponding hydroxamic acids in lowering the blood pressure. The duration of fall in the blood pressure produced by esters was also less than that produced by the hydroxamic acids in most cases.

Effects of 3-substituted amino-acids on the arterial blood pressure and heart rate of anaesthetized cats.

The 3-amino-acids i.e. 2-methyl-3-piperidino-propionic acid (K52A), 2-methyl-3-homopiperidino-propionic acid (K67A), 3-ethylamino-propionic acid (K70A) and 2-methyl-3-(3-methylpiperidino)propionic acid (K80) listed in Table VIII were the hydrolysis products of the corresponding 3-amino-esters,methyl 2-methyl-3-piperidinopropionate (K52), methyl 2-methyl-3-homopiperidinopropionate (K67), methyl 3-ethylamino-propionate (K70) and methyl 2-methyl-3-(3-methylpiperidino)propionate (K80) and were formed when these esters were stored for long periods, presumably in the presence of atmospheric moisture. It was interesting to note that these 3-amino-acids K52A, K67A, K70A and K80A did not have any effect on blood pressure and heart rate of anaesthetized cats (Table VIII) whereas the ester precursors K67, K70, and K80 produced a fall in the blood pressure with or without decreasing the heart rate and K52 produced a rise in the blood pressure. Although the change in the blood pressure caused by these esters is transient, nonetheless it is well marked. Furthermore, these 2-methyl-3-amino-propionic acids are very important in the field of metabolic breakdown of pyrimidines as shown by Fruton and Simmonds (1958). L-2-methyl-3-amino-propionic acid has been isolated from human urine (Crumpler _et al_, 1951). Hydrolysis is an important reaction which is known to occur in human body. Thus it is quite possible that these 3-amino-esters might be metabolised in the body to the corresponding 3-amino-acids, which are inactive in lowering the blood pressure and heart rate.

The preliminary study showed that _2-methyl-3-(4-phenylpiperidino)-propiono-hydroxamic acid hydrochloride_ (K76) was the most potent hypotensive agent and this compound, therefore, was selected for a detailed investigation of the mechanism of its hypotensive action. There are a few other hydroxamic acids such as _2-methyl-3-(4-n-propylpiperidino) propiono-hydroxamic acid hydrochloride_ (Table VII), _3-benzylamino-propiono-_hydroxamic acid hydrochloride (Table VI), _2-methyl-3-phenethylamino-propiono-hydroxamic acid hydrochloride_ (Table VI) and _3-n-hexylamino-propiono-hydroxamic acid hydrochloride_ (Table IV) which also need a detailed investigation of the mechanism by which they cause a fall in blood pressure. A detailed pharmacological investigation of some of the esters such as methyl 2-methyl-3-dimethylamino-propionate hydrochloride (Table V), methyl 3-dimethylamino-butyrate hydrochloride (Table V), methyl 3-n-hexylamino-propionate hydrochloride (Table IV) is also warranted.

A detailed study of compound K76 showed that the extent of fall in the blood pressure produced by this compound was dose-dependent. The fall in blood pressure was associated with bradycardia, which was not dose-dependent. This compound might produce a fall in the blood pressure by a mechanism resembling that of a parasympathomimetic, or a sympotholytic, or a ganglion-blocking, or a histamine-like, or a direct acting or a polypeptide-like agent.

The possibility of this compound having parasympathomimetic actions was ruled out because:

(a) the bradycardia associated with the fall in blood pressure was not marked, even with the highest dose; (Table IX).

(b) although atropine (4 mg/kg) completely blocked the effect of acetylcholine, it was unable to block the effect of K76 (Fig. 5);

(c) the compound, in various concentrations was ineffective in producing contractions in isolated guinea-pig ileum (Table XV).

This compound was not sympatholytic in nature because it did not block an epinephrine-induced rise in the blood pressure. It rather, potentiated the extent and duration of epinephrine-induced rise in the blood pressure (Fig. 6). This might be due either to its interference in epinephrine metabolism or due to its ganglion-blocking activity. No study has been made as far as its interference with epinephrine metabolism is concerned. When there is a rise in the blood pressure, the baroreceptors reflexes come into play to lower the blood pressure. This reflex-mediated lowering of the blood pressure is mediated ultimately through the ganglia. When the ganglia are blocked, although the baroreceptors are affected by the rise in blood pressure, the stimuli from the baroreceptors do not pass through the ganglia and hence the extent and duration of the epinephrine-induced rise in blood pressure is potentiated. That this potentiation of epinephrine-induced rise in blood pressure might be due to the ganglion-blocking effect of compound K76 is further substantiated by the results discussed later in this chapter.

Compound K76 is not a histamine-like substance or a polypeptide-like compound because it was not effective in causing contractions in isolated guinea-pig ileum (Table XV), although it produced a fall in the blood pressure in the cat. In addition, the fall in blood pressure was prolonged as opposed to a histamine-effect where the fall in blood pressure is transient. That this compound might be producing a fall in the blood pressure by blocking the ganglia is substantiated by the following facts:

(a) It was able to block completely a nicotine-induced rise in blood pressure (Fig. 7). Nicotine-induced rise in blood pressure is known to be due to stimulation of the ganglia (Volle and Koelle, 1965).

(b) It blocked completely the rise in blood pressure produced by occluding both the common carotid arteries (Fig. 10). The rise in blood pressure produced by occluding the common carotid arteries is mediated through the stimulation of the sympathetic ganglia (Wright, 1965).

(c) Local application of 5% solution of K76 on the exposed superior cervical ganglion blocked the contraction of the nictitating membrane as a result of stimulation of preganglionic fibres but the nictitating membrane still responded, by contracting, to stimulation of the post ganglionic fibres (Fig.9II). A similar effect was observed when the drug was administered to the ganglia either through intravenous or intra-arterial route. The dose for intra-arterial injection was such that it would not produce any measurable change in the blood pressure (Fig. 9I, Table XI).

When the drug was applied locally in 5% solution, the effects lasted for as long as the cotton swab (dipped in 5% solution of K76) was in contact with the superior cervical ganglia. When the drug was injected through the carotid artery, the effect lasted for a short period. This might be due to dilution of the compound when recirculating in the body. These results indicated that K76 blocked the sympathetic ganglia.

Intravenous administeration of K76 blocked to a varying degree the cardio-decelerator effect of electrical stimulation of the cut cardiac end of the right vagus (Table XIV). This shows that the drug was able to block the parasympathetic ganglia, but the compound was very weak.

(d) This compound (K76) in various doses could produce neither contraction nor relaxation in the isolated guinea pig ileum. Although

(K76) was unable to block acetylcholine-induced contraction, it blocked nicotine-induced contraction in the guinea-pig ileum to a varying degree depending upon its concentration in the bath (Fig. 11).

The effects of compound K76 were compared with those of a known ganglion blocking agent, Ansolysen .

It was found that K76 was 1.05 times as potent as Ansolysen (Table XVII) in producing a fall in blood pressure which persisted almost as long as the fall in the blood pressure produced by Ansolysen. It was, however, interesting that K76 was only 0.083 times as potent as Ansolysen in producing bradycardia in the anaesthetized cat (Table XVIII).

It is evident from the results in Table XIX that K76 is almost ineffective in blocking the bradycardia produced by vagal stimulation, whereas, Ansolysen in equimolar doses blocked the effect of vagal stimulation almost completely. From these results it may be concluded tentatively that 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride (K76) produces a fall in cat blood pressure by blocking the sympathetic ganglia. Looking at the results of this compound on the bradycardia produced by vagal stimulation, it also seems that K76 specifically blocks the sympathetic ganglia. This conclusion is open to question, however, because on isolated guinea-pig ileum, this compound (K76) was completely effective in blocking the nicotine-induced contraction which is mediated through parasympathetic ganglia.

A further investigation must be carried out before the claim that K76 does not have an action on parasympathetic ganglia is made.

APPENDIX

Application of the transesterification reaction using hydrogen bromide in diethylether

DISCUSSION

Higher esters can also be converted with ease to ethyl esters by the transesterification reaction described on page 55. The transesterification of a pure sample of butyl acetate was studied gas-chromatographically. Within 24 hours, 43% of the butyl ester had been converted to ethylacetate. After 48 hours, the conversion was 57% complete. Maximum transesterification (63%) occurred after 150 hours. Complete transesterification was possible only if the solvent was removed and replaced with fresh diethylether containing hydrogen bromide.

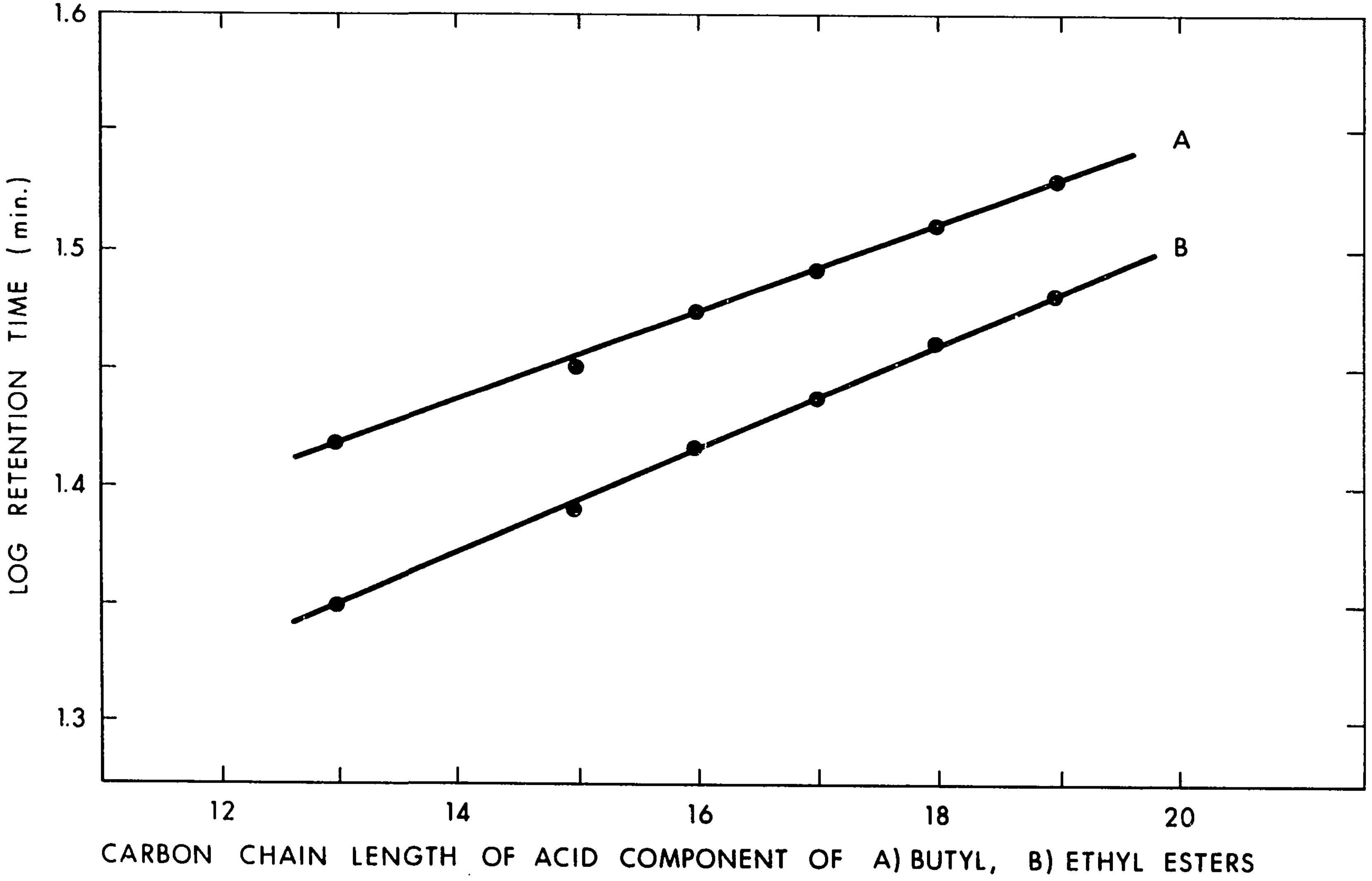
The transesterification reaction was applied to the identification of the components of mixtures of fatty esters. A commercial sample of butyl stearate was gas-chromatographed and shown to be a mixture containing two major constituents (>90% of the total) and four minor components (Table A-1). The two major constituents were collected and their mass spectra recorded. Both spectra were typical of long-chain saturated aliphatic esters (McLafferty, 1963). The more volatile component showed a parent ion, m/e 312; the parent ion of the less volatile major constituent had a mass (m/e) of 340. These two components were identified as butyl palmitate and butyl stearate respectively. A plot of the log retention time of each ester component in the mixture versus number of carbon atoms of the corresponding acid gave a straight line (Fig. A-1) and in this way the minor components of the mixture were

TABLE A-1

Composition of a Commercial Sample of Butyl Stearate

Component	Retn. Time (min.)	Percent Composition	Identity
(a) Before treatment with HBr/Et_2O.			
1	26.7	2.42	butyl tridecanoate
2	28.1	0.35	butyl pentadecanoate
3	29.9	48.26	butyl palmitate
4	30.8	2.27	butyl heptadecanoate
5	32.4	45.39	butyl stearate
6	34.1	1.31	butyl nonadecanoate
(b) After treatment with HBr/Et_2O.			
1	22.6	2.62	ethyl tridecanoate
2	24.3	0.38	ethyl pentadecanoate
3	26.1	47.55	ethyl palmitate
4	27.3	2.32	ethyl heptadecanoate
5	28.8	43.59	ethyl stearate
6	30.4	3.71	ethyl nonadecanoate

FIG. A-1 Retention times and carbon chain lengths of A) the butyl esters present in, and B) ethyl esters obtained from a commercial sample of butyl stearate.

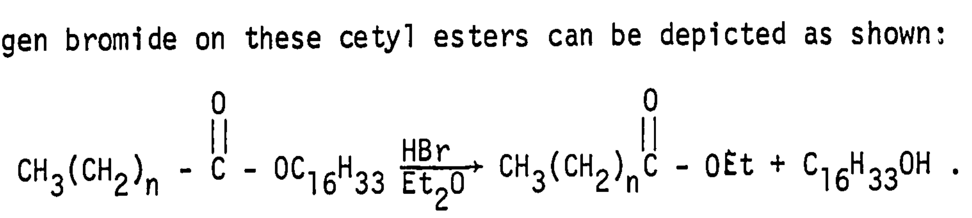


tentatively identified (Table A-1) (cf, Abramovitch et al, 1963; Abramovitch et al, 1967).

A solution of the commercial butyl stearate sample in ether was treated with hydrogen bromide. The reaction mixture was also gas-chromatographed and this confirmed that all six butyl esters were converted at approximately the same rate to the corresponding ethyl esters (Table A-1). The plot of retention time of each component versus the number of carbon atoms of the corresponding acid portion again gave a straight line (Fig. A-1) which substantiated the tentative identification of the butyl esters. The mass spectra of the two major ethyl ester components confirmed that they were ethyl palmitate (m/e 284) and ethyl stearate (m/e 312). The IR spectra of both esters were virtually identical and were typical of long chain fatty esters.

This transesterification reaction was also used to characterize a mixture of higher naturally-occurring fatty esters. The normal method employed to do this is to hydrolyse the mixture of esters, separate the acid and alcohol components, convert the acid components to their methyl or ethyl esters and then identify the acids (as their methyl or ethyl esters) and the alcohols by gas-chromatographing them separately and comparing their retention times with those of reference standards (Abramovitch et al, 1967). By employing the transesterification reaction, however, the acid and alcohol components of a mixture of two higher fatty esters were readily identified. Separation of the acid and alcohol components was not necessary.

A commercial mixture of cetyl stearate was gas-chromatographed, but due to the low volatility of these esters this was a lengthy procedure. A complete separation of one component from the other was not attained (Fig. A-2) and the presence of minor components was not revealed. A solution of the mixture in ether was treated with hydrogen bromide and the reaction mixture was gas-chromatographed. The components were readily and rapidly separated and the presence of small quantities of impurities revealed (Fig. A-2). Fractions A, B, and C were collected and a mass spectrum and an IR spectrum of each was recorded. The IR spectrum of fraction A was typical of that of a long-chain alcohol. The parent peak (m/e 242) was absent from the mass spectrum, but the presence of a relatively abundant ion of m/e 224 (M-18) readily identified (Budzikiewicz, Djerassi, and Williams, 1967) the alcohol as cetyl alcohol ($C_{16}H_{33}OH$). The IR spectra of fractions B and C showed strong carbonyl absorption bands at 1740 cm^{-1} and C-H stretching bands near 3000 cm^{-1}. Fractions B and C, therefore, were long chain fatty esters. Fraction B was ethyl palmitate and fraction C was ethyl stearate; the parent peaks in their mass spectra were located at m/e 284 and m/e 312 respectively. The action of ethereal hydrogen bromide on these cetyl esters can be depicted as shown:



$$CH_3(CH_2)_n - \overset{\overset{O}{\|}}{C} - OC_{16}H_{33} \xrightarrow[Et_2O]{HBr} CH_3(CH_2)_n\overset{\overset{O}{\|}}{C} - OEt + C_{16}H_{33}OH \ .$$

The utility of this transesterification reaction to the identification

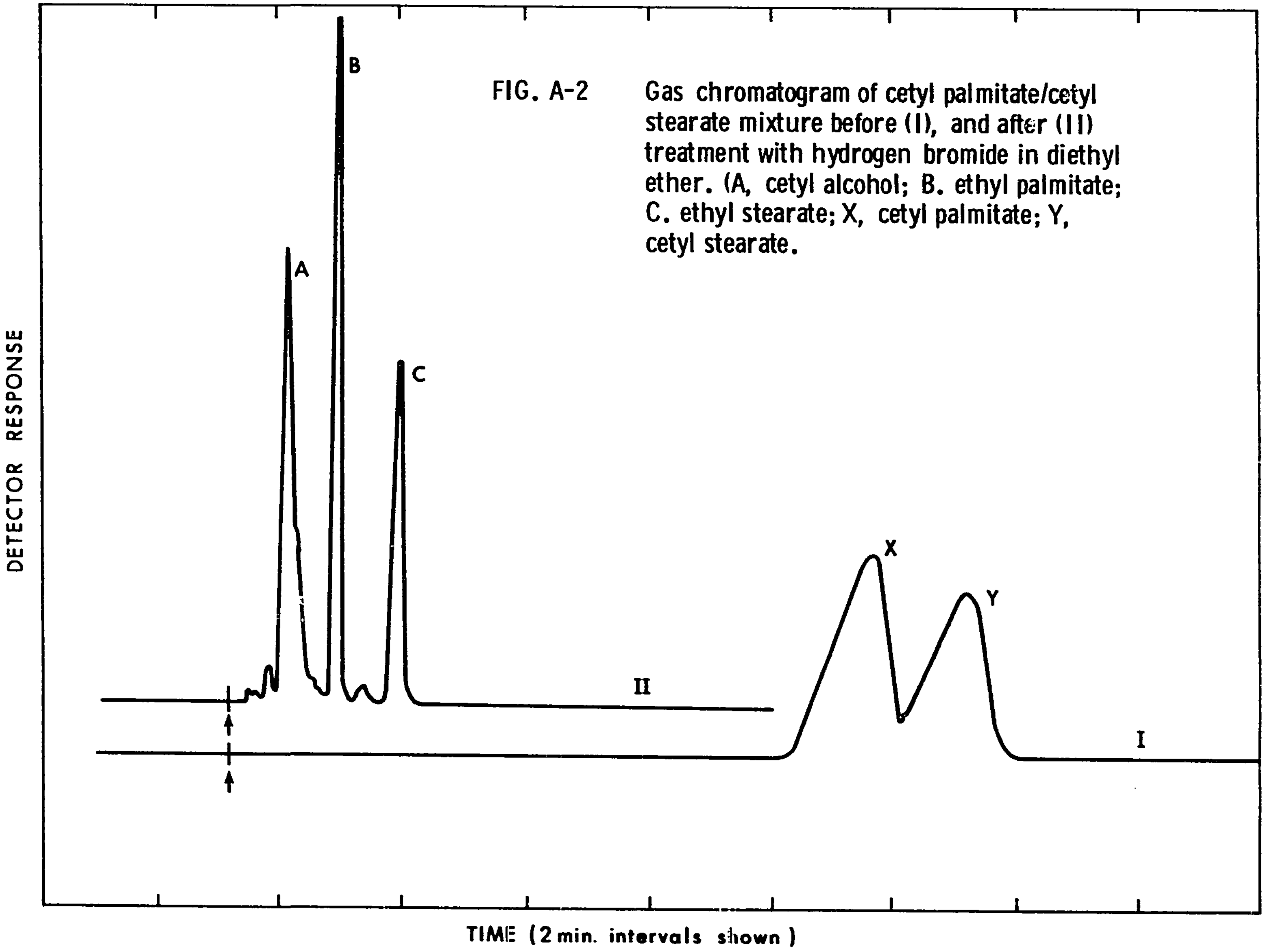


FIG. A-2 Gas chromatogram of cetyl palmitate/cetyl stearate mixture before (I), and after (II) treatment with hydrogen bromide in diethyl ether. (A, cetyl alcohol; B. ethyl palmitate; C. ethyl stearate; X, cetyl palmitate; Y, cetyl stearate.

of small quantities of natural products was demonstrated. A 50 milligram sample of the cetyl palmitate/cetyl stearate mixture was hydrobrominated in ether for 10 minutes, after which time the presence in the reaction mixture of cetyl alcohol, ethyl palmitate, and ethyl stearate could be readily established by means of GLC.

EXPERIMENTAL

Reaction of a commercial sample of butyl stearate* with hydrogen bromide

A solution of butyl stearate (12.5 g) in diethyl ether (150 ml) was cooled to 0°. Dry hydrogen bromide gas was passed through this solution for 30 minutes. The weight increase was 68.5g. The reaction mixture was kept at room temperature for 24 hours, during which time it separated into two layers. The lower layer was miscible with ether and the upper layer was miscible with water. The upper layer was diluted with water and extracted with ether. This ether extract was combined with the ethereal lower layer. The combined extract (300 ml) was washed with 10% sodium bicarbonate solution (5 x 100 ml), then with water (2 x 25 ml), and dried over anhydrous sodium sulfate. The ether was removed and a pale yellow oil (13.1 g) was obtained. Gas-chromatography using the same conditions as in the methylmethacrylate reaction (p 56) revealed that the oil was a mixture of six components (Table A-1 and Fig. A-1).

Reaction of a cetyl palmitate/cetylstearate**mixture with hydrogen bromide

A portion (25 g) of the mixture of esters in diethylether (200 ml) was treated with dry hydrogen bromide in essentially the same

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* Practical grade, purchased from Matheson, Coleman and Bell *via* Canadian Laboratory Supplies Ltd., Edmonton, Canada.

**The mixture was purchased from K & K Laboratories, Plainview, New York and was reputed to be a sample of cetyl stearate.

way as that described in the methyl methacrylate-hydrogen bromide reaction (p. 55). The final product was a pale yellow oil (21.8 g) which was gas-chromatographed using the same conditions as that described for the methyl methacrylate reaction (p. 56). Three major components were collected (Fig. A-2).

Component A was cetyl alcohol. IR spectrum (film): 3330 (OH); 2955, 2865 cm^{-1} (C-H).

Mass spectrum: 224 (84%), $[CH_3(CH_2)_{13}CH = CH_2]^{+\cdot}$, m/e (percent abundance relative to peak at m/e 57 = 100).

Component B was ethyl palmitate. IR spectrum (film): 2920, 2850 (C - H); 1742 cm^{-1} (C = 0).

Mass spectrum: 284 (14.5%), $[CH_3(CH_2)_{14}COOC_2H]^{+\cdot}$; 239 (7.5%), $[CH_3(CH_2)_{14}\overset{+}{C} = O]$, m/e (percent abundance relative to peak at m/e 88 = 100).

Component C was ethyl stearate. IR spectrum (film): 2920, 2850 (C - H); 1742 cm^{-1} (C = 0).

Mass spectrum: 312 (16%), $[CH_3(CH_2)_{16}COOC_2H_5]^{+\cdot}$; 267 (5.5%), $[CH_3(CH_2)_{16}\overset{+}{C}O]$; m/e (percent abundance relative to peak at m/e 88 = 100).

<u>Gas-chromatography of the commercial sample of butyl stearate</u>

A portion (5 μL) of the sample was gas-chromatographed using the conditions described in the methyl methacrylate-hydrogen bromide reaction (p. 56).

The two major components (retention times 29.9 minutes and 32.4 minutes) were collected. The first was butyl palmitate. IR spectrum (film): 2920, 2850 (C - H); 1743 cm^{-1} (C = 0).

Mass spectrum: 312 (23%), $[CH_3(CH_2)_{14}COOC_4H_9]^{+\cdot}$; 256 (50%), $[CH_3(CH_2)_{14}COOH]^{+\cdot}$; m/e (percent abundance relative to peak at m/e 57 = 100).

The second major component was butyl stearate. IR spectrum (film): 2920, 2850 (C - H); 1742 cm^{-1} (C = O).

Mass spectrum: 340 (11%) $[CH_3(CH_2)_{16}COOC_4H_9]^{+\cdot}$; 284 (16%) $[CH_3(CH_2)_{16}COOH]^{+\cdot}$; m/e (percent abundance relative to peak at m/e 28 = 100).

Gas-chromatography of the cetyl palmitate/cetyl stearate mixture

A small portion (5 µL) was gas-chromatographed using the instrument and column previously described in the methyl methacrylate-hydrogen bromide reaction (p.56). A temperature programmed operation was done (initial column temperature 250°; temperature increase 4°/minute; final column temperature 350° and held; detector temperature 375°) and thus two fractions, X and Y, were obtained (Fig. A-2).

Fraction X was cetyl palmitate. IR spectrum (film): 2950, 2920, 2850 (C - H); 1742 cm^{-1} (C = O).

Mass spectrum: 257 (12.5%); 256 (7%); 239 (7%); 224 (26.5%); m/e (percent abundance relative to peak at m/e 57 = 100).

Fraction Y was cetyl stearate. IR spectrum (film): 2955, 2920, 2850 (C - H); 1742 cm^{-1} (C = O).

Mass spectrum: 285 (16%); 284 (10.5%); 267 (6.5%); 224 (44.5%); m/e (percent abundance relative to peak at m/e 57 = 100).

BIBLIOGRAPHY

Abramovitch, R.A., Micetich, R.G., and Smith, S.J., Tappi, 46, 37 (1963).

Abramovitch, R.A., Coutts, R.T., and Knaus, E.E., Can. J. Pharm. Sci., 2, 71 (1967).

Adamson, D.W., J. Chem. Soc., S144 (1949).

Adamson, D.W., British Patent 624, 118 (1949); Chem. Abstr., 44, 662h (1950).

Adamson, D.W., J. Chem. Soc., 885 (1950).

Adamson, D.W., British Patent 702, 269 (1954); Chem. Abstr., 49, 4026h (1955).

Adamson, D.W., United States Patent 2, 698, 325 (1954); Chem. Abstr., 50, 1919d (1956).

Adamson, D.W., and Billinghurst, J.E.W., British Patent 788, 914 (1958); Chem. Abstr., 53, 5299b (1959).

Adamson, D.W., and Billinghurst, J.E.W., British Patent 788, 915 (1958); Chem. Abstr., 53, 5300b (1959).

Arens, J.F., Koerts, D.H., and Plieger, P., Rec. Trav. Chim., 75, 1454 (1956).

Asano, M., and Tomita, K., J. Pharm. Soc., (Japan) 68, 221 (1948); Chem. Abstr., 48, 3978i (1954).

Baltzly, R., Dvorkovitz, V., and Phillips, A.P., J. Am. Chem. Soc., 71, 1162 (1949).

Baltzly, R., and Phillips, A.P., J. Am. Chem. Soc., 71, 3419 (1949).

Barrass, B.C., Brimblecombe, R.W., Parkes, D.C., and Rich, P., Brit. J. Pharmacol., 34, 345 (1968).

Barron, D.I., Hall, G.H., Natoff, I.L., Ridley, H.F., Spickett, R.G.W., and Vallance, D.K., J. Med. Chem., 8, 836 (1965).

Barron, D.I., Hall, G.H., Natoff, I.L., and Vallance, D.K., J. Pharm. Pharmacol., 17, 509 (1965).

Beckett, A.H., First International Pharmacological Meeting, Stockholm 1961, Volume 7, The MacMillan Co., New York, 1963.

Bergmann, F., Wilson, I.B., and Nachmansohn, J., J. Biol. Chem., 186, 693 (1950).

Bergmann, F., and Shimoni, E., Biochem. J., 55, 50 (1953).

Bieber, P., Ann. Chim., 9, 674 (1954); Chem. Abstr., 50, 169c (1956).

Biel, J.H., United States Patent 3, 221,017 (1965).

Biel, J.H., and Hopps, H.B., United States Patent 3, 221, 019 (1965).

Breckpot, R., Bull. Soc. Chim., (Belgium) 32, 412 (1923); Chem. Abstr. 18, 1113 (1924).

Brimblecombe, R.W., and Sutton, J.V., Brit. J. Pharmacol.,34, 358 (1968).

British Patent 1, 018, 810 (1966); Chem. Abstr., 64, 12860a (1966).

British Patent 782, 935 (1957); Chem. Abstr., 52, 9921c (1958).

Budzikiewicz, H., Djerassi, C., and Williams, D.H., Mass Spectroscopy of Organic Compounds, Holden-Day, San Francisco, 1967, p. 101.

Cantatore, G., Ann. Chim., (Rome) 54, 549 (1964); Chem. Abstr.,61, 8308b (1964).

Clemo, G.R., and Melrose, T.A., J. Chem. Soc., 424 (1942).

Clemo, G.R., and Popli, S.P., J. Chem. Soc., 1406 (1951).

Coe, D.G., J. Org. Chem., 24, 882 (1959).

Coutts, R.T., Can. J. Pharm. Sci., 2, 1 (1967).

Coutts, R.T., Can. J. Pharm. Sci., 2, 27 (1967).

Cruickshank, P.A., and Sheehan, J.C., J. Am. Chem. Soc., 83, 2891 (1961).

Cruickshank, P.A., and Sheehan, J.C., United States Patent 3, 143, 547 (1961); Chem. Abstr., 61, 9406h (1964).

Crumpler, H.R., Dent, C.E., Harris, H., and Westall, R.G., Nature, 167, 307 (1951).

David, S., and Sinay, P., Bull. Soc. Chim. France, 2301 (1965).

Decombe, J., Ann. Chim., 18, 81 (1932); Chem. Abstr., 27, 2135^8 (1933).

Dickermann, S.E., and Besozzi, A.J., J. Org. Chem., 19, 1855 (1954).

Drake, W.V., and McElvain, S.M., J. Am. Chem. Soc., 56, 697 (1934).

Edward, J.E., and Marsh, D.F., J. Pharmacol. Exptl. Therap., 103, 196 (1951).

Edwards, Hamer, D., and Stewart, W.H., J. Pharm. Pharmacol., 16, 618 (1964).

Farquharson, M.E., and Maclean, J.A.R., J. Med. Pharm. Chem., 4, 31 (1961).

Fischer, E., and Speier, A., Chem. Ber., 28, 3252 (1895).

Flurscheim, J. Prakt. Chem., [2] 68, 348 (1903).

French Patent 1, 167, 510 (1958); Chem. Abstr., 55, 8444c (1958).

Friedman, H.L., Medicinal Research Series I (ed. Burger, A.), Marcel Dekker, Inc., New York, 167, p. 95.

Fruton, J.S., and Simmonds, S., *General Biochemistry*, 3rd edition, Wiley & Sons, New York, 1958, p. 858.

Fuson, R.C., and Bradley, R.L., *J. Am. Chem. Soc.*, *51*, 599 (1929).

Ganellin, C.R., and Spickett, R.G.W., *J. Med. Chem.*, *8*, 619 (1965).

Gearien, J.E., and Binkley, S.B., *J. Org. Chem.*, *23*, 491 (1958).

Gerjovich, H.J., and Harrison, J.B., United States Patent 3, 251, 875 (1966); *Chem. Abstr.*, *65*, 3758d (1966).

Graf, R., German Patent 955, 509 (1957); *Chem. Abstr.*, *53*, 6090a (1959).

Gresham, T.L., Jansen, J.E., Shaver, F.W., Bankert, R.A., and Fiedorek, F.T., *J. Am. Chem. Soc.*, *73*, 3168 (1951).

Halverstadt, I.F., Hardie, W.R., and Williams, A.R., *J. Am. Chem. Soc.*, *81*, 3618 (1959).

Harvieu, J., Parot, J., and Paty, M., *Compt. Rend.*, *257*, 2300 (1963); *Chem. Abstr.*, *60*, 496a (1964).

Hashimoto, K., Kumakura, S., and Hashimoto, K., *Nature*, *197*, 500 (1963).

Hazard, R., Cheymoe, J., Chabaier, P., Eche-Fialaire, R., and Bouvillet, F., *Bull. Soc. Chim. France*, *1963*, 2279 (1964); *Chem. Abstr.*, *60*, 14505a (1964).

Howton, D.R., *J. Org. Chem.*, *10*, 277 (1945).

Hromatka, O., and Eiles, E., *Monatsh*, *78*, 129 (1948); *Chem. Abstr.*, *43*, 575h (1949).

Hughes, R.A., United States Patent 2, 965, 671 (1960); *Chem. Abstr.*, *55*, 16423e (1961).

Humphreys, K.W., Michael, H.G., Ratcliffe, and Hinton, I.G., British Patent 960, 622 (1964); *Chem. Abstr.*, *61*, 4224f (1964).

Ing., H.R., Kordik, P., Tudor Williams, D.P.H., *Brit. J. Pharmacol.*, 103 (1952).

Kirk, G., *Ph.D. Thesis*, London (1958).

Kumei, K., *Nippon Seirigaku Zasshi*, *23*, 252 (1961); *Chem. Abstr.*, *55*, 20229h (1961).

Kumura, S., One, H., and Sano, K., British Patent 1, 008, 177 (1965); *Chem. Abstr.*, *64*, 5238f (1966).

Kuznetsov, S.G., and Ioffe, D.V., *Biol. Aktivn. Soedin., Akad. Nauk. SSSR*, *144* (1965); *Chem. Abstr.*, *64*, 4975d (1966).

Lands, A.M., and Cavillito, C.J., *J. Pharmacol. Exptl. Therap.*, *110*, 369 (1954).

Lightowler, J.E., and Maclean, J.A.R., *Arch. Int. Pharmacodyn.*, *145*, 233 (1963).

Lindsay, R.D., and Cheldelin, V.H., *J. Am. Chem. Soc.*, *72*, 828 (1950).

Lukeš, R., Zvonkova, J.N., Mironov, A.F., and Ferles, M., *Collect. Czech. Chem. Communs.*, *25*, 2668 (1960); *Chem. Abstr.*, *55*, 4501e (1961).

Malhotra, C.L., Das, P.K., Dhalla, N.S., and Prasad, K., *Ind. J. Med. Res.*, *49*, 448 (1961).

Matkovics, B., Foldeak, S., Pórszász, J., and Sipos, G., *Acta. Pharm. Hung.*, *31*, 113 (1961); *Chem. Abstr.*, *55*, 22321h (1961).

Matkovics, B., Foldeak, S., and Pórszász, K., Acta. Univ. Szegediensis Acta Phys. et. Chem., 7, 60 (1961); Chem. Abstr., 56, 1535h (1962).

Matveev, B.V., and Tsybaeva, G.G., J. Gen. Chem. U.S.S.R., 34, 2512 (1964).

McLafferty, F.W., Mass Spectroscopy of Organic Ions, Academic Press, New York and London, 1963, Chapter 9.

Michaelis, L., and Schubert, M.P., J. Biol. Chem., 115, 221 (1936).

Mikhlina, E.E., and Rubtsov, M.V., Zh. Obshch. Khim.,32, 2177 (1962); Chem. Abstr., 58, 9024g (1963).

Moffet, R.B., J. Org. Chem., 14, 862 (1949).

Morsch, K., Monatsh, 60, 50 (1932); Chem. Abstr., 26, 4030 (1932).

Morsch, K., Monatsh, 61, 299 (1932); Chem. Abstr., 27, 713 (1933).

Morsch, K., Monatsh, 63, 220 (1933); Chem. Abstr., 28, 2680^1 (1933).

Mozingo, R., and Patterson, L.A., Org. Syn., 20, 64 (1940).

Muehle, H., Ernst, L., Meier, W., and Renker, H., Arzneim.-Forsch., 18, 850 (1968): Index Chemicus, 30, 101214 (1968).

Murakoshi, I., Yakugaku Zasshi, 78, 594 (1958); Chem. Abstr., 52, 18409b (1958).

Murakoshi, I., Yakugaku Zasshi, 78, 598 (1958); Chem. Abstr., 52, 18409g (1958).

Namba, T., Naikano Ryôiki, 6, 442 (1958).

Nazarov, I.N., and Kazaryan, L.Z., Zhur. Obshch Khim.,27, 3302 (1957); Chem. Abstr., 52, 8953a (1958).

Nazarov, I.N., and Sorokin, O.I., Izvest. Akad. Nauk. S.S.S.R. Otdel Khim. Nauk, 872 (1960); Chem. Abstr., 55, 24720e (1961).

Netherland Patent 6,410,438 (1965); *Chem. Abstr.*, 63, 13479e (1965).

Nikitskaya, E.S., and Rubtsov, M.V., *Zhur. Obshch. Khim.*, 26, 3119 (1956); *Chem. Abstr.*, 51, 8084h (1957).

Olomucki, M., and Marszak, I., *Compt. Rend.*, 246, 1714 (1958); *Chem. Abstr.*, 52, 17097a (1958).

Pacheco, H., Dreux, M., and Beauvillain, A., *Bull. Soc. Chim. France*, 1379 (1962); *Chem. Abstr.*, 58, 7933h (1963).

Pelz, K., and Protiva, M., *Collect. Czech. Chem. Commun.*, 32, 2840 (1967); *Chem. Abstr.*, 67, 90643x (1967).

Perrine, T.D., *J. Org. Chem.*, 18, 898 (1953).

Phillippi, E., and Galter, E., *Monatsh*, 51, 253 (1929); *Chem. Abstr.*, 23, 3207 (1929).

Phillips, A.P., *J. Am. Chem. Soc.*, 72, 3298 (1950).

Pollack, M.A., *J. Am. Chem. Soc.*, 65, 1335 (1943).

Pollard, C.B., Rietz, E.G., and Robbins, R., *J. Am. Chem. Soc.*, 75, 2989 (1953).

Pórszász, J., Foldeak, S., Matkovics, B., Barankay, T., and Gibiszer-Pórzász, K., *Acta. Physiol. Acad. Sci. Hung.*, 19, 235 (1961).

Price, C.C., and Coyner, E.C., *J. Am. Chem. Soc.*, 62, 1306 (1940).

Rosen, H., Bluementhal, A., Townsend, P.N., Tislow, R., and Seifter, J., *J. Pharmacol. Exptl. Therap.*, 117, 488 (1956).

Rydon, H.N., *J. Chem. Soc.*, 1444 (1936).

Sam, J., Minor, W.F., and Perron, Y.G., *J. Am. Chem. Soc.*, 81, 710 (1959).

Shiraishi, T., Usuda, S., and Ebara, S., *Nippon Seirigaku Zasshi*, 25, 146 (1963); *Chem. Abstr.*, 59, 10672c (1963).

Skita, A., and Wulf, C., Ann. Chim., 453, 190 (1927); Chem. Abstr., 21, 2876 (1927).

Smith, R.C., and Binkley, S.B., J. Org. Chem., 24, 249 (1959).

Solov'ev, V.M., Arendaruk, A.P., and Skoldinov, A.P., Zhur. Obshch. Khim., 31, 2577 (1961); Chem. Abstr., 56, 10027f (1962).

Southwick, P.L., and Crouch, R.T., J. Am. Chem. Soc., 75, 3413 (1953).

Spickett, R.G.W., Ridley, H.F., and Hall, G.H., British Patent 1, 039, 454 (1966); Chem. Abstr., 65, 15272b (1966).

Suminov, S.I., Vestn. Mosk. Univ., Ser. II22, 75 (1967); Chem. Abstr., 67, 21425r (1967).

Surrey, A.R., and Olivet, A.J., United States Patent 3, 097, 211 (1963); Chem. Abstr., 59, 13830c (1963).

Takagi, K., Kasuya, Y., and Hattori, K., J. Pharm. Soc. Japan, 72, 1592 (1952).

Takahashi, H., Nippon Seirigaku Zasshi, 23, 417 (1961); Chem. Abstr., 55, 23837f (1961).

Takahashi, H., Uchikura, K., Takahashi, H., and Ikeda, O., Japan J. Physiol., 11, 229 (1961).

Takahashi, H., Ikeda, O., Itaya, T., and Koshino, F., Japan J. Physiol., 11, 476 (1961).

Takahashi, H., and Kohiga, M., Japanese Patent 17, 960 (1962); Chem. Abstr., 59, 11269d (1963).

Takahashi, H., and Kohiga, M., Japanese Patent 17, 961 (1962); Chem. Abstr., 59, 11269d (1963).

Thiele, K., Koberstein, E., and Nonnenmacher, G., *Arzneimi-Forsch.*, *18*, 1255 (1968); *Chem. Abstr.*, *70*, 28564f (1969).

Thompson, W.E., Warren, R.J., Eisdorfer, I.B., and Zarembo, J.E., *J. Pharm. Sci.*, *54*, 1819 (1965).

Toivonen, T., Okela, K., and Kaipainen, W.J., *Lancet*, 175II (1959).

Traynelis, V.J., and Dadura, J.G., *J. Org. Chem.*, *26*, 686 (1961).

Treibs, W., and Reinheckel, H., *Chem. Ber.*, *89*, 51 (1956); *Chem. Abstr.*, *50*, 14526a (1956).

Tsunetsugu, T., Tanaka, M., and Murata, N., *Nippon Kagaku Zasshi*, *83*, 1111 (1962); *Chem. Abstr.*, *59*, 11401d (1963).

Viscontini, M., and Meier, J., *Helv. Chim. Acta.*, *33*, 1773 (1950); *Chem. Abstr.*, *45*, 3802e (1951).

Volle, R.L., and Koelle, G.B., *The Pharmacological Basis of Therapeutics*, (eds. Goodman, L.S., and Gilman, A.) 3rd edition, The Macmillan Co., New York, 1965, p. 578.

Vystrčil, A., and Hudeček, S., *Chem. Listy*, *44*, 262 (1950).

Weisel, C.A., Taylor, R.B., Mosher, H.S., and Whitmore, F.C., *J. Am. Chem. Soc.*, *67*, 1071 (1945).

Whitemore, F.C., Mosher, H.S., Adams, R.R., Taylor, R.B., Chapin, E.C., Weisel, C., and Yanko, W., *J. Am. Chem. Soc.*, *66*, 725 (1944).

Wilson, I.B., and Bergmann, F., *J. Biol. Chem.*, *185*, 479 (1950).

Wilson, I.B., and Bergmann, F., *J. Biol. Chem.*, *186*, 683 (1950).

Wilson, I.B., Bergmann, F., and Nachmansohn, D., *J. Biol. Chem.*, *186*, 781 (1950).

Wilson, I.B., Biochem. Biophys. Acta., 7, 520 (1951).

Wilson, I.B., J. Biol. Chem., 190, 111 (1951).

Wilson, I.B., J. Biol. Chem., 197, 215 (1952).

Wilson, I.B., J. Biol. Chem., 199, 113 (1952).

Wilson, I.B., Discussions Faraday Soc., 20, 119 (1955).

Wright, S., Applied Physiology, 11th edition, Oxford University Press, London, 1965, p. 131.

Zilka, A., and Rivlin, J., J. Org. Chem., 23, 95 (1958).

Zilka, A., Rachman, E.S., and Rivlin, J., J. Org. Chem., 26, 376 (1961).