NOTICE

AVIS

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canadä

UNIVERSITY OF ALBERTA

CHIRALITY AND METABOLISM: STUDIES ON THE

ANTIDEPRESSANTS *rac*-TRANYLCYPROMINE AND *rac*-FLUOXETINE

by

LAUNA J. ASPESLET   ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES

(NEUROCHEMISTRY)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

AND FACULTY OF MEDICINE (PSYCHIATRY)

EDMONTON, ALBERTA

SPRING, 1994

Canada

University of Alberta
Edmonton

Neurochemical Research Unit
Department of Psychiatry and
Faculty of Pharmacy and Pharmaceutical Sciences

Mailing Address: Department of Psychiatry,
1E7 H.W. Mackenzie Health Sciences Centre
Telephone (403) 492-5664 Fax (403) 492-6841

March 8, 1994

Mr. Ronald E. McMillen
Director, Publications and Marketing
American Psychiatric Association
1400 K Street NW
Washington, DC   20005
USA

Dear Mr. McMillen,

By this letter I am requesting permission to include the following information from the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised,* (DSM-III-R) in the Introduction of my Ph.D. thesis entitled "Chirality and metabolism: studies on the antidepressants *rac*-tranylcypromine and *rac*-fluoxetine". I will, of course, acknowledge the source of this material in my thesis

Diagnostic Criteria for Major Depressive Episode (pages 222 and 223 of DSM-III-R).

Thank you for your consideration of this matter. I look forward to your reply.

Yours sincerely,

Launa J. Aspeslet

... one time use. English language only. The correct
... American Psychiatric Association: Diagnostic
... of Mental Disorders, Third Edition, Revised,
Washington, DC: American Psychiatric Association, 1987.

Ronald E. McMillen   3/24/94   N/C
                                         Date   Fee
Dir., Publications and Marketing

Please send a copy of this letter/form along with check payable to APA,
Attn: Publications and Marketing

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Launa J. Aspeslet

TITLE OF THESIS: Chirality and metabolism: studies on the

antidepressants *rac*-tranylcypromine and

rac-fluoxetine

DEGREE FOR WHICH THESIS WAS PRESENTED: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1994

Permission is hereby granted to the UNIVERSITY OF ALBERTA

LIBRARY to reproduce single copies of this thesis and to lend or sell such

copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis

nor extensive extracts from it may be printed or otherwise reproduced

without the author's written permission.

_____

10729 - 71 Avenue

Edmonton, Alberta
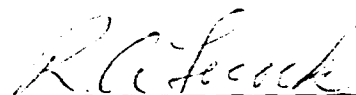
T6E 0X5

DATED:....*April 21*.............., 1994

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH
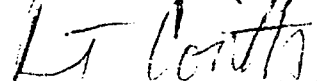
The undersigned certify that they have read, and recommend to the

Faculty of Graduate Studies and Research for acceptance, a thesis entitled

CHIRALITY AND METABOLISM: STUDIES ON THE

ANTIDEPRESSANTS rac-TRANYLCYPROMINE AND rac-FLUOXETINE

by LAUNA J. ASPESLET in partial fulfillment of the requirements for the

degree of DOCTOR OF PHILOSOPHY in Pharmaceutical Sciences.
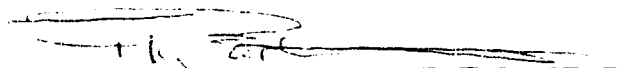
R.A. Locock (Chair and Examiner)

G.B. Baker (Cosupervisor)
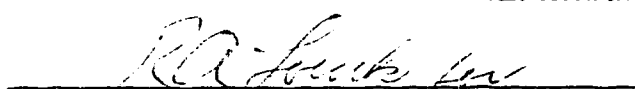
R.T. Coutts (Cosupervisor)

F.M. Pasutto

I.L. Martin

P.L. Wood (External Examiner)

DATED:......ArR.T.L...../.9......, 1994

ACKNOWLEDGEMENTS

ABSTRACT

The importance of chirality and metabolism in the actions of antidepressant drugs is often neglected. This thesis describes studies on the analysis of individual enantiomers of the antidepressant drugs tranylcypromine (TCP) and fluoxetine (FLU). The effects of drugs, which inhibit drug metabolizing enzymes, on brain levels of the enantiomers of these antidepressants were investigated. In addition, an assay was developed for a potentially important metabolite of FLU.

A novel assay developed for the separation and quantitation of the enantiomers of TCP was used to measure levels of TCP enantiomers in brain and liver tissue obtained from rats administered rac-TCP with or without pretreatment with iprindole (IPR), an inhibitor of aromatic ring hydroxylation.

The above assay was adapted for the simultaneous separation and quantitation of the enantiomers of FLU and norfluoxetine (NFLU). Levels of FLU and NFLU enantiomers in brain regions and whole brain from rats treated with rac-FLU with and without IPR pretreatment were determined. Enantiomers of FLU and NFLU were also measured in brains from rats treated with rac-FLU alone or in combination with desipramine (DMI) (the combination of FLU and DMI has been reported to be useful in the treatment of depression).

Pretreatment with IPR resulted in an elevation of brain and liver levels of (+)- and (-)-TCP, with a slightly greater increase of (-)-TCP than of (+)-TCP in brain. IPR pretreatment caused an elevation of brain levels of both R- and S-FLU, with a significantly greater effect on the R-enantiomer; an increase in brain levels of R- or S-NFLU was not observed. Co-administration of DMI with rac-FLU to rats produced an increase in brain levels of both FLU and NFLU over those observed when rac-FLU was

administered alone; the increase was similar with both enantiomers of FLU and NFLU.

In an attempt to identify metabolites of FLU other than NFLU, two screening procedures, using reaction with pentafluorobenzoyl chloride or pentafluorobenzenesulfonyl chloride, were utilized. The latter assay resulted in the identification and quantification of p-trifluoromethylphenol in urine samples from rats and humans treated with rac-FLU.

TABLE OF CONTENTS

FIGURE LEGENDS

LIST OF TABLES

ABBREVIATIONS

(-)	levorotatory
(+)	dextrorotatory
μg	microgram
μl	microlitre
2-OH-DMI	2-hydroxydesipramine
5-HT	5-hydroxytryptamine
cAMP	cyclic adenosine monophosphate
CI	chemical ionization
CYP2D6	cytochrome P450IID6
d-	dextrorotatory
DA	dopamine
DDMI	didesmethyldesipramine
DMI	desipramine
DMSO	dimethylsulfoxide
dopa	3,4-dihydroxyphenylalanine
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised
ECD	electron capture detector
ECT	electroconvulsive therapy

EDTA	ethylenediamine tetraacetate, disodium salt
EI	electron impact
FID	flame ionization detector
FLU	fluoxetine
GABA	γ-aminobutyric acid
GC	gas chromatography
h	hour
HP	Hewlett-Packard
HPLC	high-pressure liquid chromatography
i.p.	intraperitoneally
IMI	imipramine
IPR	iprindole
kg	kilogram
l-	levorotatory
M	molar
MAOIs	monoamine oxidase inhibitors
mg	milligram
min	minute
ml	millilitre
MS	mass spectrometer
m/z	mass-to-charge ratio

N	normal
NA	noradrenaline
NCI	negative chemical ionization
NFLU	norfluoxetine
ng	nanogram
NPD	nitrogen phosphorus detector
p	probability
p-	*para-*
PEA	2-phenylethylamine
PFBC	pentafluorobenzoyl chloride
PFBSC	pentafluorobenzenesulfonyl chloride
R	rectus
rac-	racemic
S	sinister
SAL	saline
SCOT	support-coated open tubular
TCAs	tricyclic antidepressants
TCD	thermal conductivity detector
TCP	tranylcypromine [*trans*-(±)-2-phenylcylclopropylamine]
TFMP	*p*-trifluoromethylphenol
v	volume

WCOT   wall-coated open tubular

1.0 INTRODUCTION

Mood disorders are common, with epidemiological studies suggesting that they occur in 3-6% of the general population at any given time (Boyd and Weissman, 1982; Holden, 1986; Tomb, 1988). The Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) recognizes major depression, categorizing it by the symptoms listed in Table 1. Major depression has been said to be a heterogenous disorder both in terms of origin and presenting symptoms. That is, the sequence of events contributing to the clinical state of depression will be influenced by the biological predisposition of the individual and, in turn, environmental factors will have a marked impact on the individual (Jesberger and Richardson, 1985). This heterogeneity is also indicated in the observation that any one antidepressant drug does not work in all types of depressions or in all individuals. There is now a significant body of evidence indicating that biochemical abnormalities in the brain are directly involved in the pathology of depression and other psychiatric disorders (Barchas *et al.*, 1978; McGeer & McGeer, 1980).

Effective drug treatments for depression were not available until the middle of this century. It was the discovery of the antidepressant

A "Major Depressive Syndrome" is defined as:

A. At least 5 of the following symptoms have been present for a 2-week period. At least one symptom is either #1 or #2.

    1. depressed mood.
    2. markedly diminished interest or pleasure.
    3. significant weight loss or weight gain or decrease or increase in appetite.
    4. insomnia or hypersomnia.
    5. psychomotor agitation or retardation.
    6. fatigue or loss of energy.
    7. feelings of worthlessness or excessive or inappropriate guilt.
    8. diminished ability to think or concentrate or indecisiveness.
    9. recurrent thoughts of death, suicidal ideation, suicide attempts.

B. 1. It cannot be established that an organic factor initiated and maintained the disturbance.
    2. The disturbance is not a normal reaction to the death of a loved one.

C. At no time during the disturbance have there been delusions or hallucinations for as long as 2 weeks in the absence of prominent mood symptoms.

D. The disorder is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder.

Table 1: An outline of the symptoms used by the DSM-III-R to classify a major depressive episode (extracted from DSM-III-R with permission of the publisher, the American Psychiatric Association).

properties of the monoamine oxidase inhibitors (MAOIs) that led to major

advances in the treatment of depression. During the search for a drug with

which to treat tuberculosis, isonicotinyl hydrazide (isoniazid) was

discovered. This substance was reported to show marked tuberculostatic

action (Bernstein et al., 1952; Grunberg and Schnitzer, 1952) and became

widely used in medical practice. In an attempt to find even more effective

antitubercular drugs, Fox and Gibas (1953) synthesized some monoalkyl

derivatives, including isopropyl-isonicotinyl hydrazide (iproniazid). The

availability of this compound served as a major impetus into research on

the chemotherapy of depression.

In the early 1950s, clinicians reported unexpected side effects of

hydrazine derivatives, especially of iproniazid (Pletscher et al., 1960). In

addition to showing tuberculostatic action, iproniazid also caused euphoria

and psychostimulation, leading in some cases to psychomotor excitation,

psychotic states, insomnia, increase of appetite, and changes in sexual

behaviour. Later, the drug was used intentionally to improve the mood of

chronically ill patients.

In addition to the clinical observations, Zeller and his colleagues

(1952) discovered that iproniazid was a potent inhibitor of MAO. This

enzyme is involved in the oxidative deamination of biogenic monoamines

and plays a role in their biological inactivation (Fowler and Ross, 1984). At

the same time as Zeller's discovery, additional ideas about the possible functional role of biogenic amines were beginning to emerge. Reserpine was a drug being used clinically as an antihypertensive and as a tranquilizing agent. It was discovered that reserpine caused a marked, long-lasting depletion of the neurotransmitters 5-hydroxytryptamine [serotonin; 5-HT] (Brodie *et al.*, 1955; Shore *et al.*, 1955), noradrenaline [NA] and dopamine [DA] (Carlsson *et al.*, 1957; Shore & Brodie, 1957) in animal brains. This drug also decreased locomotor activity and induced autonomic symptoms (e.g. miosis and diarrhea) in animals. Further studies revealed that iproniazid was able to reverse the depressant actions of reserpine (Brodie *et al.*, 1956; Chessin *et al.*, 1956).

The discovery of the tricyclic antidepressants (TCAs), drugs with three fused rings in their structure, was made almost simultaneously with the discovery of the MAOIs. The iminodibenzyl derivative imipramine (IMI), very similar structurally to the neuroleptic chlorpromazine and devoid of MAO-inhibitory effects, was found to have beneficial effect in patients with mental depression (Kuhn, 1970). It was also shown that, like MAOIs, these tricyclic compounds, reversed the depressant effects of reserpine (Sulser and Mishra, 1983).

The common effect of the MAOIs and TCAs is an increase in the

4

content of free neurotransmitters such as catecholamines and 5-HT in the synaptic cleft. The mechanisms of action are different, inhibition of reuptake into presynaptic nerve terminals by the TCAs and inhibition of metabolic breakdown by the MAOIs, but ultimately similar changes in neurotransmitter dynamics are the result.

Further interest in the antidepressant drugs was stimulated with the introduction of the biogenic amine hypothesis of depression (Schildkraut and Kety, 1967; Lapin and Oxenkrug, 1969). This theory states that depression is associated with a functional deficit of NA and/or 5-HT at central synapses. The biochemical actions of the MAOIs and the TCAs provide pharmacological support for this theory.

Several observations about depression do not support this theory (Dewhurst, 1969; Sulser, 1982; Johnstone, 1982; Sugrue, 1983; Maj *et al.*, 1984):

1. High plasma levels of the TCAs are achieved almost immediately but there appears to be a 2 to 3 week delay for the antidepressant effect to be observed (Oswald *et al.*, 1972; Quitkin *et al.*, 1986; Post *et al.*, 1987).

2. There have been novel antidepressants developed (i.e. iprindole, mianserin, and trazodone) that do not inhibit MAO or

the uptake of biogenic amines yet are still effective antidepressants (Freeman and Sulser, 1972; Rosloff and Davis, 1974; Sanghvi and Gershon, 1975; Coppen et al., 1976; Sulser et al., 1978).

3. Cocaine is a strong inhibitor of NA uptake but is not an effective antidepressant.

4. Inconsistent antidepressant effects have been obtained by administering precursor amino acids (e.g. tryptophan, tyrosine, phenylalanine) of NA or 5-HT to patients (Baker and Dewhurst, 1985).

Despite these findings, the biogenic amine hypothesis of depression is still the focus of much research in this area. The theory of depression has focused mainly on NA and/or 5-HT. There has also been work done suggesting the involvement of DA in the etiology of depression (Randrup et al., 1975; Randrup and Braestrup, 1977; Willner, 1983a,b,c; Fibiger et al., 1990; Brown and Gershon, 1993). The brain also contains a number of amines referred to as trace amines [so called because they are present in small absolute quantities in mammalian brain (<5ng/g whole brain)] that have been implicated in depression; these include octopamines, tyramines,

6

tryptamine, and 2-phenylethylamine (PEA) (Dewhurst, 1968; Boulton, 1976; Boulton and Juorio, 1982; Paulos and Tessel, 1982; Sabelli et al., 1983).

The delay in onset of action of almost all antidepressant treatments despite the observation of immediate biochemical effects on neurotransmitters suggests that there may be some additional delayed biochemical changes responsible for the therapeutic effect of these treatments. Research has shown that some delayed effects involving receptors in the brain are observed only after 2 to 3 weeks of chronic exposure in rodents, which is consistent with the time course of antidepressant activity in humans. These changes have been observed with TCAs, MAOIs, some of the novel antidepressants, and electroconvulsive therapy (ECT). The most consistently reported changes are a decrease in NA-stimulated cyclic adenosine monophosphate (cAMP) production and in β-adrenergic and 5-HT_2 receptor density; an increase in α_1-adrenergic and in 5-HT receptor sensitivity demonstrated electrophysiologically; and potentiation of α_1-adrenergic-mediated and DA-mediated behavioral responses (Sulser et al., 1978; Sugrue, 1983; Charney and Heninger, 1986; Baker and Greenshaw, 1989). These pre- and post-synaptic receptor changes are not apparent after acute treatment.

There is now a large body of literature suggesting that the inhibitory amino acid γ-aminobutyric acid (GABA) may have an important role in the

7

etiology and pharmacotherapy of depression, but this area of research continues to be surrounded by controversy. Reports have indicated that chronic administration of antidepressants of every class (TCAs, MAOIs and novel antidepressants) as well as ECT have resulted in an up-regulation of $GABA_B$ receptors in rat cortex (Lloyd et al., 1985, 1989; Gray and Green, 1987). This effect of antidepressant drugs has also been observed by Suzdak and Gianutsos (1986), but has been disputed by others (Cross and Horton, 1987, 1988; Szekely et al., 1987; McManus and Greenshaw, 1991a,b). This research has recently been extended to include the $GABA_A$/benzodiazepine receptor-chloride ionophore complex. It has been known for some time that many actions of the benzodiazepines are mediated through the $GABA_A$ receptor (Martin, 1987). Cheetham et al. (1988) found an increased number of benzodiazepine binding sites in frontal cortex from depressed suicide victims. Suzdak and Gianutsos (1986) reported a reduction in the density of $GABA_A$ binding sites with chronic administration of antidepresants. Other authors claim that $GABA_A$ function is unaltered by antidepressants (Lloyd et al., 1989). Suranyi-Cadotte et al. (1984, 1990) reported that chronic administration of several antidepressants (DMI, zimelidine, bupropion and adinazolam) to rats resulted in a decreased number of [^{3}H]flunitrazepam binding sites in rat

8

brain. Kimber et al. (1987) did not find this decrease after chronic administration of DMI, tranylcypromine or zimelidine, and workers in the Neurochemical Research Unit have been unable to find any changes in [^{3}H]flunitrazepam binding in cortex taken from rats treated chronically with phenelzine or tranylcypromine (McKenna et al., 1992, Todd et al., 1992). GABA agonists (e.g. progabide, baclofen, muscimol, fengabine) have shown antidepressant effects in animal models and psychiatric patients (Lloyd et al., 1989; Nielsen et al., 1991) but there is still some controversy about the clinical effectiveness of these agents (Paykel et al., 1991).

1.1 THE CLASSES OF ANTIDEPRESSANT DRUGS

The antidepressant drugs currently available can be divided into three main classes--the MAOIs, the TCAs and the "novel" antidepressants.

1.1.1 MONOAMINE OXIDASE INHIBITORS

As previously mentioned, MAOIs work by inhibiting the enzyme MAO which carries out oxidative deamination of biogenic amines. This inhibition leads to an increase in the levels of these biogenic amines in the brain.

There are two forms of MAO, which can be defined by the preference of the enzyme for substrates and by the selectivity of certain inhibitors (Johnson, 1968). The preferential substrate for MAO-A is 5-HT and a selective inhibitor is clorgyline (Johnson, 1968). PEA is the preferential substrate for MAO-B and a selective inhibitor is (-)-deprenyl (Finberg and Youdim, 1983).

It is important to note that in addition to inhibiting MAO, these antidepressants have an effect on many other systems within the body which may lead to an array of side effects. The most commonly occurring side effect of MAOIs is othostatic hypotension (Murphy *et al.*, 1985; Keck *et al.*, 1991). Other side effects involve anticholinergic effects such as dry mouth, constipation, sexual dysfunction, and hepatotoxicity (Baldessarini, 1985). The most serious toxic effect of these drugs is known as the "cheese effect" which can lead to headaches, a hypertensive crisis and stroke (Sjoqvist, 1965; Baldessarini, 1985). This effect occurs when patients taking MAOIs consume food containing sympathomimetic amines such as *p*-tyramine (i.e. aged cheese, red wine, brewer's yeast). In these patients *p*-tyramine cannot be broken down by MAO in the gut wall, resulting in an increased amount of this sympathomimetic amine in the circulation. *p*-Tyramine then displaces NA (which is also normally

catabolized by MAO) from its storage sites in adrenergic nerves, leading to an over-stimulation of adrenergic receptors and a dramatic elevation in blood pressure (Marley and Blackwell, 1970; Sandler, 1981). This toxic side effect can be avoided by giving the patients a list of foodstuffs to avoid. The "cheese effect" does not seem to be a problem with the recently introduced reversible MAO-A inhibitors such as moclobemide and brofaromine (Bieck et al., 1993). Another problem may arise when an MAOI is combined with a TCA; a syndrome of severe CNS toxicity, characterized by hyperpyrexia, convulsions, and coma (Baldessarini, 1990) may occur. This problem may be avoided provided the drugs are given properly with regard to dose and sequence, but clomipramine should be avoided in such a combination (Schukit et al., 1971; Sethna, 1974; Ananth and Luchins, 1977; White and Simpson, 1981; Pare et al., 1982; Schmauss et al., 1988).

According to Nies (1983), the typical symptom profile of a patient responsive to MAOI treatment is:

1. Psychopathological symptoms: mood reactivity retained, irritability, panic episodes, agoraphobia, social fears, hypochondriasis, obsessive preoccupation

11

2. Interpersonal reactions: self pity/blaming others, communicative suicidal actions, rejection sensitive, vanity/applause seeking, histrionic personality

3. Vegetative symptoms: initial insomnia, hypersomnia, weight gain, hyperphagia, craving for sweets, lethargy and fatigue, tremulousness

4. Historical features: personal loss before intensification, poor response to electroconvulsive shock therapy, liking amphetamines, dysphoric tricyclic responses, alcohol/sedative abuse.

Such depressions are referred to as 'atypical depressions', but a review by Murphy *et al.* (1987) indicates that the MAOIs are as effective as the TCAs in treating major depression. It has been reported that MAOIs are also effective in the therapeutic management of panic disorder, atypical facial pain, the phobic depersonalization syndrome, refractory thought disorders, neurodermatitis, treatment-resistant narcoleptic states, recalcitrant migrainous headache (associated with reduced plasma 5-HT) and idiopathic orthostatic hypotension (review: Tollefson, 1983).

12

1.1.1.1   TRANYLCYPROMINE

Of the MAOIs, tranylcypromine (trans-(±)-2-phenylcyclopropylamine sulfate; TCP) [Figure 1], has been one of the most frequently prescribed and is of particular interest to this thesis because it has two chiral centers. This drug was synthesized in 1948 by Burger and Yost for Smith Kline & French Laboratories in an effort to find a clinically useful analog of amphetamine (Burger and Yost, 1948). It was later shown to inhibit MAO (Maass and Nimmo, 1959; Tedeschi et al., 1959) and was marketed as an antidepressant drug in 1961 under the name of Parnate®. In 1964, the U.S. Food and Drug Administration ordered its removal from the market for a few months after reports of severe hypertensive reactions in some patients. It was later reinstated in the U.S. under strict guidelines (Sadusk, 1964).

The efficacy of TCP as an antidepressant has been extensively documented (Quitkin et al., 1979). It has been reported to be particularly efficacious in depressions associated with anxiety, agitation and phobias (Lesse, 1978; Sheehan et al., 1980; Tyrer, 1976), and anergia (Himmelhoch et al., 1982). There have also been several reports indicating that high doses of TCP are effective in treating refractory depression

13
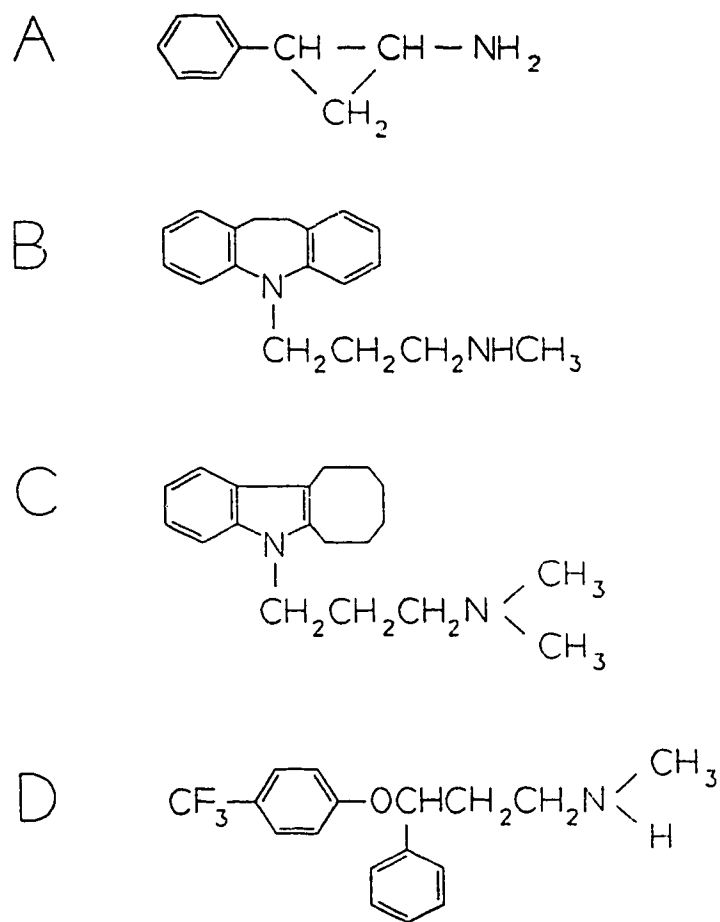
Figure 1: Chemical structures of the antidepressants of interest in this thesis: A) the monoamine oxidase inhibitor, tranylcypromine, B) the tricyclic antidepressant, desipramine, and the novel antidepressants, C) iprindole and D) fluoxetine.

(Robinson, 1983; Pearlman, 1987; Guze *et al.*, 1987; Amsterdam and Berwish, 1989).

TCP is classified as an irreversible and nonspecific (i.e. it inhibits both the A and B forms of MAO) MAOI. Within an hour after administration, amine levels throughout the body are reported to be elevated (Philips and Boulton, 1979). Brain levels of 5-HT are increased on a proportionately greater basis than the catecholamines, and trace amines such as PEA and tryptamine are increased to a much greater extent above their baseline levels than are the catecholamines or 5-HT (Philips and Boulton, 1979; McKim *et al.*, 1980).

In addition to inhibiting MAO, TCP has also been shown to inhibit the uptake of catecholamines (Hendley and Snyder, 1968; Escobar *et al.*, 1974) and cause release of NA (Schildkraut, 1970), DA and 5-HT (Baker *et al.*, 1980).

TCP is rapidly absorbed and extensively metabolized within 24 h of its administration (Lader, 1980; Mallinger *et al.*, 1990; Keck *et al.*, 1991). In the rat it undergoes acetylation to give N-acetyl-TCP (Calverley *et al.*, 1981; Kang and Chung, 1984) and ring hydroxylation to give 4-hydroxy-TCP (Baker *et al.*, 1986; Nazarali *et al.*, 1987a). N-Acetyl-4-hydroxy-TCP has also been identified as a metabolite of TCP (Kang and Chung, 1984).

The cyclopropyl ring may be broken to potentially yield amphetamine (Youdim *et al.*, 1979) although this route of metabolism remains controversial (Reynolds *et al.*, 1980; Mutschler and Mohrke, 1983; Mallinger *et al.*, 1990; Keck *et al.*, 1991; Jefferson, 1992; Sherry-McKenna, personal communication).

TCP is administered as a racemic mixture of its two enantiomers, 1R,2S-(-)-*trans*-phenylcyclopropylamine [(-)-TCP] and 1S,2R-(+)-*trans*-phenylcyclopropylamine [(+)-TCP] (Riley and Brier, 1972). 2-Phenylcyclo-propylamine can adopt either a *cis* or a *trans* form. TCP, as the name implies, is the *trans* form of 2-phenylcyclopropylamine, which can exist as one of two possible enantiomers.

Much research has been done on the individual enantiomers of TCP and it has been shown that there is a significant difference between the actions of the two. Reports have shown that (-)-TCP is a stronger inhibitor of NA and DA uptake into nerve terminals (Horn and Snyder, 1972; Tuomisto and Smith, 1986) while (+)-TCP is the more potent inhibitor of MAO (Zirkle *et al.*, 1962; Fuentes *et al.*, 1976; Reynolds *et al.*, 1980) and dramatically elevates the levels of the MAO-B substrate, PEA (Hampson *et al.*, 1986). (-)-TCP has been shown to attain a higher peak level than (+)-TCP in rat brain and human plasma (Hampson *et al.*, 1986; Reynolds *et al.*,

1980; Weber-Grandke *et al.*, 1993). Some preliminary reports conducted at short time intervals after administration of *rac*-TCP indicated that (-)-TCP is cleared faster from brain than is (+)-TCP (Hampson *et al.*, 1986; Reynolds *et al.*, 1980), but a more recent comprehensive report by Weber-Grandke et al. (1993) suggests that (+)-TCP has a shorter half-life than (-)-TCP. Fischer (1991) has reported that (+)-TCP is more potent than (-)-TCP as an anticonvulsant.

1.1.2 TRICYCLIC ANTIDEPRESSANTS

TCAs are so-named because of their structure. The majority of the TCAs consist of two benzene rings joined by a central seven member ring (Figure 1). The TCAs are well absorbed after oral administration and are then widely distributed. They are lipophilic and strongly bound to tissue and plasma proteins (Baldessarini, 1985). Metabolism of these drugs is extensive and results in both active and inactive metabolites. This metabolism occurs *via* four main routes:

1.   desmethylation of a side chain,

2.   N-oxidation of a side chain,

3.   hydroxylation of various positions of the ring structure and

4.   glucuronide formation (Rudorfer and Potter, 1985).

Tertiary amines, such as IMI, are N-demethylated at the side chain

leading to the formation of a secondary amine [desmethylimipramine

(desipramine, DMI) in the case of IMI]. Several of these secondary amines

are themselves active antidepressant agents (Mindham, 1979). IMI also

undergoes N-oxidation and IMI and DMI undergo 2-hydroxylation, forming

active metabolites (Potter and Manji, 1990; Young, 1991).

The side-effects of TCAs include anticholinergic effects such as

blurred vision, dry mouth and constipation. Side effects due to blockade of

adrenergic receptors include tachycardia, arrhythmias and orthostatic

hypotension. Interactions with other drugs may also present a problem as

many other drugs have an effect on the same liver enzymes involved in

metabolism of TCAs. Some drugs, such as phenothiazines, methylpheni-

date and fluoxetine, may be competitors for the enzymes while other drugs,

such as barbiturates and anticonvulsants, may stimulate or induce the

enzymes (Rudorfer and Potter, 1985; Dubovsky, 1987; Bergstrom et al.,

1992). Interaction between MAOIs and TCAs may be potentially lethal, as

already mentioned.

It has been widely assumed that the mechanism of action of the

TCAs is to increase the concentration of NA and/or 5-HT at the receptor

site by inhibition of the neuronal uptake for these amine neurotransmitters

18

(Glowinski & Axelrod, 1964; Carlsson *et al.*, 1969; Schildkraut, 1970;

Davis, 1970). The tertiary amine TCAs, such as IMI, primarily block 5-HT

uptake whereas those that are secondary amines, such as DMI, primarily

block the uptake of NA (Potter and Manji, 1990). TCAs usually affect DA to

a much smaller extent. TCAs block a number of neurotransmitter receptors

including muscarinic (Snyder and Yamamura, 1977), histaminergic (Diffley

et al., 1980; Green and Maayani, 1977), adrenergic (U'Prichard *et al.*,

1978) and serotonergic receptors (Snyder and Peroutka, 1984; Richelson,

1984). It is the tertiary amines, rather than the secondary amines, that tend

to be most potent at blocking these receptors (Potter and Manji, 1990).

1.1.2.1   DESIPRAMINE

Desipramine (Figure 1) is a TCA that is of particular interest to this

thesis since it is a substrate for the catabolic isozyme cytochrome

P450IID6 (CYP2D6) and is often given in combination with fluoxetine,

another substrate for this isozyme. See later sections of this introduction

for discussions on CYP2D6 and fluoxetine. As already mentioned, DMI is a

metabolite of the antidepressant IMI and is itself an effective

antidepressant.

The major metabolite of DMI in the human is 2-hydroxydesipramine (2-OH-DMI). After hydroxylation, DMI undergoes inactivation by glucuronidation and is excreted by the kidneys (Risch et al., 1981). In addition, the following metabolites have been identified in the urine of patients taking DMI: 10-hydroxy-DMI, didesmethyl-DMI (DDMI), 2-hydroxy-DDMI, 10-hydroxy-DDMI, iminodibenzyl (which resulted from the destruction of the tricyclic skeleton of the drug), and 10-hydroxy-DDMI-glucuronide (Crammer and Scott, 1966). Studies using synaptosomal preparations have shown that 2-OH-DMI is also a potent inhibitor of NA uptake, and in patients it has been shown that this metabolite accumulates in significant proportions in CSF (Potter et al., 1979, 1980; De Vane et al., 1981).

DMI and its hydroxylated metabolites are absorbed into many body compartments, and their respective plasma levels represent only a small percentage of the administered dose (Risch et al., 1981). DMI is extensively bound to plasma protein (Gram and Christiansen, 1975). It may reach steady state levels within one week with daily oral administration (Hammer and Brodie, 1967; Hammer and Sjoqvist, 1967; Alexanderson, 1972), and has a mean half-life of 32.7 hours (Ziegler et al., 1978).

1.1.3 "NOVEL" ANTIDEPRESSANTS

Despite the success of the MAOIs and the TCAs over the past 30 years, their significant side-effect profiles and their delayed onset of action have lead researche,s to search for new classes of antidepressants. The search was on for antidepressants that produce a better side-effect profile, a more rapid onset of action, and greater efficacy in a higher percentage of patients (Fuller, 1981). In some instances, this meant the discovery of antidepressants that neither inhibited MAO nor, in several cases, blocked the uptake of NA, 5-HT, or DA (Baldessarini, 1985), leading to the use of the term "novel" antidepressants. Examples of such antidepressants are iprindole (Rudorfer and Potter, 1989) and mianserin (Zis and Goodwin, 1979).

Another driving force behind the search for "novel" antidepressants was largely based on the theory that if a functional disturbance of one monoamine system is an underlying factor in depression, a better response may be seen with an antidepressant which affected that particular system selectively. This has led to the introduction of several selective 5-HT uptake inhibitors, including zimelidine, fluvoxamine, fluoxetine, sertraline and paroxetine (Feighner, 1980; Hyttel and Larsen, 1985; Montgomery, 1985; Leonard, 1988). Also introduced were the selective NA and DA

uptake blocker nomifensine and the selective DA uptake inhibitor bupropion (Feighner, 1980; Rudorfer and Potter, 1989).

Two novel antidepressants, iprindole and fluoxetine, are of specific interest to this thesis and are discussed in detail below.

1.1.3.1   IPRINDOLE

Iprindole [5-(3-dimethylaminopropyl)-6,7,8,9,10,11-hexahydro-5H-cyclooct(b)indole] was introduced clinically in 1967 with the claim that it was as efficient as, and less toxic than, other antidepressants (Cassidy and Henry, 1987). The structure of iprindole (IPR) is very similar to that of the TCAs except that an indole group replaces two rings of the standard tricyclic structure (Rudorfer and Potter, 1989) [Figure 1]. Its mechanism of action is still unclear but it has been shown that it is not an inhibitor of NA uptake (Lahti and Maickel, 1971) and appears to enhance adrenoceptor activity by an unknown mechanism (Gluckman and Baum, 1969). It has been shown in clinical trials that IPR produces only mild anticholinergic effects and is relatively safe in overdose cases (Cassidy and Henry, 1987); it demonstrates a time course for onset of antidepressant effects similar to that for the TCAs (Rudorfer and Potter, 1989). The antidepressant efficacy

of IPR has been questioned (Tait and Todrick, 1975) and its use in recent years has therefore rapidly declined.

The relevance of IPR to this thesis is its ability to inhibit metabolic aromatic hydroxylation of various substrates in rat. This ability has been amply displayed in studies with amphetamine (Freeman and Sulser, 1972; Fuller and Hemrick-Luecke, 1980; Steranka, 1982; Fuller et al., 1992; Henderson et al., 1992; Nash and Yamamoto, 1993), TCP (Baker et al., 1986) and trimipramine (Coutts et al., 1991). IPR also blocks some N-dealkylation reactions, as it has been reported to block N-deethylation of fenfluramine (Hegadoren et al., 1990), but this aspect of its action has not been extensively studied.

1.1.3.2   FLUOXETINE

Fluoxetine (FLU) [Figure 1], a selective 5-HT uptake inhibitor (Wong et al., 1975; Fuller et al., 1991), is of particular interest to this thesis because of the presence of a chiral center in its structure. This selective antidepressant was introduced in North America approximately five years ago under the trade name of Prozac®. Since that time it has received a great deal of attention both from the scientific community and the general

23

public and has even been touted as the "wonder drug of the '90s". The main reasons for its initial appeal are its broad range of applications and its fewer number of adverse effects relative to the TCAs. In 1990, after FLU had only been on the market for a short period of time, The Medical Letter (Abramowicz, 1990) described it as "the most frequently prescribed of all antidepressants". Even today, after all the negative publicity it has received regarding its possible involvement in suicidal ideation (Teicher et al., 1990; Hamilton and Opler, 1992), FLU is still one of the most frequently prescribed antidepressants.

FLU acts by selectively inhibiting the uptake of 5-HT, thus increasing 5-HT neurotransmission (Richelson and Nelson, 1984; Richelson and Pfenning, 1984). Research has shown that FLU has the ability to inhibit competitively the uptake of radiolabelled 5-HT in synaptosomes prepared from rat brain with a potency equal to that of clomipramine (Wong et al., 1975). FLU has also been shown to antagonize brain 5-HT depletion by p-chloroamphetamine in mice and in rats (Fuller et al., 1974, 1975). Using a cytofluorimetric technique called "fading", Geyer and his colleagues (1978) were able to provide evidence that FLU increases 5-HT levels in the synaptic cleft. More evidence that synaptic levels of 5-HT were increased by FLU was provided by in vivo voltammetry (Marsden et al., 1979) and

24

push-pull cannulae (Guan and McBride, 1986). The decrease in 5-HT turnover following FLU treatment is seen as a compensatory device due to the increased activation by 5-HT of presynaptic autoreceptors that modulate 5-HT synthesis and release (Fuller and Wong, 1989).

The selectivity of FLU for 5-HT uptake has been extensively reported in the literature. Wong and his colleagues (1975) reported that the FLU-induced inhibition of uptake is very selective for 5-HT, with a Ki value for the inhibition of NA uptake 170 times higher than the Ki value for 5-HT uptake. Another study reported that by using the measurement of blood pressure responses to administration of tyramine and NA, FLU had no effect on uptake of these two amines at noradrenergic neurons (Lemberger *et al.*, 1978). FLU does not antagonize the destruction of brain NA nerve terminals by 6-hydroxydopamine in rats (Wong and Bymaster, 1976). Other studies have shown that FLU, unlike the TCAs, has relatively low affinity for multiple neurotransmitter receptors at concentrations necessary to inhibit 5-HT. FLU has a low affinity for muscarinic cholinergic, histaminergic (H1 and H2), α_1- and α_2-adrenergic, $5-HT_2$, opioid, γ-aminobutyric acid (GABA), benzodiazepine, and dopamine (D_1 and D_2) receptors (Stark *et al.*, 1985; Wong *et al.*, 1983).

The selectivity of FLU is maintained upon N-demethylation to its major metabolite, norfluoxetine [NFLU] (Fuller, 1984). NFLU is as potent

as FLU at inhibiting the uptake of 5-HT *in vivo* and *in vitro* (Fuller *et al.*, 1978, 1991; Wong *et al.*, 1975). This aspect sets FLU apart from 5-HT uptake inhibiting TCAs such as clomipramine. The N-demethylated metabolite of clomipramine is a potent inhibitor of NA uptake (Fuller *et al.*, 1978).

FLU is shown to be well absorbed in both rat and man (Caccia *et al.*, 1990) and reaches steady state after 2 to 4 weeks of long term administration (Benfield *et al.*, 1986). It has been shown to be approximately 95% protein bound in plasma from normal subjects (Aronoff *et al.*, 1984). Both FLU and NFLU are extensively distributed in tissues, particularly the lung, liver, and brain, and slowly released (Parli and Hicks, 1974). This has been indicated by very large volumes of distribution for both FLU and NFLU (Aronoff *et al.*, 1984). The average elimination half-life of FLU is 1 to 4 days while that of NFLU is 7 to 15 days (Lemberger *et al.*, 1985). Upon administration, FLU is extensively metabolized by the body so that very little of the unchanged drug is excreted in the urine (Benfield *et al.*, 1986). It is of great interest that approximately 70% of the administered FLU is still unaccounted for (Lemberger *et al.*, 1985). NFLU is the only metabolite of FLU that has been extensively studied (Lemberger *et al.*, 1985; Parli and Hicks, 1974; Schmidt *et al.*, 1988). It has been reported

26

that there are interindividual differences in the metabolism of FLU to NFLU and that a higher ratio of FLU/NFLU after 3 weeks of treatment appeared to correlate with a better antidepressant response (Montgomery *et al.*, 1990; Tyrer *et al.*, 1990). It has been reported that *p*-trifluoromethylphenol (TFMP) may also be a metabolite of FLU and NFLU (Benfield *et al.*, 1986). After the formation of TFMP, the remainder of the molecule may further be metabolized to hippuric acid. However, there are no reports on the quantitation of this potential metabolite in the literature (R. Bergstrom, Lilly Research Laboratories, personal communication).

FLU was originally introduced for the treatment of depression, and much research has proven its effectiveness. It has been shown to be more effective than placebo in the treatment of a variety of forms of depression, including mild depression (Dunlop *et al.*, 1990), major depression (Byerley *et al.*, 1988), refractory depression (Beasley *et al.*, 1990a; Solyom and Gibson, 1990), depression in the elderly (Feighner and Cohn, 1985), and depression in adolescents (Simeon *et al.*, 1990). FLU has given positive results in the treatment of panic attacks (Brady *et al.*, 1989; Schneir *et al.*, 1990) and obsessive-compulsive disorders (Turner *et al.*, 1985; Fontaine and Choinard, 1986; Murphy and Pigott, 1990; Pigott *et al.*, 1990) and shows promise in the treatment of obesity (Willner *et al.*, 1990), substance

27

abuse (Batki et al., 1993; Polson et al., 1993), borderline personality disorder (Cornelius et al., 1990), bulimia nervousa (Freeman and Hampson, 1987; Marcus et al., 1990; Goldbloom and Olmsted, 1993), and post-traumatic stress disorder (Nagy et al., 1993).

The limited side-effect profile of FLU includes nausea, nervousness, and insomnia (Wernicke, 1985). These effects tend to be mild and occur early in the treatment. In addition, in less than 20% of patients, headache, tremor, anxiety, drowsiness, dry mouth, sweating, and diarrhea may occur (Wernicke, 1985). All reports of side-effects increased with higher doses (Beasley et al., 1990b). Overall there is a much lower incidence of anticholinergic, hypotensive, and sedative side effects observed when compared with the TCAs (Schatzberg et al., 1987; Wernicke, 1985; Young et al., 1987).

Recently, both FLU and NFLU have been shown to be inhibitors of CYP2D6 (Brosen and Skjelbo, 1991; Crewe et al., 1992; Otton et al., 1993), an enzyme that is responsible for the hydroxylation of many drugs. This is of great importance when FLU is administered with other drugs and will be discussed in more detail in following sections.

FLU is yet another example of a drug that is administered as a racemic mixture. Although the eudismic ratio (see Section 1.4.1) is very

near unity, research has shown that S-FLU is slightly more potent than R-FLU at inhibiting [^{3}H]5-HT uptake in cortical synaptosomes, at inhibiting [^{3}H]FLU binding (indicative of binding to the 5-HT carrier) in cortical membranes, at inhibiting 5-HT uptake *ex vivo* in synaptosomes from brain stem and cortex, and at antagonizing *p*-chloroamphetamine-induced depletion of brain 5-HT (Fuller and Snoddy, 1986; Fuller *et al.*, 1991; Robertson *et al.*, 1988; Wong *et al.*, 1985). It has also been demonstrated that the time courses of R- and S-FLU are different with regard to inhibition of 5-HT uptake, with inhibition lasting up to 24 h after a single dose of S-FLU but only 8 h after a single dose of R-FLU (Wong *et al.*, 1985). This latter observation is consistent with the finding that S-FLU is more tightly associated with serum proteins than R-FLU (Peyton *et al.*, 1991).

The metabolite NFLU is also a chiral compound. It has been shown that S-NFLU is more potent than R-NFLU at inhibiting uptake of 5-HT in rat brain synaptosomes *in vitro*, at antagonizing *p*-chloroamphetamine-induced depletion of rat brain 5-HT (Fuller *et al.*, 1991), and at inhibiting [^{3}H]paroxetine binding to 5-HT uptake sites (Wong *et al.*, 1993). These differences in potencies observed between the enantiomers of NFLU are more pronounced than those observed between the enantiomers of FLU. It is also of interest that Stevens and Wrighton (1993) recently reported that

the S-enantiomers of both FLU and NFLU were 5 to 6 times more potent than the R-enantiomers as competitive inhibitors of CYP2D6-mediated bufuralol 1'-hydroxylation *in vitro*.

1.2   DRUG-DRUG INTERACTIONS

In the treatment of mental illness, there is the widespread occurance of polypharmacy (Grahame-Smith, 1977). This use of multiple drugs leads to the potential for drug interactions which can either be of benefit or be the cause of adverse effects. In extreme cases, the potentiation of the pharmacological effects caused by drug interactions can lead to toxic effects that are life-threatening (Shen and Lin, 1991). In the opposite situation, the decrease in activity of one drug caused by another drug may lead to a relapse or worsening of the psychiatric problems of the patients (Shen and Lin, 1991).

Drug interactions can be pharmacodynamic or pharmacokinetic (Grahame-Smith, 1977). A pharmacodynamic drug interaction is an interaction that involves either a quantitative or a qualitative change in the action of one of the drugs (Grahame-Smith, 1977). This interaction is very difficult to assess in a clinical setting. A pharmacokinetic interaction is one

that results in an alteration in the expected plasma level of a drug through some effect on absorption, distribution, metabolism and/or elimination. An example of this with relevance to this thesis is the interaction between FLU and DMI.

The combination of DMI and FLU is often used because of reports that it achieves a more rapid antidepressant response than the drugs given individually (Baron et al., 1988; Nelson et al., 1991). However, there are also reports that elevated blood levels of DMI had been associated with adverse clinical effects in depressed patients who were being treated with FLU and DMI simultaneously (Bell and Cole, 1988; Vaughan, 1988; Aranow et al., 1989; Downs et al., 1989; Goodnick, 1989; Westermeyer, 1991). Research has shown that FLU can inhibit the N-demethylation and 2-hydroxylation of DMI by rat liver microsomes in vitro and can lead to increased blood and tissue concentrations of DMI in rats in vivo (Fuller and Perry, 1989). In humans, the combination leads to an increased amount of DMI and a decreased amount of 2-OH-DMI being excreted in the urine (Bergstrom et al., 1992). NFLU has also been shown to inhibit the metabolism of DMI, although to a lesser extent than FLU (Fuller and Snoddy, 1991). This increase in DMI levels in the presence of FLU is seen to be reponsible for the adverse clinical effects but can easily

be controlled if plasma levels of drugs are monitored (Nelson *et al.*, 1991; Weilburg *et al.*, 1989).

The reason suggested for this drug interaction is the involvement of the cytochrome P450 system of enzymes (Bergstrom *et al.*, 1992) which will be discussed in more detail in the following section. It has been shown that the 2-hydroxylation of DMI is mediated by CYP2D6 (Spina *et al.*, 1984). FLU is metabolized by the P450 system as well. Although the specific P450 isozymes involved are not yet known (Bergstrom *et al.*, 1992), it has been suggested that CYP2D6 is not the enzyme responsible for the majority of FLU N-demethylation (Stevens and Wrighton, 1993). Research has shown that both FLU and NFLU are inhibitors of CYP2D6 activity and would therefore affect the metabolism of other drugs metabolized by this isozyme (Brosen and Skjelbo, 1991; Crewe *et al.*, 1992; Otton *et al.*, 1993).

It is also possible that the individual enantiomers of a racemic mixture have the potential to be a source of drug interactions, or the individual enantiomers may be involved to different extents in interactions with other drugs (Jamali *et al.*, 1989). Such an interaction has been observed when cimetidine is coadministered with verapamil. An increase in the bioavailability of both verapamil enantiomers is observed. However,

twice that of the (+)-enantiomer. This then leads to a more pronounced effect of verapamil in the presence of cimetidine even at identical total concentrations of verapamil (Mikus et al., 1988).

Another example of a drug interaction showing stereoselectivity is that between warfarin and cimetidine. It has been shown in humans that cimetidine interacts only with R-warfarin, leaving the more active S-warfarin unaffected (Choonara et al., 1986).

1.3 METABOLISM OF ANTIDEPRESSANTS AND THE CYTOCHROME P450 ENZYME SYSTEM

There are two basic purposes of the metabolism of drugs:

1. to convert drugs to products (metabolites) that are less pharmacologically active, or in other words, to terminate a pharmacological action, and

2. to convert drugs to products that are much more water soluble (i.e. more polar or ionized) than the parent drug and therefore more readily and rapidly excreted.

33

less pharmacologically active and more polar than the parent drug. However, there are also many examples of metabolism resulting in the production of active metabolites (reviews: Rudorfer and Potter, 1985; Young, 1991; Baker *et al.* 1994). In other words, metabolites that possess a pharmacological activity equal to, greater than, or different from the parent drug may be formed. In fact, such metabolism may be the basis of the actions of certain prodrugs, i.e. a compound that is inactive in the administered form becomes an active compound by being metabolized by the biological system (Albert, 1958; Harper, 1959; Poznansky and Juliano, 1984; Gardner and Alaxander, 1985).

Drug metabolism occurs mainly in the liver, but has also been shown to occur to a lesser extent in the kidney, blood, brain, lungs, gastro-intestinal tract, skin and other tissues (Gibson and Skett, 1986). One of the most important drug metabolism reactions is oxidation, and it occurs mainly in the liver. The oxidase enzymes, also referred to as the cytochrome P450 isozymes, are located in the smooth endoplasmic reticulum. Cytochrome P450 is involved in the metabolism of numerous structurally diverse basic drugs (Coutts, 1994).

The exact number of cytochrome P450 isozymes is unknown. The cytochrome P450 super family is classified into families, subfamilies, and

individual enzymes. Human P450 isozymes have been allocated to ten

different gene families (1, 2, 3, 4, 7, 11, 17, 19, 21 and 27) based on the

similarity in their amino acid sequences. Each gene family displays less

than 40% amino acid similarity with the other gene families and all

members of a particular family display 40% or greater homology (Coutts *et*

al., 1994). Families 1 to 4 are said to be involved in the metabolism of

numerous drugs (Gonzalez, 1992). Within these different gene families,

there is only one (family 2) that contains a large number of subfamilies,

each of which is designated by a different capital letter. Individual P450s

within a subfamily have greater than 59% homology and are distinguished

by the terminal arabic number (Nebert *et al.*, 1989, 1991; Nelson *et al.*,

1993). Five human P450 isozymes that have recieved most attention are

CYP1A1, CYP1A2, CYP2D6, CYP3A4 and CYP_{MP}. Of these, CYP2D6 is

the most studied by far (Brosen and Gram, 1989; Eichelbaum and Gross,

1990; Gaedigk *et al.*, 1991). CYP2D6 (also referred to as debrisoquine 4-

hydroxylase, sparteine dehydrogenase or debrisoquine/sparteine oxidase)

and CYP_{MP} are two isozymes that exhibit polymorphism (Coutts *et al.*,

1994). Approximately 5-10% of Caucasians and 2% of Orientals are poor

metabolizers of debrisoquine/sparteine because they do not have the

ability to synthesize CYP2D6 (Gaedigk *et al.*, 1991). Numerous drugs,

35

such as amitriptyline, DMI and IMI have been identified as important substrates for CYP2D6 (Coutts, 1994). Therefore, the metabolism of these drugs will be impaired in individuals who cannot synthesize this isozyme.

It is at the level of the CYP isozymes that drug interactions frequently occur. It has been shown that administration of certain drugs can cause an induction, or increase in the levels, of some CYP isozymes (Gibson and Skett, 1986; Coon *et al.*, 1992). This alteration in levels may occur at any of several modes of regulation such as transcription activation, or mRNA or protein stabilization (Coon *et al.*, 1992; Guengerich, 1992). An example is the induction of CYP1A1 caused by the polycyclic hydrocarbons found in cigarette smoke. Some CYP isozymes have also been shown to be inhibited (Guengerich, 1992). This can be competitive or non-competitive inhibition. Competitive inhibition may be seen when two drugs, administered at the same time, compete for a particular isozyme. This competition will cause differences in the pharmacokinetic properties of each drug when compared to those properties observed when the drugs are administered alone. Competitive inhibition is also observed when one of two drugs administered is a non-substrate (is not metabolized by the enzyme) inhibitor. Quinidine is a non-substrate inhibitor of CYP2D6 (Otton *et al.*, 1984). A compound may also be oxidized by a particular CYP

36

isozyme to form a product that can inhibit that enzyme by binding tightly to it (Guengerich, 1992).

1.4 CHIRALITY

The concepts of chirality and optical activity were brought forth by two key researchers in the 1800s. They were Jean-Baptiste Biot in 1815 and Louis Pasteur in 1848 (Krstulovic, 1989). Later these concepts were included in the general theory of organic structure in three dimensions put forth by Van't Hoff and Le Bel (Richardson, 1901).

Chirality is a word that comes from the Greek word "cheiros" which refers to handedness, i.e. being left-handed or right-handed (Witte et al., 1993). Approximately 40% of synthetic drugs on the market today contain a chiral center or center of asymmetry (Lennard, 1991). In most cases, this centre is a carbon atom, but it may also be a nitrogen, a sulfur, or a phosphorus, among others.

An asymmetric carbon is one to which is attached four different atoms or groups of atoms. Under these conditions two different structural arrangements are possible. These two structures are not superimposable but are mirror images of one another and have different optical activities,

with one rotating plane polarized light to the right [(+) or dextrorotatory] and the other to the left [(-) or levorotatory]. These enantiomers have identical chemical and physical properties in a non-chiral environment.

1.4.1 TERMINOLOGY

A brief explanation of the various nomenclature systems for enantiomers is necessary here as this is an area leading to much confusion. As already mentioned, (+) and (-) are indications of the direction in which the enantiomer rotates plane polarized light. In the past, lower case *d* and *l* were also used for indicating rotation to the right [dextrorotatory or (+)] and rotation to the left [levorotatory or (-)], respectively. It is important to realize that the optical rotation caused by the enantiomer is not an independent variable and can be changed by a number of factors such as the solvent the enantiomer is dissolved in, the temperature at which the measurement was taken, the sample concentration, the light wavelength, and the pH of the aqueous solutions. Changes in optical activity that may take place as a result of altering one of these variables are not the result of a change in the spatial orientation of the atoms in the molecule.

Enantiomers may also be described according to their 3-dimensional

38

structure. The individual structures can be determined by a number of different methods, including anomalous X-ray dispersion or spectro-photometric correlations (Klyne and Buckingham, 1978; Kagan, 1977). Unlike its optical rotation, the configuration of the enantiomer can only be altered by the breaking and making of the atomic bonds. The method used to indicate the 3-dimensional structure of the enantiomers is the Cahn-Ingold-Prelog method (Cahn et al., 1956). This method uses upper case R (rectus) and S (sinister) as an indication of the locations of the substituents around the chiral center. When naming amino acids, carbohydrates and their derivatives according to their absolute configuration, upper case L and D are sometimes used according to the Fischer convention (Allinger et al., 1976). It is important to note that R and S or L and D give no indication as to the effect the enantiomer would have on plane polarized light. At present there is no way of connecting absolute configuration with optical rotation.

Another set of terms used in describing enantiomers is "eutomer" and "distomer". With regard to a specific pharmacological activity, the eutomer refers to the more active enantiomer while the distomer refers to the less active enantiomer (Ariens, 1983). The ratio of pharmacological activity, the eudismic ratio, is an indication of the degree of enantio-selectivity (Lehmann, 1976).

1.4.2 THE ROLE OF CHIRALITY IN PHARMACEUTICALS

Those drugs of natural origin containing a chiral center are usually optically active, meaning that they exist as only one of the two possible enantiomers (Kondepudi and Nelson, 1985; Mason, 1986). In contrast, those drugs of synthetic origin containing a chiral center are usually produced as an optically inactive racemate, or an equal mixture of enantiomers. The enantiomeric composition of pharmaceuticals is becoming an important issue with regard to drug development as well as in drug approval and clinical use. It has not, however, been possible until recent years to easily separate the enantiomers (Davies, 1990; Scott, 1988). As a consequence, approximately 90% of chiral drugs throughout the world have been marketed as racemic mixtures (Lennard, 1991).

Whether or not a drug is administered as a pure enantiomer or a racemic mixture is of importance because the enantiomers may interact differentially with living systems, which are themselves formed of chiral constituents. In other words, enantiomers may produce distinct pharmacodynamic responses because of their interactions with membrane components involved in transport and with receptors, and may differ in their pharmacokinetic behavior because of their interactions with enzymes (Ariens, 1984; Mason, 1984; Simonyi, 1984; Williams and Lee, 1985;

Drayer, 1986; Walle and Walle, 1986; Eichelbaum, 1988; Evans *et al.*, 1988; Coutts and Baker, 1989; Jamali *et al.*, 1989; Testa, 1990; Tucker and Lennard, 1990; Mehvar, 1992).

It is important to realize that in theory a racemic mixture could potentially act as three different drugs: the (+)-isomer, the (-)-isomer, and the racemic mixture. It may be that one enantiomer is active while the other is inactive. In this situation some researchers believe there may be no harm in administering the racemic mixture. However this poses a problem when total blood levels of the drug are being measured because a portion of that measured is not contributing to the therapeutic effect. For this reason, it was proposed by Drayer (1988) that therapeutic monitoring of enantiomers be conducted. Many researchers hold the view of Ariens (1989) who states that "inactive isomers should be regarded as impurities, isomeric ballast, not contributing to the effect aimed at" and argue that the single enantiomer should be administered.

Another situation that may arise with a racemic mixture is that one enantiomer may be the cause of the majority of the side effects. In this case it may also be efficacious to administer the drug as a single enantiomer. There are numerous examples of this situation: R-timolol, L-dopa, (+)-ketamine, (+)-ethambutol, and (+)-penicillamine all exhibit fewer

adverse effects compared to their respective antipodes (Williams and Lee, 1985; Drayer, 1986; Ariens, 1989; Lennard, 1991).

It is also possible that both enantiomers contribute to the therapeutic effect. It may be that a racemic drug has equipotent enantiomers. Examples of such drugs are the antiasthmatic drug proxyphylline, and the antiarrhythmic agent propafenone (Jamali, 1993). In such situations, it is generally not disadvantageous to administer the racemic mixture.

It is also important to consider that when a drug is administered as a racemic mixture there is the potential to have a certain amount of interaction between the two enantiomers (Jamali *et al.*, 1989). This interaction may alter metabolism kinetics (Jamali *et al.*, 1989; Testa, 1986). For example, one enantiomer may compete with the other for binding at plasma protein binding sites, at the active sites of enzymes, or at receptors. It has been suggested that the underlying mechanism for several enantiomer interactions is competition for plasma protein binding sites. An example of this is seen with ibuprofen enantiomers. Greater plasma concentrations of the enantiomers are observed when they are given alone than when administered as a racemate (Lee *et al.*, 1985).

Pharmacodynamics may also be affected by enantiomer interactions (Jamali *et al.*, 1989). In the case of methadone, the S-enantiomer reduces

the respiratory and miotic activities of the R-enantiomer to a considerable extent (Olson *et al.*, 1977).

When research on racemic drugs is conducted, the selection of the animal species is very important if comparisons are to be made to humans because there are species differences in enantioselectivity. One example of this is oxazepam. On the basis of plasma and/or urinary concentrations of R- and S-oxazepam glucuronides, it has been observed that racemic oxazepam undergoes enantioselective glucuronidation. In the human, dog and rabbit the S-oxazepam glucuronide predominates, while in the rhesus monkey it is R-oxazepam glucuronide that predominates (Sisenwine *et al.*, 1982).

In the final analysis, the choice between enantiomerically pure drugs and racemic drugs will have to be based on the risk-benefit and cost-benefit factors. The risk-benefit factors include toxicity, potency, pharmacology and pharmacokinetics. In dealing with the cost-benefit factor, it has been estimated that the actual production costs of an enantio-mer are at least twice as high as those for a racemate (Carter, 1988).

1.4.3 QUANTITATIVE ANALYSIS OF RACEMIC MIXTURES USING GC

When racemic mixtures are studied, it is often of interest to analyze the two enantiomers separately. Conventional analytical methods do not distinguish between enantiomers, but rather measure the sum of the enantiomers. However, over the past decade or so, chirally selective methods have become simpler, more generally available and applicable to many chiral compounds (Wozniak et al., 1991).

The first model for analysis of chiral molecules chromatographically was put forth by Dalgliesh in 1952. Since that time this aspect has been re-examined by many other researchers (reviews: Testa and Jenner, 1978; Souter, 1985; Testa, 1986; Konig, 1987; Allenmark, 1988; Armstrong and Han, 1988; Zief and Crane, 1988; Holmstedt et al., 1989; Hutt, 1990; Pasutto, 1992).

Chromatographic analysis can be done in two ways, indirectly (Gal, 1988; Pasutto, 1992) or directly (Allenmark, 1984; Pasutto, 1992). The indirect method requires that the racemic mixture be reacted with a chiral derivatizing reagent (an enantiomerically pure reagent) which will form diastereomers. Since the diastereomers have different physical and chemical properties, they can then be analyzed on a conventional

stationary phase. One of the potential problems with this method is the possibility of enantiomeric impurity of the chiral derivatizing reagent (Gal, 1988; Pasutto, 1992). Another problem that may arise is the possibility of unequal rates of reaction between the derivatizing reagent and the individual enantiomers (Krstulovic, 1989).

The direct method of analyzing the racemic mixture chromatographically is to separate the mixture on a suitable chiral stationary phase. In some cases, it may be necessary to first treat the racemic mixture with a conventional derivatizing reagent prior to GC analysis. This direct method eliminates the potential problem of enantiomeric impurity. However, the chiral columns are more costly than conventional columns and often are not as rugged or efficient (Pasutto, 1992). In addition, there is often a great deal of variability from column to column (Geisslinger *et al.*, 1989; Matuszewski *et al.*, 1990; Straka *et al.*, 1990; Takahashi *et al.*, 1990).

In addition to chromatographic analysis, racemic mixtures can be analyzed using competitive binding assays (Cook, 1993). This type of analysis is performed with the use of receptors and antibodies that are enantioselective.

1.5 GAS CHROMATOGRAPHY

Since gas chromatography (GC) plays such an important part in this thesis, a brief introduction to this analytical technique is presented here.

1.5.1 THE PRINCIPLES OF GAS CHROMATOGRAPHY

GC is an analytical procedure used to separate the components of an organic mixture such that the components can be identified and/or quantitated. Theoretically, any compound that can be vaporized or converted to a volatile derivative can be analyzed by this procedure (Poklis, 1989).

The procedure for separating and quantitating the components of a mixture consists of partitioning them as vapors between two phases; the stationary and the mobile phase. The stationary phase is contained in a column through which the mixture components to be separated and the mobile phase flow. Stationary phases are most often a high-boiling, virtually nonvolatile liquid. The mobile phase (or carrier gas) is a stream of gas. Commonly used carrier gases are helium, nitrogen, and hydrogen.

Components of the mixture will separate according to their partition coefficients between the stationary and the mobile phases.

A basic GC system consists of a heated inlet to permit entry of both the mixture to be analyzed and the inert carrier gas, an oven in which the column is contained with a variable temperature, normally over a 20-300°C range, and a detector that detects each component as it elutes from the column (Coutts and Baker, 1982; Coutts et al., 1985).

1.5.2 COLUMNS

GC columns are available commercially in a number of different materials, including glass, stainless steel, nickel, copper, aluminum and nylon (Baker et al., 1982; Coutts and Baker, 1982; Coutts et al., 1985). The columns used for the experiments described in this thesis were fused silica capillary columns, so only this type of column will be discussed here. This type of column has become popular because of its inertness, flexibility and durability.

There are two principal types of capillary columns: wall-coated open tubular [WCOT] and support-coated open tubular [SCOT] (Baker et al., 1982; Coutts and Baker, 1982; Coutts et al., 1985). In the WCOT type,

the liquid phase has been deposited directly onto the inner surface of the column. In the SCOT type, the inner surface of the column is covered with a thin layer of a solid support that is coated with the liquid phase. The WCOT columns generally permit analysis of larger volumes of samples and were used in the projects described in this thesis because of the relatively low concentrations of drugs being investigated.

1.5.3 DETECTORS

There are five different detectors that are generally used for GC: the thermal conductivity detector (TCD), the flame ionization detector (FID), the electron capture detector (ECD), the nitrogen-phophorus detector (NPD) and the mass spectrometer (MS) as a detector. For this thesis, only the last three detectors were employed.

1.5.3.1   THE ELECTRON CAPTURE DETECTOR

In the ECD, a radioactive isotope, usually ^{63}Ni, releases β-particles (high-energy electrons) that collide with the carrier gas molecules, producing many low-energy electrons (Baker *et al.*, 1982; Coutts and

Baker, 1982; Coutts *et al.*, 1985; Poklis, 1989). These electrons are collected on electrodes and produce a small, measurable, standing current. When sample components containing chemical groups with high electron affinity flow through, they capture the low-energy electrons generated by the isotope. The detector measures the disturbance in the standing current, and the reduction in current is amplified and recorded as a peak.

The ECD is selective for electronegative compounds, including those that contain halogen atoms, conjugated ketones, nitro compounds, nitriles and organometals (Baker *et al.*, 1982; Coutts and Baker, 1982; Coutts *et al.*, 1985). This detector is extremely sensitive and can detect and quantitate subpicogram levels of analytes (Coutts *et al.*, 1985).

1.5.3.2   THE NITROGEN-PHOSPHORUS DETECTOR

The NPD operates by first mixing the effluent from the GC column with a smaller volume of hydrogen and then entering it into an electrically heated detector chamber which contains an alkali source [often a rubidium salt] (Baker *et al.*, 1982; Coutts and Baker, 1982). A low-temperature plasma is formed that emits a minute current that is amplified and recorded as a peak.

The NPD is a relatively selective detector and, as its name suggests, it is sensitive to components containing nitrogen and/or phosphorus. It is capable of detecting low picogram quantities of nitrogen- and phosphorus-containing compounds (Coutts and Baker, 1982).

1.5.3.3 THE MASS SPECTROMETER AS A DETECTOR

When a gas chromatograph is coupled to a mass spectrometer, it becomes a very powerful analytical system capable of chemical structure elucidation and quantitation. The three main steps involved in mass spectrometry are: ionization, mass filtration and detection (Poklis, 1989). The three modes of ionization commonly used for biological specimens are electron impact (EI), chemical ionization (CI) and negative chemical ionization (NCI) (Poklis, 1989). The EI mode was used for all analyses conducted for this thesis so only this mode of ionization will be discussed here.

To use a mass spectrometer as a detector, most of the carrier gas is removed from the column effluent before it is passed into the ionization chamber of the mass spectrometer, where ionization of the compound occurs as a result of bombardment by high energy electrons (Baker *et al.,*

1982; Coutts et al., 1985; Poklis, 1989). The ionized molecule then undergoes fragmentation into positive, negative, and neutral fragments. The fragment ions are separated according to their atomic masses. Usually the positively charged fragments are passed into an electron multiplier that generates an electric current which is amplified and recorded. The trace obtained (total ion current vs time) appears much like a GC trace (detector response vs time). The mass spectrometer is also capable of measuring the current produced by a single charged ion (usually a positively charged ion) of particular mass (Coutts et al., 1985). This is referred to as single (or selected) ion monitoring. The mass spectrometer becomes very selective in this situation, allowing for the detection and quantification of a single compound even though it may coelute with other compounds (Baker et al., 1982; Coutts et al., 1985; Wood and Rao, 1990).

By using a mass spectrometer as a detector, levels in the high picogram range can be detected (Coutts et al., 1985).

1.5.4 INJECTION SYSTEMS

The two main types of injection systems are the splitless system and the splitter system (Coutts et al., 1985). The splitless system was utilized

51

for the experiments described in this thesis. In this system, the sample is introduced and vaporized in a glass-lined tube that extends from the septum to the column. This method is recommended for analysis of very dilute and wide boiling-range samples.

The splitter system involves splitting the carrier gas stream such that only a small proportion of the injected sample enters the column. This ensures that the column will not be overloaded and that the deposition of the sample on the column is in a very narrow bandwidth. This system is recommended for the analysis of concentrated samples.

1.5.5 DERIVATIZATION

In many instances, the derivatization of the substance of interest is required before GC analysis can be conducted (reviews: Coutts and Baker, 1982; Coutts *et al.*, 1985). The main reasons for derivatization are:

1.   to increase volatility,

2.   to increase stability,

3.   to reduce the polarity of the substance to improve chromatographic properties,

4.   to improve extraction efficiency from aqueous solutions, and

5. to introduce a functional group that is sensitive to the particular detector being employed.

Derivatizations typically involve the replacement of the active hydrogen in polar groups (i.e. NH, OH, SH). Examples of the more common derivatization reactions are alkylation, acylation, silylation, and condensation. The general reactions for each derivatization are shown in Figure 2.

acylation: R – NH – R $\xrightarrow{\text{(R'CO)}_2\text{O}}$ R – N – R · R'COOH
                                                                 |
                                                                 COR'

alkylation: R – OH $\xrightarrow[\text{base}]{\text{R'X}}$ R – O – R' · HX

silylation: R – OH $\xrightarrow{\text{(CH}_3)_3\text{SiX}}$ R – O – Si(CH$_3$)$_3$ · HX

condensation: R – NH$_2$ $\xrightarrow{\substack{\text{R'}\\ \text{R''}}\text{CO}}$ R – N = C$\substack{\text{R'}\\ \text{R''}}$ · H$_2$O
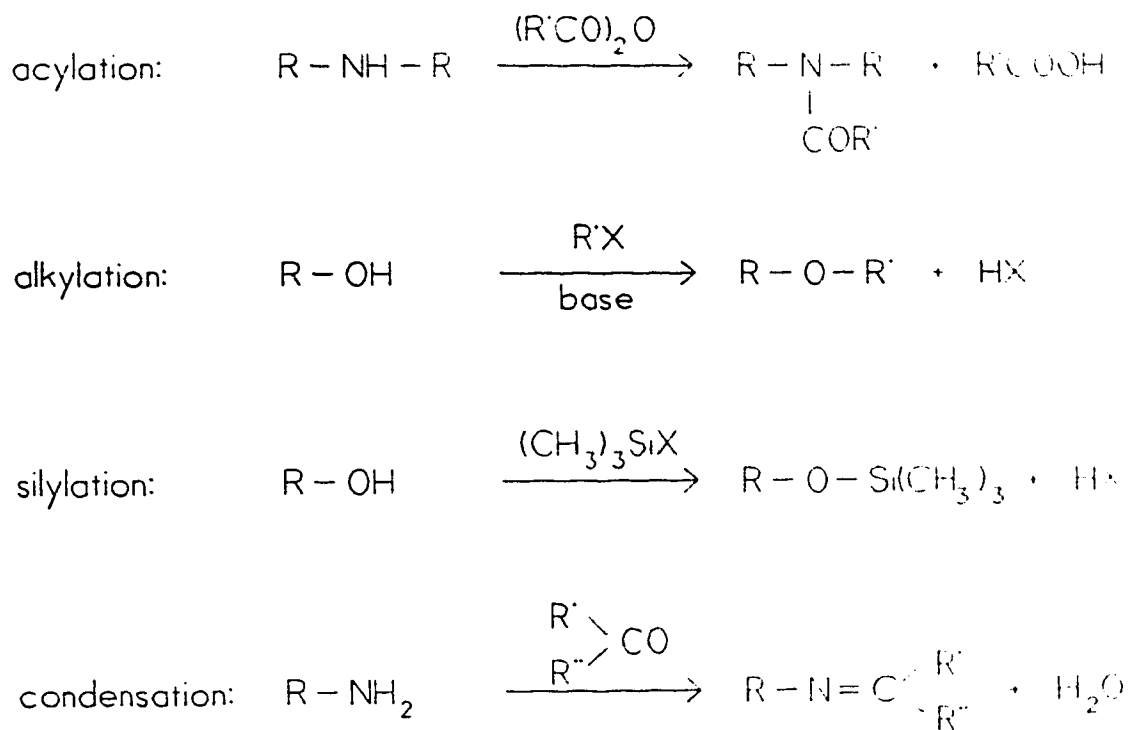
Figure 2:   Four common types of derivatization reactions used for GC analysis [adapted from Baker *et al.* (1982)].

2.0 RATIONALE FOR EXPERIMENTS DESCRIBED IN THIS THESIS

2.1 DEVELOPMENT OF ENANTIOSELECTIVE ASSAYS

As mentioned in the previous section, it is often of interest in therapy with drugs administered as racemic mixtures that the enantiomers be quantitated individually. Fassihi (1993) summarized the reasons as follows:

a) The kinetics of the enantiomers are often different, leading to differing plasma levels of the enantiomers.

b) The pharmacological properties of the enantiomers may be drastically different. As mentioned earlier, one enantiomer may be responsible for the therapeutic effect while the other enantiomer may be responsible for the side effects.

c) Enantiomers may act as competitive antagonists. They may have opposite effects and different plasma protein binding properties.

2.1.1 ANALYSIS OF TCP ENANTIOMERS

The antidepressant drug TCP was used as a prototypical amine drug with which to develop a method that would allow for the separation and quantitation cf the enantiomers of a racemic mixture (Aspeslet *et al.*, 1992). It is also well known that the individual enantiomers of TCP differ markedly with regard to pharmacological properties, the (+) enantiomer being a much better inhibitor of MAO and the (-) enantiomer being a more potent inhibitor of catecholamine uptake into nerve terminals (Smith, 1980). Numerous methods have been developed for the analysis of TCP (Fuentes *et al.*, 1975; Lang *et al.*, 1979; Youdim *et al.*, 1979; Bailey and Barron, 1980; Reynolds *et al.*, 1980; Calverley *et al.*, 1981; Hampson *et al.*, 1984; Edwards *et al.*, 1985; Rao *et al.*, 1986; Mutschler *et al.*, 1990), and an HPLC method which allows for the separation of TCP enantiomers has recently been reported (Spahnlangguth *et al.*, 1992; Weber-Grandke *et al.*, 1993). The gas chromatographic method presented in this thesis allows for the extraction, derivatization and separation of the enantiomers of TCP by GC after administration of the racemate to rats.

2.1.2 ANALYSIS OF FLU AND NFLU ENANTIOMERS

FLU is an antidepressant drug that is in widespread use today. Despite this fact, there has not been much attention paid to the importance of analyzing the individual enantiomers. Using a modification of the assay developed for TCP, a novel gas chromatographic assay was developed that would allow for the simultaneous separation and quantitation of the enantiomers of both FLU and NFLU (Torok-Both et al., 1992). Very few other assays have been reported that achieve this separation, and these are all HPLC assays (Prperaki and Parissipoulou, 1993; Peyton et al., 1992; Potts and Parli, 1992).

Using the assay developed, the distribution of both FLU and NFLU enantiomers was examined in the whole brain and various brain regions of the rat. The reason for determining the regional distribution was the possibility of enantioselectivity in drug disposition.

2.2 EFFECT OF IPR PRETREATMENT ON TCP ENANTIOMERS

IPR, as previously mentioned, is of particular interest here because of its reported ability to inhibit ring hydroxylation of other drugs. One route

57

of metabolism of TCP is 4-hydroxylation. It is proposed that by pretreating animals with IPR, an increase in the level of TCP will be observed. The assay used should indicate whether or not the effect caused by IPR is enantioselective.

2.3 EFFECT OF IPR PRETREATMENT ON FLU AND NFLU ENANTIOMERS

To date, there is very little known about the metabolism of FLU. IPR is a drug that has been shown to block ring hydroxylation. By observing the effects that IPR has on the levels of the enantiomers of both FLU and NFLU, more insight into other possible routes of metabolism for FLU may be gained. In addition, it will be observed whether or not the effect caused by IPR is enantioselective.

2.4 SCREENING PROCEDURES FOR SELECTED POTENTIAL METABOLITES OF FLU

In an attempt to identify potential metabolites of FLU other than NFLU, two general screens were used, involving reaction with either

pentafluorobenzoyl chloride (PFBC) or pentafluorobenzenesulfonyl chloride (PFBSC). These two procedures have been utilized extensively in the Neurochemical Research Unit for derivatization of phenol- and amine-containing drugs and metabolites (Baker et al., 1986, 1987a,b; Coutts et al., 1986; Nazarali et al., 1984, 1986, 1987b,c; Rao et al., 1986, 1987a,b).

2.5   COMBINATION OF DMI AND FLU

Previous work that has been reported on drug-drug metabolic interactions of FLU with other drugs, such as DMI (Fuller et al., 1991), did not take into account the possible effects of the drug combination on levels of FLU or NFLU.

The combination of FLU and DMI is a treatment which is commonly used in depressed patients. It has been reported to be effective in treating major depression and to produce more rapid antidepressant effects than either drug alone (Weilburg et al., 1989; Nelson et al., 1991).

The combination of DMI and FLU has been shown to produce a more rapid and greater down-regulation of β-adrenergic receptors in rat brain than DMI alone (Baron et al., 1988; Goodnough and Baker, 1994). Down-regulation of β-adrenergic receptors is a property shared by many

antidepressants (Baker and Greenshaw, 1989), so the marked effects of the drug combination on this down-regulation may be important clinically. In a study by Nelson et al. (1991), it was shown that patients receiving DMI and FLU responded more rapidly and completely than patients treated with DMI alone. The combination of DMI and FLU leads to an increase in the plasma levels of DMI in patients (Aranow et al., 1989; Downs et al., 1989; Goodnick, 1989; Vaughan, 1988) and to an increase in DMI levels in rat brain (Fuller and Perry, 1989; Goodnough and Baker, 1994). Nelson et al. (1991) dispute the claim that the increase in plasma DMI levels alone is responsible for the more rapid β-adrenergic down-regulation since at one week of combination treatment clinical improvement is observed but at this time interval there is no significant difference in the levels of DMI achieved over those noted when DMI was administered alone. These workers thus suggest that the more rapid clinical response may be due to a combination of two effects, the more rapid β-adrenergic down-regulation and an enhancement of 5-HT transmission. Thus, it would be important to know what effects the drug combination is having on levels of FLU and NFLU, both potent inhibitors of 5-HT uptake.

3.0 MATERIALS AND METHODS

3.1 CHEMICALS

Table 2:   Chemicals used in the studies described in this thesis.

Chemicals	Suppliers
acetonitrile	British Drug Houses [BDH] (Toronto, ON)
(-)-alprenolol HCl	Sigma (St. Louis, MO)
(-)-ascorbic acid	Fisher Scientific (Edmonton, AB)
p-chlorophentermine	Warner-Lambert Canada Inc. (Scarborough, ON)
2,4-dichlorophenol	Sigma
ethyl acetate	BDH
ethylenediamine tetraacetate, disodium salt	Fisher Scientific
rac-fluoxetine HCl	Lilly Research Laboratories (Indianapolis, IN)
R-fluoxetine HCl	Lilly Research Labs
S-fluoxetine HCl	Lilly Research Labs
hydrochloric acid, 37-38%	Fisher Scientific
iprindole HCl	Wyeth Research UK Ltd. (Berkshire, UK)
isopentane	BDH
rac-norfluoxetine HCl	Lilly Research Labs
R-norfluoxetine HCl	Lilly Research Labs
S-norfluoxetine HCl	Lilly Research Labs

61

pentafluorobenzenesulfonyl chloride	Aldrich (Milwaukee, WI)
pentafluorobenzoyl chloride	Aldrich
perchloric acid, 60%	Fisher Scientific
potassium bicarbonate	Fisher Scientific
potassium carbonate anhydrous	Fisher Scientific
sodium borate	Fisher Scientific
toluene, glass-distilled	BDH
rac-tranylcypromine HCl	Sigma
(+)-tranylcypromine HCl	Smith Kline & French Research Laboratories (Philadelphia, PA)
(-)-tranylcypromine HCl	Smith Kline & French Research Labs
S-(-)-N-(trifluoroacetyl)prolyl chloride	Aldrich
p-trifluoromethylphenol	Aldrich

3.2   INSTRUMENTATION AND APPARATUS

3.2.1 GAS CHROMATOGRAPHY (GC)

3.2.1.1   NITROGEN PHOSPHORUS DETECTION

Analysis of TCP enantiomers was conducted on a Hewlett-Packard (HP) model 5890A gas chromatograph (Hewlett Packard Co., Palo Alto, CA, USA) equipped with a nitrogen-phosphorus detector and linked to an HP 3392A integrator. A fused silica capillary column (25 m X 0.32 mm i.d.) coated with a 0.52 μm film thickness of 5% phenyl methyl silicone (Hewlett

Packard Co.) was employed. The carrier gas was pure helium (Linde,

Union Carbide, Edmonton, AB, Canada) at a flow rate of 2 ml/min. The

detector was purged with pure hydrogen (Linde, Union Carbide) at 3.5

ml/min mixed with dry air (Linde, Union Carbide) at 80 ml/min. The

injection port temperature was 250°C and the detector temperature was

325°C. A splitless injection system was employed.

3.2.1.2 ELECTRON CAPTURE DETECTION

Analyses of FLU and NFLU as well as the enantiomers of each were

conducted on a HP 5880 gas chromatograph equipped with a ^{63}Ni electron-

capture detector and linked to a HP 5880A integrator/printer. The

chromatographic column installed was a 15 m X 0.25 mm i.d. DB-5 cross-

linked fused-silica capillary (0.22 μm thickness; J & W Scientific, Palo Alto,

CA, USA). The carrier gas was helium (Linde, Union Carbide) at a flow

rate of 1 ml/min and the make-up gas was argon-methane [95:5] (Linde,

Union Carbide) at a flow rate of 30 ml/min. The injection port temperature

was 270°C and the detector temperature was 325°C.

Analysis of TFMP was conducted on the same gas chromatograph

as described for FLU and NFLU but with use of a different column. The

column used for this analysis was a fused silica capillary column (25 m X 0.32 mm i.d.) coated with a 0.52 μm film thickness of 5% phenyl methyl silicone (Hewlett Packard Co.). The carrier gas was helium at a flow rate of 3 ml/min and the make-up gas was argon-methane (95:5) at a flow rate of 30 ml/min. The injection port temperature was 200°C and the detector temperature was 325°C. A splitless injection system was employed.

3.2.1.5 MASS SPECTROMETERIC (MS) DETECTION

Chemical structures of all the derivatives utilized in development of GC assays for this project were confirmed by combined GC-MS. The GC-MS system used was a HP 5840A gas chromatograph inlet coupled to a HP 5985A mass spectrometer with dual EI/CI sources and an HP 7920 data system. The GC-MS system also consisted of an HP 2648A graphics terminal, HP 9876A printer, HP 7920 disc drive (software) and HP 21MX series E computer (hardware). Operating conditions were as follows: ion source temperature, 200°C; interface temperature, 275°C; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 100 amu/sec; and dwell time, 200 msec. The same column and oven conditions as those described for the GC were used.

3.2.2 TISSUE HOMOGENIZER

A combination of a TRI-R S63C (TRI-R Instruments, Rockville

Center, NY, USA) variable speed laboratory motor with a Teflon glass

pestle and a glass grinding tube was used for homogenizing tissue

samples.

3.2.3 CENTRIFUGES

A Sorvall GLC-2B or Sorvall GLC-1 General Laboratory Centrifuge

(Dupont Instruments, Wilmington, DE, USA) was used for low-speed, small

volume centrifugations. Higher speed and/or larger volumes

centrifugations were carried out in a Damon-IEC B-20 (Needham Heights,

MA, USA) refrigerated high-speed centrifuge or a Beckman L755 (Palo

Alto, CA, USA) vacuum refrigerated ultracentrifuge.

3.2.4 SAVANT EVAPORATOR

A Savant Speed Vac SS1 (Savant Instruments, Inc., Farmingdale,

NY, USA) was used for evaporating samples. The instrument consists of a

concentrator (a rotor chamber with a heater), a chemical trap that accepts

disposable cartridges, a refrigerated condensation trap, and a vacuum

pump. The process of drying involves combining centrifugal force, vacuum

and applied heat.

3.2.5 SHAKER-MIXER

Two types of vortex-shakers were used: Ika-Vibrax VXR2 Shaker

(Janke and Kunkel GMBH and Co., Staufen, Germany) and a Thermolyne

Maxi Mix vortex mixer (Thermolyne Corp., Dubuque, IO, USA).

3.2.6 WEIGHING BALANCES

A Mettler AE 160 (Zurich, Switzerland) electronic balance was used

for weighing chemicals and biological samples.

3.2.7 GLASSWARE CLEANING

All glassware was rinsed with tap water. Further cleaning was

completed using a Miele Electronic 6715 dishwasher and biodegradable

Sparkleen (Fisher Scientific Co.). For test tubes, an additional cleaning step was added. The tubes were sonicated (Ultra-sonic cleaner, Mettler Electronics) in a solution of Decon 75 concentrate (BDH Chemicals). Tubes were then cleaned in the dishwasher without Sparkleen. All glassware was then air-dried in a mechanical convection oven (Model 28, Precision Scientific Group, Chicago, IL, USA).

3.3 ANIMALS

The male Sprague-Dawley rats (250-300g) used for all experiments described in this thesis were obtained from Bio-Science Animal Services, Ellerslie, Alberta, Canada. The animals were housed in pairs in plastic cages on cedar chip bedding in a temperature controlled room (21 ± 1°C). They were subjected to a 12 h light/dark cycle (lights on at 7:30 a.m.) throughc experiments. Water and lab chow were provided *ad libitum*. The lab chow (Lab-Blox feed, Wayne Feed Division, Continental Grain Company, Chicago, IL, USA) was 4.0% (min) crude fat, 4.5% (max) crude fibre and 24% (min) crude protein. During urine collection time periods, the rats were housed individually in plastic metabolic cages (Nalgene, model E

1000, Maryland Plastic Inc., New York, NY, USA) designed for separate collection of urine and feces.

Procedures involving the use of animals were approved by the University of Alberta Health Sciences Animal Welfare Committee and were conducted according to the guidelines put forth by the Canadian Council on Animal Care.

3.3.1 ADMINISTRATION OF DRUGS

Animals were randomly allocated to drug or vehicle treatment groups. All drugs were administered intraperitoneally (i.p.) and in a volume of 2 ml/kg. rac-TCP and IPR were dissolved in 0.9% saline. rac-FLU and DMI were dissolved in a solution of 9 parts saline and 1 part dimethyl-sulfoxide (DMSO). DMSO was necessary to dissolve rac-FLU in saline at the required concentration. In each study the control animals were injected with the corresponding vehicle solution.

For the experiment involving TCP with and without IPR pretreatment, rats were injected with either vehicle or IPR (10 mg/kg). After 1 h, the rats were injected with either vehicle or rac-TCP (2.5 mg/kg). Rats were then sacrificed at 1 h and 3 h post-TCP injection.

68

For the experiment involving FLU with and without IPR pretreatment, rats were injected with either vehicle or IPR (10 mg/kg). After 1 h, the rats were injected with either vehicle or rac-FLU (10 mg/kg). Rats were then sacrificed at 5 h post-FLU injection.

The experiment involving the combination of FLU and DMI consisted of administering vehicle, rac-FLU (10 mg/kg), DMI (5 mg/kg), or the combination of rac-FLU and DMI (10 mg/kg and 5 mg/kg, respectively). The drugs were administered once daily for a period of 1 or 14 days. The doses and the 14-day period were the same as those used by Baron et al. (1988) when they reported a more rapid β-adrenergic down-regulation with the drug combination than with DMI alone. Rats were sacrificed 24 h after the last dose.

3.3.2 SAMPLE COLLECTION AND STORAGE

Following drug administration, animals were sacrificed by guillotine decapitation. The brains were rapidly removed and immediately frozen in dry ice-cooled isopentane. Livers were removed and immediately placed on dry ice. Until the time of analysis, brain samples were stored at -80°C and liver samples were stored at -20°C.

For experiments involving the analysis of urine samples the rats were placed in the metabolic cages for the 24 h preceding decapitation. The volume of the urine collected was measured and the samples were frozen at -20°C until the time of analysis.

3.4 HUMAN SUBJECTS

The human subjects involved in this study were outpatients of the Department of Psychiatry at the University of Alberta, Edmonton, Alberta, Canada and were under the care of Dr. K.F. McKenna.

3.4.1 ADMINISTRATION OF DRUGS

The patients involved were being treated for major depressive disorder using *rac*-FLU (Prozac®). These participants received 20 mg of *rac*-FLU once daily, usually taken between 7:00 and 8:00 a.m.

3.4.2 SAMPLE COLLECTION AND STORAGE

Blood and urine samples were taken from the patients before FLU

treatment began and again after three and six weeks of treatment. A blood sample was collected between 9:00 and 10:00 a.m. and centrifuged at 1000 x g for 10 min to obtain plasma. Blood was collected in Vacutainer® brand evacuated blood collection tubes containing EDTA (lavender stoppers). During collection and mixing, care was taken to ensure that the blood did not contact the stopper. Urine was collected for the previous 24 h, ending at 8:00 a.m. on the day of blood collection. The plasma and urine samples were frozen at -20°C until analyzed.

3.5   ASSAY PROCEDURES

3.5.1 ANALYSIS OF TCP ENANTIOMERS

A novel GC procedure was developed for the analysis of TCP enantiomers and is depicted in Figure 3. Tissue samples were allowed to partially thaw and then were weighed and homogenized in 5-10 volumes of ice-cold 0.1 N perchloric acid containing EDTA (10 mg/100 ml) and (-)-ascorbic acid (0.88 mg/100 ml). The homogenates were centrifuged at 10,000 x g for 15 min at 0-4°C to remove the protein precipitate. Aliquots (2 ml) of each supernatent were transferred to clean glass test tubes and to each was added 200 ng of the internal standard, p-chlorophentermine.

TISSUE

| homogenize in $HClO_4$
| centrifuge

SUPERNATANT

| basify with K_2CO_3
| shake with ethyl acetate
| centrifuge

RETAIN TOP LAYER

| evaporate to dryness

RESIDUE

| react with S-(-)-N-(trifluoro-
| acetyl)prolyl chloride
| wash with sodium borate
| centrifuge

RETAIN TOP LAYER

ALIQUOT ON GC-NPD
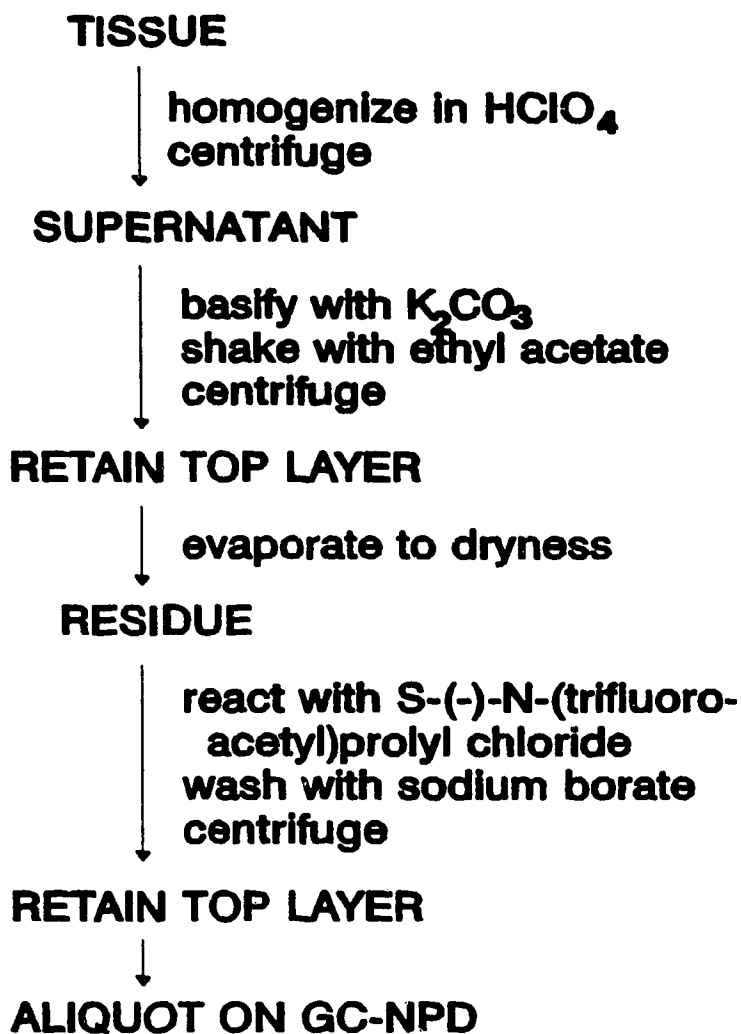
Figure 3:   The procedure used for the simultaneous analysis of the enantiomers of TCP.

Potassium carbonate (25%) (400 μl) was added to all samples and the pH

value was checked to ensure basicity (pH 10.5-11.5). Ethyl acetate (4 ml)

was added to each tube, and the tubes were shaken vigorously for 5 min

and centrifuged at 1000 x g for 5 min. The organic phase was carefully

transferred to another tube and taken to dryness in a warm water bath

under a gentle stream of nitrogen. The residue was dissolved in a mixture

of toluene (100 μl) and the chiral derivatizing reagent S-(-)-N-

(trifluoroacetyl)prolyl chloride (2 μl). The samples were vortexed briefly

and allowed to sit at room temperature for 15 min. Saturated sodium

borate (1 ml) was added to each sample, and each was vortexed and

centrifuged. The toluene layer was retained from each sample for injection

of aliquots (1 μl) on the gas chromatograph described in section 3.2.1.1.

Chromatographic separation was accomplished using the following oven

temperature program: initial temperature of 105°C for 0.5 min, increasing

at a rate of 10°C/min to 270°C where it was held until the end of the run.

In this and the other GC procedures used in this thesis, a standard

(calibration) curve was prepared with each assay run to permit

quantification of the drug of interest in the brain or liver homogenates, or

the urine or plasma samples. The curves were constructed by adding

known, varying amounts of authentic standards and the fixed amount of the

appropriate internal standard to a series of tubes and carrying these tubes through the assay procedure in parallel with the sample tubes. Each curve was prepared using the appropriate tissue homogenate or biological fluid obtained from control animals or drug-naive humans. The ratio of the peak height of the derivatized drug to that of the derivatized internal standard was calculated and plotted against the concentration of the drug.

3.5.2 ANALYSIS OF THE ENANTIOMERS OF FLU AND NFLU

The GC assay used for the analysis of the enantiomers of FLU and NFLU (Figure 4) was adapted from the above TCP assay (Torok-Both *et al.*, 1992). Rat brain was allowed to partially thaw and then weighed and homogenized in five volumes of ice-cold 0.1 N perchloric acid. An aliquot (300-600 μl) of the homogenate was removed to be used in the analysis. The appropriate aliquot was placed into a clean glass test tube and diluted to a final volume of 2 ml with distilled water. To this solution was added 20 μg of the internal standard, (-)-alprenolol. The samples were then basified by adding 500 μl of a 25% potassium carbonate solution and the pH value was checked to ensure basicity (pH 10.5-11.5). The basic solution was then shaken briefly, and 4 ml of toluene were added. The samples were

74

BRAIN HOMOGENATE IN HClO$_4$

basify with K$_2$CO$_3$
shake with toluene
centrifuge

RETAIN TOP LAYER

shake with HCl
centrifuge

RETAIN BOTTOM LAYER

basify with K$_2$CO$_3$
shake with toluene
centrifuge

RETAIN TOP LAYER

evaporate to dryness

RESIDUE

react with S-(-)-N-(trifluoro-
acetyl)prolyl chloride
evaporate to dryness

RESIDUE

reconstitute in toluene

ALIQUOT ON GC-ECD
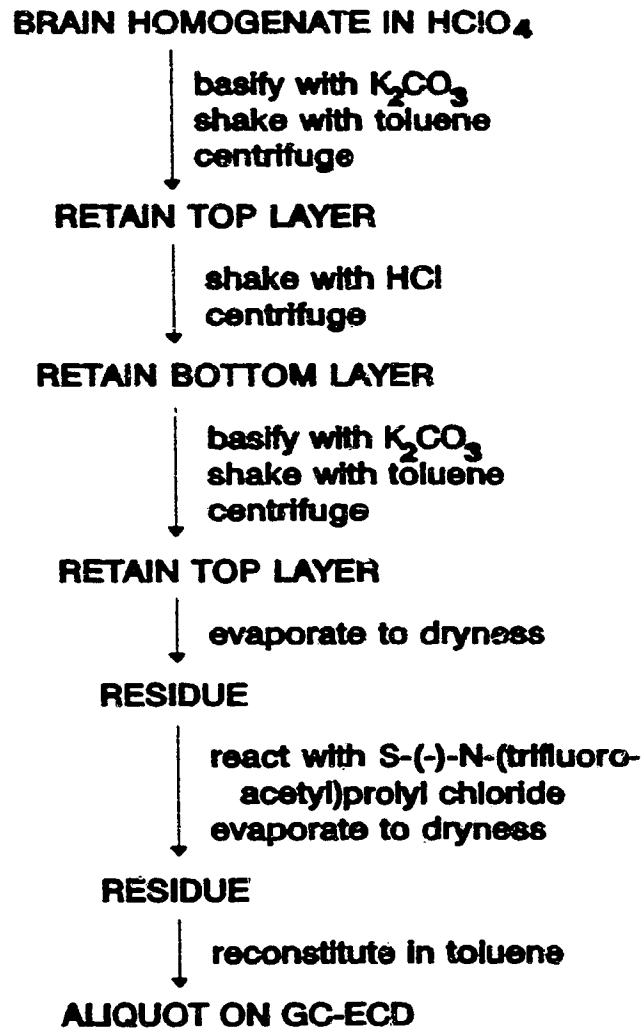
Figure 4:   The procedure used for the simultaneous analysis of the enantiomers of FLU and NFLU.

75

shaken for 15 min and centrifuged for 15 min at 1000 x *g* to separate the aqueous and organic phases.

The toluene layer was transferred to a second glass test tube to which was added 2 ml of 0.5 M HCl. The samples were shaken for 10 min and centrifuged for 10 min at 1000 x *g*. The toluene layer was aspirated off and discarded. The remaining aqueous layer was basified with 1000 μl of 25% potassium carbonate in addition to a small amount of solid potassium carbonate and the pH value was checked (pH 10.5-11.5). Toluene (4 ml) was again added to the basic solution and the samples were shaken for 10 min and centrifuged for 10 min at 1000 x *g*. The toluene layer was retained and transferred to small glass test tubes. The organic solvent was evaporated using the Savant Speed Vac evaporator. To the dry residue was added 100 μl of the derivatizing solution [40 μl of S-(-)-N-(trifluoroacetyl)prolyl chloride per ml of toluene]. The tubes were shaken briefly on a vortex mixer and the derivatization reaction was allowed to proceed at 60°C for 60 min. Following completion of the reaction, the samples were evaporated to dryness using the Savant Speed Vac and then redissolved in 100 μl of toluene. An aliquot (1 μl) of this solution was injected on the gas chromatograph system described in section 3.2.1.2. Chromatographic separation was accomplished using the following oven

temperature program: The initial oven temperature of 105°C, held for 0.5 min, increased at a rate of 30°C/min to 207°C at which value it was maintained for 4 min. An increase at a rate of 1°C/min to 225°C followed. From 225°C, the oven temperature was increased to 300°C at a rate of 25°C/min. This final temperature of 300°C was maintained for 2 min.

In the initial stages of development of assays for the enantiomers of TCP, FLU and NFLU, after retention times for the derivatives of the enantiomers had been determined, coded samples of the enantiomers were prepared by another person in the Unit and subjected to analysis by me. After analyses were performed, the other person was informed which enantiomers were which according to the assay, and the codes were broken to check. This "blind" study ensures that enantiomers were not confused during assay development. This is a standard procedure of the Neurochemical Research Unit.

3.5.3 SEARCH FOR METABOLITES OF FLU

3.5.3.1   EXTRACTIVE DERIVATIZATION USING PENTAFLUOROBENZOYL CHLORIDE

The GC procedure developed is shown in Figure 5. An aliquot of plasma (2 ml) or urine (1 ml) was placed in a clean glass test tube and diluted, if required, to a final volume of 2 ml with distilled water. The internal standard, (-)-alprenolol (1000 ng), was added to each sample. Each sample was basified with 500 μl of 25% potassium carbonate and the pH value was checked to ensure basicity (pH 10.5-11.5). The samples were briefly mixed on a vortex mixer. Toluene (4 ml) and pentafluoro-benzoyl chloride (10 μl) were added to each sample and they were shaken vigorously for 5 min and centrifuged for 5 min at 1000 x g. The organic layer was carefully transferred to another test tube and taken to dryness using a Savant Speed Vac evaporator. The residue was reconstituted in 150 μl toluene and retained for injection (1 μl aliquot) on the gas chromato-graph system described in section 3.2.1.2. Chromatographic separation was accomplished using the following oven temperature program: The initial oven temperature of 105°C (maintained for 0.5 min) increased to 200°C at a rate of 30°C/min. An increase to 206°C at a rate of 1°C/min

PLASMA OR URINE SAMPLE

basify with K_2CO_3
shake with toluene and pentafluoro-
   benzoyl chloride
centrifuge

RETAIN TOP LAYER

evaporate to dryness

RESIDUE

reconstitute in toluene

ALIQUOT ON GC-ECD

Figure 5:   The procedure used for the analysis of FLU and NFLU in
             human body fluids.

followed. The temperature was held at 206°C for 4 min, and increased at a rate of 5°C/min to the final temperature of 300°C, at which value it was held for 60 min.

3.5.3.2 EXTRACTIVE DERIVATIZATION WITH PENTAFLUOROBENZENESULFONYL CHLORIDE

This study resulted in a novel assay procedure for the analysis of TFMP (Figure 6), a metabolite of FLU which had been previously identified but never quantitated. Urine samples were diluted 1:2 (v/v) with distilled water. Aliquots of 1.5 ml were transferred to clean glass test tubes. To each tube was added 1 μg of the internal standard, 2,4-dichlorophenol. Each sample was basified by the addition of solid potassium bicarbonate (400 mg). The samples were mixed briefly on a vortex mixer and to each was added 4.5 ml of a solution made up of 9 parts of ethyl acetate and 1 part acetonitrile and the derivatizing reagent, PFBSC, at a concentration of 0.1% v/v. Samples were shaken for 15 min and centrifuged for 2 min at 1000 x g. The upper organic layers were transferred to small glass test tubes and the samples were taken to dryness using the Savant Speed Vac evaporator. The residues were reconstituted in 300 μl of toluene and an

URINE SAMPLE

basify with $KHCO_3$
shake with solution of ethyl acetate,
    acetonitrile, & pentafluorobenzene-
    sulfonyl chloride
centrifuge

RETAIN TOP LAYER

evaporate to dryness

RESIDUE

reconstitute in toluene

ALIQUOT ON GC-ECD
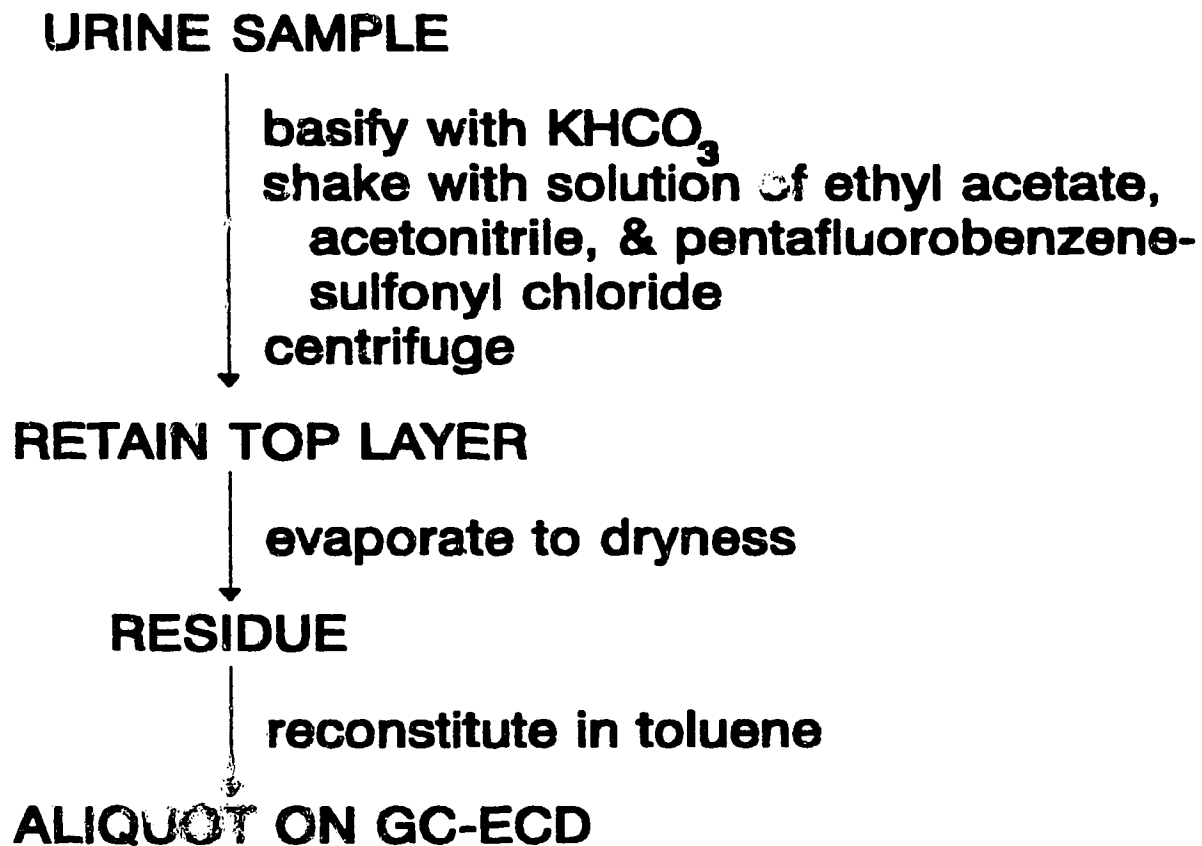
Figure 6:   The procedure used for the analysis of TFMP.

aliquot of 1 μl was used for analysis on the gas chromatograph system described in section 3.2.1.2. Chromatographic separation was accomplished using the following oven temperature program: initial temperature of 80°C for 0.5 min, increasing at a rate of 10°C/min to 270°C where it was held for 60 min.

3.5.4 REPRODUCIBILITY OF RETENTION TIMES

As a further check on reproducibility of assays, retention times of individual derivatives were monitored closely in standard curves and samples. Ratios of these retention times to those of the corresponding derivatives of the internal standards were also monitored. With the capillary columns used in the studies described in this thesis, the retention times and ratios were virtually unchanged within and between assays for all the drugs and metabolites under consideration.

3.6   STATISTICAL ANALYSIS

Data were analyzed by analysis of variance followed, when necessary, by the Newman-Keuls multiple comparisons test. A two-tailed

probability distribution was used, and the general convention of a

probability value of p<0.05 was used to establish statistical significance.

4.0 RESULTS

4.1 ANALYSIS OF ENANTIOMERS OF TCP

The novel GC procedure developed for the analysis of the enantiomers of TCP in rat brain and liver provided derivatives with good chromatographic properties. Typical GC traces obtained with this assay are shown in Figure 7. The standard curve was linear over the range of 5-500 ng and a correlation coefficient > 0.99 was obtained routinely. Mean interassay coefficients of variation for 50 and 100 ng samples of the enantiomers were 3.5 and 4.8%, respectively, for (-)-TCP and 4.0 and 3.8%, respectively, for (+)-TCP (N=8). The practical limits of sensitivity (signal:noise ratio >3) for (-)-TCP and (+)-TCP were 20 and 5 ng/g (67 and 17 pg "on-column"), respectively, in both brain and liver extracts; the difference in sensitivities was the result of a noisier baseline in the area of the retention time of the (-)-enantiomer. Mean percent recoveries of (-)-TCP and (+)-TCP from brain extracts were 82.7 ± 7.1% and 75.2 ± 6.3% (N=6), respectively.

The structures of the derivatives of (-)- and (+)-TCP were confirmed by combined GC-MS. The proposed structures of all major ions in the mass spectrum of the derivative of TCP are shown in Figure 8.

Figure 7:   Typical GC traces obtained in the separation of TCP
             enantiomers. Shown are derivatives of: (A) extract of whole
             brain from a rat treated with vehicle; (B) extract of liver tissue
             from a rat treated with rac-TCP; and (C) extract of whole brain
             from a rat treated with rac-TCP. The peaks represent
             derivatives of: (-)-TCP (1); (+)-TCP (2); and added internal
             standard, p-chlorophentermine (3). Retention times for these
             peaks were 13.77 min (1), 14.00 min (2) and 14.19 min (3).
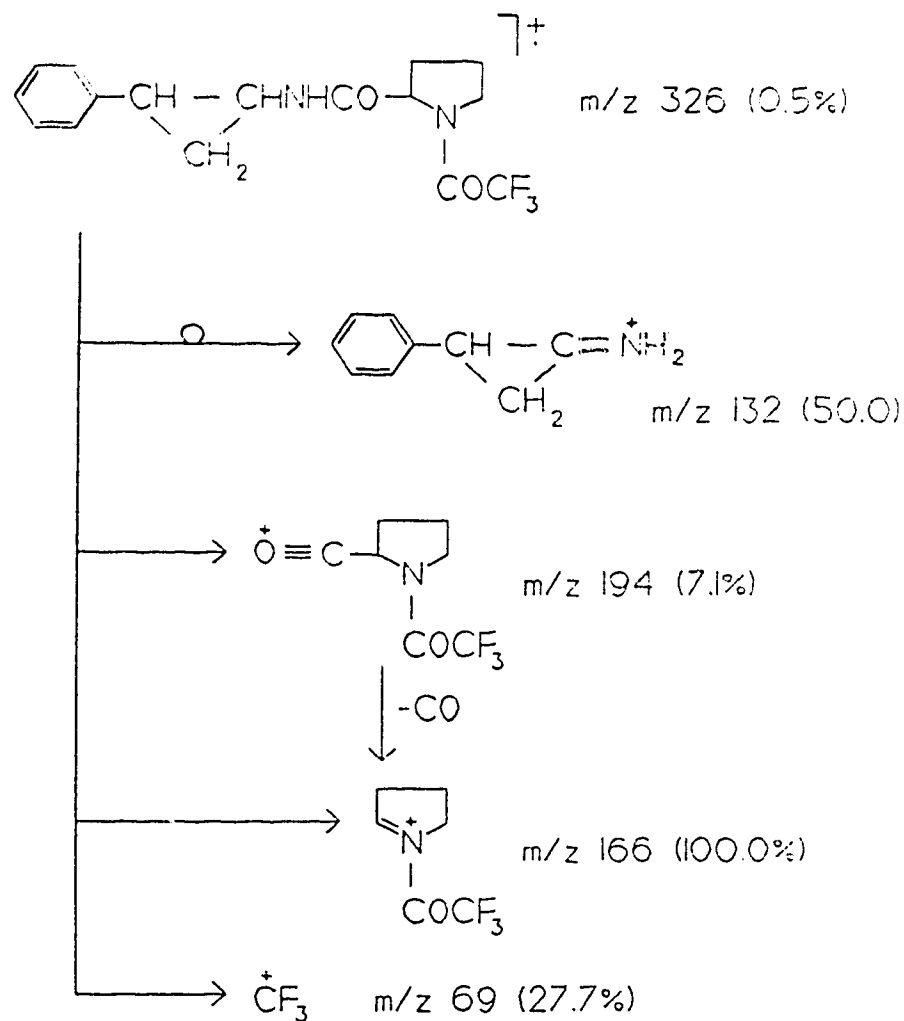
Figure 8:   Proposed electron impact mass spectrometric fragmentation
pattern for the TCP derivative. The % relative abundance
values are shown in parentheses.

86

IPR/TCP EXPERIMENT

Levels of the enantiomers of TCP were measured in the rat brain and liver with and without IPR pretreatment (Figures 9-12). Times of 1 and 3 h after treatment with rac-TCP were chosen based on acute studies conducted by Hampson et al. (1986) which showed that at these time intervals brain levels of both enantiomers of TCP were still relatively high after injection of the individual enantiomers. In the brain of vehicle-pretreated rats in the present study, the levels of (+)- and (-)-TCP were similar at 1 h (Figure 9) but after 3 h (Figure 10) the concentration of (+)-TCP was higher than that of (-)-TCP. A similar pattern was also noted in the liver samples (Figures 11 and 12).

The levels of both enantiomers of TCP in the brain as well as the liver showed an increase after pretreatment with IPR. This elevation was observed at both 1 h and 3 h. In the brain at 1 h the ratio of (+)-TCP:(-)-TCP is 1.07 without IPR pretreatment. This value drops slightly, but significantly, to 0.95 with IPR pretreatment. The same situation is observed at 3 h in the brain, with the ratio dropping significantly from 1.60 to 1.32 with IPR pretreatment. In the liver, however, significant changes are not observed in the ratios of (+)-TCP:(-)-TCP at either time interval

.
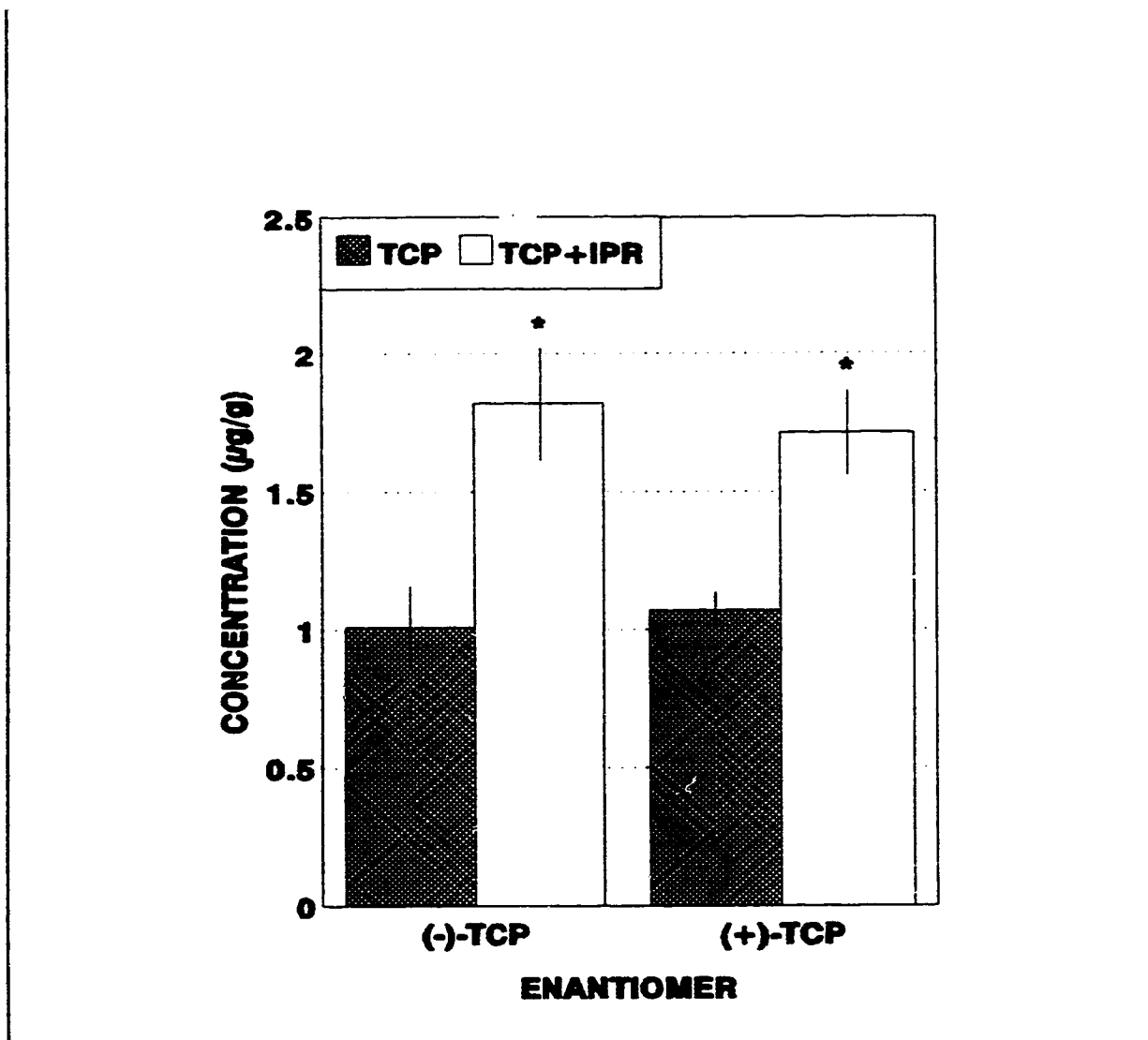
Figure 9:   Levels of (-)- and (+)-TCP found in rat whole brain at 1 h post-
             rac-TCP treatment with IPR pretreatment or without (vehicle-
             pretreated). Rats were injected with saline or with IPR (10
             mg/kg i.p.) and 1 h later with rac-TCP (2.5 mg/kg i.p.). The
             rats were sacrificed at 1 or 3 h following rac-TCP injection.
             Values represent mean ± SEM (N=5-10). * Significant
             difference (p<0.05) from the levels of the corresponding
             enantiomers in the rats not pretreated with IPR. In both the
             IPR-pretreated and the vehicle-pretreated rats, there was no
             significant difference between the levels of the (-)- and (+)-
             enantiomers.

88
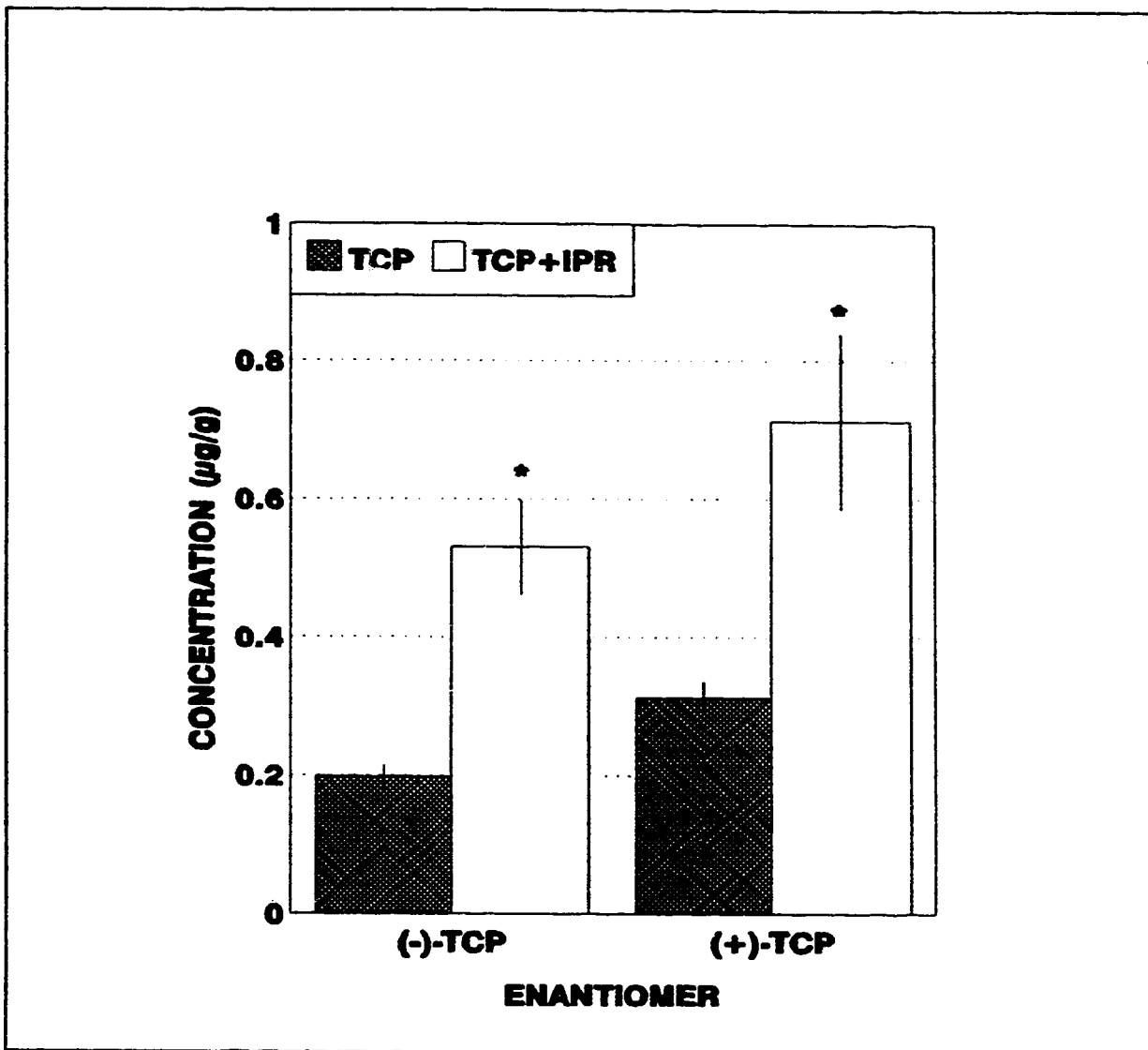
Figure 10:   Levels of (-)- and (+)-TCP found in rat whole brain at 3 h post-
rac-TCP treatment with and without IPR pretreatment. Values
represent mean ± SEM (N=5-10). * Significant difference
(p<0.05) with IPR pretreatment. (-)-TCP levels are lower than
(+)-TCP levels in the vehicle-pretreated rats but not in the IPR-
pretreated rats, although the ratio of (-)-TCP to (+)-TCP is
significantly lower (p<0.05) in the latter than in the former.
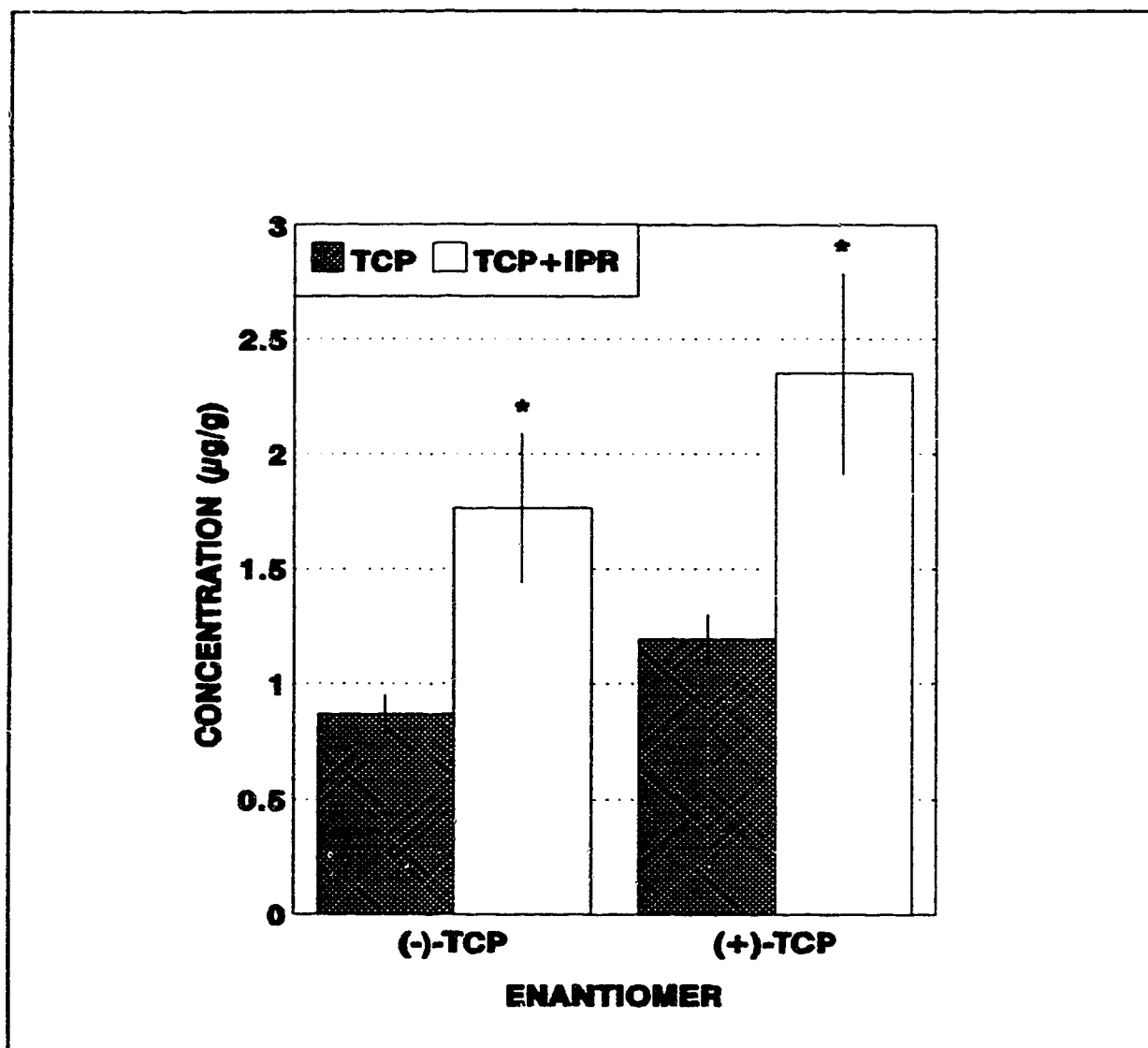
Figure 11: Levels of (-)- and (+)-TCP found in rat liver at 1 h post-*rac*-TCP treatment with and without IPR pretreatment. Values represent mean ± SEM (N=5-10). * Significant difference (p<0.05) with IPR pretreatment. There were no significant differences in levels between the two enantiomers in either the vehicle-pretreated or IPR-pretreated rats.
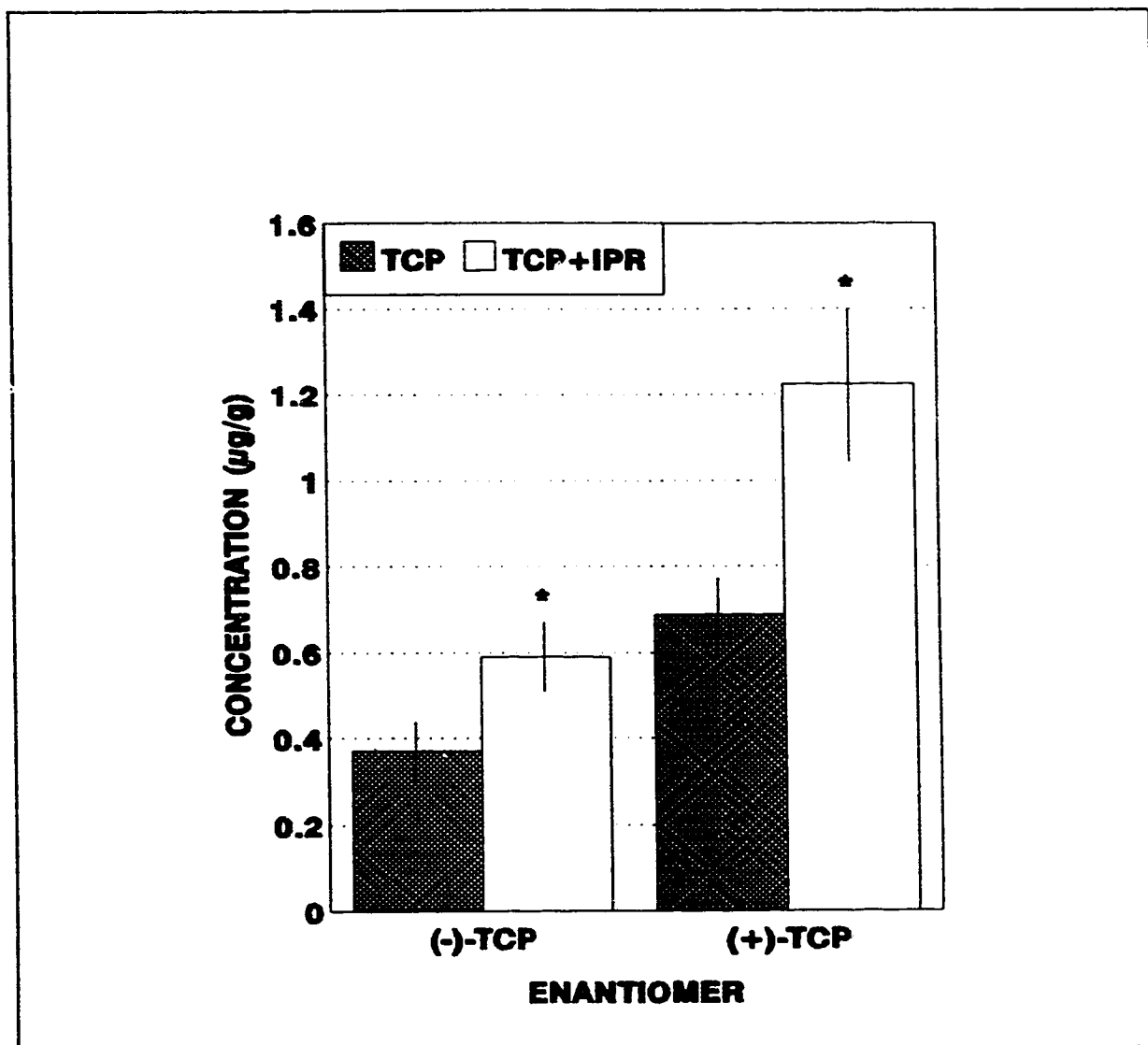
Figure 12: Levels of (-)- and (+)-TCP found in rat liver at 3 h post-*rac*-TCP treatment with and without IPR pretreatment. Values represent mean ± SEM (N=5-10). * Significant difference (p<0.05) with IPR pretreatment. The levels of (-)-TCP are lower than those of (+)-TCP in both the vehicle- and IPR-pretreated rats.

91

studied.

4.2   ANALYSIS OF ENANTIOMERS OF FLU AND NFLU

The novel GC procedure developed for the simultaneous analysis of the enantiomers of FLU and NFLU provides derivatives with good chromatographic properties. Typical GC traces obtained are shown in Figure 13. The standard curve was linear over the range of 10 ng to 1.0 μg, with a correlation coefficient > 0.99 obtained routinely. Calibration curves generated on different days were reproducible with coefficients of variation ranging from 3.0 to 5.5% (N=6). Repeated analysis of spiked brain samples produced coefficients of variation ranging from 5.3 to 8.2% (N=6). Practical limits of sensitivity (signal:noise ratio > 3) were < 10 ng/g and < 40 ng/g for the FLU enantiomers and the NFLU enantiomers, respectively. These values represent < 10 and < 40 pg "on-column", respectively.

The structures of the derivatives of R- and S-FLU and R- and S-NFLU were confirmed by combined GC-MS. The proposed structures of all major ions in the spectrum of the derivatives of FLU and NFLU are identified in Figure 14.
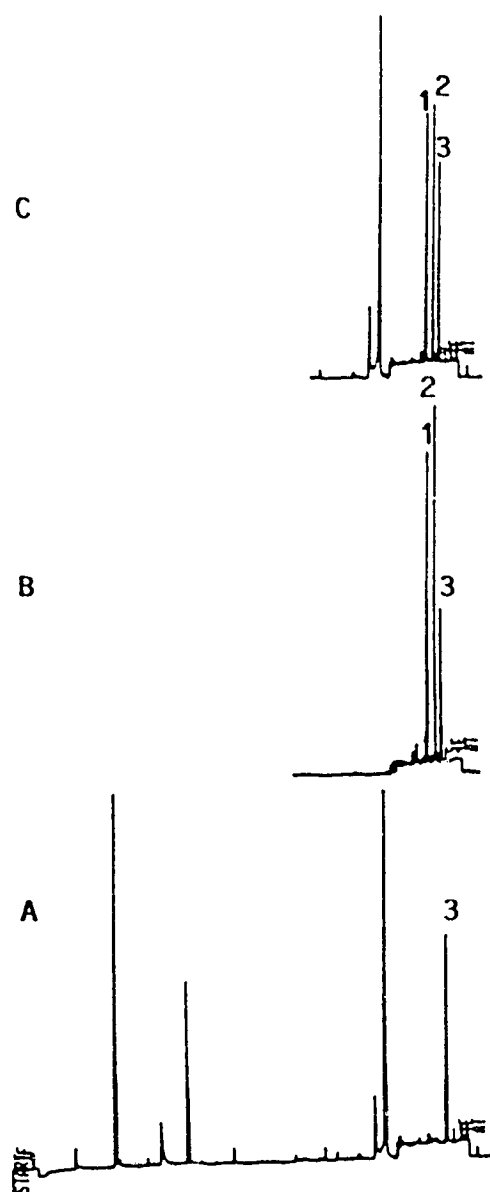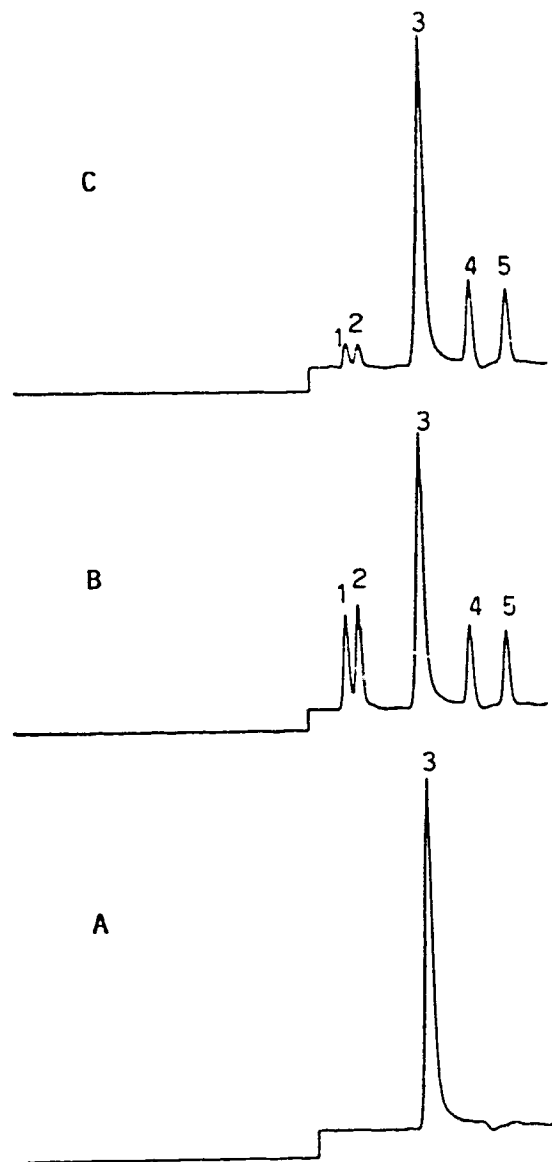
Figure 13: Typical GC traces obtained in the separation of FLU and NFLU enantiomers. Shown are derivatives of: (A) extract of whole brain from a control rat; (B) authentic standard of FLU and NFLU; and (C) extract of whole brain from a rat treated with rac-FLU. The peaks represent derivatives of: S-NFLU (1); R-NFLU (2); added internal standard, (-)-alprenolol (3); R-FLU (4); and S-FLU (5). Retention times for these peaks were 24.69 min (1), 25.03 min (2), 26.84 min (3), 28.20 min (4), and 29.22 min (5).

m/z 502 absent (R-CH₃)
m/z 488 (R-H)

Figure text (fragmentation scheme):

CF_3—⬡—OCHCH₂CH₂N< R / CO—pyrrolidine—N—COCF₃]⁺·   m/z 502 absent (R-CH₃); m/z 488 (R-H)

-CF₃—⬡—OCHCH₂CH₂NR

→ Ō≡C—N—COCF₃   m/z 194   —CO→   N⁺—COCF₃   m/z 166

-CF₃—⬡—OCHĊH₂

→ CH₂=N⁺< R / CO—N—COCF₃   m/z 237 (R-CH₃); m/z 223 (R-H)

-CF₃—⬡—Ȯ

→ ⁺CHCH₂CH₂N< R / CO—N—COCF₃   m/z 341 (R-CH₃); m/z 327 (R-H)

—CH₃NRCO—N—COCF₃→ ⬡—⁺CHCH=CH₂   m/z 117

-C₂H₂ → C₇H₇⁺   m/z 91
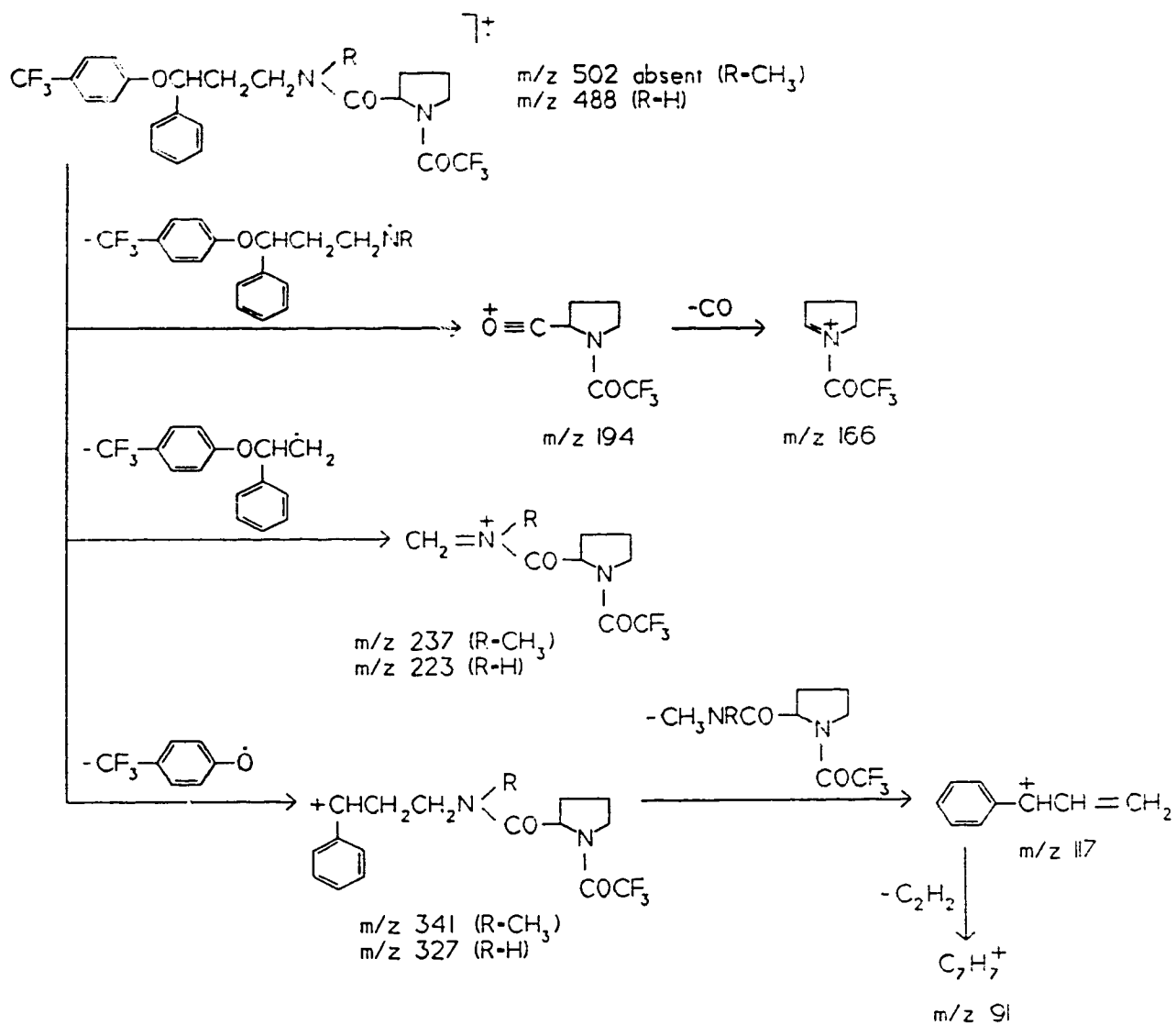
Figure 14: Proposed electron impact mass spectrometric fragmentation pattern for the FLU (R=CH₃) and NFLU (R=H) derivatives.

94

4.2.1 IPR/FLU EXPERIMENT

Levels of the enantiomers of FLU and NFLU were measured in whole rat brain and rat brain regions 5 h after pretreatment with vehicle or IPR. This time interval (5 h) was chosen after performing detailed time studies on brain levels of R- and S-FLU and R- and S-NFLU in brains of rats (Torok-Both *et al.*, 1992). The results of the present study show that IPR significantly increased concentrations of both R- and S-FLU in rat whole brain (Figure 15) and in the brain regions studied (Figures 16 and 17). The increase is significantly greater in the case of R-FLU than of S-FLU in the whole brain, the cortex, the pons medulla and the striatum (Table 3). The combination of IPR and *rac*-FLU did not cause a significant increase in the levels of either enantiomer of NFLU in rat brain. For this reason, only the results obtained from the whole brain analysis are depicted here for R- and S-NFLU (Figure 18).

Results from the regional data show a consistency in concentration of FLU in the cerebellum, cortex, pons medulla and striatum. The hypothalamus showed a relatively higher concentration of FLU than observed in other brain areas, both in the vehicle- and IPR-pretreated rats. The distribution of both R- and S-FLU also appears to be consistent across

95
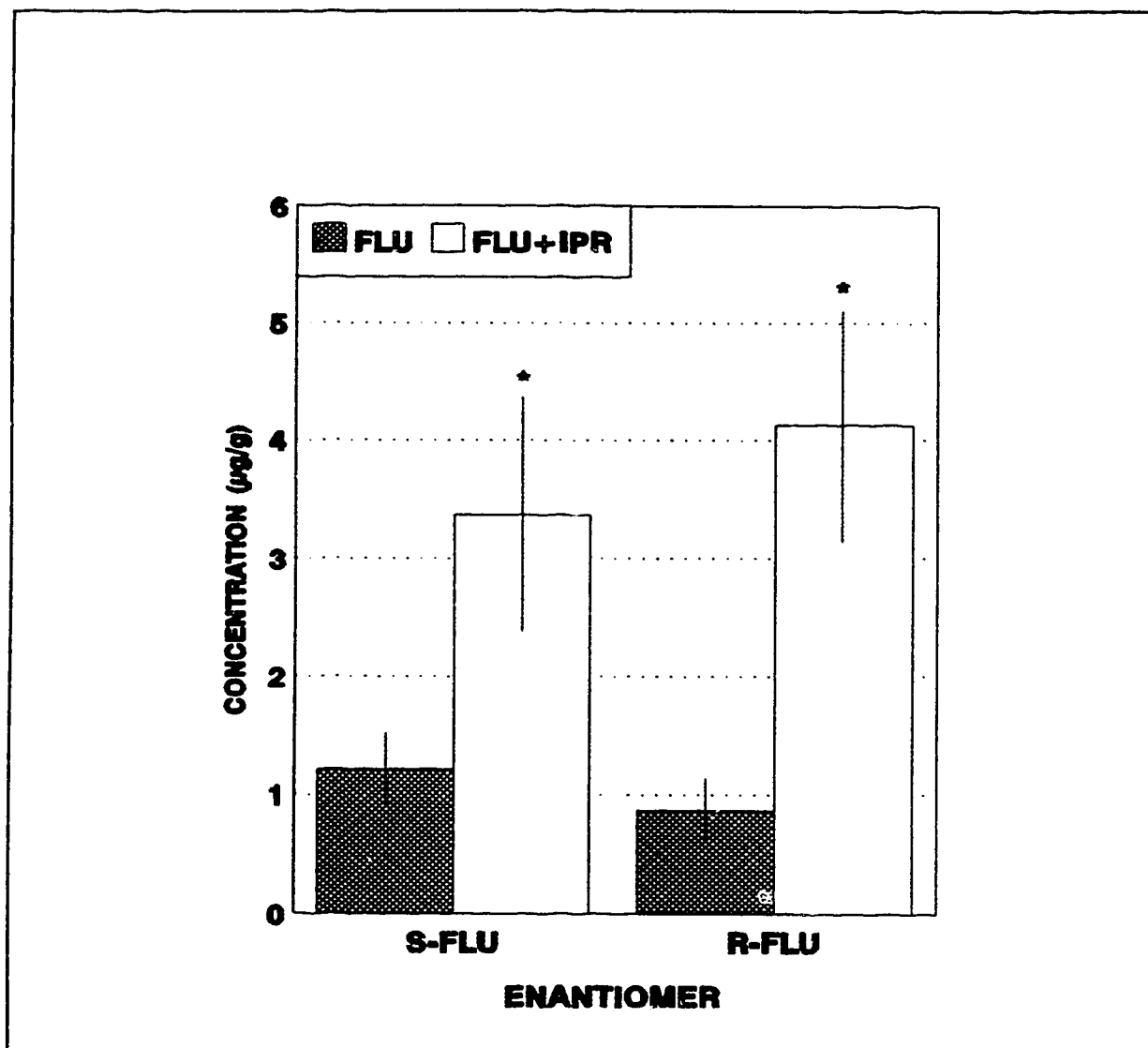
Figure 15: Levels of R- and S-FLU found in rat whole brain at 5h post-*rac*-FLU treatment with and without IPR pretreatment. Rats were injected with saline or IPR (10 mg/kg i.p.) and 1 h later with *rac*-FLU (10 mg/kg i.p.). The rats were sacrificed 5 h following the *rac*-FLU injection. Values represent mean ± SEM (N=5). * Significant difference (p<0.05) with IPR pretreatment.
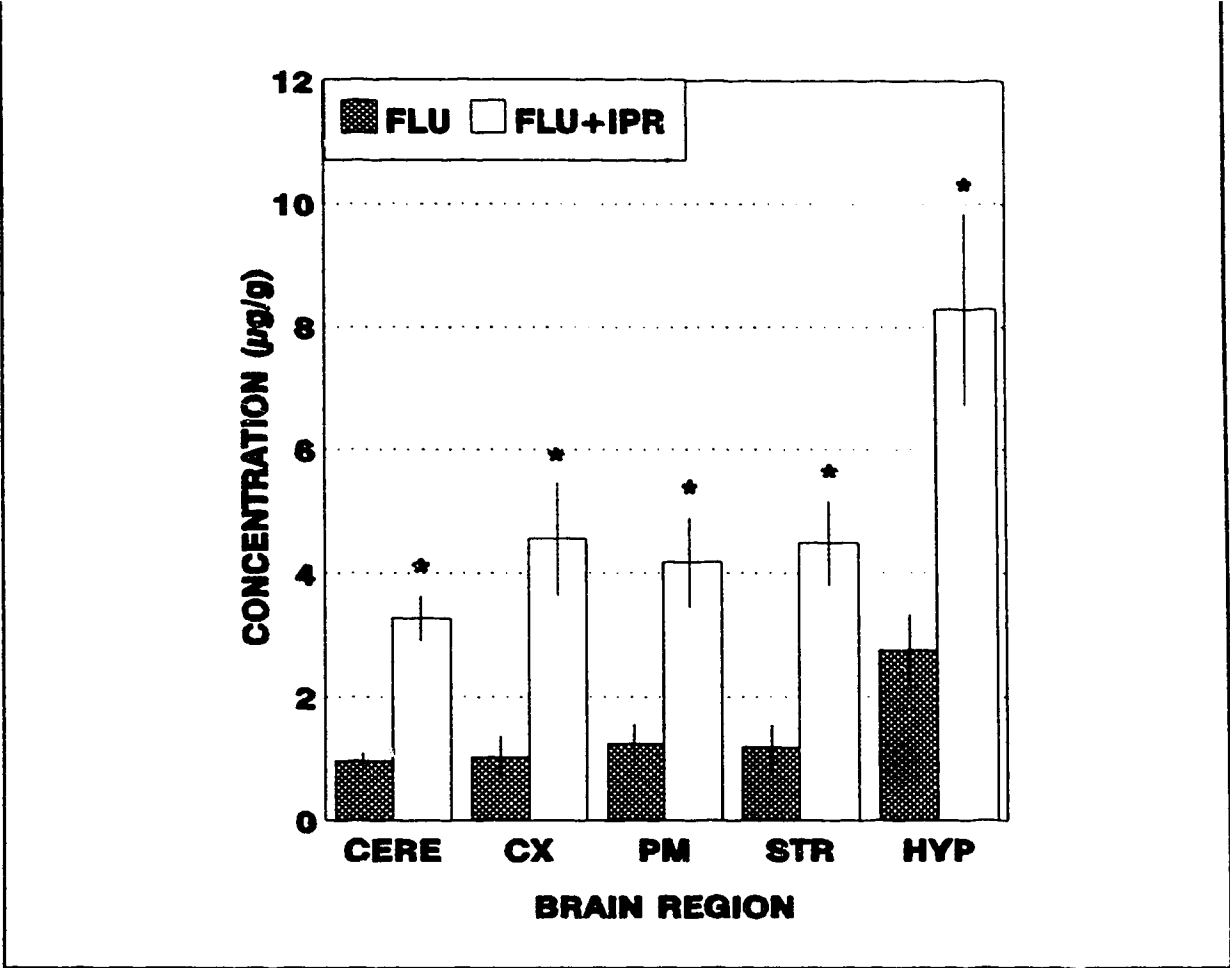
Figure 16: Level of R-FLU found in various brain regions at 5h post-*rac*-FLU treatment with and without IPR pretreatment. Values represent mean ± SEM (N=5). Regions: CERE=cerebellum; CX=cortex; PM=pons medulla; STR=striatum; HYP=hypothalamus. * Significant difference (p<0.05) with IPR pretreatment.

97
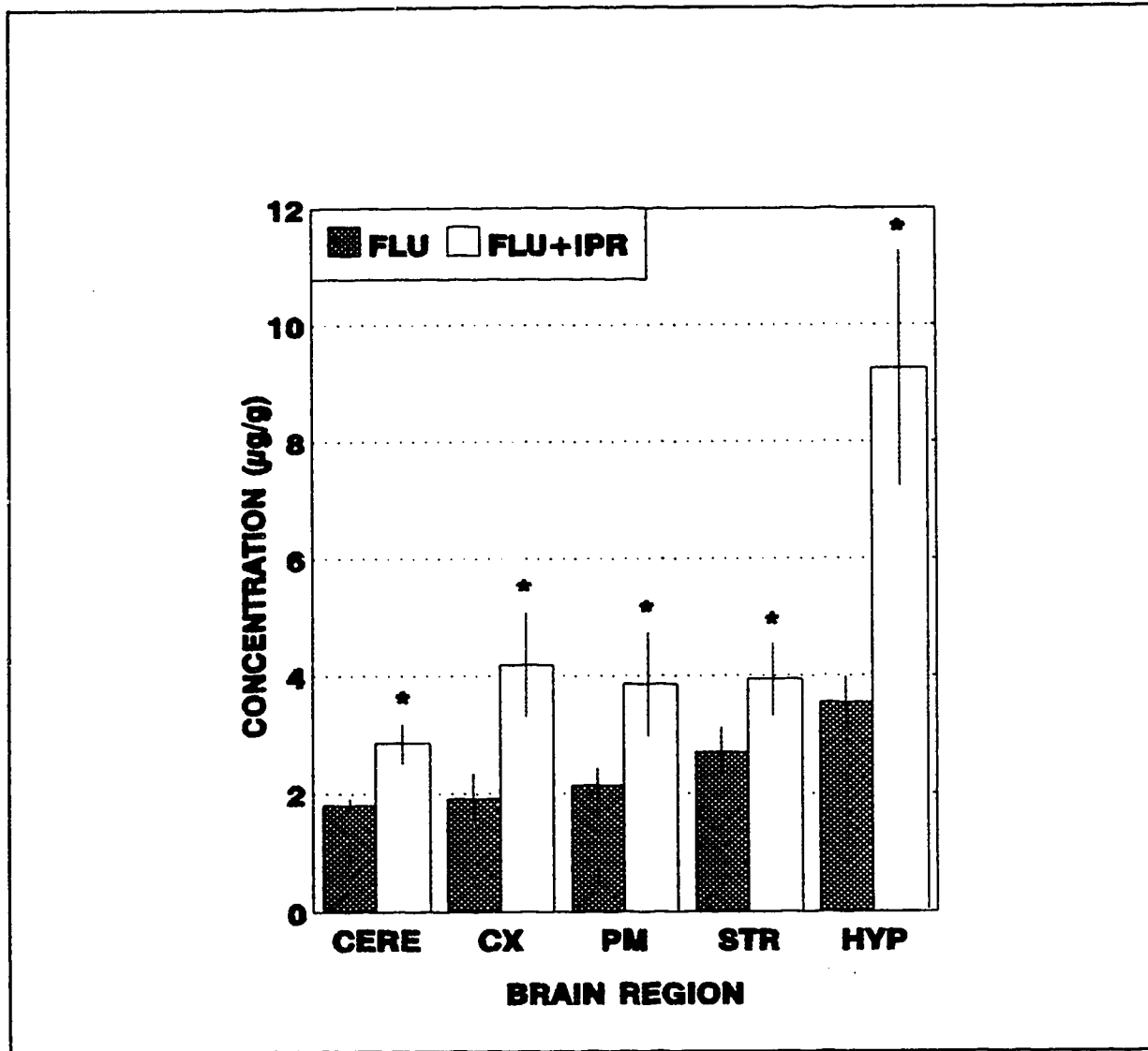
Figure 17:   Level of S-FLU found in various brain regions at 5h post-*rac*-
FLU treatment with and without IPR pretreatment. Values
represent mean ± SEM (N=5). Regions: CERE = cerebellum;
CX = cortex; PM = pons medulla; STR = striatum; HYP =
hypothalamus. * Significant difference (p<0.05) with IPR
pretreatment.

Table 3: The ratio of S-FLU to R-FLU in rat whole brain and brain regions after injection of rac-FLU with and without IPR pretreatment. Rats were injected with saline (SAL) vehicle (A) or IPR (10 mg/kg i.p.) (B) and 1 h later with rac-FLU (10 mg/kg i.p.). The rats were sacrificed 5 h following the rac-FLU injection. Values represent mean ± SEM (N=5). * Significant difference ($p < 0.05$) from values in vehicle-pretreated rats.

	RATIO S-FLU:R-FLU	
	A: SAL/rac-FLU	B: IPR/rac-FLU
whole brain	1.52 ± 0.13	0.78 ± 0.06 *
cerebellum	1.56 ± 0.38	0.88 ± 0.04
cortex	2.39 ± 0.40	0.92 ± 0.03 *
pons medulla	2.12 ± 0.41	0.90 ± 0.05 *
striatum	2.79 ± 0.49	0.88 ± 0.04 *
hypothalamus	1.64 ± 0.46	1.10 ± 0.11
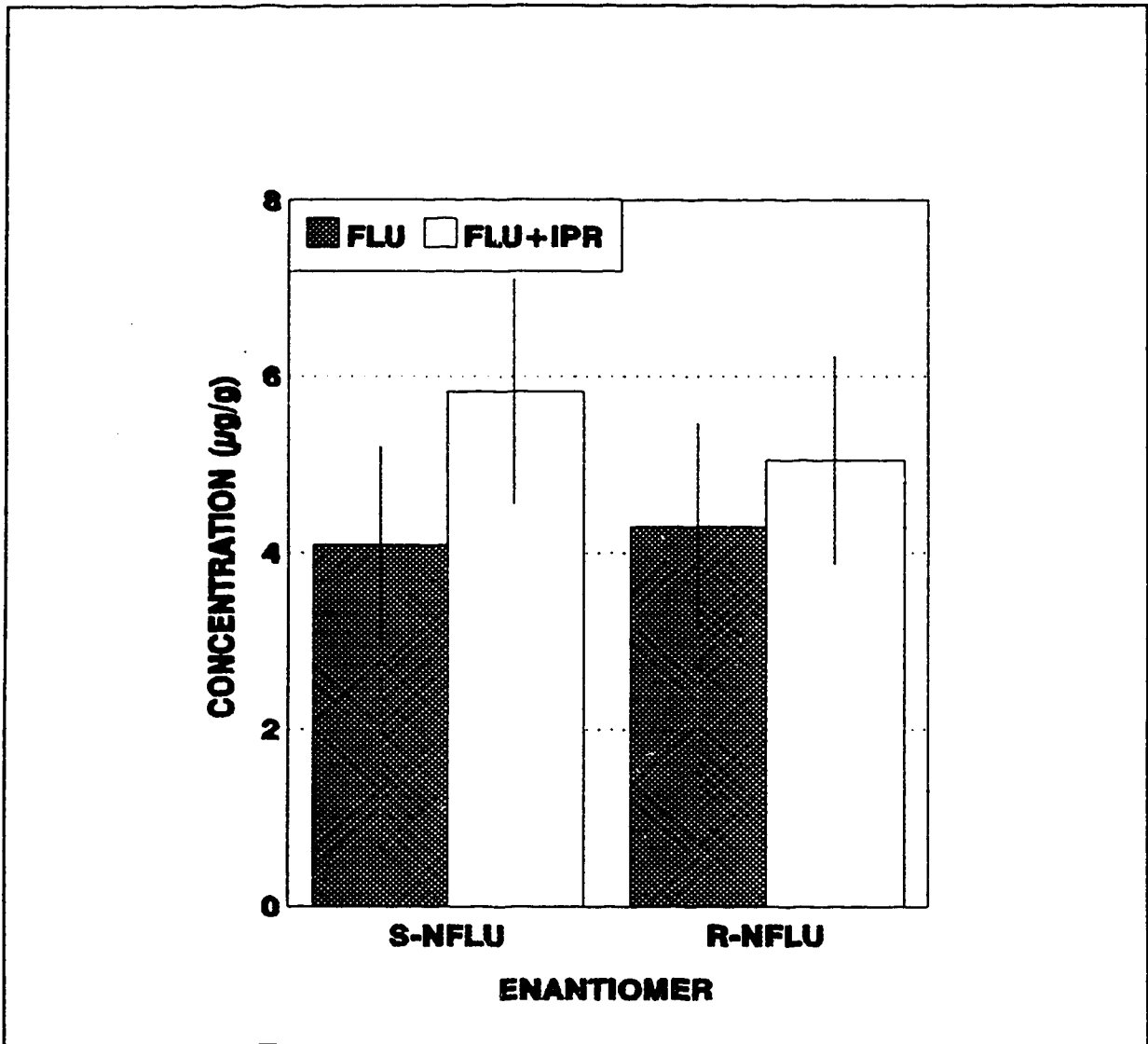
Figure 18: Levels of R- and S-NFLU found in rat whole brain at 5h post-
rac-FLU treatment with and without IPR pretreatment. Values
represent mean ± SEM (N=5). IPR pretreatment caused no
significant change in levels of either S- or R-NFLU.

all the regions, with S-FLU levels higher than those of R-FLU in the vehicle pretreated rats. However, in the IPR-pretreated rats, the greater increase in R-FLU than in S-FLU resulted in S/R ratios of 1 or slightly less in the brain regions. These results are summarized in Table 3.

4.2.2 DMI/FLU EXPERIMENT

Levels of the enantiomers of FLU and NFLU were measured in brains from rats that had been treated with rac-FLU alone or in combination with DMI. The doses used in this study, rac-FLU (10 mg/kg) and DMI (5 mg/kg), are identical to those used in a study by Baron et al. (1988). Baron and his colleagues observed that at these doses, a more rapid down-regulation of β-adrenergic receptors was observed with the drug combination than with either drugs alone. Their study included 4 and 14 day time periods. As in the Baron et al. (1988) study, the rats were killed 24 h after the last dose.

In the present study, it was observed that the combination of DMI and rac-FLU caused a significant increase in the levels of both R- and S-FLU over the levels achieved when rac-FLU was administered alone at both day 1 and day 14 (Figures 19 and 20). This combination also caused

an increase in the levels of R- and S-NFLU at both times (Figures 21 to 22).

In rats treated with rac-FLU alone, brain levels of the enantiomers of FLU are quite similar at day 1 with a ratio of S-FLU to R-FLU of 1.39 (Table 4). After 14 days, the level of S-FLU has increased to a greater extent than R-FLU, resulting in a ratio of 2.34 (Table 4). In the case of NFLU, there is very little difference observed between the ratios of S-NFLU to R-NFLU at day 1 and day 14 (Table 4). Although the drug combination of rac-FLU and DMI produced marked increases in levels of all four enantiomers, the drug combination produces no overall effect on the ratios S-FLU to R-FLU or S-NFLU to R-NFLU compared to those observed when rac-FLU is administered alone (Table 4). In contrast to the situation with IPR pretreatment, where there was a preferential increase in brain levels of R-FLU over S-FLU, with DMI pretreatment, levels of both enantiomers increased to an equivalent extent.
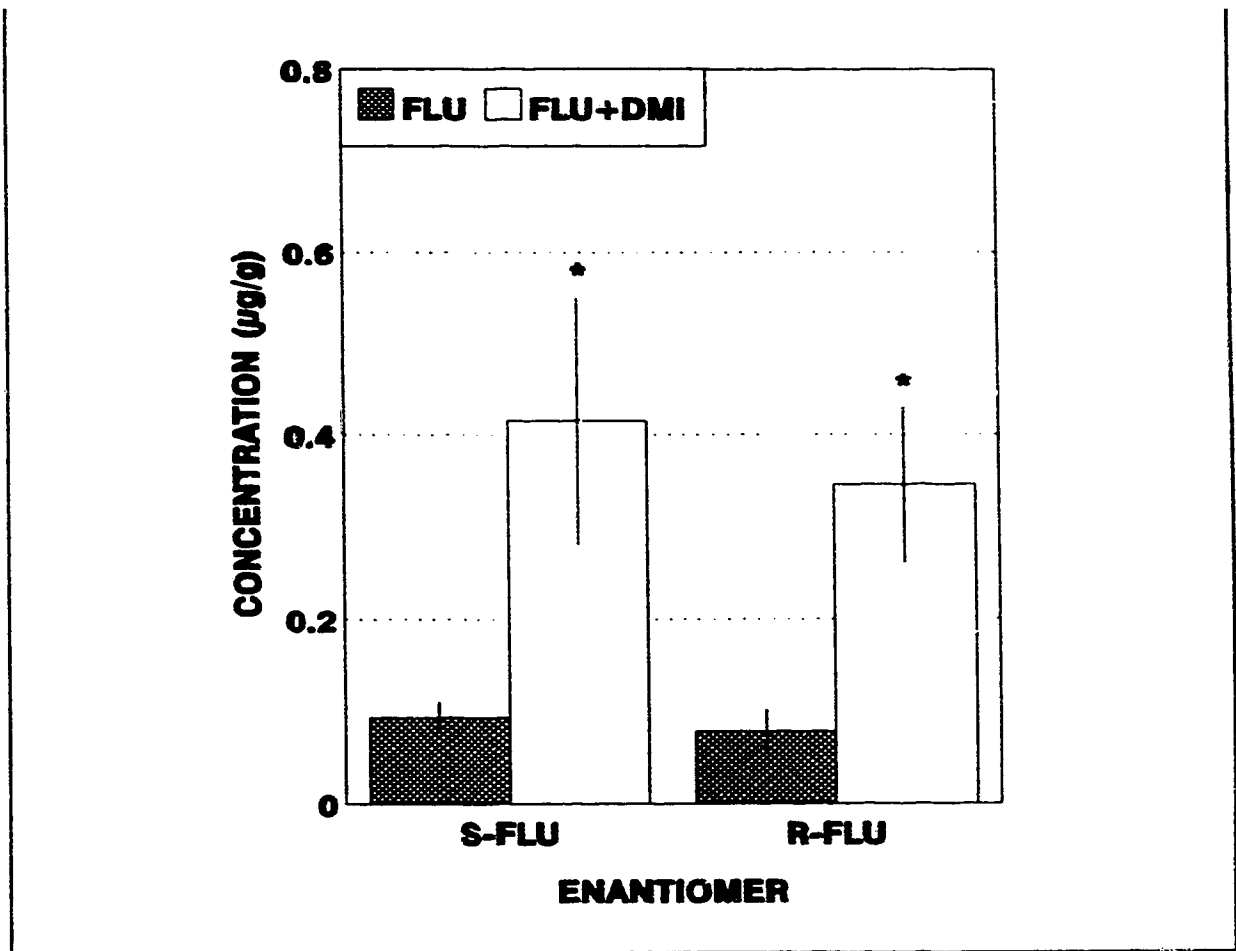
Figure 19:   Levels of R- and S-FLU found in rat brain after 1 day of
treatment with either rac-FLU alone or in combination with
DMI. Rats were injected once daily with either rac-FLU (10
mg/kg i.p.) or the combination of rac-FLU and DMI (10 mg/kg
and 5 mg/kg i.p., respectively). The rats were sacrificed after
either 1 or 14 days of treatment. Values represent mean ±
SEM (N=8). * Significant difference (p<0.05) from values in
rats treated with rac-FLU alone.
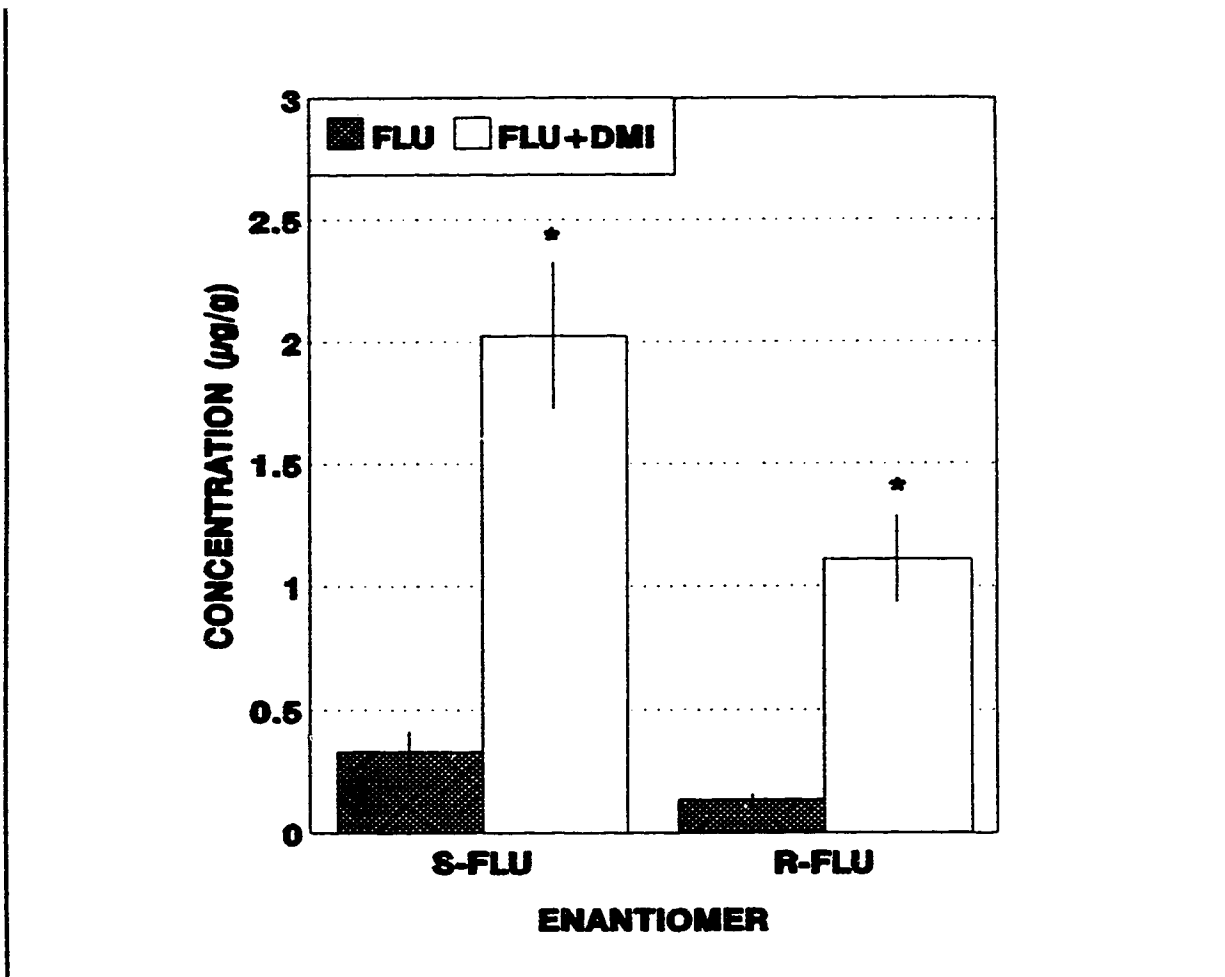
Figure 20: Levels of R- and S-FLU found in rat brain after 14 days of treatment with either rac-FLU alone or in combination with DMI. Values represent mean ± SEM (N=8). * Significant difference (p<0.05) from values in rats treated with rac-FLU alone.

104
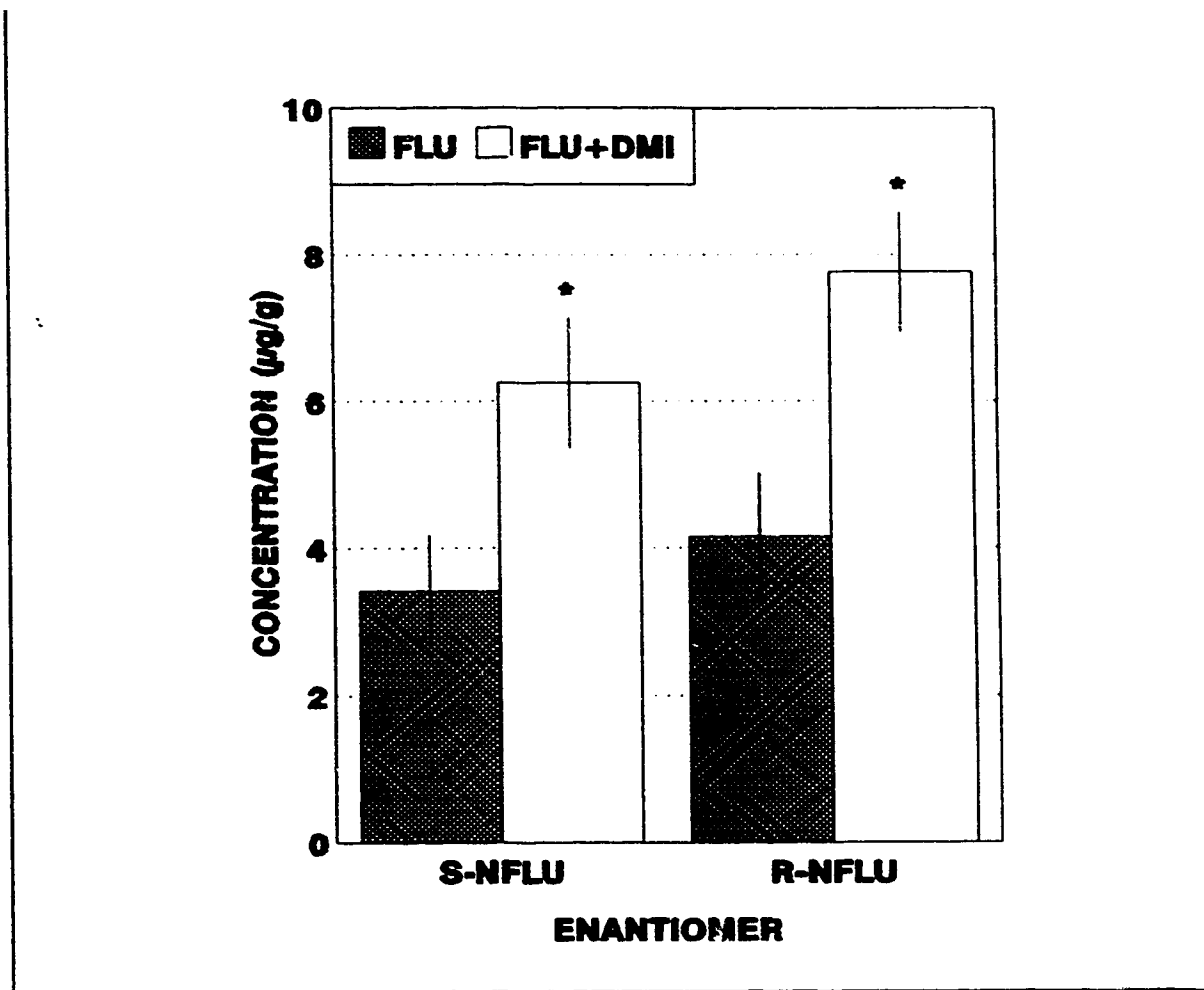
Figure 21:   Levels of R- and S-NFLU found in rat brain after 1 day of treatment with either *rac*-FLU alone or in combination with DMI. Values represent mean ± SEM (N=8). * Significant difference (p<0.05) from values in rats treated with *rac*-FLU alone.
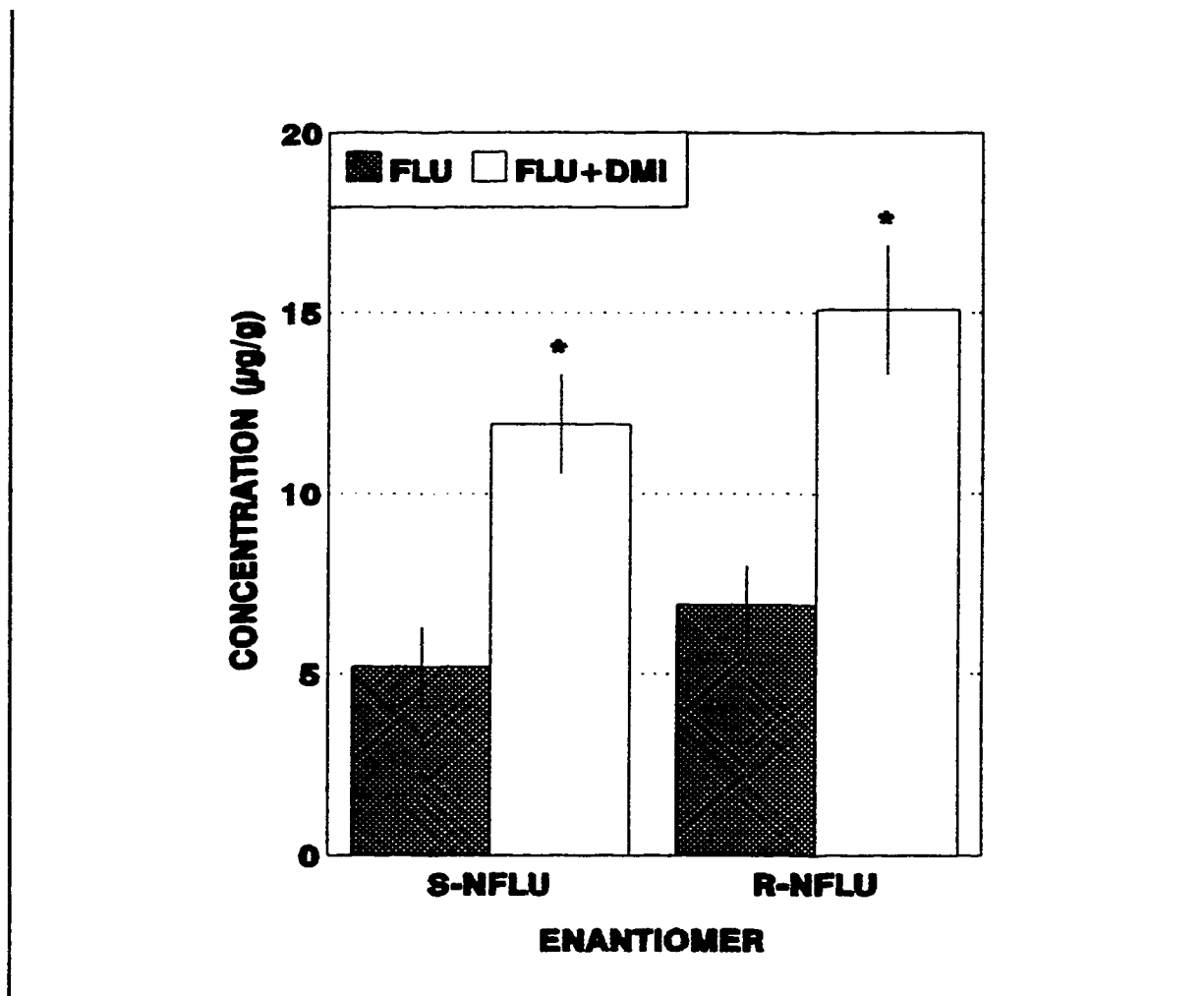
Figure 22:   Levels of R- and S-NFLU found in rat brain after 14 days of treatment with either *rac*-FLU alone or in combination with DMI. Values represent mean ± SEM (N=8). * Significant difference (p<0.05) from values in rats treated with *rac*-FLU alone.

106

Table 4: The ratios of S-FLU to R-FLU and S-NFLU to R-NFLU in rat whole brain after injection of *rac*-FLU alone or in combination with DMI. Rats were injected with *rac*-FLU (10 mg/kg i.p.) or the combination of *rac*-FLU and DMI (10 mg/kg and 5 mg/kg i.p., respectively). The rats were sacrificed after 1 or 14 days of treatment. Values represent mean ± SEM (N=8).

	DRUG	S-FLU:R-FLU	S-NFLU:R-NFLU
DAY 1	FLU	1.39 ± 0.12	0.81 ± 0.03
	FLU+DMI	1.27 ± 0.21	0.78 ± 0.04
DAY 14	FLU	2.34 ± 0.33	0.71 ± 0.06
	FLU+DMI	1.92 ± 0.13	0.79 ± 0.02

4.3 INVESTIGATION OF SELECTED POTENTIAL METABOLITES OF FLU

4.3.1 SCREEN USING THE PFBC ASSAY

In an attempt to find possible metabolites of FLU in addition to NFLU, chromatograms obtained from this experiment were closely examined for any peaks other than those corresponding to FLU and NFLU, and that were present only in the urine and plasma samples obtained from patients after 3 weeks of treatment and not in the pretreatment samples. Additional peaks were not found even after letting samples run through the column for more than an hour. The assay did, however, prove very useful for the simultaneous analysis of FLU and NFLU.

4.3.1.1 ANALYSIS OF FLU AND NFLU USING THE PFBC ASSAY

This gas chromatographic method provides a relatively simple procedure for the simultaneous analysis of FLU and NFLU in human body fluids and produces derivatives with good chromatographic properties. Typical chromatograms of plasma and urine extracts from drug-naive

individuals and patients receiving FLU are shown in Figure 23. The standard curve was linear over the range of 50-1000 ng, with a correlation coefficient of > 0.99 obtained routinely. Mean interassay coefficients of variation for 200 ng samples were 7.2% for FLU and 6.3% for NFLU in urine and 4.9% for FLU and 3.0% for NFLU in plasma (N=6). The practical limits of sensitivity (signal:noise ratio > 3) for both FLU and NFLU were 25 ng/ml in plasma extracts and 50 ng/ml in urine extracts (333 pg "on-column").

The structures of the derivatives of FLU and NFLU were confirmed by combined GC-MS. The proposed structures of the major ions in the mass spectra of the derivatives of FLU and NFLU are identified in Figure 24.

4.3.1.2   APPLICATION OF THE PFBC ASSAY TO HUMAN BODY FLUIDS

The results obtained by measuring the levels of FLU and NFLU in plasma and urine samples obtained from patients receiving 20 mg *rac*-FLU per day are depicted in Table 5. After 3 weeks, the mean plasma FLU
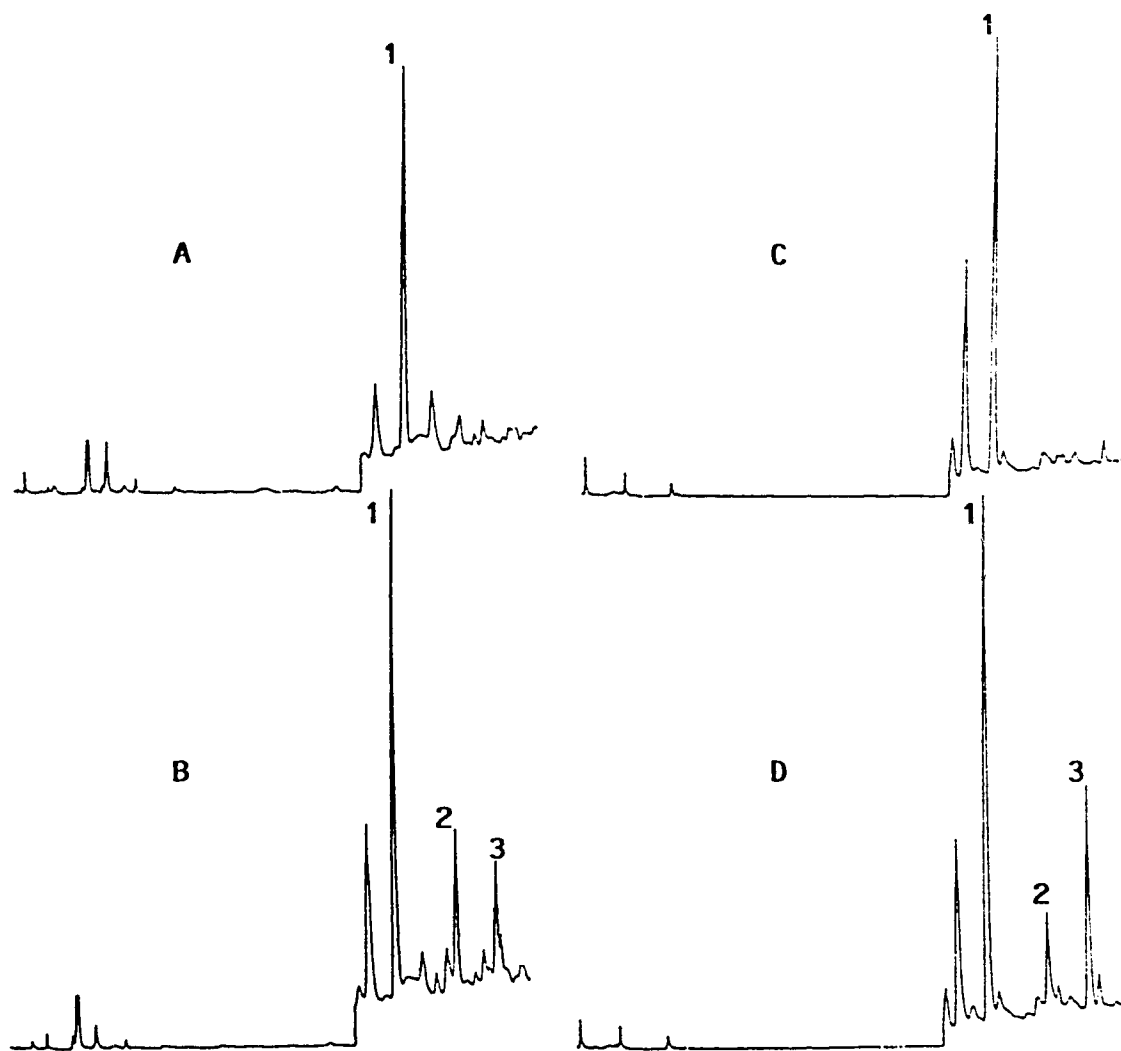
Figure 23: Typical GC traces obtained in the analysis of FLU and NFLU in human body fluids using the extractive pentafluorobenzoylation technique. Shown are derivatives of: (A) extract of a urine sample from a drug-naive individual; (B) extract of a urine sample from a patient treated with FLU for 3 weeks; (C) extract of a plasma sample from a drug-naive individual; and (D) extract of a plasma sample from a patient treated with *rac*-FLU (20 mg/day) for 3 weeks. The peaks represent the derivatives of: added internal standard, (-)-alprenolol (1); FLU (2); and NFLU (3). Retention times for these peaks were 15.25 min (1), 16.94 min (2), and 18.08 min (3).

110
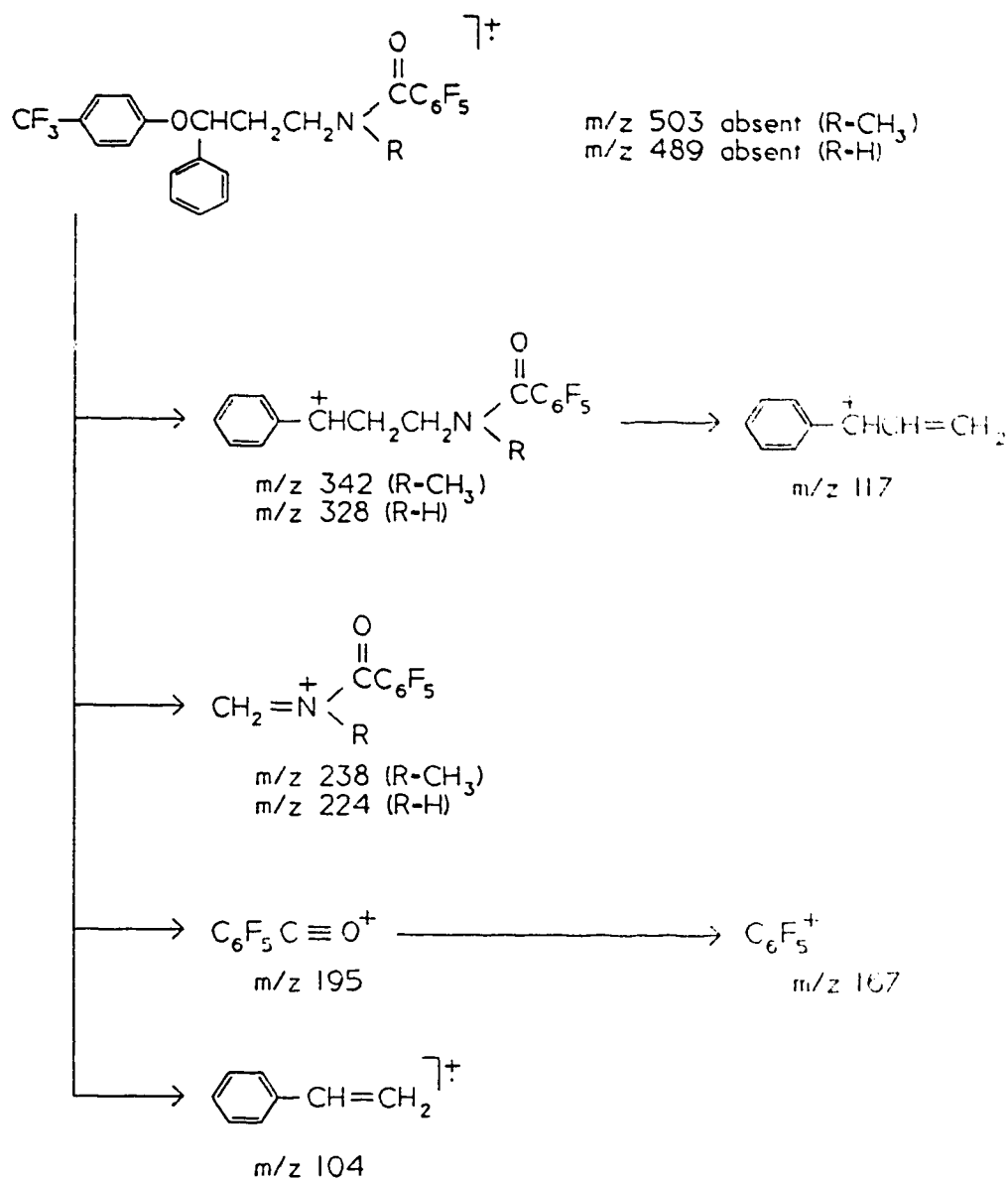
Figure 24: Proposed electron impact mass spectrometric fragmentation patterns for the pentafluorobenzoyl derivatives of FLU (R=CH3) and the NFLU (R=H).

111

Table 5: Urine and plasma concentrations of FLU and NFLU in patients taking 20 mg FLU per day for a period of three weeks.

	URINE			PLASMA		
patient	FLU μg/24h	NFLU μg/24h	FLU/ NFLU	FLU ng/ml	NFLU ng/ml	FLU/ NFLU
A	315.5	364.0	0.867	48.3	135.5	0.356
B	311.6	417.4	0.746	39.5	116.5	0.339
C	926.4	1037.4	0.893	65.4	117.5	0.557
D	721.9	814.9	0.886	86.4	186.6	0.463
E	524.8	639.0	0.822	18.9	31.9	0.592
F	282.3	593.1	0.476	99.2	83.2	1.192
G	415.3	737.3	0.563	104.6	94.8	1.104
H	285.6	495.2	0.577	28.0	72.9	0.384
MEAN	472.9	637.3	0.729	61.3	104.9	0.623
SEM	84.1	78.7	0.059	11.6	16.3	0.119

and NFLU concentrations were determined to be 64.1 and 105.3 ng/ml, respectively. The mean urine levels of FLU and NFLU at the same time interval were 274.0 and 391.7 μg/24 hr, respectively. In all urine samples and in all but two of the plasma samples, levels of NFLU were higher than levels of FLU after 3 weeks of FLU treatment. These findings are in good agreement with levels found using the procedure of Torok-Both *et al.* (1992) in a previous study to measure levels in patients receiving similar doses of *rac*-FLU.

4.3.2 SCREEN USING THE PFBSC ASSAY

4.3.2.1   ANALYSIS OF TFMP

The screen using PFBSC as a derivatizing reagent resulted in the identification and quantification of TFMP in 24 h urines from patients treated with *rac*-FLU (20 mg/day for 6 weeks) and in rats treated with *rac*-FLU (10 mg/day) for 1 or 14 days. The gas chromatographic method described for the analysis of TFMP produces derivatives with good chromatographic properties. Typical chromatograms of urine extracts from drug-naive individuals and patients receiving *rac*-FLU, and vehicle and *rac*-FLU-treated rats are shown in Figure 25. The standard curve was linear
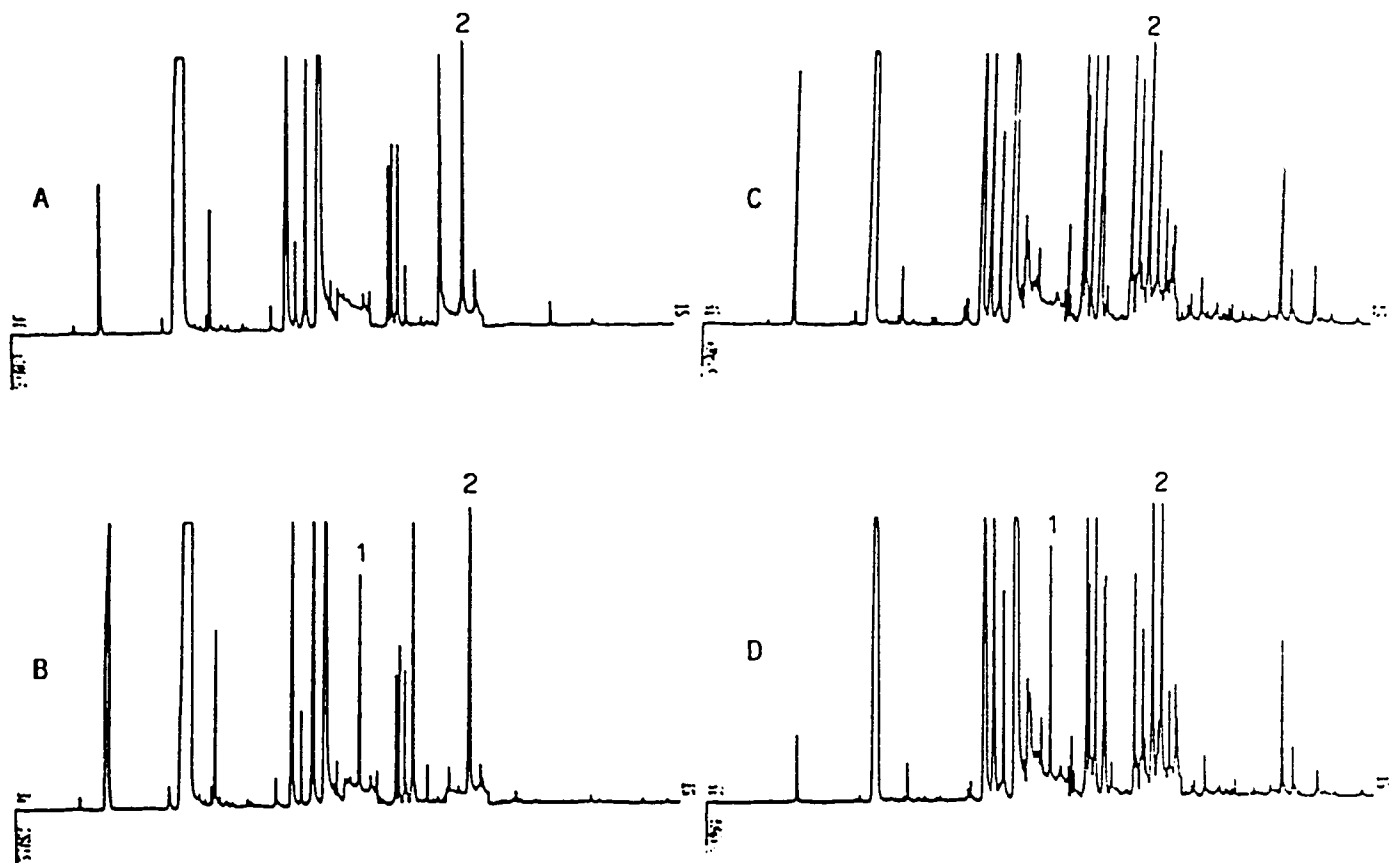
Figure 25: Typical GC traces obtained in the analysis of TFMP in human and rat urine. Shown are derivatives of: (A) extract of a urine sample from a drug-naive individual; (B) extract of a urine sample from a patient treated with rac-FLU for 6 weeks; (C) extract of a urine sample from a vehicle-treated rat; and (D) extract of a urine sample from a rat treated with rac-FLU for 14 days. The peaks represent derivatives of: TFMP (1) and added internal standard, 2,4-dichlorophenol, (2). Retention times for these peaks were 12.32 min (1) and 16.27 min (2).

114

over the range of 50-1000 ng and a correlation coefficient of > 0.99 was obtained routinely. Mean interassay coefficient of variation for 500 ng samples was 7.4% (N=5) for TFMP in rat urine. The practical limit of sensitivity (signal:noise ratio > 3) for TFMP was < 25 ng/ml (< 62.5 pg "on-column") in rat urine extracts. The mean percent recovery of TFMP from rat urine extracts was 85.6 ± 2.9% (N=5).

The structures of the derivative of TFMP and the internal standard, 2,4-dichlorophenol, were confirmed by combined GC-MS. The proposed structures of the major ions in the mass spectra of the derivatives of TFMP and 2,4-dichlorophenol are indicated in Figures 26 and 27, respectively.

The results obtained from the analysis of the human and rat urine are shown in Table 6 and Figure 28. No additional peaks were found in the urines from humans or rats treated with rac-FLU relative to the drug-naive samples.

The urinary level of TFMP increased from day 1 to day 14 in the rat (Figure 28). In rats that were treated with a combination of rac-FLU and DMI there was a significant decrease in urine levels of TFMP at day 1. Although there was also a trend to decrease in the drug combination rats at 14 days, the results were more variable at this time interval and the decrease was not significant (Figure 28).

CF$_3$—◯—O—SO$_2$—(C$_6$F$_5$)]$^+$ $\xrightarrow{\ -\dot{F}\ }$ m/z 373 (7.8%)

M$^+$ m/z 392 (23.2%)

$\xrightarrow{\ -CF_3-◯-\dot{O}\ }$ F—(C$_6$F$_4$)—$\overset{+}{S}$(=O)O $\longleftrightarrow$ F—(C$_6$F$_4$)$^+$=SO$_2$ $\longleftrightarrow$ etc.

m/z 231 (100.0%)

$\xrightarrow{\ -SO_2\ }$ F—(C$_6$F$_4$)$^+$ m/z 167 (48.1%)

$\xrightarrow{\ -SO\ }$ F—(C$_6$F$_4$)—$\overset{+}{O}$ $\longleftrightarrow$ F—(C$_6$F$_4$)$^+$=O $\longleftrightarrow$ etc.

m/z 183 (20.7%)

$\downarrow$ -CO

m/z 155 C$_5$F$_5^+$ $\xrightarrow{\ -C_2F_2\ }$ C$_3$F$_3^+$
(2.3%)   m/z 93 (4.3%)

$\downarrow$ -F$_2$

C$_5$F$_3^+$
m/z 117 (16.1%)

$\xrightarrow{\ -C_6F_5\dot{S}O_2\ }$ CF$_3$—◯—$\overset{+}{O}$ $\longleftrightarrow$ CF$_3$—◯=O $\longleftrightarrow$ etc.

m/z 161 (9.9%)

$\downarrow$ -CO

CF$_3$—▱$^+$ $\longleftrightarrow$ etc.

m/z 133 (36.1%)

$\downarrow$ -HF

CF$_2$=▱$^+$

m/z 113 (16.6%)
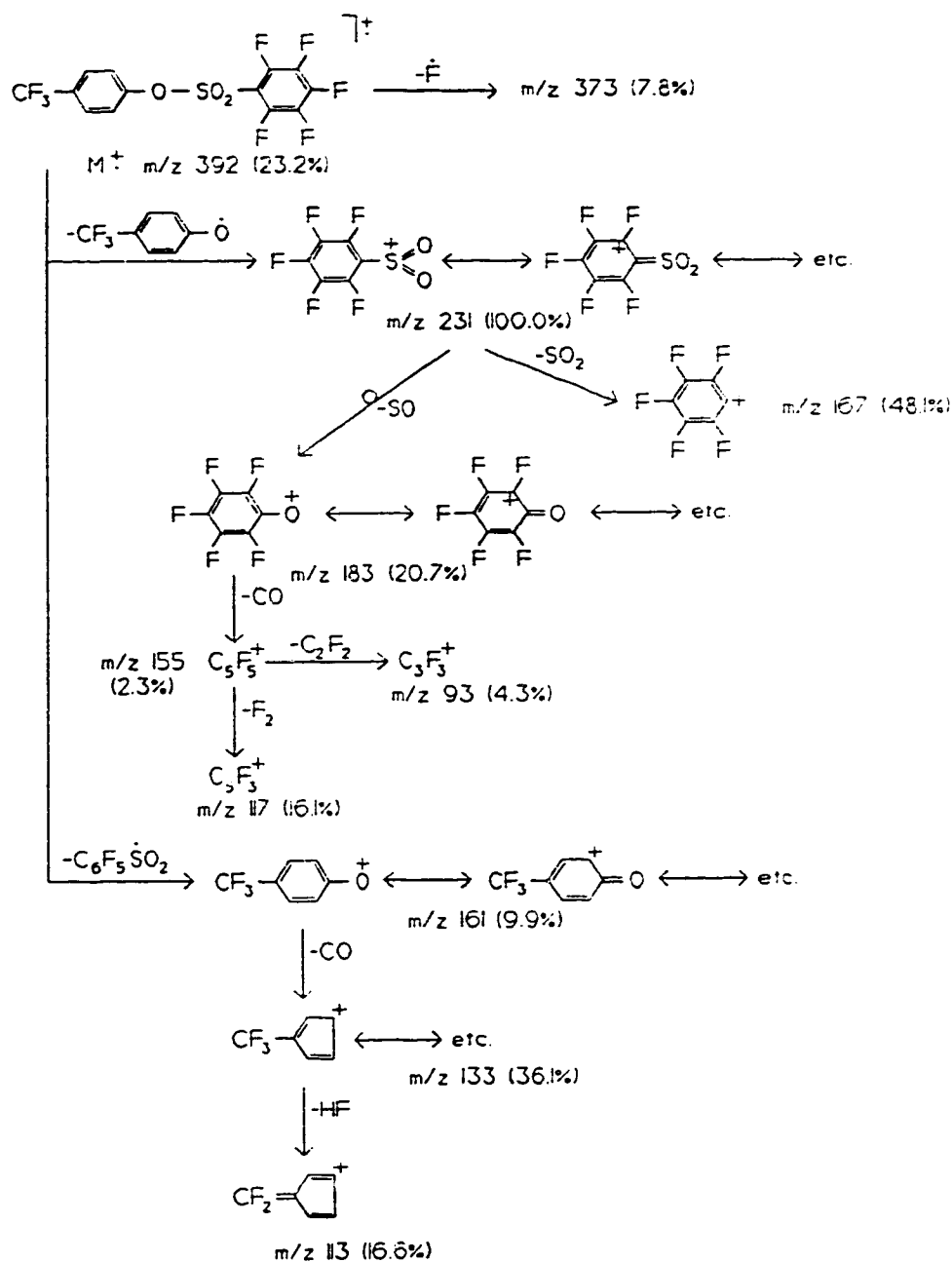
Figure 26: Proposed electron impact mass spectrometric fragmentation pattern for the pentafluorobenzenesulfonyl derivative of TFMP. The % relative abundance values are shown in parentheses.

116
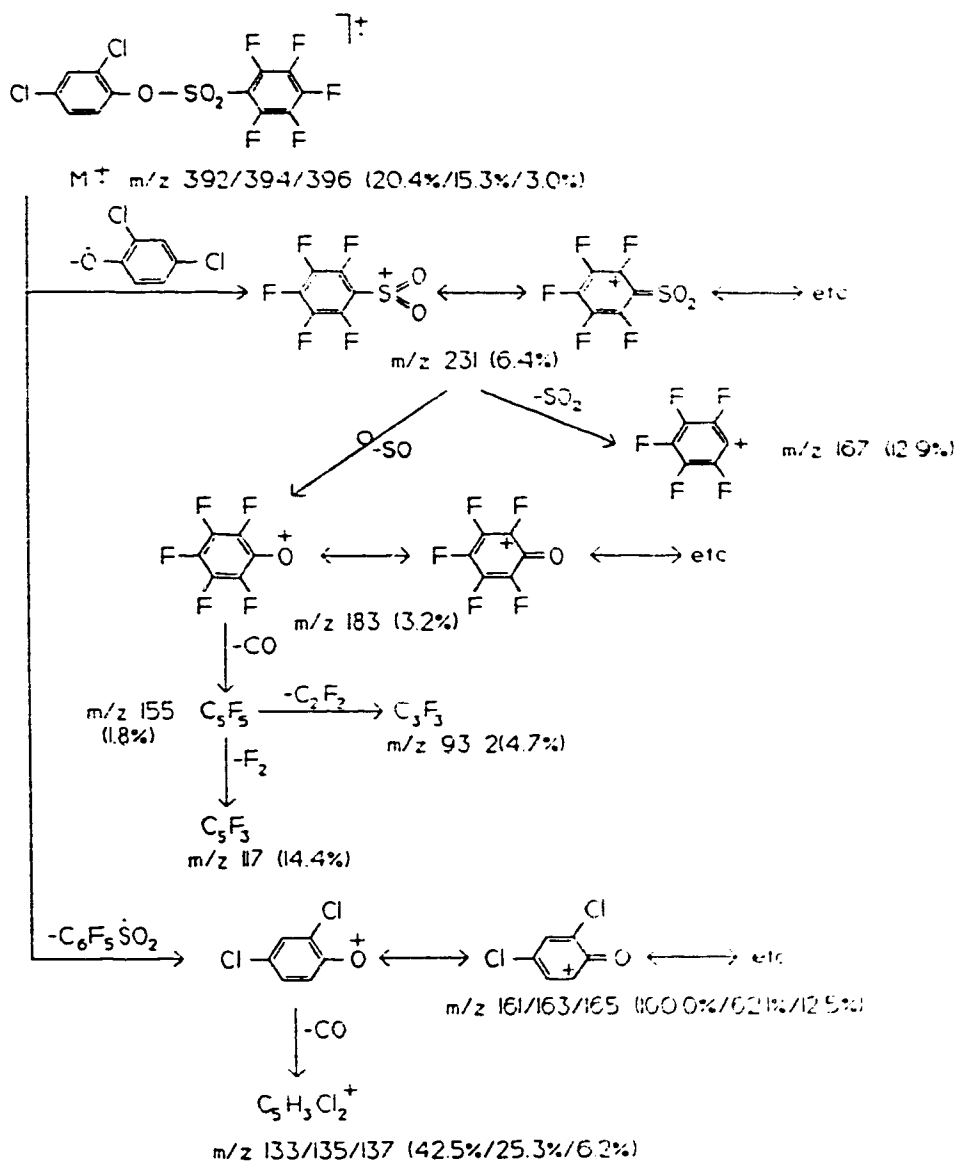
Figure 27: Proposed electron impact mass spectrometric fragmentation pattern for the pentafluorbenzenesulfonyl derivative of 2,4-dichlorophenol. The % relative abundance values are shown in parentheses.

117

Table 6:   Urine concentrations of TFMP in patients taking 20 mg FLU
             per day for a period of six weeks.

patient	TFMP $\mu g/24h$
C	34.7
D	765.9
E	40.2
F	240.9
G	31.6
H	68.7
MEAN	197.0
SEM	118.4
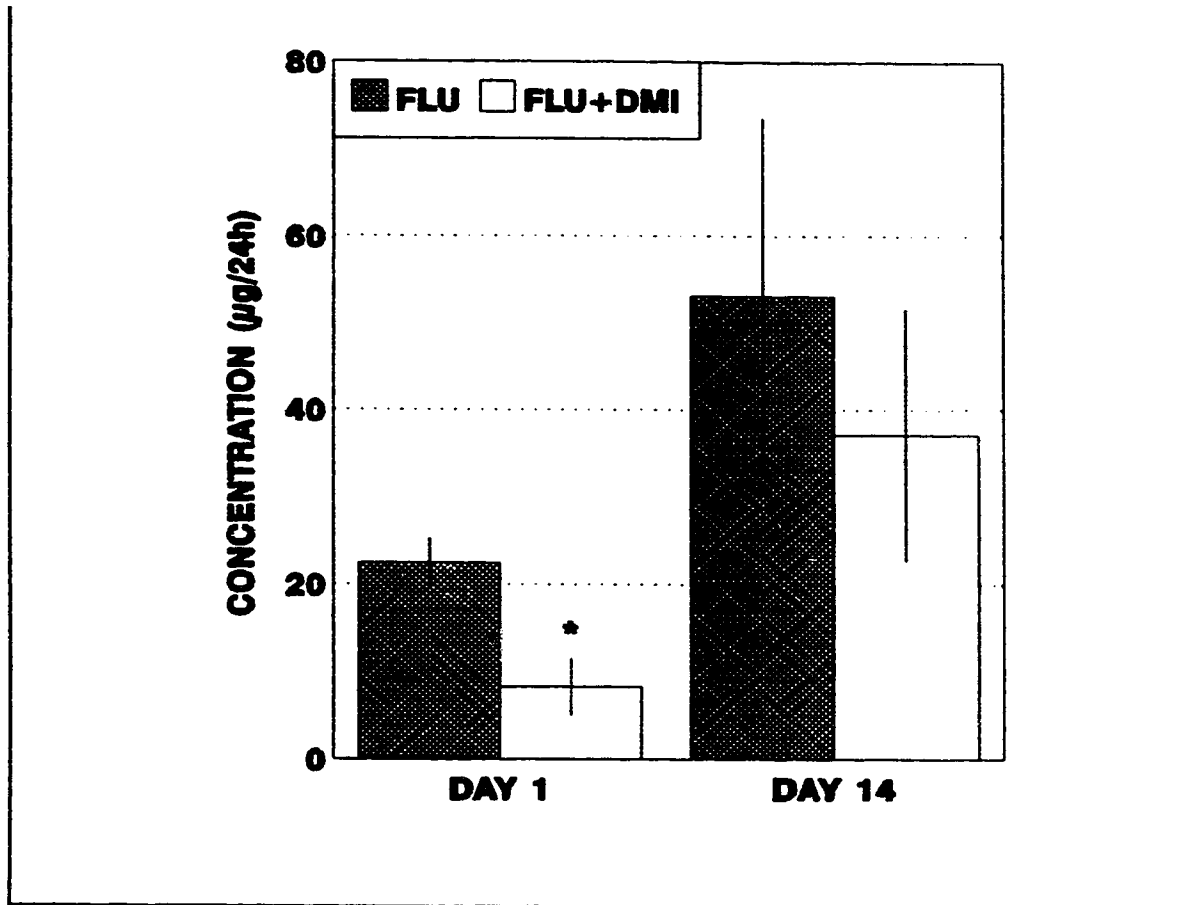
Figure 28: Levels of TFMP found in 24 h rat urine after 1 or 14 days of treatment with either rac-FLU alone or in combination with DMI. Values represent mean ± SEM (n=6). * Significant difference (p<0.05) from values in rats treated with rac-FLU alone.

119

5.0 DISCUSSION

5.1 ANALYSIS OF TCP ENANTIOMERS

The gas chromatographic procedure developed for the simultaneous analysis of the enantiomers of TCP is both rapid and relatively simple. The method is readily applicable to tissues from rats and should prove to be useful in future studies on the pharmacodynamics and pharmacokinetics of (+)- and (-)-TCP.

The doses of TCP administered to rats reported in the literature vary widely (approximately 0.5 to 25 mg/kg). For the present investigation, a dose of 2.5 mg/kg was chosen since this is similar, on a mg/kg basis, to clinical doses which have been reported recently to be useful in treating refractory depressives (Amsterdam and Berwish, 1989).

The results from this study obtained by measuring the concentrations of each enantiomer after administration of rac-TCP suggest that there are differences in the pharmacokinetics of (+)-TCP and (-)-TCP in rat brain and liver, but future longer term studies will be necessary to clarify this situation. The increase in the level of (+)-TCP over that of (-)-TCP observed after 3 h is in agreement with findings of Hampson et al. (1986) who examined rat brain levels of each enantiomer after intraperitoneal

administration of the individual enantiomers and found, in a short term study, that the (-)-enantiomer is cleared faster than the (+)-enantiomer from rat brains. Fuentes and his colleagues (1976) reported that rat brain levels of (-)-TCP are higher than those of (+)-TCP 15 min after separate administration of the enantiomers but that by 60 min, concentrations of the enantiomers are similar. Reynolds *et al.* (1980), in a study in which human subjects were administered (+)- and (-)-TCP on separate occasions found that plasma levels of (-)-TCP are higher than those of (+)-TCP after a single dose (3 h) whereas the reverse effect is apparent after drug administration for 3 days. Mutschler *et al.* (1990) reported that in humans (-)-TCP plasma levels are higher than those of (+)-TCP for up to 8 h after drug administration, whether the enantiomers are given separately or the TCP is given as the racemate; interestingly, administration of racemic TCP resulted in higher plasma levels of both enantiomers when compared to levels observed after administering the individual enantiomers. This higher plasma level of both enantiomers when the racemate is administered instead of the individual enantiomers was also reported by Weber-Grandke *et al.* (1993). This latter group also reported that the plasma and urine concentrations of (-)-TCP exceeded those of (+)-TCP.

It appears that IPR, a drug known to block ring hydroxylation, has

very little enantioselectivity in its effects on the enantiomers of TCP

although longer time studies on coadministration of IPR and TCP are

warranted. In the brain in the present study, IPR caused a very small

increase in the (+)-TCP/(-)-TCP ratio, but this effect was not evident in the

liver at the time intervals studied.

An elevation of TCP levels was observed with IPR pretreatment at

both time intervals studied. This is in agreement with a previous study

conducted by Baker *et al.* (1986) in which an increase in whole brain levels

of TCP (enantiomers were not separated) after pretreatment with IPR was

reported, but the present report appears to be the first time that the effects

of IPR on individual TCP enantiomers have been investigated. It is of

interest the IPR seems to have a similar effect on both enantiomers of

TCP. Although it has been assumed here that the elevation of TCP was

the result of the known effects of IPR on aromatic ring hydroxylation (Fuller

and Hemrick-Luecke, 1980; Freeman and Sulser, 1972; Steranka, 1982), a

possible direct effect of IPR on the clearance of the TCP enantiomers or an

effect on another metabolic pathway of TCP cannot be ruled out at this

time.

There have been reports in the past regarding problems encountered

with the chiral derivatizing reagent, S-(-)-N-(trifluoroacetyl)prolyl chloride,

due to optical impurity (presence of small amounts of the R-enantiomer)

and racemization of the reagent during storage (Hermansson and von Bahr, 1980; Silber and Riegelman, 1980). In the experiments conducted here, such problems were not encountered. The reagent used by the above mentioned researchers was obtained from a different supplier than that employed for the experiments presented in this thesis. In my studies, the reagent was stored in a desiccator at 0-4°C and the derivatizing solution was prepared fresh immediately prior to the derivatization step. In experiments designed to ensure stability and enantiomeric purity of the reagent, the reagent was used to derivatize the individual pure enantiomers of the drugs of interest. In such experiments, an additional small peak would be observed next to the peak cf interest if the R-enantiomer was present in small amounts in the reagent; no such additional peak was noted.

5.2   ANALYSIS OF THE ENANTIOMERS OF FLU AND NFLU
5.2.1   IPR/FLU EXPERIMENT

The gas chromatographic procedure developed for the simultaneous analysis of the enantiomers of FLU and NFLU is readily applicable to biological fluids and tissues, and it should prove to be useful for future

studies into differences between the enantiomers of FLU and NFLU.

The regional study in the rat brain revealed a relative consistency in concentration of FLU in the cerebellum, cortex, pons medulla and striatum. Such a finding is in agreement with previous reports on FLU (Caccia *et al.*, 1990).

Pretreatment of the animals with IPR significantly increased concentrations of both R- and S-FLU in rat whole brain and in brain regions. It appears that IPR shows some enantioselectivity in this regard. It has a greater effect on R-FLU which, in control animals treated with *rac*-FLU, is eliminated faster than S-FLU (Fuller and Snoddy, 1991; Torok-Both *et al.*, 1992). The combination of IPR and *rac*-FLU did not cause a significant increase in the levels of either enantiomer of NFLU compared to the situation when *rac*-FLU is administered in the absence of IPR.

The increase in FLU levels caused by pretreatment with IPR suggests that FLU may undergo hydroxylation. This observation is important since, as already mentioned, 70% of the metabolism of FLU is still unaccounted for (Lemberger *et al.*, 1985), and it has been suggested that an as yet unidentified metabolite of FLU may account for the excitatory effects of FLU observed in some patients (Lemberger *et al.*, 1985). If hydroxylation does occur, it would presumably be the result of the actions

of the enzyme CYP2D6 (Coutts and Baker, 1989), and FLU and NFLU

have both been reported to inhibit this isozyme, thus affecting the

metabolism of other drugs involving CYP2D6 (Brosen and Skjelbo, 1991;

Westermeyer, 1991; Bergstrom *et al.*, 1992; Crewe *et al.*, 1992; Greenblatt

et al., 1992; Vandel *et al.*, 1992; Otton *et al.*, 1993; Stevens and Wrighton,

1993). If FLU and NFLU are substrates for CYP2D6, IPR's effect may be

due to an inhibition of this metabolic pathway.

As indicated later in this thesis, O-dealkylation of FLU (to TFMP)

also occurs. Such a metabolic route may well be *via* the actions of a CYP

isozyme, but probably not CYP2D6. Based on studies done on CYP2D6,

the distance between the drug site of metabolic oxidation and the

quaternary nitrogen (at physiological pH) is always between 0.5 and 0.7 nm

(Guengerich *et al.*, 1986). In the case of FLU and NFLU, this distance

does not fall within this range. Whatever the nature of the metabolic route

resulting in TFMP formation, it is possible that IPR is an inhibitor, resulting

in increased brain levels of FLU.

It is of interest that short-term administration of the CYP2D6 inhibitor,

IPR, increases brain levels of FLU and not of NFLU while long-term

administration of DMI, another inhibitor of CYP2D6, results in an elevation

of brain levels of both FLU and NFLU. Such an observation suggests that

IPR may have other effects, such as inhibition of N-demethylation, which would prevent or reduce the formation of NFLU from the increased FLU available in brain after pretreatment with IPR. In addition, in a very recent study, Paslowski and Baker (personal communication) found that short-term treatment with DMI and FLU, at the time interval reported here for IPR, resulted in an increase only in FLU, but not NFLU levels. Such an observation indicates that future long-term experiments with IPR are warranted to observe its effects on levels of both FLU and NFLU.

5.2.2 DMI/FLU EXPERIMENT

The drug combination of DMI and *rac*-FLU caused a significant increase in the levels of the enantiomers of both FLU and NFLU in the rat brain at both time intervals studied (days 1 and 14). The increase caused when DMI was administered in combination with *rac*-FLU does not appear to show any enantioselectivity in its effect on FLU levels in rat brain at either time period studied. This result is in contrast to the findings with short-term IPR pretreatment, where there was a greater increase in brain levels of R-FLU than of S-FLU. The increase in the levels of R- and S-

NFLU observed with the addition of DMI to *rac*-FLU also did not appear to be an enantioselective effect.

Although considerable work has been done on the effects of this drug combination on DMI levels, it appears that this is only the second time that the effect of the drug combination on FLU and NFLU has been investigated; in the first study (Goodnough and Baker, 1994), effects on the individual enantiomers of FLU and NFLU were not investigated. Previous reports have indicated an increase in DMI plasma levels when DMI was administered in combination with FLU to patients (Aranow *et al.*, 1989; Bell and Cole, 1988; Bergstrom *et al.*, 1992; Goodnick, 1989; Nelson *et al.*, 1991; Preskorn *et al.*, 1990; Vaughan, 1988; Westermeyer, 1991). Fuller and Perry (1989) found that FLU could inhibit N-demethylation and ring hydroxylation of DMI by rat liver microsomes *in vitro* and can cause an increase in blood and tissue concentrations of DMI in rats *in vivo*. Fuller and Snoddy (1991) found that NFLU also contributed to the inhibition of DMI metabolism but to a lesser extent than FLU. This increase in DMI levels may be due to the inhibitory influence that FLU and NFLU have on the cytochrome P450 isozymes. More specifically, FLU and NFLU have been shown to inhibit CYP2D6 (Brosen and Skjelbo, 1991; Crewe *et al.*, 1992; Otton *et al.*, 1993). The increase in levels of FLU and NFLU in the

present study may be due, at least in part, to an inhibitory influence of DMI on ring hydroxylation of FLU and/or NFLU, but to date such a metabolite of FLU or NFLU has not been identified. As mentioned elsewhere in this thesis, TFMP is a metabolite of FLU (and presumably also of NFLU); this metabolite may arise as the result of O-dealkylation. Although this metabolic route is mediated by CYP2D6 in the case of drugs such as dextromethorphan (Schmidt et al., 1985), it may be mediated by another unknown isozyme in the case of FLU and NFLU due to their structures (see section 5.2.1). The increase in brain levels of FLU and NFLU observed with DMI pretreatment could be the result of decreased O-dealkylation (and of subsequent TFMP formation), but this situation has not yet been investigated in rat brain. Goodnough and Baker (1994) recently reported that coadministration of DMI and rac-FLU by osmotic minipumps also resulted in markedly increased brain levels of FLU and NFLU in the rat compared to the situation when rac-FLU was administered alone; these workers did not analyze the individual enantiomers of FLU or NFLU.

5.3 SEARCH FOR METABOLITES OF FLU

5.3.1 ANALYSIS OF FLU AND NFLU USING EXTRACTIVE PENTAFLUOROBENZOYLATION

As mentioned in the Rationale section of this thesis, pentafluoro-benzoylation can be conducted readily under aqueous conditions and has proven very useful for analysis of phenol- and amine-containing compounds. Pentafluorobenzoylation was thus utilized for the purpose of the present project as a screen for potential metabolites of FLU or NFLU containing phenol or amine groups. This assay would not, however, identify metabolites such as those containing carboxylic acid groups. Although the procedure did not reveal any metabolites, it would be useful as a rapid assay for simultaneous analysis of FLU and NFLU for laboratories who were not interested in, or could not afford the extra time and expense of, analyzing the separate enantiomers of FLU and NFLU.

The levels of FLU and NFLU found, using this assay procedure, in the urine and plasma samples of patients receiving FLU for 3 weeks showed marked interindividual differences, as has been previously reported in other studies (Nash *et al.*, 1982; Aronoff *et al.*, 1984; Tyrer *et al.*, 1990; Wong *et al.*, 1990; Torok-Both *et al.*, 1992). The levels reported

similar doses as assayed using different procedures (Lemberger *et al.*, 1978; Torok-Both *et al.*, 1992).

Some research suggests that there is no association between concentration of FLU in plasma and clinical efficacy (Beasley *et al.*, 1990b; Fava *et al.*, 1992; Kelly *et al.*, 1989). However, other work (in which individual enantiomers were not measured) has shown that the level of NFLU found in plasma has a negative relationship with antidepressant response (Montgomery *et al.*, 1990). Work by Tyrer *et al.*, (1990) suggests that differences in the metabolism of FLU to NFLU may account for differences found in the response of the patients to FLU. Keck and McElroy (1992) indicate that the unexpected side effects of sedation seen in some patients taking FLU may be due to a relative excess in NFLU. For these reasons, it is of value to monitor a patient's level of the parent drug and metabolite.

The popularity of FLU has led to the development of a number of assays that allow for the quantitation of FLU and NFLU (Nash *et al.*, 1982; Wong *et al.*, 1990; Dixit *et al.*, 1991; Suckow *et al.*, 1992; Thomare *et al.*, 1992; Elmaanni *et al.*, 1993; Elyazigi and Raines, 1993; Lantz *et al.*, 1993). Most of these procedures are based on HPLC analysis. An advantage of

130

as part of a combined GC-MS system, providing ready confirmation of structures of derivatives and permitting GC-MS analysis if required. HPLC-MS analysis is also possible, but such methodology is still in its infancy and often not readily available. The three gas chromatographic methods for FLU and NFLU mentioned above (Nash *et al.*, 1982; Dixit *et al.*, 1991; Lantz *et al.*, 1993) all involve quite lengthy and involved extraction procedures. The method described here is a very rapid and simple procedure allowing for the simultaneous analysis of FLU and NFLU in human body fluids by employing GC.

5.3.2 ANALYSIS OF TFMP USING PFBSC UNDER AQUEOUS CONDITIONS

Extractive derivatization with PFBSC under aqueous conditions permitted the identification and quantification of TFMP as a metabolite of FLU in human and rat urine. Although this proposed metabolite of FLU was briefly mentioned previously in a report from 1986 (Benfield *et al.*, 1986), no quantitative measurement has been reported to our knowledge (personal communication with Dr. R. Bergstrom, Lilly Research

compound (Table 6) and levels of FLU and NFLU(Table 5) in human urine were converted to μmol/24 h (Table 7), the mean TFMP level excreted in the urine is actually 79.5% and 56.3% of the level of FLU and NFLU, respectively,excreted in the urine. Thus it appears that TFMP may be an important metabolite of FLU. This is of great importance since to date only 30% of the administered dose of FLU has been accounted for metabolically (Lemberger et al., 1978). The development of this assay thus permits further investigation to be done on the metabolites of FLU. An examination of the structure of NFLU also indicates that TFMP is also a potential metabolite of NFLU.

It is also of interest to note that in rats that had been administered a combination of DMI and rac-FLU, the urinary levels of TFMP achieved at day 1 with the drug combination were significantly decreased over those achieved when rac-FLU was administered alone (Figure 26). TFMP is presumably formed by O-dealkylation, a process which in this case is probably mediated by an as yet unknown CYP (see section 5.2.1). Many psychotropic drugs, including DMI, that are given in combination with FLU can affect activity of CYP isozymes (Coutts and Baker, 1989), so the present finding may be of interest for future studies on drug-drug

Table 7: Urine concentrations of FLU, NFLU and TFMP in patients taking 20 mg FLU per day for a period of six weeks.

	FLU μmol/24h	NFLU μmol/24h	TFMP μmol/24h
MEAN	1.529	2.158	1.215

133

interactions with FLU in human plasma samples and in rat brain.

Through the use of molecular biological techniques (Crespi, 1991), pure preparations of human CYP2D6 are now available (Gentest Corporation) and have been used extensively by other members of the Neurochemical Research Unit to investigate metabolism of TCAs (Coutts *et al.*, 1993; Su *et al.*, 1993; Bolaji *et al.*, 1993). It now appears that studies with such preparations on TFMP formation (in the presence and absence of IPR and DMI) may represent an exciting avenue for future research on metabolism of FLU.

The results presented here have indicated that TFMP may be a very important metabolite of FLU and NFLU. This is of great interest because of the 70% of the administered dose of FLU that is still unaccounted for. It is now important to carry on studies on this metabolite to characterize it with regard to pharmacological activity (e.g. effects on 5-HT uptake in synaptosomes) and potential antidepressant utility and side effect profile (e.g. by testing in animal models of depression).

6.0 CONCLUSIONS

1. A novel GC method was developed for the separation and quantitation of the enantiomers of the antidepressant TCP. The procedure is rapid and simple and is readily applicable to biological tissues and fluids.

2. IPR, a drug known to block ring hydroxylation, causes an increase in levels of both enantiomers of TCP in rat brain and liver when it is administered one hour before rac-TCP. This may be due to the inhibition of aromatic ring hydroxylation of TCP but other direct effects that IPR may have on the clearance of TCP cannot be ruled out. In the brain, the elevation of (-)-TCP caused by IPR is only slightly greater than that for (+)-TCP. This small difference was not observed in the liver.

3. A novel GC method was developed for the simultaneous separation and quantitation of the enantiomers of FLU and its metabolite, NFLU. This procedure is readily applicable to biological tissues and fluids.

4. IPR pretreatment caused an increase in the levels of both enantiomers of FLU but had no significant effect on the levels of the enantiomers of NFLU in rat brain when measured 5 h after

administration of *rac*-FLU to the rats. IPR's effect on FLU enantiomers exhibited enantioselectivity, with a greater increase being observed with R-FLU.

5. The drug combination of DMI and FLU, one which is being used increasingly clinically, lead to an increase in the levels of both enantiomers of FLU and NFLU in rat brain over those obtained when FLU is administered alone. Enantioselectivity was not observed. These observations are important because previous studies in the literature on the drug combination of DMI and FLU reported elevated levels of DMI, but neglected to look at the effects the combination has on the levels of the enantiomers of FLU and NFLU.

6. It has been shown that both DMI and IPR have an effect on the level of FLU achieved when they are administered in combination with FLU. This is of importance because drugs such as DMI and IPR may provide indirect information about possible metabolic pathways for FLU. Although both DMI and IPR are known inhibitors of CYP2D6 and ring hydroxylation, only DMI caused an elevation of NFLU levels. This observation implies that IPR may have additional effects (e.g. on N-demethylation of FLU), and this aspect is worthy of further investigation. As indicated earlier in this thesis, such studies utilizing chronic administration of IPR are also warranted.

7.	In an attempt to screen for possible additional metabolites of FLU and NFLU, a novel GC procedure for simultaneous analysis of FLU and NFLU was developed utilizing aqueous pentafluorobenzoylation. This method is rapid and simple and provides good separation of FLU and NFLU, although it does not separate the individual enantiomers. Additional peaks other than those corresponding to derivatized FLU and NFLU were not identified in plasma or urine samples from humans taking rac-FLU or urine samples from rats treated with rac-FLU.

8.	A screening procedure using derivatization with pentafluorobenzene-sulfonyl chloride resulted in the identification and quantification of TFMP, which had previously been proposed as a metabolite of FLU but had never been quantitated, in urine from both humans and rats treated with rac-FLU. The procedure did not result in the identification of any other potential metabolite of FLU or NFLU.

7.0 REFERENCES

Abramowicz M. (ed.) (1990) Fluoxetine (Prozac) revisited. *The Medical Letter on Drugs and Therapeutics.* The Medical Letter, Inc., New Rochelle, NY 32: 83-85.

Albert A. (1958) Chemical aspects of selective toxicity. *Nature* 182: 421-423.

Alexanderson B. (1972) Pharmacokinetics of desmethylimipramine and nortriptyline in man after single and multiple oral doses - a cross-over study. *Eur. J. Clin. Pharmacol.* 6: 44-53.

Allenmark S. (1984) Recent advances in methods of direct optical resolution. *J. Biochem. Biophys. Methods* 9: 1-25.

Allenmark S.G. ed. (1988) *Chromatographic Enantioseparation: Methods and Applications.* Ellis Horwood, Chichester.

Allinger N.L., Cava M.P., De Jongh D.C., Johnson C.R., Lebel N.A. and Stevens C.L. (1976) Organic Chemistry. Worth Publishers, New York, N.Y., pp. 98-101.

Amsterdam J.D. and Berwish N.J. (1989) High dose tranylcypromine therapy for refractory depression. *Pharmacopsychiatry* 22: 21-25.

Ananth J. and Luchins D. (1977) A review of combined tricyclic and MAOI therapy. *Compr. Psychiatry* 18: 221-230.

Aranow R.B., Hudson J.I., Pope H.G., Grady T.A., Laage T.A., Bell I.R. and Cole J.O. (1989) Elevated antidepressant plasma levels after addition of fluoxetine. *Am. J. Psychiatr.* 147: 911-913.

Ariens E.J. (1983) Stereoselective bioactive agents. In: *Stereochemistry and Biological Activity of Drugs.* Ariens, E.J., Soudijn W. and Timmermans P.B.M.W.M. eds. Blackwell, Oxford. pp. 11-32.

138

Ariens E.J. (1984) Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur. J. Clin. Pharmacol.* 26: 663-668.

Ariens E.J. (1989) Racemates-an impediment in the use of drugs and agrochemicals. In: *Chiral Separations by HPLC: Applications to Pharmaceutical Compounds.* Krstulovic A.M. ed. Ellis Horwood Ltd., Chichester, West Sussex, pp. 31-68.

Armstrong D.W. and Han S.M. (1988) Enantiomeric separations in chromatography. *CRC Crit. Rev. Anal. Chem.* 19: 175-224.

Aronoff G.R., Bergstrom R.F., Pottratz S.T., Sloan R.S., Wolen R.L. and Lemberger L. (1984) Fluoxetine kinetics and protein binding in normal and impaired renal function. *Clin. Pharmacol. Ther.* 36: 138-144.

Aspeslet L.J., Baker G.B., Coutts R.T. and Mousseau D.D. (1992) A gas chromatographic procedure for separation and quantitation of the enantiomers of the antidepressant tranylcypromine. *Biochem. Pharmacol.* 44: 1894-1897.

Bailey E. and Barron E.J. (1980) Determination of tranylcypromine in human plasma and urine using high-resolution gas-liquid chromatography with nitrogen-sensitive detection. *J. Chromatogr. Biomed. Appl.* 183: 25-31.

Baker G.B., Coutts R.T. and Holt A. (1994) Capsule review: the importance of metabolism and chirality in the actions of drugs used in psychiatry. *Biol. Psychiatry* (in press).

Baker G.B., Coutts R.T. and LeGatt D.F. (1982) Gas chromatographic analysis of amines in biological systems. In: *Analysis of Biogenic Amines.* Baker G.B. and Coutts R.T., eds. Elsevier Scientific Publishing Company, New York, NY pp 109-128.

Baker G.B. and Dewhurst W.G. (1985) Biochemical theories of affective disorders. In: *Pharmacotherapy of Affective Disorders.* Dewhurst W.G. and Baker G.B., eds. Croon Helm Ltd., Beckenham, UK, pp 1-59.

Baker G.B. and Greenshaw A.G. (1989) Effects of long-term administration of antidepressants and neuroleptics on receptors in the central nervous system. *Cell Mol. Neurobiol.* 9: 1-44.

Baker G.B., Hampson D.R., Coutts R.T., Micetich R.G., Hall T.W. and Rao T.S. (1986) Detection and quantitation of a ring-hydroxylated metabolite of the antidepressant drug tranylcypromine. *J. Neural Transm.* 65: 233-243.

Baker G.B., Wong J.T.F., Coutts R.T. and Pasutto F.M. (1987a) Simultaneous extraction and quantitation of several bioactive amines in cheese and chocolate. *J. Chromatogr.* 392: 317-331.

Baker G.B., Coutts R.T. and Rao T.S. (1987b) Neuropharmacological and neurochemical properties of N-(cyanoethyl)-β-phenylethylamine, a prodrug of β-phenylethylamine. *Br. J. Pharmacol.* 92: 243-256.

Baker G.B., Hiob L.A. and Dewhurst W.G. (1980) Effects of monoamine oxidase inhibitors on release of dopamine and 5-hydroxytryptamine from rat striatum in vitro. *Cell. Mol. Biol.* 26: 182-186.

Baldessarini R.J. (1985) *Chemotherapy in Psychiatry: Principles and Practice.* Harvard University Press, Cambridge, MA.

Baldessarini R.J. (1990) Chapter 18: Drugs and the treatment of psychiatric disorders. *The Pharmacological Basis of Therapeutics.* Gilman A.G., Rall T.W., Nies A.S. and Taylor P. eds. Pergamon Press, Elmsford, NY, pp. 383-435.

Barchas J.D., Akil H., Elliott G.R., Holman R.B. and Watson S.J. (1978) Behavioural neurochemistry: Neuroregulators and behavioral states. *Science* 200: 964-973.

Baron B.M., Ogden A.M., Siegel B.W., Stegeman J., Ursillo R.C. and Dudley M.W. (1988) Rapid down regulation of β-adrenoceptors by co-administration of desipramine and fluoxetine. *Eur J Pharmacol* 154: 125-134.

Batki S.L., Manfredi L.B., Jacob P. and Jones R.T. (1993) Fluoxetine for cocaine dependence in methadone maintenance - quantitative

plasma and urine cocaine/benzoylecgonine concentrations. *J. Clin. Psychopharmacol.* 13: 243-250.

Beasley C.M., Bosomworth J.C. and Wernicke J.F. (1990b) Fluoxetine: Relationships among dose, response, adverse events, and plasma concentrations in the treatment of depression. *Psychopharmacol. Bull.* 26: 18-24.

Beasley C.M., Sayler M.E., Cunningham G.E., Weiss A.M. and Masica D.N. (1990a) Fluoxetine in tricyclic refractory major depressive disorder. *J. Affec. Disord.* 20: 193-200.

Bell I. and Cole J. (1988) Fluoxetine induces elevation of desipramine level and exacerbation of geriatric nonpsychotic depression. *J. Clin. Psychopharmacol.* 8: 447-448.

Benfield P., Heel R.C. and Lewis S.P. (1986) Fluoxetine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drug Evaluation* 32: 481-508.

Bergstrom R.F., Peyton A. and Lemberger L. (1992) Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clin. Pharmacol. Ther.* 51: 239-248.

Bernstein J., Lott W.A., Steinberg B.A. and Yale H.L. (1952) Chemotherapy of experimental tuberculosis. B. Isonicotinic acid hydrazide (Nydrazid) and related compounds. *Ann. Rev. Tuberculosis* 65: 357-364.

Bieck P.R., Antonin K.-H. and Schmidt E. (1993) Clinical pharmacology of reversible monoamine oxidase-A inhibitors. *Clin. Neuropharmacol.* 16: S34-S41.

Bolaji O., Coutts R.T. and Baker G.B. (1993) Metabolism of trimipramine *in vitro* by human CYP2D6 isozyme. *Res. Commun. Chem. Path. Pharmacol.* 82: 111-120.

Boulton A.A. (1976) Cerebral aryl alkyl aminergic mechanisms. In: *Trace Amines and the Brain.* Usdin E. and Sandler M. eds. Marcel Dekker, New York, NY, pp. 22-39.

Boulton A.A. and Juorio A.V. (1982) Brain trace amines. In: *Handbook of Neurochemistry, Vol. 1*. Lajtha A. ed. Plenum Publishing Corp., New York, NY. pp. 189-222.

Boyd F.H. and Weissman M.M. (1982) Epidemiology. In: *Handbook of Affective Disorders*. Paykel E.S. ed. The Guilford Press, New York, NY, pp. 109-125.

Brady K., Zarzar M. and Lydiard R.B. (1989) Fluoxetine in panic disorder patients with imipramine-associated weight gain. *J. Clin. Psychopharmacol.* 9: 66-67.

Brodie B.B., Pletscher A. and Shore P.A. (1956) Possible role of serotonin in brain function and reserpine action. *J. Pharmacol. Exp. Ther.* 116: 9.

Brodie B.B., Pletscher A. and Shore P.A. (1955) Evidence that serotonin has a role in brain function. *Science* 122: 968.

Brosen K. and Gram L.F. (1989) Clinical significance of the sparteine/ debrisoquine oxidation polymorphism. *Eur. J. Clin. Pharmacol.* 36: 537-547.

Brosen K. and Skjelbo E. (1991) Fluoxetine and norfluoxetine are potent inhibitors of P450IID6 - The source of the sparteine/debrisoquine oxidation polymorphism. *Br. J. Clin. Pharmacol.* 32: 136-137.

Brown A.S. and Gershon S. (1993) Dopamine and depression. *J. Neural Transm. [Gen. Sect.]* 91: 75-109.

Burger A. and Yost W.L. (1948) Arylcycloalkylamines. I. 2-phenylcyclopropylamine. *J. Am. Chem. Soc.* 70: 2198-2201.

Byerley W.F., Reimherr F.W., Wood D.R. and Grosser B.I. (1988) Fluoxetine, a selective serotonin uptake inhibitor, for the treatment of outpatients with major depression. *J. Clin. Psychopharmacol.* 8: 112-115.

Caccia S., Cappi M., Fracasso C. and Garattini S. (1990) Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. *Psychopharmacol.* 100: 509-514.

Cahn R.S., Ingold C.K. and Prelog V. (1956) The specification of asymmetric configuration in organic chemistry. *Experientia* 12: 81-124.

Calverley D.G., Baker G.B., Coutts R.T. and Dewhurst W.G. (1981) A method for measurement of tranylcypromine in rat brain regions using gas chromatography with electron capture detection. *Biochem. Pharmacol.* 30: 861-867.

Carlsson A., Corrodi H., Fuxe K., and Hokfelt T. (1969) Effects of some antidepressant drugs in the depletion of intraneuronal brain catecholamine stores caused by 4,α—dimethyl-meta-tyramine. *Eur. J. Pharmacol.* 5: 367-373.

Carlsson A., Lindquist M. and Magnusson T. (1957) 3,4-Dihydroxyphenyl-alanine and 5-hydroxytryptophan as reserpine antagonists. *Nature (Lond.)* 180: 1200.

Carter J.E. (1988) The development of stereochemically pure drug substances. In: *Drug Stereochemistry, Anayltical Methods and Pharmacology.* Wainer I.W. and Drayer D.E. eds. Marcel Dekker, New York, N.Y., pp. 357-363.

Cassidy S. and Henry J. (1987) Fatal toxicity of antidepressant drugs in overdose. *Br. Med. J. [Clin. Res.]* 295: 1021-1024.

Charney D.S. and Heninger G.R. (1986) Receptor sensitivity hypothesis of antidepressant action. *Proc. Ann. Meet. Am. Coll. Neuropsycho-pharmacol.* 1986: 49.

Cheetham S.C., Crompton M.R., Katona C.L.E. and Horton R.W. (1988) Brain $GABA_A$/benzodiazepine binding sites and glutamic acid decarboxylase activity in depressed suicide victims. *Brain Res.* 460: 114-123.

143

Choonara, I.A., Cholerton S., Haynes B.P., Breckenridge A.M. and Park B.K. (1986) Stereoselective interaction between the R enantiomer of warfarin and cimetidine. *Br. J. Clin. Pharmacol.* 21: 271-277.

Cook C.E. (1993) Enantiomer analysis by competitive binding methods. In: *Drug Stereochemistry: Analytical Methods and Pharmacology.* Wainer I.W. ed., Marcel Dekker, Inc., New York, NY, pp. 35-64.

Coon M.J., Ding X., Pernecky S.J. and Vaz A.D.N. (1992) Cytochrome P450: progress and predictions. *The FASEB J.* 6: 669-673.

Coppen A., Gupta R. and Montgomery S. (1976) Mianserin hydrochloride: A novel antidepressant. *Br. J. Psychiat.* 129: 342-345.

Cornelius J.R., Soloff P.H., Perel J.M. and Ulrich R.F. (1990) Fluoxetine trial in borderline personality disorder. *Psychopharmacol. Bull.* 26: 151-154.

Coutts R.T. (1994) Polymorphism in the metabolism of drugs, including antidepressant drugs: Comments on phenotyping. *J. Psychiatry Neurosci.* 19: 30-44.

Coutts R.T. and Baker G.B. (1982) Gas Chromatography. In: *Handbook of Neurochemistry, 2nd ed., Volume 2: Experimental Neurochemistry.* Lajtha A., ed. Plenum Press, New York, NY pp 429-448.

Coutts R.T. and Baker G.B. (1989) Implications of chirality and geometric isomerism in some psychoactive drugs and their metabolites. *Chirality* 1: 99-120.

Coutts R.T., Baker G.B. and Nazarali A.J. (1985) Gas chromatography of amines and their metabolites in tissues and body fluids. In: *Neuromethods 2: Amines and Their Metabolites.* Boulton A.A., Baker G.B. and Baker J.M., eds. The Humana Press Inc., Clifton, NJ, pp 45-85.

144

Coutts R.T., Nazarali A.J., Baker G.B. and Pasutto F.M. (1986) Metabolism and disposition of N-(2-cyanoethyl)amphetamine (fenproporex) and amphetamine: study in the rat brain. *Can. J. Physiol. Pharmacol.* 64: 724-728.

Coutts R.T., Su P. and Baker G.B. (1994) Involvement of CYP2D6, CYP3A4 and other cytochrome P-450 isozymes in N-dealkylation reactions. *J. Pharmacol. Toxicol. Methods.* (in press).

Coutts R.T., Su P., Baker G.B. and Daneshtalab (1993) Metabolism of imipramine *in vitro* by CYP2D6 expressed in a human cell line, and observations on metabolite stability. *J. Chromatogr. Biomed. Appl.* 615: 265-272.

Crammer J.L. and Scott B. (1966) New metabolites of imipramine. *Psychopharmacol.* 8:461.

Crespi C.L. (1991) Expression of cytochrome P450 cDNAs in human B-lymphoblastoid cells: application to toxicology and metabolite analysis. In: *Cytochrome P450, Methods in Enzymology, 206.* Waterman M.R. and Johnson E.F. eds. Academic Press Inc., San Diego, CA, pp. 123-129.

Crewe H.K., Lennard M.S., Tucker G.T., Woods F.R. and Haddock R.E. (1992) The effect of selective serotonin re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. *Br. J. Clin. Pharmacol.* 34: 262-265.

Cross J.A. and Horton R.W. (1987) Are increases in $GABA_B$ receptors consistent findings following chronic antidepressant administration? *Eur. J. Pharmacol.* 141: 159-162.

Cross J.A. and Horton R.W. (1988) Effects of chronic oral administration of the antidepressants, desmethylimipramine and zimelidine on rat cortical $GABA_B$ binding sites: a comparison with $5-HT_2$ binding site changes. *Br. J. Pharmacol.* 93: 331-336.

145

Davies S.G. (1990) Chiral recognition in synthesis. In: *Chirality in Drug Design and Synthesis*. Brown C. ed. Academic Press, San Diego, pp. 181-198.

Davis J.M. (1970) Theories of biological etiology of affective disorders. *Int. Rev. Neurobiol.* 122: 509-522.

De Vane C.L., Savett M. and Jusko W.J. (1981) Desipramine and 2-hydroxydesipramine pharmacokinetics in normal volunteers. *Eur. J. Clin. Pharmacol.* 19: 61-64.

Dewhurst W.G. (1969) Amines and abnormal mood. *Proc. Roy. Soc. Med.* 62: 1102-1107.

Dewhurst W.G. (1968) New theory of cerebral amine function and its clinical application. *Nature (Lond.)* 218: 1130-1133.

Diffley D., Tran V.T. and Snyder S.H. (1980) Histamine H1-receptors labelled in vivo: antidepressant and antihistamine interactions. *Eur. J. Pharmacol.* 64: 177-181.

Dixit K., Nguyen H. and Dixit V.M. (1991) Solid-phase extraction of fluoxetine and norfluoxetine. *J. Chromatogr. Biomed. Appl.* 563: 379-384.

Downs J.M., Downs A.D., Rosenthal T.L., Deal N. and Akiskal H.S. (1989) Increased plasma tricyclic antidepressant concentrations in two patients concurrently treated with fluoxetine. *J. Clin. Psychiatry* 50: 226-227.

Drayer D.E. (1986) Protein binding of drugs, drug enanticmers, active drug metabolites and therapeutic drug monitoring. In: *Drug-Protein Binding*. Praeger Publishers, New York, N.Y., pp. 332-347.

Drayer D.E. (1988) Problems in therapeutic drug monitoring: the dilemma of enantiomeric drugs in man. *Ther. Drug Monit.* 10: 1-7.

In: *Textbook of Neuropsychiatry*. Hales R.E. and Yudofsky S.C. eds. American Psychiatric Press Inc., Washington, D.C., pp. 411-438.

Dunlop S.R., Dornseif B.E., Wernicke J.F. and Potvin J.H. (1990) Pattern analysis shows beneficial effect of fluoxetine treatment in mild depression. *Psychopharmacol. Bull.* 26: 173-180.

Edwards D.J., Mallinger A.G., Knopf S. and Himmelhoch J.M. (1985) Determination of tranylcypromine in plasma using gas chromatography-chemical-ionization mass spectrometry. J. Chromatogr. Biomed. Appl. 344: 356-361.

Eichelbaum M. and Gross A.S. (1990) The genetic polymorphism of debrisoquine/sparteine metabolism - clinical aspects. *Pharmacol. Ther.* 46: 377-394.

Eichelbaum M. (1988) Pharmacokinetic and pharmacodynamic consequences of stereoselective drug metabolism in man. *Biochem. Pharmacol.* 37: 93-96.

Elmaanni A., Combourieu I., Bonini M. and Creppy E.E. (1993) Fluoxetine, an antidepressant, and norfluoxetine, its metabolite, determined by HPLC with a C-8 column and ultraviolet detection. *Clin. Chem.* 39: 1749-1750.

Elyazigi A. and Raines D.A. (1993) Concurrent liquid chromatographic measurement of fluoxetine, amitriptyline, imipramine, and their active metabolites norfluoxetine, nortriptyline, and desipramine in plasma. *Ther. Drug. Monit.* 15: 305-309.

Escobar J.E., Schiele B.C. and Zimmerman R. (1974) The tranylcypromine isomers: A controlled clinical trial. *Am. J. Psychiat.* 131: 1025-1026.

Evans A.M., Nation R.L., Sansom L.N., Bochner F. and Somogyi A.A. (1988) Stereoselective drug disposition: Potential for misinterpretation of drug disposition data. *Br. J. Clin. Pharmacol.* 26: 771-780.

Fassihi A.R. (1993) Racemates and enantiomers in drug development. *Int. J. Pharmaceut.* 92: 1-14.

...., ..., ..., ..., ..., ...
and Clancy K. (1992) High-dose fluoxetine in the treatment of depressed patients not responsive to a standard dose of fluoxetine. *J. Affect. Disord.* 25: 229-234.

Feighner J.P. (1980) Pharmacology: New Antidepressants. *Psychiatric Annals* 10: 388-395.

Feighner J.P. and Cohn J.B. (1985) Double-blind comparative trial of fluoxetine and doxepin in geriatric patients with major depressive disorder. *J. Clin. Psychiat.* 46: 20-25.

Fibiger H.C., Phillips A.G. and Blaha C.D. (1990) Dopamine and the neural substrates of reward: implications for the mechanisms of action of antidepressant drugs. In: *Dopamine and Mental Depression*. Gessa G.L. and Serra G. eds. Pergamon Press, New York, NY, pps. 51-62.

Finberg J.P.M. and Youdim M.B.H. (1983) Selective MAO A and MAO B inhibitors: their mechanism of action and pharmacology. *Neuropharmacol.* 22: 441-446.

Fischer W. (1991) Zur Antikonvulsiven Wirksamkeit von (+)- und (-)-Tranylcypromin. *Pharmazie* 46: 357-358.

Fontaine R. and Chouinard G. (1986) An open clinical trial of fluoxetine in the treatment of obsessive-compulsive disorder. *J. Clin. Psychopharmacol.* 6: 98-101.

Fowler C.J. and Ross S.B. (1984) Selective inhibitors of monoamine oxidase A and B: biochemical, pharmacological and clinical properties. *Med. Res. Rev.* 4: 323-358.

Fox H.H. and Gibas J.T. (1953) Synthetic tuberculostats, VII. Monoalkyl derivates of isonicotinylhydrazine. *J. Org. Chem.* 18: 994-1002.

Freeman C.P. and Hampson M. (1987) Fluoxetine as a treatment for bulimia nervousa. *Int. J. Obes.* 11 (suppl.) 171-177.

the rat: The role of aromatic hydroxylation of amphetamine in its mode of action. *J. Pharmacol. Exp. Ther.* 183: 307-315.

Fuentes J.A., Oleshansky M.A. and Neff N.H. (1976) Comparison of the apparent antidepressant activity of (-) and (+) tranylcypromine in an animal model. *Biochem. Pharmacol.* 25: 801-804.

Fuentes J.A., Oleshansky M.A. and Neff N.H. (1975) A sensitive enzymatic assay for *dextro-* or *levo-*tranylcypromine in brain. *Biochem. Pharmacol.* 24: 1971-1973.

Fuller R.W. (1981) Enhancement of monoaminergic neurotransmission by antidepressant drugs. In: *Antidepressants: Neurochemical, Behavioral, and Clinical Perspectives.* Enna S.J.,Malick J.B. and Richelson E. eds. Raven Press, New York, NY, pps 1-12.

Fuller R. and Hemrick-Luecke S. (1980) Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. *Science* 209: 305-307.

Fuller R.W., Perry K.W. and Molloy B.B. (1975) Effect of 3-(p-trifluoro-methylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. *J. Pharmacol. Exp. Ther.* 193: 796-803.

Fuller R.W., Perry K.W. and Molloy B.B. (1974) Effect of an uptake inhibitor on serotonin metabolism in rat brain: Studies with 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine (LY110140). *Life Sci.* 15: 1161-1171.

Fuller R.W., Snoddy H.D., Perry K.W., Bymaster F.P. and Wong D.T. (1978) Importance of duration of drug action in the antagonism of p-chloroamphetamine depletion of brain serotonin-comparison of fluoxetine and chlorimipramine. *Biochem. Pharmacol.* 27: 193-198.

Fuller R.W. and Wong D.T. (1989) Fluoxetine: A serotonergic appetite suppressant drug. *Drug Dev. Res.* 17: 1-15.

Fuller R.W. and Perry K.W. (1989) Effect of fluoxetine pretreatment on plasma and tissue concentrations of desipramine in rats. *Res. Commun. Chem. Pathol. Pharmacol.* 66: 375-384.

Fuller R.W., Wong D.T. and Robertson D.W. (1991) Fluoxetine, a selective inhibitor of serotonin uptake. *Med. Res. Rev.* 11: 17-34.

Fuller R.W. and Snoddy H.D. (1991) Role of norfluoxetine in the inhibition of desipramine metabolism and the inhibition of serotonin uptake after fluoxetine administration to rats. *Res. Commun. Chem. Path. Pharmacol.* 73: 31-40.

Fuller R.W. and Snoddy H.D. (1986) Fluoxetine enantiomers as antagonists of p-chloroamphetamine effects in rats. *Pharmacol. Biochem. Behav.* 24: 281-284.

Fuller R.W. (1984) Specificity in the inhibition of monoamine uptake. *Clin. Neuropharmacol.* 7 (suppl. 1): 310-311.

Fuller R.W., Hemrick-Luecke S.K. and Ornstein P.L. (1992) Protection against amphetamine-induced neurotoxicity toward striatal dopamine neurons in rodents by LY274614, an excitatory amino acid antagonist. *Neuropharmacol.* 31: 1027-1032.

Gaedigk A., Blum M., Gaedigk R., Eichelbaum M. and Meyer U.A. (1991) Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. *Am. J. Hum. Genet.* 48: 943-950.

Gal J. (1988) Indirect chromatographic methods for resolution of drug enantiomers - Synthesis and separation of diastereomeric derivatives. In: *Drug Stereochemistry: Analytical Methods and Pharmacology.* Wainer W.I. and Drayer D.E. eds. Marcel Dekker, New York, pp. 81-113.

Gardner C.R. and Alaxander J. (1985) Pro-drug approaches to drug targetting: past accomplishment and future potential. In: *Drug Targetting.* Buri P. and Gumma A. eds. Elsevier Science, Amsterdam, pp. 145-178.

150

Geisslinger G., Dietzel K., Loew D., Schuster O., Rau G., Lachmann G. and Brune K. (1989) High-performance liquid chromatographic determination of ibuprofen, its metabolites and enantiomers in biological fluids. *J. Chromotogr.* 491-149.

Geyer M.A., Dawsey W.J. and Mandell A.J. (1978) Fading: A new cytofluorimetric measure quantifying serotonin in the presence of catecholamines at the cellular level in brain. *J. Pharmacol. Exp. Ther.* 207: 650-667.

Gibson G.G. and Skett P. eds. (1986) *Introduction to Drug Metabolism.* Chapman and Hall, New York, NY.

Glowinski S. and Axelrod J. (1964) Inhibition of uptake of tritiated noradrenaline in the intact rat brain by imipramine and structurally related compounds. *Nature (Lond.)* 204: 1318-1319.

Gluckman M.I. and Baum T. (1969) The pharmacology of iprindole, a new antidepressant. *Psychopharmacologia (Berlin)* 15: 169-185.

Goldbloom D.S. and Olmsted M.P. (1993) Pharmacotherapy of bulimia nervosa with fluoxetine – assessment of clinically significant attitudinal change. *Am. J.* Psychiatry 150: 770-774.

Gonzalez F.J. (1992) Human cytochrome P450: problems and prospects. *Trends Pharmacol. Sci.* 13: 346-352.

Goodnick P.J. (1989) Influence of fluoxetine on plasma levels of desipramine. *Am. J. Psychiatry* 146: 1478.

Goodnough D.B. and Baker G.B. (1994) 5-HT$_2$ and beta-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. *J. Neurochem.* (in press).

Grahame-Smith D.G. (1977) Chapter 11: General aspects of drug interactions in psychopharmacology. In: *Drug Interactions.* Grahame-Smith D.G. ed. University Park Press, Baltimore, MD, pp. 147-157.

Gram L.F. and Christiansen J. (1975) First pass metabolism of imipramine in man. *Clin. Pha.macol. Ther.* 17: 555-563.

Gray J.A. and Green A.R. (1987) Increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs and electroconvulsive shocks. *Br. J. Pharmacol.* 92: 357-362.

Green J.P. and Maayani S. (1977) Tricyclic antidepressant drugs block histamine H2 receptor in brain. *Nature (Lond.)* 269: 163-165.

Greenblatt D.J., Preskorn S.H., Cotreau M.M., Horst W.D. and Harmatz J.S. (1992) Fluoxetine impairs clearance of alprazolam but not of clonazepam. *Clin. Pharmacol. Ther.* 52: 479-486.

Grunberg E. and Schnitzer R.J. (1952) Studies on the activity of hydrazine derivatives of isonicotinic acid in the experimental tuberculosis of mice. *Quart. Bull. Sea View Hospital.* 13: 3-11.

Guan X.-M. and McBride W.J. (1986) Fluoxetine increases the extracellular levels of serotonin in the nucleus accumbens. *Brain Res. Bull.* 21: 43-46.

Guengerich F.P. (1992) Minireview: Human cytochrome P-450 enzymes. *Life Sci.* 50: 1471-1478.

Guze B.H., Baxter L.R. Jr. and Rego J. (1987) Refractory depression treated with high doses of monoamine oxidase inhibitor. *J. Clin. Psychiatry* 48: 31-32.

Hamilton M.S. and Opler L.A. (1992) Akathisia, suicidality, and fluoxetine. *J. Clin. Psychiat.* 53: 401-406.

Hammer W. and Brodie B.B. (1967) Application of isotope derivative technique to assay of secondary amines. Estimation of desipramine by acetylation with ^3H-acetic anhydride. *J. Pharmacol. Exp. Ther.* 157: 503-508.

Hammer W. and Sjoqvist F. (1967) Plasma levels of monomethylated tricyclic antidepressants during treatment with imipramine-like compounds. *Life Sci.* 6: 1895-1903.

Hampson D.R., Baker G.B. and Coutts R.T. (1986) A comparison of the neurochemical properties of the stereoisomers of tranylcypromine in the central nervous system. *Cell. Mol. Biol.* 32: 593-599.

Hampson D.R., Baker G.B., Nazarali A.J. and Coutts R.T. (1984) A rapid and sensitive electron-capture gas chromatographic method for the analysis of tranylcypromine in brain tissue using acetylation and pentafluorobenzoylation. *J. Biochem. Biophys. Methods* 9: 85-87.

Harper N.J. (1959) Drug latentiation. *J. Med. Pharm. Chem.* 1: 467-500.

Hegadoren K.M., Baker G.B., Coutts R.T. and Dewhurst W.G. (1990) Interactions of iprindole with fenfluramine metabolism in rat brain and liver. *J. Psychiat. Neurosci.* 11: 5-11.

Henderson M.G., Hemrick-Luecke S. and Fuller R.W. (1992) MK-801 protects against amphetamine-induced striatal dopamine depletion in iprindole-treated rats, but not against brain serotonin depletion after *p*-chloroamphetamine administraion. *Ann. N.Y. Acad. Sci.* 648: 286-288.

Hendley E.D. and Snyder S.H. (1968) Relationship between the action of monoamine oxidase inhibitors on the noradrenaline uptake system and their antidepressant efficacy. *Nature (Lond.)* 220: 1330-1331.

Hermansson J. and Von Bahr C. (1980) Simultaneous determination of *d*- and *l*-propranolol in human plasma by high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* 221: 109-117.

Himmelhoch J.M., Fuchs C.Z. and Symons B.J. (1982) A double-blind study of tranylcypromine treatment of major anergic depression. *J. Nerv. Men. Dis.* 170: 628-634.

Holden C. (1986) Depression research advances, treatment lags. *Science* 133: 723-726.

Holmstedt B., Frank H. and Testa B. eds. (1989) *Chirality and Biological Activity.* Alan R. Liss, Inc., New York, NY.

Horn A.S. and Snyder S.H. (1972) Steric requirements for catecholamine uptake by rat brain synaptosomes: studies with rigid analogs of amphetamine. *J. Pharmacol. Exp. Ther.* 180: 523-530.

Hutt A.J. (1990) Enantiospecific analytical methodology: Applications in drug metabolism and pharmacokinetics. Chapter 6. In: *Progress in Drug Metabolism.* Taylor & Francis, London, pp. 257-361.

Hyttel J. and Larsen J.-J. (1985) Serotonin-selective antidepressants. *Acta. Pharmacol. Toxicol.* 56: 146-153.

Jamali F. (1993) Stereochemically pure drugs: An overview. In: *Drug Stereochemistry: Analytical Methods and Pharmacology.* Wainer I.W. ed. Marcel Dekker, Inc., New York, NY, pp. 375-384.

Jamali F., Mehvar R. and Pasutto F.M. (1989) Enantioselective aspects of drug action and disposition: therapeutic pitfalls. *J. Pharm. Sci.* 78: 695-715.

Jefferson J.W. (1992) Is tranylcypromine really metabolized to amphetamine? *J.Clin. Psychiatry* 53: 450-451.

Jesberger J.A. and Richardson J.S. (1985) Neurochemical aspects of depression: The past and the future? *Int. J. Neurosci.* 27: 19-47.

Johnson J.P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* 17: 1285-1297.

Johnstone E.C. (1982) Affective disorders. In: *Disorders of Neurohumoral Transmission.* Crow T.J. ed. Academic Press, New York, NY, pp. 255-286.

Kagan H.B. ed. (1977) *Stereochemistry Vol. 4: Absolute Configurations of 6000 Compounds with One Asymmetric Carbon Atom.* Thieme, Stuttgart.

Kang G.I. and Chung S.Y. (1984) Identification of N-acetyl and hydroxlated N-acetyltranylcypromine from tranylcypromine-dosed rat urine. *Arch. Pharm. Res. (Korea)* 7: 65-68.

Keck P.E., Carter W.P., Nierenberg A.A., Cooper T.B., Potter W.Z. and Rothschild A.J. (1991) Acute cardiovascular effects of tranylcypromine: correlation with plasma drug, metabolite, norepinephrine and MHPG levels. *J. Clin. Psychiat.* 52: 250-254.

Keck P.E. and McElroy S.L. (1992) Ratio of plasma fluoxetine to norfluoxetine concentrations and associated sedation. *J. Clin. Psychiatry* 53: 127-129

Kelly M.W., Perry P.J., Holstad S.G. and Garvey M.J. (1989) Serum fluoxetine and norfluoxetine concentration and antidepressant response. *Ther. Drug Monit.* 11: 165-170.

Kimber J.R., Cross J.A. and Horton R.W. (1987) Benzodiazepine and GABA$_A$ receptors in rat brain following chronic antidepressant drug administration. *Biochem. Pharmacol.* 4175-4176.

Klyne W. and Buckingham J. (1978) *Atlas of Organic Molecules*, 2nd ed., Vols 1 and 2. Chapman & Hill.

Kondepudi D.K. and Nelson G.W. (1985) Weak neutral currents and the origin of biomolecular chirality. *Nature* 314: 438-441.

Konig W.A. ed. (1987) *The Practice of Enantiomer Separation by Capillary Gas Chromatography.* Huthig Verlag, Heidelberg.

Krstulovic A.M. (1989) Racemates versus enantiomerically pure drugs: Putting high-performance liquid chromatography to work in the selection process. *J. Chromatogr.* 488: 53-72.

Kuhn R. (1970) The imipramine story. In: *Discoveries in Biological Psychiatry.* Ayd F.J. and Blackwell B. eds. J.B. Lipincott, Philadelphia/Toronto. pp 205-217.

Lader M. (1980) Sleep and hypnotics. *Scott. Med. J.* 24: 59-63.

Lahti R.A. and Maickel R.P. (1971) The tricyclic antidepressants--inhibition of norepinephrine uptake as related to potentiation of norepinephrine and clinical efficacy. *Biochem. Pharmacol.* 20: 482-486.

Lang V.A., Geissler H.E. and Mutschler E. (1979) Bestimmung und Vergleich der plasma and urinkonzentrationen nach gabe von (+)- and (-)-tranylcypromin. *Arzneimittel Forschung/Drug Res.* 29: 154-157.

Lantz R.J., Farid K.Z., Koons J., Tenbarge J.B. and Bopp R.J. (1993) Determination of fluoxetine and norfluoxetine in human plasma by capillary gas chromatography with electron-capture detection. *J. Chromatogr. Biomed. Appl.* 614: 175-179.

Lapin I.P. and Oxenkrug G.F. (1969) Intensification of the central serotonin-ergic processes as a possible determinant of the thymoleptic effect. *Lancet* i: 132-136.

Lee E.J., Williams K., Day R., Graham G. and Champion D. (1985) Stereo-selective disposition of ibuprofen enantiomers in man. *Br. J. Clin. Pharmacol.* 19: 669-674.

Lehmann F.P.A. (1976) Stereoselectivity and affinity in molecular-pharmacology. In: *Progress in Drug Research.* Jucker E. ed. Birkhauser, Stuttgart, pp. 101-142.

Lemberger L., Bergstrom R.F., Wolen R.L., Farid N.A., Enas G.G. and Aronoff G.R. (1985) Fluoxetine: clinical pharmacology and physiologic disposition. *J. Clin. Psychiatry* 46: 14-19.

Lemberger L., Rowe H., Carmichael R., Crabtree R., Horng J.S., Bymaster F. and Wong D. (1978) Fluoxetine, a selective serotonin uptake inhibitor. *Clin. Pharmacol. Ther.* 23: 421-429.

Lennard M.S. (1991) Clinical pharmacology through the looking glass: reflections on the racemate *vs* enantiomer debate. *Br. J. Clin. Pharmacol.* 31: 623-625.

Leonard B.E. (1988) Pharmacological effects of serotonin reuptake inhibitors. *J. Clin. Psychiatry* 49: 12-17.

Lesse S. (1978) Tranylcypromine (Parnate)--A study of 1000 patients with severe agitated depressions. *Am. J. Psychother.* 32: 220-242.

Lloyd K.G., Thuret F. and Pilc A. (1985) Upregulation of gamma-aminobutyric acid GABA$_B$ binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. *J. Pharmacol. Exp. Ther.* 235: 191-199.

Lloyd K.G., Zivkovic B., Scatton B., Morselli P.L. and Bartholini G. (1989) The GABAergic hypothesis of depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 13: 341-351.

Maass A.R. and Nimmo M.J. (1959) A new inhibitor of serotonin metabolism. *Nature (Lond.)* 184(Suppl): 547-548.

Maj J., Przegalinski E. and Mogilnicka E. (1984) Hypothesis concerning the mechanism of action of antidepressant drugs. *Rev. Physiol. Biochem. Pharmacol.* 100: 1-74.

Mallinger A.G., Himmelhoch J.M., Thase M.E., Edwards D.J. and Knopf S. (1990) Plasma tranylcypromine: Relationship to pharmacokinetic variables and clinical antidepressant actions. *J. Clin. Psychopharmacol.* 10: 176-183.

Marcus M.D., Wing R.R., Ewing L., Kern E., McDermott M. and Gooding W. (1990) A double-blind, placebo-controlled trial of fluoxetine plus behavior modification in the treatment of obese binge-eaters and non-binge eaters. *Am. J. Psychiatry* 47: 876-881.

Marley E. and Blackwell B. (1970) Interactions of monoamine oxidase inhibitors, amines and foodstuffs. *Adv. Pharmacol. Chemother.* 8: 185-239.

Marsden C.A., Conti J., Strope E., Curzon G. and Adams R.N. (1979) Monitoring 5-hydroxytryptamine release in the brain of the freely moving unanaesthetized rat using in vivo voltammetry. *Brain Res.* 171: 85-99.

Martin I.L. (1987) The benzodiazepines and their receptors: 25 years of progress. *Neuropharmacology* 26: 957-970.

Mason S. (1984) The left hand of nature. *New Scientist* 101: 10-14.

Matuszewski B.K., Constanzer M.L., Hessey G.A. and Bayne W.F. (1990) Development of direct stereoselective and non-stereoselective assays in biological fluids for the enantiomers of thieno[2,3-b]thiopyran-2-sulfonamide, a topically effective carbonic anhydrase inhibitor. *J. Chromatogr.* 526: 461-473.

McGeer P.L. and McGeer E.G. (1980) Chemistry of mood and emotion. *Ann. Rev. Psychol.* 31: 273-307.

McKenna K.F., McManus D.J., Baker G.B. and Coutts R.T. (1994) Chronic administration of the antidepressant phenelzine and its N-acetyl analogue: e.'ects on GABAergic function. *J. Neural Transm.* (in press).

McKim H.R., Calverley D.G., Baker G.B. and Dewhurst W.G. (1980) The effects of tranylcypromine on the levels of some cerebral amines in rat diencephalon. In: *Recent Advances in Canadian Neuropsycho-pharmacology.* Grof P. and Saxena B. eds. S. Karger, Basel, pp. 7-13.

McManus D.J. and Greenshaw A.J. (1991a) Differential effects of chronic antidepressants in behavioral tests of β-adrenergic and GABA$_B$ receptor function. *Psychopharmacol.* 103: 50-54.

McManus D.J. and Greenshaw A.J. (1991b) Differential effects of anti-depressants on GABA$_B$ and β-adrenergic receptors in rat cerebral cortex. *Biochem. Pharmacol.* 42: 1525-1528.

Mehvar R. (1992) Stereochemical considerations in pharmacodynamic modeling of chiral drugs. *J. Pharm. Sci.* 81: 199-200.

Mikus B., Kroemer H.K., Klotz U. and Eichelbaum M. (1988) The effect of cimetidine on verapamil metabolism and renal excretion of metabolites. *Clin. Pharmacol. Ther.* 43: 134.

158

Coppen A. eds. Oxford University Press, Oxford. pp. 123-158.

Montgomery S.A. (1985) Development of new treatments for depression. J. Clin. Psychiatry 46: 3-6.

Montgomery S.A., Baldwin D., Shah A., Green M., Fineberg N. and Montgomery D. (1990) Plasma-level response relationships with fluoxetine and zimelidine. Clin. Neuropharmacol. 13(suppl): 571-575.

Murphy D.L. and Pigott T.A. (1990) A comparative examination of a role for serotonin in obsessive-compulsive disorder, panic disorder, and anxiety. J. Clin. Psychiatry 51 (suppl): 53-58.

Murphy D.L., Sunderland T., Garrick N.A., Aulakh C.S. and Cohen R.M. (1987) Selective amine oxidase inhibitors: basic to clinical studies and back. Psychopharmacol. (Berlin) 3: 135-146.

Murphy D.L., Sunderland T., Campbell I. and Cohen R.M. (1985) Monoamine oxidase inhibitors as antidepressants. In: Pharmacotherapy of Affective Disorders. Dewhurst W.G. and Baker G.B. eds. New York University Press, New York, NY, pp. 238-261.

Mutschler E., Gietyl Y., Krauss D., Martin E., Pflugmann G. and Weber H. (1990) Stereospecific analysis and human pharmacokinetics of the enantiomers of drugs administered as racemates. In: Chirality and Biological Activity. Holmstedt B., Frank H. and Testa B. eds. Alan R. Liss, New York, NY, pp. 199-219.

Mutschler E. and Mohrke W. (1983) Kinetics of MAO inhibitors. Mod. Probl. Pharmacopsychiatry 19: 126-134.

Nagy L.M., Morgan C.A., Southwick S.M. and Charney D.S. (1993) Open prospective trial of fluoxetine for posttraumatic stress disorder. J. Clin. Psychopharmacol. 13: 107-113.

Nash J.F. and Yamamoto B.K. (1993) Effect of d-amphetamine on the extracellular concentraions of glutamate and dopamine in iprindole-treated rats. Brain Res. 627: 1-8.

Determination of fluoxetine and norfluoxetine in plasma by gas chromatography with electron-capture detection. *Clin. Chem.* 28: 2100-2102.

Jazarali A.J., Baker G.B., Coutts R.T., Pasutto F.M. and Cristofoli W.A. (1984) Simultaneous analysis of N-(2-cyanoethyl)amphetamine (Fenproporex), amphetamine and *para*-hydroxyamphetamine in rat brain. *Res. Commun. Subst. of Abuse* 5: 317-320.

Jazarali A.J., Baker G.B. and Boisvert D.P. (1986) Analysis of clonidine in biological tissues and body fluids by gas chromatography with electron-capture detection. *J. Chromatogr. Biomed. Appl.* 380: 393-400.

Jazarali A.J., Baker G.B., Coutts R.T. and Greenshaw A.J. (1987a) *Para*-hydroxytranylcypromine: Presence in rat brain and heart following administration of tranylcypromine and an N-cyanoethyl analogue. *Eur. J. Drug Metab. Pharmacokinet.* 12: 207-214.

Jazarali A.J., Baker G.B., Coutts R.T. and Wong T.F.J. (1987b) *N*-(2-Cyanoethyl)tranylcypromine, a potential prodrug of tranylcypromine: Its disposition and interaction with catecholamine neurotransmitters in brain. *Pharmaceut. Res.* 4: 16-20.

Jazarali A.J., Baker G.B., Coutts R.T., Yeung J.M. and Rao T.S. (1987c) Rapid analysis of β-phenylethylamine in tissues and body fluids utilizing pentafluorobenzoylation followed by electron-capture gas chromatography. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 11: 251-258.

Jebert D.W., Nelson D.R., Adesnik M., Coon M.J., Estabrook R.W., Gonzalez F.J., Guengerich F.P., Gunsalus I.C., Johnson E.F., Kemper B., Levin W., Philips I.R., Sato F. and Waterman M.R. (1989) The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* 8: 1-13.

Jebert D.W., Nelson D.R., Coon M.J., Estabrook R.W., Feyereisen R., Fujii-Kuriyama Y., Gonzalez F.J., Guengerich F.P., Gunsalus I.C., Johnson E.F., Loper J.C., Sato R., Waterman M.R and Waxman D.J.

mapping, and recommended nomenclature. *DNA Cell Biol.* 10: 1-14.

Ielson D.R., Kamataki T., Waxman D.J., Guengerich F.P., Estabrook R.W., Feyereisen R., Gonzalez F.J., Coon M.J., Gunsalus I.C., Gotoh O., Okuda K. and Nebert D.W. (1993) The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol.* 12: 1-51.

Ielson J.C., Mazure C.M., Bowers M.B. and Jatlow P.I. (1991) A preliminary, open study of the combination of fluoxetine and desipramine for rapid treatment of major depression. *Arch Gen Psychiatry* 48: 303-307.

Nielsen E.B., Suzdak P.D., Andersen K.E., Knutsen L.J.S., Sonnewald U. and Braestrup C. (1991) Characterization of tiagabine (NO-328), a new potent and selective GABA-uptake inhibitor. *Eur. J. Pharmacol.* 196: 257-266.

Nies A. (1983) Clinical applications of MAOIs. In: *Drugs in Psychiatry, Vol. 1*, Burrows G.D., Norman T.R. and Davies B. eds. Elsevier, Amsterdam, pp. 229-247.

Olson G.D., Wendle H.A., Livermore J.D., Leger R.M., Lynn R.K. and Gerber N. (1977) Clinical effects and pharmacokinetics of racemic methadone and its optical isomers. *Clin. Pharmacol. Ther.* 21: 147-157.

Oswald J. Brezinova V. and Dunleavy D.L.F. (1972) On the slowness of action of tricyclic antidepressant drugs. *Br. J. Psychiat.* 120: 673-677.

Otton S.V., Inaba T. and Kalow W. (1984) Competitive inhibition of sparteine oxidation in human liver by β-adrenoreceptor antagonists and other cardiovascular drugs. *Life Sci.* 34: 73-80.

Otton S.V., Wu D., Joffe R.T., Cheung S.W. and Sellers E.M. (1993) Inhibition by fluoxetine of cytochrome P450 2D6 activity. *Clin. Pharmacol. Ther.* 53: 401-409.

161

Pare C.M.D., Kline N., Hallstrom C. and Cooper T.B. (1982) Will amitryp-
tyline prevent the "cheese" reaction of monoamine oxidase inhibitors? *Lancet* ii: 183-186.

Parli C.J. and Hicks J. (1974) In vivo demethylation of Lilly 110140: 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine to an active metabolite - Lilly 103947. *Fed Proc.* 33: 560.

Pasutto F.M. (1992) Mirror images: The analysis of pharmaceutical enantiomers. *J. Clin. Pharmacol.* 32: 917-924.

Paulos M.A. and Tessel R.E. (1982) Excretion of β-phenylethylamine is elevated in humans after profound stress. *Science* 215: 1127-1129.

Paykel E.S., Van Woerkom A.E., Walters D.W., White W. and Mercer J. (1991) Fengabine in depression: A placebo-controlled study of a GABA agonist. *Human Psychopharmacol.* 6: 147-154.

Pearlman C. (1987) High dose tranylcypromine in refractory depression. *J. Clin. Psychiatry* 48: 423-424.

Peyton A.L., Carpenter R. and Rutkowski K. (1992) The stereospecific determination of fluoxetine and norfluoxetine enantiomers in human plasma by high-pressure liquid chromatography (HPLC) with fluorescence detection. *Pharmaceut. Res.* 8: 1528-1532.

Peyton A.L., Lemberger L., Rutkowski K., Wolen R.L., Bergstrom R.F., Haynes T. and Ziege E.A. (1991) Differential disposition and protein binding of fluoxetine and norfluoxetine enantiomers after administration of racemic fluoxetine. *Pharmaceut. Res.* 8: S-308.

Philips S.R. and Boulton A.A. (1979) The effect of monoamine oxidase inhibitors on some arylalkylamines in rat striatum. *J. Neurochem.* 33: 159-167.

Pigott T.A., Pato M.T., Bernstein S.E., Grover G.N., Hill J.L., Tolliver T.J. and Murphy D.L. (1990) Controlled comparisons of clomipramine and fluoxetine in the treatment of obsessive-compulsive disorder. Behavioural and biological results. *Arch. Gen. Psychiatry* 47: 926-932.

162

Pletscher A., Gey K.F., and Keller P. (1960) Monoaminooxydase-Hemmer: Biochemie, Chemie, Pharmakologie, Klinik. In: *Progress in Drug Research, Vol. 2.* Jucker E. ed. Birkhauser Verlag, Basel/Stuttgart, pp. 417-590.

Poklis A. (1989) Gas Chromatography. In: *Clinical Chemistry: Theory, analysis and correlation.* Kaplin L.A. and Pesce A.J. eds. The C.V. Mosby Company, Toronto, ON, pp. 110-125.

Polson R., Fleming P.M. and O'Shea J.K. (1993) Fluoxetine in the treatment of amphetamine dependence. *Human Psychopharmacol.* 8:55-58.

Post R.M., Uhde T.W., Runinow D.R. and Huggins T. (1987) Differential time course of antidepressant effects after sleep deprivation, ECT, and carbamazepine: clinical and theoretical implications. *Psychiat. Res.* 22: 11-19.

Potter W.Z., Calil H.M., Manian A.A., Zavadil A.P. and Goodwin F.K. (1979) Hydroxylated metabolites of tricyclic antidepressants: preclinical assessment of activity. *Biol Psychiatry* 14: 601-613.

Potter W.Z., Calil H.M., Zavadil A.P., Jusko W.J., Sutfin T., Rapaport J.L. and Goodwin F.K. (1980) Steady-state concentrations of hydroxylated metabolites of tricyclic antidepressants in patients: relationship to clinical effect. *Psychopharmacol. Bull.* 16: 32-34.

Potter W.Z. and Manji H.K. (1990) Antidepressants, metabolites and apparent drug resistence. *Clin. Neuropharmacol.* 13: S45-S53.

Potts B.D. and Parli C.J. (1992) Analysis of the enantiomers of fluoxetine and norfluoxetine in plasma and tissue using chiral derivatization and normal-phase liquid chromatography. *J. Liq. Chromatogr.* 15: 665-681.

Poznansky M.J. and Juliano R.L. (1984) Biological approaches to the controlled delivery of drugs. A critical review. *Pharmacol. Rev.* 36: 277-336.

Preskorn S.H., Beber J.H., Fual J.C. and Hirschfeld R.M.A. (1990) Serious adverse effects of combining fluoxetine and tricyclic antidepressants. *Am. J. Psychiatry* 147: 532.

Prperaki S. and Parissipoulou M. (1993) Use of cyclodextrins as chiral selectors for direct resolution of the enantiomers of fluoxetine and its metabolite norfluoxetine by HPLC. *Chirality* 5: 258-266.

Quitkin F., Rifkin A. and Klein D.F. (1979) Monoamine oxidase inhibitors. A review of antidepressant effectiveness. *Arch. Gen. Psychiat.* 36: 749-760.

Quitkin F.M., Rabkin J.G., Stewart J.W. McGrath P.J. and Harrison W. (1986) Study duration in antidepressant research: advantages of a 12-week trial. *J. Psychiat. Res.* 20: 211-216.

Randrup A., Munkvad I., Fog R., Gerlach J., Molander L., Kjellberg B. and Scheel-Kruger J. (1975) Mania, depression and brain dopamine. In: *Current Developments in Psychopharmacology, vol. 2.* Essman W.B. and Valzelli L. eds. Spectrum, New York, NY, pp 206-248.

Randrup A. and Braestrup C. (1977) Uptake inhibition of biogenic amines by newer antidepressant drugs: relevance to the dopamine hypothesis of depression. *Psychopharmacol. (Berl.)* 53: 309-314.

Rao T.S., Baker G.B. and Coutts R.T. (1986) Pentafluorobenzenesulfonyl chloride as a sensitive reagent for the rapid gas chromatographic analysis of tranylcypromine in tissues and body fluids. *Biochem. Pharmacol.* 35: 1925-1928.

.ao T.S., Baker G.B., Coutts R.T., Yeung J.M., McIntosh G.J.A. and Torok-Both G.A. (1987a) Analysis of the antidepressant phenelzine in brain tissue and urine using electron-capture gas chromatography. *J. Pharmacol. Methods* 17: 297-304.

Rao T.S., Baker G.B. and Coutts R.T. (1987b) N-(3-chloropropyl)-phenylethylamine as a possible prodrug of β-phenylethylamine: studies in the rat brain. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 11: 301-308.

Reynolds G.P., Rausch W.-D. and Riederer P. (1980) Effects of tranyl-cypromine stereoisomers on monoamine oxidation in man. *Br. J. Clin. Pharmacol.* 9: 521-523.

Richardson G.M. (1901) *Memoirs by Pasteur, Van't Hoff, Le Bel, and Wislicenus.* American Book, New York, NY.

Richelson E. and Pfenning M. (1984) Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepine-phrine uptake. *Eur. J. Pharmacol.* 104: 277-286.

Richelson E. (1984) The newer antidepressants: structures, pharmaco-kinetics, pharmacodynamics and proposed mechanism of action. *Psychopharmacol. Bull.* 20: 213-223.

Richelson E. and Nelson A. (1984) Antagonism by antidepressants of neurotransmitter receptors of normal human brain in vitro. *J. Pharmacol. Exp. Ther.* 230: 94-102.

Riley T.N. and Brier C.G. (1972) Absolute configuration of (+) and (-) trans-2-phenylcyclopropylamine hydrochloride. *J. Med. Chem.* 15: 1187-1188.

Risch S.C., Janowsky D.S. and Huey L.Y. (1981) Plasma levels of tricyclic antidepressants and clinical efficacy. In: *Antidepressants: Neurochemical, Behavioral and Clinical Perspectives.* Enna S.J.,Malick J.B. and Richelson E. eds. Raven Press, New York, NY, pp. 183-217.

Robertson D.W., Krushinski J.H., Fuller R.W. and Leander J.D. (1988) Absolute configurations and pharmacological activities of the optical isomers of fluoxetine, a selective serotonin-uptake inhibitor. *J. Med. Chem.* 31: 1412-1417.

Robinson D.S. (1983) High-dose monoamine oxidase-inhibitor therapy. *J. Am. Med. Assoc.* 250: 2212.

Rosloff B.N. and Davis J.M. (1974) Effect of iprindole on norepinephrine turnover and transport. *Psychopharmacol.* 40: 53-64.

Rudorfer M.V. and Potter W.Z. (1989) Antidepressants - a comparative review of the clinical pharmacology and therapeutic use of the "newer" versus the "older" drugs. *Drugs* 37: 713-738.

Rudorfer M.V. and Potter W.Z. (1985) Metabolism of drugs used in affective disorders. In: *Pharmacotherapy of Affective Disorders*. Dewhurst W.G. and Baker G.B. eds. New York University Press, New York, NY, pp. 382-448.

Sabelli H.C., Fawcett J., Gusovsky F., Javaid J., Edwards J. and Jeffries H. (1983) Urinary phenylacetate: A diagnostic test for depression? *Science* 220: 1187-1188.

Sadusk J.F., Jr. (1964) The physician and the Food and Drug Administration. *J. A. M. A.* 190: 907-909.

Sandler M. (1981) Monoamine oxidase inhibitor efficacy in depression and the "cheese effect". *Psychol. Med.* 11: 455-458.

Sanghvi I. and Gershon S. (1975) Effect of acute and chronic iprindole on serotonin turnover in mouse brain. *Biochem. Pharmacol.* 24: 2103-2104.

Schatzberg A.F., Dessain E., O'Neil P., Katz D.L. and Cole J.O. (1987) Recent studies on selective serotonergic antidepressants: trazodone, fluoxetine, and fluvoxamine. *J. Clin. Psychopharmacol.* 7: 44S-49S.

Schildkraut J.J. (1970) Tranylcypromine: Effects on norepinephrine metabolism in rat brain. *Am. J. Psychiat.* 126: 925-931.

Schildkraut J.J. and Kety S.S. (1967) Biogenic amines and emotions. *Science* 156: 21-30.

Schmauss M., Kapfhammer H., Meyr P. and Hoff P. (1988) Combined MAO-inhibitor and tri-(tetra)cyclic antidepressant treatment in therapy resistant depression. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 12: 523-532.

Schmidt B., Bircher J., Preisig R. and Kupfer A. (1985) Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-

demethylation with debrisoquine hydroxylation. *Clin. Pharmacol. Ther.* 38: 618-624.

Schmidt M.J., Fuller R.W. and Wong D.T. (1988) Fluoxetine, a highly selective serotonin reuptake inhibitor: A review of preclinical studies. *Br. J. Psychiatr.* 153: 40S-46S.

Schneir F.R., Liebowitz M.R., Davies S.O., Fairbanks J., Hollander E., Campeas R. and Klein D.F. (1990) Fluoxetine in panic disorder. *J. Clin. Psychopharmacol.* 10: 119-121.

Schukit M., Robins E. and Feighner J. (1971) Tricyclic antidepressants and monoamine oxidase inhibitors. *Arch. Gen. Psychiatry* 24: 509-514.

Scott J.W. (1988) Synthesis of enantiomerically pure drugs. In: *Drug Stereochemistry: Analytical Methods and Pharmacology.* Wainer I.W. and Drayer D.E. eds. Marcel Dekker, New York, NY, pp. 175-206.

Sethna E.R. (1974) A study of refractory cases of depressive illnesses and their response to combined antidepressant treatment. *Br. J. Psychiatry* 124: 265-272.

Sheehan D.V., Ballenger J. and Jacobsen G. (1980) Treatment of endogenous anxiety with phobic, hysterical, and hypochondriacal symptoms. *Arch. Gen. Psychiat.* 37: 51-59.

Shen W.W. and Lin K.-M. (1991) Cytochrome P-450 monooxygenases and interactions of psychotropic drugs. *Int. J. Psychiatry Med.* 21: 47-56.

Shore P.A., Silver S.L. and Brodie B.B. (1955) Interaction of reserpine, serotonin and lysergic acid diethylamide in brain. *Science* 122: 284-285.

Shore P.A. and Brodie B.B. (1957) Influence of various drugs on serotonin and norepinephrine in the brain. In: *Psychotropic Drugs.* Garattini S. and Ghetti U. eds. Elsevier, Amsterdam, pp. 423-427.

Silber B. and Riegelman S. (1980) Stereospecific assay for (-)- and (+)-propranolol in human and dog plasma. *J. Pharmacol. Exp. Ther.* 215: 643-648.

Simeon J.G., Dinicola V.F., Ferguson H.B. and Copping W. (1990) Adolescent depression: a placebo-controlled fluoxetine treatment study and follow-up. *Progr. Neuropsychopharmacol. and Biol. Psychiatry* 14: 791-795.

Simonyi M. (1984) On chiral drug action. In: *Medicinal Research Reviews.* John Wiley & Sons, Inc, New York, NY, 4: 359-413.

Sisenwine S.F., Tio C.O., Hadley F.V., Liu A. L., Kimmel H.B. and Ruelius H.W. (1982) Species-related differences in the stereoselective glucuronidation of oxazepam. *Drug Metab. Dispos.* 7: 40-43.

Sjoqvist F. (1965) Psychotropic drugs (2). Interaction between monoamine oxidase (MAO) inhibitors and other substances. *Proc. Roy. Soc. Med.* 58: 967-975.

Smith D.F. (1980) Tranylcypromine stereoisomers, monoaminergic transmissions and behaviour. A review. *Pharmakopsychiat.* 13: 130-136.

Snyder S.H. and Peroutka S.J. (1984) Antidepressants and neurotransmitter receptors. In: *Neurobiology of Mood Disorders* Post R.M. and Ballenger J.C. eds. Williams & Wilkins, Baltimore, MA, pp. 686-697.

Snyder S.H. and Yamamura H.T. (1977) Antidepressants and the muscarinic acetylcholine receptor. *Arch. Gen. Psychiat.* 34: 236-239.

Solyom L. and Gibson R.E. (1990) Fluoxetine in refractory depression. *Biol. Psychiat.* 27:261-463.

Souter R.W. ed. (1985) *Chromatographic Separations of Stereoisomers.* CRC Press, Boca Raton, pp. 11-195.

Spahnlangguth H., Hahn G., Mutschler E., Mohrke W. and Langguth P. (1992) Enantiospecific high-performance liquid chromatographic assay with fluorescence detection for the monoamine oxidase

inhibitor tranylcypromine and its applicability in pharmacokinetic studies. *J. Chromatogr.* 584: 229-237.

Spina E., Birgersson C., von Bahr C., Ericsson E., Mellstrom B., Steiner E. and Sjoqvist F. (1984) Phenotypic consistency in hydroxylation of desmethylimipramine and debrisoquine in healthy subjects and in human liver microsomes. *Clin. Pharmacol. Ther.* 36: 677-682.

Stark P., Fuller R.W. and Wong D.T. (1985) The pharmacologic profile of fluoxetine. *J. Clin. Psychiat.* 46: 7-13.

Steranka L.R. (1982) Long-term decreases in striatal dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid after a single injection of amphetamine in iprindole-treated rats: Time course and time-dependent interactions with amfonelic acid. *Brain Res.* 234: 123-136.

Stevens J.C. and Wrighton S.A. (1993) Interaction of the enantiomers of fluoxetine and norfluoxetine with human liver cytochromes-P450. *J. Pharmacol. Exp. Ther.* 266: 964-971.

Straka R.J., Johnson K.A., Marshall P.S. and Remmel R.P. (1990) Analysis of metoprolol enantiomers in human serum by liquid chromatography on a cellulose-based chial stationary phase. *J. Chromatogr.* 530: 83-93.

Su P., Coutts R.T., Baker G.B. and Daneshtalab M. (1993) The quantitative analysis of three metabolites of imipramine produced by cytochrome P450IID6 expressed in a human cell line. *Xenobiotica* 23: 1289-1298.

Suckow R.F., Zhang M.F. and Cooper T.B. (1992) Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization. *Clin. Chem.* 38: 1756-1761.

Sugrue M.F. (1983) Do antidepressants possess a common mechanism of action? *Biochem. Pharmacol.* 32: 1811-1817.

Sulser F., Vetulani J. and Mobley P.L. (1978) Mode of action of antidepressant drugs. *Biochem. Pharmacol.* 27: 257-261.

Sulser F. (1982) Giovanni Lorenzini Foundation Lecture - Antidepressant drug research: its impact on neurobiology and psychobiology. In: *Typical and Atypical Antidepressants: Molecular Mechanisms.* Costa E. and Racagni G. eds. Raven Press, New York, NY pp. 1-20.

Sulser F. and Mishra R. (1983) The discovery of tricyclic antidepressants and their mode of action. In: *Discoveries in Pharmacology, Vol I: Psycho- and Neuropharmacology.* Parnham M.J. and Brunivels J. eds. Elsevier Science Publishers B.V., Amsterdam, pp 233-247.

Suranyi-Cadotte B.E., Bodnoff S.R. and Welner S.E. (1990) Antidepressant-anxiolytic interactions: Involvement of the benzodiazepine-GABA and serotonin systems. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 14: 633-654.

Suranyi-Cadotte B.E., Dam T.V. and Quirion R. (1984) Antidepressant-anxiolytic interaction density of benzodiazepine receptors in rat brain following chronic administration of antidepressants. *Eur. J. Pharmacol.* 106: 673-675.

Suzdak P.D. and Gianutsos G. (1986) Effect of chronic imipramine or baclofen on $GABA_B$ binding and cyclic AMP production in cerebral cortex. *Eur. J. Pharmacol.* 131: 129-133.

Szekely A.M., Barbaccia M.L. and Costa E. (1987) Effect of a protracted antidepressant treatment of signal transduction and 3H(-)-baclofen binding at $GABA_B$ receptors. *J. Pharmacol. Exp. Ther.* 243: 155-159.

Tait A.C. and Todrick A. (1975) What are tricyclic antidepressant drugs? *Br. J. Pharmacol.* 2: 93-94.

Takahashi H., Tamura A., Ogata H. and Masuhara K. (1990) Simultaneous determination of disopyramide and its mono-N-dealkylated metabolite enantiomers in human plasma and urine by enantioselective HPLC. *J. Chromatogr.* 529: 347-358.

Tedeschi R.E., Tedeschi D.H., Cook L., Mattis P.A. and Fellows E.J. (1959) Some neuropharmacological observations on SKF trans 385 (2-phenylcyclopropylamine hydrochloride), a potent inhibitor of monoamine oxidase. *Fed. Proc.* 18: 451.

Teicher M.H., Glod C. and Cole J.O. (1990) Emergence of intense suicidal preoccupation during fluoxetine treatment. *Am. J. Psychiat.* 147: 207-210.

Testa B. (1986) The chromatographic analysis of enantiomers in drug metabolism studies. *Xenobiotica* 16: 265-279.

Testa B. (1990) Mechanisms of chiral recognition in pharmacology. *Acta Pharm. Nord.* 2:139-144.

Testa B. and Jenner P. (1978) Stereochemical methodology. In: *Drug Fate and Metabolism: Methods and Techniques*. Garrett E.R. and Hirtz J.L. eds. Dekker, New York, NY, p. 143-193.

Thomare P., Wang K., Vandermeerschmougeot V. and Diquet B. (1992) Sensitive micromethod for column liquid chromatographic determination of fluoxetine and norfluoxetine in human plasma. *J. Chromatogr.* 583: 217-221.

Todd K.G., McManus D.J. and Baker G.B. (1992) Effects of chronic administration of antidepressants on ^{3}H-flunitrazepam binding in rat brain. *Proc. 9th Gen. Meet. Eur. Soc. Neurochem.*, Dublin, Ireland, *Neurochem. Int.* 21(suppl): C2.

Tollefson G.D. (1983) Monoamine oxidase inhibitors: A review. *J. Clin. Psychiatry* 44: 280-288.

Tomb D.A. (1988) *Psychiatry for the House Officer, 3rd ed.* Williams & Wilkins, Baltimore, MD, p. 36.

Torok-Both G.A., Baker G.B., Coutts R.T., McKenna K.F. and Aspeslet L.J. (1992) Simultaneous determination of fluoxetine and norfluoxetine enantiomers in biological samples by gas chromatography with electron-capture detection. *J. Chromatogr. Biomed. Appl.* 579: 99-106.

Tucker G.T. and Lennard M.S. (1990) Enantiomer specific pharmaco-
kinetics. *Pharmacol. Ther.* 45: 309-329.

Tuomisto J. and Smith D.F. (1986) Effects of tranylcypromine enantiomers
on monoamine uptake and release and imipramine binding. *J. Neural
Transm.* 65: 135-145.

Turner S.M., Jacob R.B., Beidel D.C. and Griffin S. (1985) A second case
of mania associated with fluoxetine. *Am. J. Psychiat.* 142: 274-275.

Tyrer J. (1976) Towards a rational therapy with monoamine oxidase
inhibitors. *Brit. J. Psychiat.* 128: 354-360.

Tyrer S.P., Marshall E.F. and Griffiths H.W. (1990) The relationship
between response to fluoxetine, plasma drug levels, imipramine
binding to platelet membranes and whole-blood 5-HT. *Prog. Neuro-
Psychopharmacol. Biol. Psychiat.* 14: 797-805.

U'Prichard D.C., Greenberg D.A., Sheehan P.P. and Snyder S.H. (1978)
Tricyclic antidepressants: therapeutic properties and affinity for
alpha-noradrenergic receptor binding sites in the brain. *Science* 199:
197-198.

Vandel S., Bertschy G., Bonin B., Nezelof S., Francois T.H., Vandel B.,
Sechter D. and Bizouard P. (1992) Tricyclic antidepressant plasma
levels after fluoxetine addition. *Neuropsychobiology* 25: 202-207.

Vaughan D.A. (1988) Interaction of fluoxetine with tricyclic antidepressants.
Am. J. Psychiatr. 145: 1478.

Walle T. and Walle U.K. (1986) Pharmacokinetic parameters obtained with
racemates. *Trends Pharmacol. Sci.* 7: 155-158.

Weber-Grandke H., Hahn G., Mutschler E., Mohrke W., Langguth P. and
Spahn-Langguth (1993) The pharmacokinetics of tranylcypromine
enantiomers in healthy subjects after oral administration of racemic
drug and the single enantiomers. *Br. J. Clin. Pharmacol.* 36: 363-
365.

Weilburg J.B., Rosenbaum J.F., Biederman J., Sachs G.S., Pollack M.H. and Kelly K. (1989) Fluoxetine added to non-MAOI antidepressants converts nonresponders to responders: A preliminary report. *J. Clin. Psychiatry* 50: 447-449.

Wernicke J.F. (1985) The side effect profile and safety of fluoxetine. *J. Clin. Psychiat.* 46: 59-67.

Westermeyer J. (1991) Fluoxetine-induced tricyclic toxicity: extent and duration. *J. Clin. Pharmacol.* 31: 388-392.

White K. and Simpson G. (1981) Combined MAOI-tricyclic antidepressant treatment: A reevaluation. *J. Clin. Psychopharmacol.* 1: 264-282.

Williams K. and Lee E. (1985) Importance of drug enantiomers in clinical pharmacology. *Drugs* 30: 333-354.

Willner P. (1983a) Dopamine and depression: a review of recent evidence. I. Empirical studies. *Brain Res. Rev.* 6: 211-224.

Willner P. (1983b) Dopamine and depression: a review of recent evidence. II. Theoretical approaches. *Brain Res. Rev.* 6: 225-236.

Willner P. (1983c) Dopamine and depression: a review of recent evidence. III. The effects of antidepressant treatments. *Brain Res. Rev.* 6: 237-246.

Willner P., McGuirk J., Phillips G. and Muscat R. (1990) Behavioural analysis of the anorectic effects of fluoxetine and fenfluramine. *Psychopharmacol. (Berl)* 102: 273-277.

Witte D.T., Ensing K., Franke J.-P. and De Zeeuw R.A. (1993) Development and registration of chiral drugs. *Pharm. World Sci.* 15: 10-16.

Wong D.T. and Bymaster F.P. (1976) The comparison of fluoxetine and nisoxetine with tricyclic antidepressants in blocking the neurotoxicity of p-chloroamphetamine and 6-hydroxydopamine in the rat brain. *Res. Commun. Chem. Pathol. Pharmacol.* 15: 221-231.

Wong S.H.Y., Dellafera S.S., Fernandes R. and Kranzler H. (1990) Determination of fluoxetine and norfluoxetine by high-performance liquid chromatography. *J. Chromatogr.* 499: 601-608.

Wong D.T., Bymaster F.P., Horng J.S. and Molloy B.B. (1975) A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *J. Pharmacol. Exp. Ther.* 193: 804-811.

Wong D.T., Bymaster F.P., Reid L.R., Fuller R.W. and Perry D.W. (1985) Inhibition of serotonin uptake by optical isomers of fluoxetine. *Drug Dev. Res.* 6: 397-403.

Wong D.T., Bymaster F.P., Reid L.R., Mayle D.A., Krushinski J.H. and Roberson D.W. (1993) Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain. *Neuropsychopharmacol.* 8: 337-344.

Wong D.T., Bymaster F.P., Reid L.R. and Threlkeld P.G. (1983) Fluoxetine and two other serotonin uptake inhibitors without affinity for neuronal receptors. *Biochem. Pharmacol.* 32: 1287-1293.

Wood P.L. and Tadimeti S.R. (1990) GC/MS and GC/MS/MS analysis of monoamines, indoles, trace amines, and their associated precursors and metabolites. In: *Mass Spectrometry of Biological Materials.* McEwen C.N. and Larsen B.S. eds. Marcel Dekker, Inc., New York, NY, pp 217-256.

Wozniak T.J., Bopp R.J. and Jensen E.C. (1991) Chiral drugs: an industrial analytical perspective. *J. Pharm. Biomed Anal.* 9: 363-382.

Youdim M.B.H., Aronson J.K., Blau K., Green A.R. and Grahame-Smith D.G. (1979) Tranylcypromine ("Parnate") overdose: Measurement of tranylcypromine concentrations and MAO inhibitory activity and identification of amphetamines in plasma. *Psychol. Med.* 9: 377-382.

Young R.C. (1991) Hydroxylated metabolites of antidepressants. *Psychopharmacol. Bull.* 27: 521-532.

Young J.P., Coleman A. and Lader M.H. (1987) A controlled comparison of fluoxetine and amitriptyline in depressed out-patients. *Br. J. Psychiatry* 151: 337-340.

Zeller E.A. and Barsky J. (1952) In vivo inhibition of liver and brain monoamine oxidase by 1-isonicotinyl-2-isopropyl hydrazine. *Proc. Soc. Exp. Biol.* 81: 459-461.

Zief M. and Crane L.J. eds. (1988) *Chromatographic Chiral Separations.* Marcel Dekker, New York, NY.

Ziegler V.E., Biggs J.T., Rosen S.H., Meyer D.A. and Preskorn S.H. (1978) Imipramine and desipramine plasma levels: Relationship to dosage schedule and sampling time. *J. Clin. Psychiatry* 39: 660-663.

Zirkle C.L., Kaiser C., Tedeschi D.H., Tedeschi R.E. and Burger A. (1962) 2-Substituted cyclopropylamines-II. Effect of structure on monoamine oxidase-inhibitory activity as measured by in vivo potentiation of tryptamine convulsions. *J. Med. Chem.* 5: 1265-1284.

Zis A.P. and Goodwin F.K. (1979) Novel antidepressants and the biogenic amine hypothesis of depression. *Arch. Gen. Psychiatry* 36: 1097-1107.