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## Neurochemical Studies on the Metabolism and Effects of Catecholic Isoquinolines

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NEUROCHEMICAL STUDIES ON THE METABOLISM AND  
EFFECTS OF CATECHOLIC ISOQUINOLINES

by

Thomas C. Origitano

A Dissertation Submitted to the Faculty of the Graduate School  
of Loyola University of Chicago in Partial Fulfillment  
of the Requirements for the Degree of  
Doctor of Philosophy

January

1981

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I wish to acknowledge the inspiration and love that has been my good fortune to gain from Judith Melaine Hines, my wife.

Above all, I wish to thank God for His Son and His grace which made my work worthwhile.

## VITA

Thomas Charles Origitano was born in Chicago, Illinois, on May 15, 1954, the son of Thomas C. Origitano and Marian D. Origitano. After graduating from St. Joseph's High School, Westchester, Illinois, in 1972, he received the Bachelor of Science degree in Chemistry and Biology from Mac Murray College in 1976. At that time he enrolled in the Department of Biochemistry and Biophysics of Loyola University of Chicago.

At Loyola, he began his doctoral research under the tutelage of Dr. Michael Collins studying aspects of neurochemical metabolism and effects of catechol isoquinolines associated with alcoholism. In August of 1976, Mr. Origitano was awarded a Basic Science Fellowship. In July of 1978, he was granted a research assistantship in the laboratory of Dr. Collins covering the period until the completion of his doctoral work. In August of 1980, he began studies leading to the degree of Doctor of Medicine in the Stritch School of Medicine of Loyola University of Chicago.

Mr. Origitano was married to Judith Melaine Hines on January 21, 1978.

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## CHAPTER I

### INTRODUCTION AND REVIEW OF THE LITERATURE

#### A. Objectives

In recent years it has become evident that endogenous compounds are produced from the condensation of catecholamines (CAs) with aldehydes or  $\alpha$ -keto acids in several pathological conditions. Of interest in this dissertation are the simple, 1-alkyl, CA-derived condensation products, the tetrahydroisoquinolines (THIQs) which are associated with ethanol (EtOH) metabolism. The close structural similarities between CA-derived THIQ alkaloids and CA neurotransmitters (NTs) suggests that these alkaloids may be uniquely qualified to modify biogenic amine function and metabolism.

Little is specifically known about catechol-THIQ metabolic routes in the nervous system and to what extent these pathways differ from conventional CA metabolic routes. It is believed that O-methylation and, possibly, conjugation are the major metabolic routes for THIQs. However, it is not known whether the in vivo O-methylation of isoquinolines is

similar or identical to that of their open-chain precursors, or whether significant stereoselective differences might exist between isoquinolines and CAs. These factors were investigated in this dissertation.

A second possible alternative mode of THIQ metabolism, oxidation to isoquinolines, was surveyed in vivo as a route to the potential formation of intermediates which could bind covalently to cellular nucleophiles.

In view of known formation of THIQs and lack of knowledge about the metabolic consequences of these now-recognized endogenous CA derivatives, studies of their effects on the steady state levels of biogenic amines and acid metabolites in vivo also were pursued.

In order to study THIQ metabolism and to evaluate the pharmacodynamic events initiated by the presence of THIQ alkaloids and their possible metabolites, highly sensitive and specific assays were developed for routine, rapid, analysis of THIQs, biogenic amines and their respective metabolites in vivo.

In summary, the purpose of the work described in this dissertation is to elucidate with greater clarity the discrete metabolic consequences of THIQs in mammalian systems, with the underlying hypothesis that these condensation products may have neuroregulatory and/or neuropathological effects.

## B. Background

### 1. Nonenzymatic Condensations: Introduction and Overview

Recent experimental findings demonstrate that non-enzymatic, bimolecular condensation reactions are definite occurrences in mammalian homeostatic and disease states. These reactions, first characterized in plants, can be generally defined as multistep processes involving an amine or amino acid and a carbonyl compound, in equilibrium with the first product, an imine (Schiff's base), which, if the amine is derived from a CA, undergo an irreversible intramolecular cyclization to form a second heterocyclic product, a THIQ molecule (Fig. 1).

Increased levels of catecholamine-carbonyl condensation products are evident in at least three pathological conditions in man: phenylketonuria, Parkinson's disease, and alcoholism. The products have been suggested to be involved in some way in the sequelae and etiology of these disease states, but their involvement is not specifically known.

Parkinson's disease, a progressive neurological disorder, is characterized by degeneration of the substantia nigra and associated subnormal striatal dopamine (DA) levels. The clinical characteristics of

tremor, bradykinesia and rigidity can be relieved to varying degrees by oral L-3,4-dihydroxyphenylalanine (L-DOPA) treatment in conjunction with peripheral L-DOPA-decarboxylase inhibitors. In 1973, Sandler et al. (1) unequivocally demonstrated in vivo biosynthesis of THIQs in Parkinsonian patients undergoing L-DOPA treatment who were given test doses of EtOH. Both salsolinol (SAL), the DA/acetaldehyde, (AcA) condensation product, and tetrahydropapaveroline (THP), the DA/3,4-dihydroxyphenylacetaldehyde (DHPAcA) condensation product, were found in significant amounts (Fig. 2).

Further evidence for THIQ synthesis was the finding of tetrahydroprotoberberines (THPBs) in the urine of Parkinsonian patients undergoing L-DOPA treatment (2). THPB alkaloids are suggested to be further "condensation" products derived from in vivo enzymatic conversion of THP via a liver S-adenosyl-methionine-N-methyl transferase, followed by apparent oxidation and ring closure (2, 4) (Fig. 3).

In 1977, Coscia et al. (3) found yet another group of THIQs associated with L-DOPA treatment and Parkinsonism. The condensation of DA with phenylpyruvates yielded the two 1-benzyl-1-carboxyl-THIQs, norlaudanosolinecarboxylic acid (NLCA) and 3-O-

methylnorlaudanosolinecarboxylic acid (MNLCA) (Fig. 4). Interestingly, MNLCA was found to be a normal constituent in control urines as well as in human and rat brain (3).

Phenylketonuria (PKU) is a well-known disease resulting from a genetic deficiency in functional phenylalanine hydroxylase. As a result, large amounts of phenylalanine, phenethylamine (PEA) and phenylpyruvate accumulate in the tissues and body fluids of PKU individuals (5). Initial studies by Y.H. Loo (6) in 1967 characterized an aldimine condensate of phenethylamine and pyridoxal in the urines of human PKU patients and in the brain and urines of rats made "phenylketonuric" (Fig. 5).

In 1979, Lasala and Coscia, utilizing computerized gas chromatography (GC)/mass spectrometry (MS), quantitated significant levels of the phenylpyruvate/DA condensation product, 3,4-deoxynorlaudanosolinecarboxylic acid (DNLCA) (Fig. 6), in the urine of PKU children and in the urine and brains of rats with experimentally-induced hyperphenylalaninemia (7).

In 1979, Collins and coworkers reported the first evidence for the endogenous formation of THIQs in alcoholic humans with the finding of SAL and O-methylated SAL (isomer unknown) in the urines

of patients undergoing detoxification (Fig. 7) (8, 9). Consistent with these findings, Borg et al. (10) have recently found SAL and O-methylated SAL (isomer unknown) in the urine and lumbar spinal fluid of alcoholic patients during and after prolonged intoxication. Earlier work by Collins and Bigdeli provided evidence for the formation of SAL in DA-rich brain regions in acute EtOH-intoxicated rats pretreated with pyrogallol, a potent catechol-O-methyl transferase (COMT) and aldehyde dehydrogenase inhibitor (11, 12). Hamilton et al. provided important additional evidence for in vivo CNS formation of THIQ alkaloids with his finding of O-methylated SAL (isomer unknown) in the corpus striatum of mice chronically exposed only to EtOH vapors (13), and no other pharmacological agents.

In summary, non-enzymatic products of CA/carbonyl condensation reactions are apparently present normally in human metabolism. However, during various pathological conditions their levels appear to be elevated (14). The possible physiological and metabolic roles of these compounds are the central questions addressed in this dissertation and will be discussed in detail. Of particular interest are the simple catechol isoquinolines formed during acute



and/or chronic alcohol ingestion.

## 2. Metabolic Considerations of EtOH Ingestion

EtOH is an unusual psychoactive drug because of its significant interactions and contributions to cellular metabolism (15). The primary metabolic fate of ingested EtOH is conversion to acetate, which is then available to enter cellular metabolism at several portals (16). EtOH is primarily oxidized in the liver by a cytosolic, nicotinamide-adenine-dinucleotide (NAD<sup>+</sup>) dependent alcohol dehydrogenase (ADH) (17 - 19) (Fig. 8). AcA produced during the oxidation of EtOH in the liver is metabolized to acetate by mitochondrial NAD<sup>+</sup> dependent aldehyde dehydrogenase (ALDH) (20 - 23). Although the liver has the enzymatic capacity to metabolize EtOH-derived acetate to carbon dioxide (CO<sub>2</sub>), most of the acetate is released into the circulation to be metabolized by other organs (24, 25).

Concomitant with the oxidation of EtOH, cytosolic NAD<sup>+</sup> is reduced to NADH by cytosolic ADH. Most of the peripheral metabolic changes associated with chronic EtOH ingestion can be traced back to the substantial decreases in the free NAD<sup>+</sup>/NADH ratio (18, 26).

The decrease in the free NAD<sup>+</sup>/NADH ratio in

hepatocytes has been shown to have profound effects on the intermediary metabolism of these cells (19, 27). This change in redox potential during chronic EtOH metabolism has been implicated as the cause of decreased lipid oxidation, increased fatty acid synthesis (28), hyperlactacidemia (29), decreased glucose utilization (30) and diminished activity of the citric acid cycle (TCA) (31). Under these conditions the reoxidation of NADH to NAD<sup>+</sup> (the requisite cofactor for cytosolic ADH) in the mitochondria is rate-limiting with regard to in vivo oxidation of EtOH (32).

In lieu of these findings, it is interesting to note that chronic ingestion of EtOH by rats (27, 33) and humans (34) results in an increased ability to oxidize EtOH. Although ADH is the major enzyme for the oxidation of EtOH in vivo (18, 19), at least two other enzyme systems, microsomal EtOH oxidizing system (MEOS) and catalase are thought to contribute to the oxidation of EtOH to AcA. Inducible changes in the activity of MEOS and catalase have been proposed to be responsible for the increases in EtOH oxidation associated with chronic EtOH ingestion (35, 36).

In addition to the metabolic consequences resulting from the oxidation of EtOH to AcA, many of

the pharmacological effects associated with EtOH ingestion have been ascribed to direct effects of AcA in animal tissues. Most AcA generated in the liver (as well as other tissues) is metabolized to acetate by ALDH (37 - 39). In normal individuals given a moderate test dose of EtOH, a relatively small portion of AcA escapes metabolism in the liver and enters circulation where it is quickly converted to acetate by other organs (40, 41). Circulating AcA levels are maintained at a steady-state level by a balance between the rate of production and catabolism (42).

Chronic consumption of EtOH has been observed to alter these "steady-state" levels of AcA in humans and animals. It has been suggested that these observed changes are the consequence of adaptive increases in EtOH metabolism with concomitant decreases in AcA metabolism (35, 43). Chronic, elevated AcA levels have been shown to produce structural damage in mitochondria which in turn leads to a decreased capacity for the metabolism of AcA (43, 44).

Human alcoholics have been found to have significantly higher circulating AcA levels compared to controls given a test dose of EtOH (40, 45). These observations led to the proposal that a "vicious" metabolic cycle may develop during chronic alcohol

abuse (Fig. 9), where, chronic EtOH ingestion leads to induced EtOH metabolism and concomitant decreases in AcA metabolism (26, 44). Subsequently, tissues of the body are perfused with increasing levels of AcA. High AcA levels are believed to contribute not only to the pathology at the primary site of AcA metabolism (i.e., the liver), but also in other peripheral tissues (46) and possibly the brain.

Little oxidation of EtOH occurs in brain tissue due to very low ADH activity (1/4000 of liver activity) (47 - 49). However, EtOH-derived metabolites and products (AcA and acetate) produced in the periphery are metabolized by brain. AcA is primarily metabolized to acetate by brain ALDH which has been noted in the cytosol, mitochondria and microsomes of brain tissue (50). Brain ALDHs located in the parenchymal cells of capillaries, glia and neurons are even suggested to form a metabolic blood-brain barrier to AcA (51, 52). Support for this concept comes from data which reveal multiphasic increases in brain AcA content corresponding to the saturation of various brain ALDHs by AcA (52, 53) as blood AcA levels increase.

### 3. Neuroamine Condensation Products and EtOH:

#### Pertinent Theories

Recently several hypotheses have attempted to

relate the potential biosynthesis of CA/aldehyde condensation products or their biotransformed products in mammalian tissue to some of the actions mediating the autonomic and behavioral effects of chronic EtOH ingestion. It has been proposed that these compounds may also take part in the underlying biochemical mechanisms of EtOH tolerance and dependence (54).

The first hypothesis envisions EtOH, through its active metabolite, Aca, inducing alterations in the metabolism of CAs to produce complex aberrant metabolites (alkaloids) having unique pharmacological activities (55, 56). The biogenesis of the proposed complex alkaloid products would be the result of the known competitive inhibition of aromatic ALDH (57, 58) by elevated Aca levels (40, 45) generated during chronic EtOH ingestion. The inhibition of NAD<sup>+</sup> linked ALDH would result in a localized increase in the highly reactive biogenic aldehydes generated by monoamine oxidase (MAO) in tissues rich in CAs. Deprived of their normal metabolic route(s), the catechol-aldehydes would be available for condensation with parent amines (Fig. 10).

This hypothesis is based on the formation of THP. THP is the requisite intermediate in the biosynthesis of morphine in the opium poppy Papaver

somniferum (59). As early as 1938, Holtz and Heise (60) speculated that biogenic amines and their aldehyde derivatives may condense in animal tissues. In 1964, Holtz et al. provided evidence for the in vitro formation of THP in rat liver homogenates incubated with DA (61). Davis and Walsh, the major proponents of the complex-THIQ theory, utilizing  $^{14}\text{C}$ -DA, provided evidence for THP formation in rat brainstem homogenates and showed that formation was enhanced by addition of increasing amounts of AcA to preparations (55, 62). In similar experiments, Davis et al. (63) incubated  $^{14}\text{C}$ -norepinephrine (NE) in rat brainstem homogenate and isolated a substance which apparently had the physical characteristics of a 4-hydroxylated-THP. Product formation was enhanced by the addition of barbiturates to the incubate. Further evidence to support complex alkaloid formation, as mentioned in section A., Background, came from the biotransformation of administered THP to complex THPBs in rat brain and liver preparations, and the detection of THPBs in the urines of Parkinson's patients undergoing L-DOPA therapy (64).

Stimulated by these initial findings, numerous investigators have studied the possible relationships between the actions of opiates and that of EtOH (65 - 68). However, experiments involving THIQ interactions

with opiate systems (through quantitation of naloxone displacement and analgesic effects) have yet to provide consistent data which would elucidate an accepted connection between the molecular mechanisms underlying EtOH and opiate "addiction."

Independently, but simultaneously, Cohen and Collins (69 - 71) put forth a second hypothesis delineating the relationship between THIQ formation and alcohol ingestion. The major premise of this hypothesis was that ACA, in competition with rapid oxidation and binding to macromolecules, can condense directly with CAs to form simple 1-alkyl-THIQ alkaloids (Fig. 11). The similarity in structure between the THIQs and parent CAs gave rise to the possibility that THIQs could interact with mechanisms that normally regulate the physiological properties of CAs. In this way the THIQs, acting as "false" NTs, might contribute to the development of EtOH dependence and/or to the general pharmacological effects of EtOH ingestion (72).

Although the remainder of this dissertation will not deal further with the hypothesis concerning "morphine-like" alkaloids and alcoholism, THP and other 1-benzyl-THIQs will be included in the discussion on the properties and interactions of THIQs

with biogenic amine systems.

#### 4. Tetrahydroisoquinoline Alkaloid Synthesis and Biosynthesis

##### a. Plant Synthesis of THIQs

The THIQ alkaloids are naturally occurring products in plants; many are known to exhibit pharmacological actions on mammalian nervous and cardiovascular systems (73). Examples of naturally occurring THIQs are anhalamine and salsoline, which are found in desert cacti (74), and the saguaro alkaloid gigantine, which causes hallucinogenic reactions in squirrel monkeys and cats (75) (Fig. 12). In plants, the formation of THIQ alkaloids is believed to be the result of the reaction of  $\alpha$ -keto acids with an amine followed by decarboxylation (76). Morphine biosynthesis in the opium poppy passes through the 1-benzyl-THIQ, THP (77). It is believed that THP is formed from the condensation of DA with DHPAcA followed by enzymatic decarboxylation (78) to a 3,4-dihydroisoquinoline (DHIQ). The DHIQs and their quaternary equivalents are established intermediates in the biosynthesis of plant alkaloids; chirality is developed through asymmetric reduction at this stage (79) (Fig. 13). Morphine and codeine are subsequently formed by a series of ring closures and



methyations followed by partial reduction of the DA-derived benzene ring.

SAL and THP formation in mammalian systems by the THIQ-1-carboxylic pathway is an interesting possibility in light of the finding of several 1-carboxy-THP derivatives in the urine of Parkinsonian and PKU individuals (3, 7).

#### b. General Synthesis and Biosynthesis In Vitro

As early as 1934 researchers showed that DA and epinine would undergo a spontaneous condensation with AcA under physiological conditions to form THIQ alkaloids (80). The formation of THIQs from the condensation of phenethylamine derivatives and aldehydes (or  $\alpha$ -keto acids) is characterized by the Pictet-Spengler condensation reaction (81) (Fig. 1). Usually, strong acid and heat have been used to catalyze the reaction (82). Pictet-Spengler condensation reactions of CAs (DA, NE, L-DOPA, and epinephrine; EPI) under physiological conditions with various aldehydes such as formaldehyde (HCHO) (70), AcA (83), DHPACA (62), and pyridoxal phosphate (84) have been studied. When a phenolic substituent is located para to the site of ring closure, the reaction was known to occur at ambient temperature and neutral pH (69, 80). This facilitation is negligible under physiological

conditions if the hydroxyl group is O-methylated or meta to the site of ring closure (84).

THIQ synthesis has been studied in tissue homogenates (62, 85), isolated intact cells, and organ preparations (70, 86). Perfusion of cow adrenals with AcA or HCHO yielded the corresponding THIQs of NE and EPI (70, 86). When high concentrations of HCHO were used, total conversion of EPI and NE to THIQs was observed (69). THIQ synthesis has also been observed when perfusion with AcA was in the range of that seen in the blood of human subjects ingesting EtOH (87).

## 5. Biogenic Amine Biosynthesis and Metabolism

Before discussing interactions of THIQs with biogenic amine metabolism, biogenic amine biosynthesis and metabolism will be considered. In the mammalian CNS the CAs, EPI, NE and DA and the indoleamine, serotonin (5HT), are putative neurotransmitters.

### a. Catecholamines

Tyrosine is the only dietary amino acid precursor of DA, NE, and EPI (88). Phenylalanine through its conversion in the liver to tyrosine by phenylalanine hydroxylase can also contribute to CA biosynthesis (89). The rate limiting step in the biosynthesis of CAs is the conversion of tyrosine to L-DOPA by tyrosine hydroxylase (TH) (90). TH is a cytoplasmic

enzyme requiring reduced bipterin (91) as a cofactor, and is under metabolic feedback inhibition by CA products (92). L-DOPA is rapidly decarboxylated to DA by L-DOPA decarboxylase (DDC) (93, 94). Because of its low specificity for aromatic amino acids, DDC has been given the name aromatic acid decarboxylase (AAD). It is known to decarboxylate a variety of aromatic amino acids such as 5-hydroxytryptophan, phenylalanine, tryptophan and tyrosine.

DA is converted to NE by the enzyme DA- $\beta$ -hydroxylase (DBH) (95), an ascorbate -requiring, copper containing oxygenase (96). NE is converted to EPI by the action of phenylethanolamine-N-methyl-transferase (PMNT) utilizing S-adenosyl-L-methionine (SAM) as a methyl donor (97). Evidence is accumulating that EPI is a putative neurotransmitter in the brainstem (98).

The only appreciable endogenous metabolites of DA in vivo are 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (4-hydroxy-3-methoxy-phenylacetic acid, HVA) and 3-O-Methyl-DA(3M-DA) (100 - 102). By use of GC, the endogenous occurrence of 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) and 3,4-dihydroxyphenylethylene glycol (DOPEG) have been demonstrated as the CNS metabolites of NE in vivo in many species

(99, 103). Little is known about central metabolism of EPI. The main metabolites of intraventricularly administered  $^3\text{H}$ -EPI were metanephrine (MET) and vanillylmandelic acid (3-methoxy-4-hydroxy-mandelic acid, VMA) (260). CAs are oxidatively deaminated to their corresponding catechol-aldehydes by a multiform, mitochondrial bound enzyme, MAO. Investigators have been able to distinguish two forms of this enzyme by the use of the selective-irreversible inhibitors clorgyline and deprenyl (104, 105). These studies indicate that specific substrates for the A species of the enzyme include 5HT, NE and normetanephrine (NORMET) whereas benzylamine and PEA are the substrates for the B species and DA is acted on by both. The aldehydes are converted by the actions of aromatic aldehyde dehydrogenases or reductases to corresponding acids or alcohols.

In addition to oxidative deamination, O-methylation plays a role in the metabolism of CAs. COMT catalyzes the transfer of a methyl group preferentially to the "meta" hydroxyl of substrates containing the catechol moiety, in the presence of SAM as the methyl donor and magnesium ions ( $\text{Mg}^{++}$ ) as activator (106). To a very limited extent para O-methylation of endogenous substrates is seen in vivo (107).

para O-methylation of CAs has been shown to be increased in such pathological conditions as Parkinson's disease (108), neuroblastoma (109, 110) and pheochromocytoma (111). COMT shows low specificity, having as some of the physiological substrates, EPI, NE, DA, L-DOPA, DOPEG, and DOPAC (112). Summaries of possible metabolic routes of NE and DA can be found in Figures 14 and 15, respectively.

b. Serotonin (5HT)

Tryptophan is the immediate precursor of 5HT and is converted by the enzyme tryptophan hydroxylase to 5-hydroxytryptophan (5HTP) (113). 5HT synthesis is controlled at the level of tryptophan hydroxylase. 5HTP is converted to 5HT by AAD. The main metabolic route for 5HT in the CNS and periphery appears to be deamination (MAO-A) and subsequent oxidation to 5-hydroxyindoleacetic acid (5HIAA) (114). However, following ingestion of large amounts of EtOH, a shift in peripheral 5HT metabolism is observed. Increases in the reductive metabolism of 5HT to 5-hydroxytryptophol (5HTOL) with a concomitant decrease in 5HIAA are seen in the liver and urine, indicating probable inhibition of aromatic aldehyde dehydrogenase by AcA (115) (Fig. 16).

### c. General Neurotransmitter Events

In general the biogenic amine NTs are stored in high concentrations in selective areas of the peripheral and central nervous systems. They are actively sequestered into dense-granular vesicles located in the presynaptic endings of neurons. Stimulation of the nerve releases only those transmitters bound within the dense-core vesicles. NTs released into the synaptic cleft (interneuronal space) can react with specific post-synaptic receptor sites, initiating a sequence of molecular events leading to a post-synaptic response. Inactivation of NTs occurs rapidly, primarily due to reuptake into the pre-synaptic nerve terminal (>80%). Neuronal uptake is an unidirectional, sodium-ion (Na<sup>+</sup>) dependent, stereospecific, energy requiring mechanism which follows Michaelis-Menten saturation kinetics. After reuptake across the neuronal membrane, NTs can be reincorporated into dense-core vesicles. Those NTs not stored become available for catabolism by MAO and COMT.

### 6. Transmitter-Like Properties of THIQs

The similarities between THIQ and CA structure led to the theory that the THIQs may act as "false" NTs (69 - 71). The THIQs would then exert their influence by interacting with mechanisms that normally

regulate the physiological properties of CAs. In support of this theory, investigators have noted that THIQs interact with axonal and vesicular transport systems leading to their uptake and storage in CA neurons, are released from nerve terminals along with CAs, and interact with pre- and post-synaptic receptors.

THIQ alkaloids have been shown to block the uptake of CAs by rat synaptosomes and tissue slice preparations. The addition of THIQs to rat brain synaptosomes or tissue slices incubated with  $^3\text{H}$ -CAs resulted in an efflux of  $^3\text{H}$ -CAs, probably due to displacement of the  $^3\text{H}$ -CAs from storage vesicles (116 - 118). 6,7-dihydroxytetrahydroisoquinoline [6,7-(OH)<sub>2</sub>THIQ] has been shown to be taken up and stored in sympathetic neurons of rat iris (119, 120). Similarly, THIQs were shown to be taken up by rat heart, salivary gland, iris, and mouse adrenal (121), by capillary endothelial cells, unidentified neuronal components of rat hypothalamus and hypophysis (122), and by synaptosomal fractions of rat brain (123). THIQs have also been shown to be weak inhibitors of 5-HT uptake into rat brain slices (124, 125). Uptake was blocked by traditional inhibitors of the axonal membrane such as cocaine and desmethyylimipramine (119 - 121). These results suggest that THIQs and CAs compete with

each other for transport sites on the neuronal presynaptic membrane (126).

THIQs formed during the perfusion of cow adrenals with ACA and HCHO were retained in the gland and were actually found to be stored in the CA-binding vesicles (127, 128). 6,7-(OH)<sub>2</sub>THIQ was shown to be stored in the CA-binding vesicles of the rat iris and pineal gland (129). THP was found to be incorporated into small granular vesicles when incubated with rat caudate slices (130). Vesicular storage of THIQs in CA-binding vesicles has been shown to be by a reserpine-resistant mechanism both in vitro (119, 129) and in vivo (120). Reserpine blocks CA uptake into storage vesicles but does not affect the axonal uptake mechanisms. Therefore, THIQs apparently share axonal transport mechanisms with CAs but binding to storage vesicles occurs by a mechanism which differs from endogenous amine binding (131).

THIQs have been shown to be released upon nerve stimulation via the same mechanisms that control release of endogenous NTs. EPI and NE condensation products formed during perfusion of cow adrenals with ACA were released with endogenous EPI and NE upon stimulation with acetylcholine or succinylcholine (132, 133). In a classical experiment, Mytilineou



et al. demonstrated the release of 6,7-(OH)<sub>2</sub> THIQ from NE nerve terminals in rat iris upon electrical stimulation in vivo (120). Rats were first depleted of endogenous stores of NE by pretreatment with  $\alpha$ -methyl-para-tyrosine methyl ester and reserpine. Subsequently rats received intravenous injections of 6,7-(OH)<sub>2</sub>THIQ which was shown to accumulate in NE nerve terminals within the CA-binding vesicles. Electrical stimulation of the preganglionic fibers of the superior cervical ganglion showed depletion of the THIQs from the storage vesicles and caused retraction of the upper eye lid, protrusion of the eyeball and dilation of the pupil, indicating activation of post-synaptic adrenergic receptors.

The THIQs have been shown to interact with CA receptors both in vitro and in vivo. This is not surprising considering the structural similarities, for example, between THP and the pharmacological DA agonists apomorphine and bulbo-capnine (Fig. 17) (134, 135). As early as 1910, THP had been shown to have  $\beta$ -agonist actions, lowering blood pressure, increasing heart rate, and causing relaxation of uterine muscle in cats (136). Several investigators have reported potent lipolytic activity for THP (137 - 140) and to a less extent SAL (138). The

S(-) isomer of both compounds exerted the most potent effects on glycerol and free fatty acid release (138).

THP has been shown to be a moderate DA-dependent adenylyl cyclase inhibitor (140 - 142). SAL also exerts adenylyl cyclase inhibition but to a lesser degree (141, 142). The S(-) isomer of both compounds was again the more effective configuration. Thus far, SAL has demonstrated relatively weak  $\beta$ -agonist effects (137, 143, 144). However, this THIQ has shown moderate  $\alpha$ -antagonism of NE on aorta and vas deferens of rat (145, 146). It has also been shown to block the effects of 5HT stimulation of smooth muscle in the vas deferens and uterus and block the effect of oxytocin and vasopressin on the uterus of rat (147).

#### 7. THIQ/Enzyme Interactions

O-methylation of DA- and NE-derived THIQ alkaloids has been studied in rat brain and liver homogenates (148) and with purified rat liver COMT (149). The O-methylation of THIQs is sensitive to inhibition by pyrogallol, a known COMT inhibitor. Intracerebroventricular (ICV) administration of THP and SAL resulted in half life's ( $T_{1/2}$ 's) of 24 and 12 minutes, respectively (150). These  $T_{1/2}$ 's were markedly prolonged by pretreatment with pyrogallol. In vitro studies have demonstrated that the

radioactivity derived from  $^{14}\text{C}$ -SAM is incorporated into the molecular structure of THP and SAL following incubation with rat liver COMT preparations (151). THIQs are both substrates and competitive inhibitors of COMT (148, 151 - 153). SAL and THP have been reported to be equal or better substrates for in vitro O-methylation by COMT than the endogenous substrates, DA and NE (149, 151, 153). Creveling et al. have reported that both the 6-(meta) and 7-(para) hydroxyls were O-methylated on two DA derived THIQs by purified rat liver COMT (154). Bail et al. (155) has presented interesting preliminary evidence indicating that SAL and 6,7-(OH)<sub>2</sub> THIQ are largely or exclusively O-methylated on the 7-(para) hydroxyl in rat tissues in vivo. Extensive O-methylation on the para hydroxyl is simply not observed with "normal" catecholamine and catechol acid substrates. Meyerson et al. have demonstrated that the O-methylation of the complex THIQs, THP and THPB, is on both the meta and para hydroxyls in vitro (156), the ratio (of meta/para O-methylation) being significantly influenced by the optical isomer used.

Locke et al. demonstrated that O-methylated forms of  $^3\text{H}$ -6,7-(OH)<sub>2</sub>DHIQ appeared to be retained in sympathetically innervated tissues such as heart and salivary glands in amounts greater than or equal

to the unmetabolized THIQ (121). As mentioned in section I, Collins et al. have found O-methylated-SAL (isomer unknown) in the urines of patients undergoing alcohol detoxification (8) and Hamilton et al. (13) have found O-methylated-SAL (isomer unknown) in the striata of EtOH-treated mice. In view of the rapid formation of O-methylated THIQ alkaloids, it is not surprising that O'Neill and Rahwan (157) were unable to detect SAL in the brain of alcohol-treated mice, in the absence of pretreatment with COMT inhibitors.

With the exception of earlier work by Hjort et al. (158, 159) on the gross peripheral pharmacology of phenolic THIQs, little work has been done on the physiological and pharmacological properties of O-methylated-THIQs, which are probably the primary metabolites of catechol-THIQ alkaloids in vivo.

To date there is no evidence that the cyclic THIQ alkaloids are metabolized by MAO. However, SAL and THP have been demonstrated to inhibit competitively the deamination of 5HT in rat brain and liver homogenates (151, 160). A variety of THPBs and THIQs were shown to inhibit both type A and B species of MAO in rat brain homogenates (161). It appears that type A is more sensitive to inhibition by these substrates. The THPBs proved to be relatively

potent and specific inhibitors of MAO type A. However, sequential methylation of the hydroxyls on the berberine ring system resulted in decreased selectivity and potency of MAO inhibition (161).

Compared to classical inhibitors of MAO (i.e. clorgyline, pargyline, deprenyl, etc.) (162), the THIQs appear to be relatively weak to moderate inhibitors of MAO in vitro. However, the synaptic properties of uptake and vesicular storage provide mechanisms for amplification of THIQ actions. Intra-neuronal inhibition of MAO has been investigated both in vivo and in vitro in the sympathetic nerve plexus of mouse heart. Cohen and Katz (163) utilized 6,7-(OH)<sub>2</sub>THIQ and demonstrated a 38% increase in <sup>3</sup>H-NE content with a concomitant decrease in <sup>3</sup>H-deaminated-NE in <sup>3</sup>H-NE-treated reserpine-pretreated mice, indicating in vivo inhibition of MAO. In a complementary in vitro experiment, Katz and Cohen (164) demonstrated intraneuronal inhibition of MAO by both SAL and 6,7-(OH)<sub>2</sub>THIQ. THP did not elicit potent inhibitory actions, apparently because of poor uptake into the nerve plexus.

In vivo experiments in which large doses of THP and SAL were administered ICV provided evidence that these THIQ alkaloids were able to alter the

steady-state levels of endogenous CA and 5HT (165). The exact mechanism by which their actions are exerted is still unknown. Hannigan and Collins (166) demonstrated that acute peripheral administration of 3-carboxy-sal-solinol (3-CSAL), increases 5HT levels in the corpus striatum and hypothalamus of rats, whereas chronic administration decreased the 5HT levels in these tissues. Livra et al. (167) further demonstrated that THIQ alkaloids can influence cerebral monoamine metabolism in vivo by the finding that HVA and 5HIAA levels are raised after acute or chronic intraperitoneal (i.p.) injections of THP. In addition, THP blocked the L-DOPA-induced increase in HVA as well as the p-chlorophenylalanine-induced decrease in 5HIAA. As there is some question as to whether THP can cross the blood-brain-barrier (BBB), these observed interactions with biogenic amine systems could depend on a peripheral THP metabolite which enters the CNS.

The THIQ alkaloids have been shown to be weak inhibitors of the Na<sup>+</sup>-Potassium ion (K<sup>+</sup>) ATPase and Mg<sup>++</sup>ATPase systems. These membrane-bound, active transport systems participate in modulating the Na<sup>+</sup> and K<sup>+</sup> fluxes of excitation and are fundamental to the energy-dependent reuptake and storage of NTs

released at nerve endings (168 - 170).

Evidence indicates that SAL is a good in vitro inhibitor of rat brain TH, the rate limiting step in CA biosynthesis (171). Little stereo-specificity was observed with both the S(-) and R(+) configurations inhibiting TH activity nearly equally. The inhibition was found to be competitive with the pteridine cofactor (170). THP proved to be a relatively weak inhibitor while 4,6,7-trihydroxy-THIQ [4,6,7-(OH)<sub>3</sub>THIQ] and 3-CSAL demonstrated moderate inhibition of TH activity. However, in vivo experiments utilizing SAL, 4,6,7-(OH)<sub>3</sub>THIQ and 3-CSAL did not indicate any in vivo TH inhibition by these compounds (170).

Coscia et al. (173) have recently reported on the interactions of a variety of NLCA derivatives with the enzymes involved in CA metabolism. The NLCAs inhibited TH non-competitively with respect to substrate and cofactor. DNLCA was shown to inhibit TH both in vitro and in vivo. MNLCA proved to be a potent in vitro inhibitor of DBH while DNLCA proved less inhibitory and NLCA was not effective. NLCA was found to be a competitive inhibitor of NE methylation by hepatic COMT. The NLCA derivatives showed only minor effects on MAO and AAD.

## 8. Possible Oxidative Mechanism For THIQ Metabolism

A growing amount of evidence derived from human and animal alcohol studies now documents that chronic EtOH ingestion leads to central and peripheral nerve damage despite adequate nutrition (174 - 177). The precise mechanisms by which ethanol exerts these chronic toxic effects is still unknown. Based on several lines of evidence, Collins (178) has proposed a discrete chemical mechanism to explain the cytotoxicity of ethanol, based on the potential involvement of THIQs. Considering the chronic nature of THIQ-related pathological conditions and attendant excess production of these alkaloids, it is conceivable that they may have some unique pathological effect(s). Supporting this hypothesis are experiments by Azevedo and Osswald demonstrating that administration of a NE-derived THIQ produced selective ultrastructural degeneration of adrenergic nerve terminals (179) and hepatotoxicity (180) in rats. In addition, Meyers and Melchior (181) demonstrated long-term aberrant behavioral effects (increased ethanol preference) in rats which were induced by ICV administration of THP.

While the major metabolic routes for catecholic THIQs are probably O-methylation and



conjugation, Collins proposed a minor oxidative metabolic route for THIQ metabolism which would lead to DHIQs and related N-oxides (Fig. 18). It was suggested that the DHIQs, as possible tissue binding agents, may play a role in the chronic neurotoxic effects of EtOH.

The conversion of THIQs to DHIQs is a well-established pathway in the aldehyde-induced fluorescence method for histochemical demonstration of biogenic amines in nerve (182). The decarboxylation of 1-carboxyl THIQs to afford DHIQs has been accomplished by oxidative chemical decarboxylation (183), electrochemical oxidation (184), and by horseradish peroxidase enzyme (78). In addition, DHIQs derived from 1-carboxy-THIQs have been demonstrated as reactive intermediates in the biosynthesis of alkaloids in plants (79, 185).

Because of electronic similarities, the mechanism by which the DHIQs would exert their neurotoxic effects could be analogous to that of 6-hydroxydopamine (6OHDA), a potent pharmacological neurotoxin. The actions of 6OHDA are dependent initially on uptake into catecholamine neurons and subsequent oxidation to at least two active species, the quinone and hydrogen peroxide (186) (Fig. 19). Studies of the interactions of 6OHDA with various model proteins

in vitro (187, 188) and chemical interactions with brain tissue in vivo (189, 190) clearly demonstrate that 6OHDA covalently reacts with and binds nucleophilic groups on proteins to form stable covalent bonds (Fig. 20).

At physiological pH, DHIQs are believed to exist predominately at quinoidamine tautomers (191). However, this is based on studies with formaldehyde-related DHIQs; the 1-methyl DHIQs in Fig. 19 have not actually been studied. Upon examination, the electronic and structural similarities between DHIQ quinoidamines and the oxidized electrophilic quinone of 6OHDA become strikingly apparent (Fig. 19). If two DHIQ tautomers share even a degree of the electrophilic nature of 6OHDA, the DHIQs, which are known to be taken up and stored in CA neurons (192), should be capable of producing neuronal damage via covalent attachment to electron-rich cellular ligands (sulfhydryls).

The DHIQs need not have as extensive electrophilic and sulfhydryl-binding capabilities of 6OHDA since they are postulated to form in trace amounts during chronic ingestion of EtOH. O-methylation of THIQs and DHIQs at the meta-hydroxyl (analogous to that of the open chain catecholamine precursors) would be expected to block the cytotoxic mechanism. However, as stated in section 8., evidence now

indicates that the stereoselective O-methylation patterns of THIQs differs from their catecholamine precursors (155, 156).

#### 9. Current Analytical Techniques in THIQ Research

It has been recognized that available analytical methods for the neurochemical study of biogenic amines and their metabolites are often limited, especially when dealing with the endogenous levels found in small biological samples. This problem is exacerbated when dealing with the THIQ alkaloids and their metabolites since their levels will always be significantly less than their catecholamine precursors. Early THIQ research relied heavily on radio-labeled precursor studies, with separations by thin layer chromatography (TLC), paper chromatography, paper electrophoresis and ion-exchange chromatography. Detection was by combinations of visualizing stains and radioautography (85, 87, 127, 193 - 195). These techniques do not offer a very great degree of selectivity, are not generally sensitive (with the exception of radio-labeled studies which are impractical for the study of endogenous compounds) and are complicated by long arduous isolation procedures.

More recently, a radioenzymatic method has been developed for the detection and quantitation

of SAL (196). The principle of this technique is based on enzymatic O-methylation of a catechol by COMT utilizing  $^{14}\text{C}$ -SAM as cofactor. Separation is by TLC and quantitation by liquid scintillation counting of an extract from the developed plate. Although this technique is adequately sensitive for the detection of endogenous levels of SAL (and probably other THIQs), it is inherently limited by its requirement for catechols. Therefore, endogenous O-methylated or conjugated THIQs cannot be assayed directly by this approach.

A significant recent development in the analysis of biogenic amines and their congeners is HPLC with electrochemical detection. This technique offers an inexpensive but sensitive method for the detection of picomole concentrations of a range of important phenolic and catecholic compounds (197). Initial studies utilizing HPLC with electrochemical detection for the separation and quantitation of SAL from biological samples has proven the efficacy of this technique for THIQ analysis. HPLC with electrochemical detection has been utilized for the detection of NE, DA, 5HT, 5HIAA, HVA and DOPAC in a variety of combinations in discrete brain regions (197). What has not been accomplished is the simultaneous assay of all

biogenic amines and acids in a given brain structure, such as DA, DOPAC, HVA, 5HT and 5HIAA. Separation and quantitation of these compounds would give a very complete profile of biogenic amine metabolism within the corpus striatum, for example.

GC/MS is a powerful technique which can conclusively identify neuroamines and derivatives present in tissue in femtomole amounts. Several investigators have utilized this technique to identify THIQs in discrete brain regions and biological fluids (1 - 3). This assay, which appears to be the definitive identification step in neurochemical research, is plagued by two serious drawbacks: 1) complicated and sophisticated instruments requiring expert maintenance and manipulation for both sample analysis, data processing, and interpretation; and, 2) a prohibitive cost ( $\$1.5 - 2 \times 10^5$ ). In lieu of these problems, the practicality of GC/MS in the average neurochemical laboratory is limited.

GC with electron capture (EC) detection offers a second highly sensitive and specific technique for detection of THIQs with picomole/g tissue sensitivity. Several investigators have utilized GC with EC detection, with varying degrees of success, to determine THIQs in biological samples (8, 11, 13, 155).

A limiting factor in GC/EC is the ability to separate compounds with close structural similarities, which is a function of column efficiency. To date the separation of geometrical isomers of biogenic amines and THIQS on conventional packed GC columns has not been accomplished. One possible answer to this problem is the use of capillary GC columns. Generally, capillary columns are those which have internal diameters less than 1 mm.

There are three main types of capillary columns: 1) micropacked, miniaturized versions of ordinary packed columns; 2) support-coated open tubular (SCOT), which have small particles of solid support adhered to the wall, but have an unrestricted flow path; 3) wall-coated open tubular (WCOT), in which the liquid phase is directly coated to the inner wall of the column as a thin film. With this type of column, the highest efficiency can be attained (>3,000 theoretical plates/m).

GC/EC utilizing a 25 $\mu$ m WCOT capillary chromatograph has been utilized for the separation and quantitation of the principal in vitro metabolites of prostaglandin endoperoxides (198). Detection limits equaled or exceeded those obtained for packed column separations and EC detection. The  $\beta$ -antagonists,

alprenolol and oxyprenolol, have been successfully quantitated and separated, after isolation from human blood samples, using EC/GC with a 25M X 0.25mm WCOT glass capillary column (199). The lowest detectable amount of the halogenated (heptafluoracyl) derivatives of alprenolol and oxyprenolol were 2 pg. Preliminary studies utilizing model compounds to represent various classes of biogenic amines indicated that fluoracyl derivatives of biogenic amines and their metabolites could be rapidly separated and quantitated by EC/GC with WCOT columns.

In light of the rapidly accumulating evidence of THIQ involvement in pathological conditions and the current state-of-the-art for THIQ and biogenic amine analysis, highly sensitive and specific assays to simultaneously detect these compounds and their metabolites are definitely needed. The requirements for the assays are the ability to separate a broad spectrum of closely related compounds at picomole sensitivity with minimal cost and high reproducibility. Assays that would meet these requirements would enable the investigator to assess THIQ involvement in human pathological conditions.

## CHAPTER II

### EXPERIMENTAL: MATERIALS AND METHODS

#### A. Standard Analytical Methods for the Characterization of Purity and Identity of Compounds Synthesized in Section II

Identity and purity of synthesized compounds were established by comparison of melting points to literature values, co-chromatography with authentic samples (when available), infrared (IR) spectra and nuclear magnetic resonance (NMR) spectra. HPLC, conventional and capillary GC and TLC techniques were employed where stated. HPLC and GC techniques are discussed in a separate section.

##### 1. Melting Point

Melting points were obtained (uncorrected) on dried, crystalline samples utilizing a standard melting point apparatus (Gallenkamp).

##### 2. IR Spectrum

IR spectra were performed by making a potassium bromide (KBR; Mallinckrodt) pellet with dried crystals. The spectra between 4000 and 400 nm were obtained on a grating IR spectrophotometer (Perkin Elmer, model 337).



### 3. NMR Spectrum

NMR spectra were performed utilizing deuterium oxide ( $D_2O$ ) solvent. The spectra between 10 and 0 ppm ( $H_2$ ) were obtained on a 60 MHz NMR system (Varian 360; courtesy Dr. D. Crumrine, Loyola University, Department of Chemistry).

### 4. TLC

TLC was utilized to assess the progress and completion of synthetic reactions. Two principal procedures were utilized. TLCs of CA-derived THIQs were performed by spotting 1 - 5 microgram quantities on 4 X 10 cm silica gel (Whatman) plates. The plates were developed in a butanol/acetic acid (HOAc)/water (distilled, deionized;  $dH_2O$ ) 4/1/1, (V/V/V) solvent system. Starting material, suspected product and a mixture of the two were run on the same plate simultaneously. Completed plates were air dried and visualized in an iodine ( $I_2$ ) chamber unless otherwise described.

Mono- and di-methylated THIQs, DHIQs, and CAs were separated from their dihydroxylated derivatives by utilizing aluminum oxide ( $AL_2O_3$ ) plates (Macherey-Nagel). Development was in a chloroform/methanol ( $MeOH$ )/ $dH_2O$ , 70/50/5, (V/V/V) solvent system. Starting material, suspected product, and a mixture of the

two were run on the same plate. Completed plates were air dried and visualized in an  $I_2$  chamber, or with a  $K_3 Fe (CN)_6$  (Mallinckrodt; 0.25 mg/ml in 0.2  $Na_2HPO_4$  buffer pH 8.3) spray which is allowed to dry and then counter stained with  $Fe Cl_3$  (Mallinckrodt; 2.5%  $Fe Cl_3$  freshly mixed in 1.5 volumes of acetone) (70).

## B. Synthesis of Tetrahydroisoquinolines

### 1. Synthesis of 1-Methyl-6,7-Dihydroxy-1,2,3,4-Tetrahydroisoquinoline (Salsolinol; SAL) HCl

A solution of 1.87 g (8.0 mMoles) of DA HBr (Aldrich) in 30 ml of  $dH_2O$  was adjusted to pH 4.5 with dilute  $NH_4OH$  (Mallinckrodt). Ice-cold redistilled AcA (J.T. Baker, 0.79 g; 18 mMoles) was added. The reaction was capped and allowed to stir slowly at room temperature ( $R_T^\circ$ ). After 3 days, the reaction was blown to dryness under a stream of extra dry nitrogen ( $N_2^{Ex}$ ) (Benster Welding). The residue was dissolved in a minimum volume of hot, absolute EtOH (U.S. Industrial Chem.) and brought to the cloud-point by drop-wise addition of ethyl acetate (EtOAc). The suspension was placed in a  $-20^\circ C$  freezer overnight, to yield a primary crop of white needle-like crystals. The mother liquor was not recrystallized. The crystals were dried over night in a vacuum desiccator with phosphorous pentoxide ( $P_2O_5$ ) and

characterized by NMR, IR, TLC, GC, HPLC and melting point comparison (Table 1.)

2. Synthesis of 1-Methyl-1-Carboxy-6-Hydroxy-7-Methoxy-1,2,3,4-Tetrahydroisoquinoline (7-0 Methyl -1-Carboxy-Salsolinol; 7M-1-CSAL)

A solution of 0.5 g (2.5 mmoles) of 4- $\theta$ -Methyl-dopamine (4M-DA) (HCL salt; Aldrich) and 0.264 g (4.4 mmoles) of pyruvic acid (Sigma) in 10 ml of dH<sub>2</sub>O was adjusted with concentrated (conc.) NH<sub>4</sub>OH to pH 4.5 and allowed to stir gently. After 4 days, the reaction was seeded by scratching the side of the glass vial with a glass rod and was then placed in a refrigerator for 1 hr. The crystals which separated were filtered and washed with cold dH<sub>2</sub>O to remove any excess pyruvic acid. The product was dried under vacuum in the presence of P<sub>2</sub>O<sub>5</sub> and characterized by NMR, IR, TLC, GC, HPLC and melting point comparison (Table 1).

3. Synthesis of 1-Methyl-1-Carboxy-6,7-Dihydroxy 1,2,3,4-Tetrahydroisoquinoline (1-Carboxy-Salsolinol; 1-CSAL)

A solution of 3.0 g (16 mmoles) of DA (HCl salt; Sigma) and 1.85 g (42 mmoles) pyruvic acid in 10 ml of dH<sub>2</sub>O was adjusted to pH 4.0 with conc. NH<sub>4</sub>OH. After gentle stirring for 4 days at R<sub>T</sub><sup>o</sup>,

reaction was seeded by scratching the side of the reaction vial. The reaction mixture was placed in a refrigerator for 1 hr. The white powdery crystals which separated were isolated over a sintered glass filter and washed with cold  $\text{dH}_2\text{O}$  to remove excess pyruvic acid. The product was dried overnight under vacuum in the presence of  $\text{P}_2\text{O}_5$  and characterized by NMR, IR, GC, HPLC, TLC and melting point comparison (Table 1).

#### 4. Synthesis of 6,7-Dihydroxy 1,2,3,4-Tetrahydro-isoquinoline [6,7-(OH)<sub>2</sub>THIQ]HBr

A suspension of DA(HBr salt; 0.233 g; 1 mMole) in 5 ml of  $\text{dH}_2\text{O}$  was adjusted to pH 4.5 with dilute  $\text{NH}_4\text{OH}$ . HCHO (37% solution; Mallinckrodt; 1.23 ml) was added and the reaction mixture was sealed and allowed to stir gently at  $R_T^\circ$ . After 24 hr, the reaction mixture was brought to dryness under a stream of  $\text{N}_2^{\text{Ex}}$ . The residue was dissolved in a minimum volume of hot, absolute EtOH and brought to the cloud-point by dropwise addition of EtOAc. This suspension was placed in a  $-20^\circ\text{C}$  freezer overnight yielding a primary crop of white needle-like crystals. The mother liquor was not recrystallized. The crystals were dried over night under vacuum in the presence of  $\text{P}_2\text{O}_5$  and characterized by NMR, IR, TLC, HPLC, GC, and melting point comparison (Table 1).

5. Conversion of 1-Methyl-6,7-Dimethoxy-3,4-Dihydro-  
isoquinoline (1-ME-6,7DMDHIQ) HCl to 1-Methyl-6,  
7-Dihydroxy 3,4 Dihydroisoquinoline (1-ME-6,7-  
DHIQ) HBr

1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline HCl (1-Me-6,7-DMDHIQ; synthesized by A. Hashmi, 1978; 1 g; 4.2 m Moles) was placed in a 20 ml screw top vial. Borontribromide ( $\text{BBr}_3$ ; 10 ml; Aldrich) was added, the vial was capped with a plastic-lined top and the solution was stirred at  $R_T^\circ$  for 3 hr. The reaction mixture was blown to dryness in a hood under a stream of  $\text{N}_2^{\text{Ex}}$ . The residue was brought up in a minimum volume of hot, absolute EtOH and brought to the cloud-point by dropwise addition of EtOAc. The suspension was placed in a  $-20^\circ\text{C}$  freezer overnight to yield a crop of yellow crystals. The mother liquor was not recrystallized. The product was dried over night under vacuum in the presence of  $\text{P}_2\text{O}_5$  and characterized by NMR, IR, TLC, HPLC, GC and melting point comparison (Table 1).

6. Conversion of 1-Methyl-6-Methoxy-7-Hydroxy-  
3,4-Dihydroisoquinoline (1-Me-6M-DHIQ) HCl  
to 1-ME-6,7-DHIQ HBr

1-methyl-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline (HCl salt; 0.5 g; 2.2 m Moles; synthesized by A. Hashmi, 1978) was placed in a 20 ml screw top

vial.  $\text{BBr}_3$  (10 ml) was added, the vial was capped with a plastic lined top, and the reaction was stirred at  $R_T^\circ$ . After 3 hrs, the reaction was taken to dryness under a stream of  $\text{N}_2^{\text{EX}}$  and the residue was brought up in a minimum volume of hot, absolute EtOH. The solution was brought to the cloud point by drop-wise addition of EtOAc. The suspension was placed in a  $-20^\circ\text{C}$  freezer overnight to yield a crop of yellow crystals. No effort was made to recrystallize the mother liquor. The crystals were dried overnight, under vacuum, in the presence of  $\text{P}_2\text{O}_5$  and characterized by NMR, IR, TLC, GC, HPLC and melting point (Table 1).

A summary of selected physical data for the synthesized compounds in section B. can be seen in Table 1.

### C. Techniques for Animal Studies

Compounds utilized in the studies in this dissertation were dissolved in sterile, isotonic saline and administered to male Sprague-Dawley rats (King;  $100 \pm 5\text{g}$ ) either centrally by stereotaxic injections into the lateral cerebral ventricles or peripherally by intraperitoneal injection. Stereotaxic coordinates (200) were bregma  $\pm 0$ , lateral  $\pm 1.4$  from the midline suture and verticle  $\pm 1.4$  from the point of entry. Initial experiments utilizing black India ink dye confirmed that the

injections were in the lateral cerebral ventricles. Sodium pentobarbital (Abbott) was utilized as the surgical anesthesia. It was injected (50 mg/Kg i.p.) 10 minutes prior to central injections. At specified time points after central injections, experimental rats and their paired saline injected controls were sacrificed by decapitation. Brain areas and peripheral parts of interest were quickly dissected out according to Holman et al. (201) and placed in plastic weigh boats on dry ice. The samples were weighed by difference and placed in plastic centrifuge tubes for extraction.

D. Extraction of Biogenic Amines, THIQs and Their Metabolites From Tissue

To each plastic centrifuge tube (7 ml) containing a tissue sample was added 100  $\mu$ l of  $dH_2O$  containing 200 ng of an appropriate internal standard. The solvent of choice (acid or aqueous EtOH, vide infra) was added to the plastic centrifuge tube (5 ml/g) and the sample was homogenized with a 20 sec burst of a (Tekmar) Tissue-mizer. The blades of the Tissue-mizer were washed with an equal volume of solvent, the washings were combined and the homogenate then was centrifuged in a Sorvall refrigerated (4°C) centrifuge at 30,000 X g for 20 min. The blades of the Tissue-mizer were scrupulously washed with  $dH_2O$

and wiped dry between each tissue sample to avoid cross-contamination of samples.

### 1. Acid Extraction

Tissue samples were homogenized in either 1N HCl or 0.4N HClO<sub>4</sub> (5 ml/g tissue). After homogenization and centrifugation as described (vide supra), the supernatants were decanted into 20 ml screw-top vials, and the pHs were adjusted to 5.5 with 2.0N NaOH followed by 0.2N NaOH. The neutralized samples were then frozen, lyophilized and stored at -20°C until cation column isolation and/or direct HPLC analysis.

### 2. Ethanol Extraction

Tissue samples were homogenized in aqueous 75% EtOH (5 ml/g) which had been stored at -20°C. After homogenization and centrifugation as described (vide supra) the supernatants were decanted into 20 ml screw-top vials and the volumes were doubled with dH<sub>2</sub>O. These solutions were then frozen, lyophilized, and stored at -20°C until cation column isolation and/or HPLC analysis.

### 3. Strong Cation-Exchange Column Chromatography

#### Isolation Procedure

#### a. Resin Preparation

Dowex AG50-WX, 200/400 mesh (Bio-Rad Labs)



(H<sup>+</sup> form) was stirred with 4 volumes of dH<sub>2</sub>O. Small particles were removed from the suspension by allowing the resin to settle and aspirating any suspended particles along with the dH<sub>2</sub>O layer. This procedure was repeated until the supernatant was clear, thus indicating a homogenous resin bed. The aqueous layer was then removed and replaced with 4 volumes of 2 N NH<sub>4</sub>OH. The slurry was allowed to stir for 1 hr after which it was poured into a sintered glass filter (coarse-pore) and washed with dH<sub>2</sub>O until the pH of the effluent was 4.0. The resin was resuspended in 4 volumes of 2N HCl and stirred 1 hr. It was again washed as described (vide supra) with dH<sub>2</sub>O until the effluent pH was 4.0. Ion exchange columns (2.50 mx 0.6 cm) were prepared in plastic columns equipped with sintered plastic filters (Isolabs). The columns were washed with 5 ml of 4N HCl/MeOH, 1/1, (V/V) and with dH<sub>2</sub>O until pH 4.0.

b. Column Procedure (Dowex)

The supernatants from acid-precipitated tissues or solutions of standards (prior to neutralization and lyophilization) were decanted directly onto the resin and the columns were allowed to drain. Subsequently, the columns were washed with 25 ml of dH<sub>2</sub>O, 5 ml of 40/60 dH<sub>2</sub>O/MeOH (V/V) and eluted with 5 ml of 4N HCl/MeOH, 1/1 (V/V). The acid/MeOH elution was

then lyophilized to dryness. The dried residue was stored at  $-20^{\circ}\text{C}$  for subsequent analysis by HPLC, or was derivatized and analyzed by GC.

c. Weak Cation Exchange Column Chromatography

Isolation Procedure

1. Resin Preparation

BioRex-70, 200/400 mesh,  $\text{Na}^+$  form (Bio-Rad Labs) was sized and washed by repetitive stirring and aspiration in 4 volumes of  $\text{dH}_2\text{O}$ . Once the supernatant was clear of all fine particulate, the aqueous layer was removed and replaced with 4 volumes of 3N HCl. The resin was stirred for 1 hr, decanted over a sintered glass funnel (coarse-pore) and washed to pH 5.0 with  $\text{dH}_2\text{O}$ . The resin was then resuspended in 5 volumes of 3N NaOH and allowed to stir for 1 hr, transferred to a sintered glass funnel (coarse-pore) and washed to pH 5.0 with  $\text{dH}_2\text{O}$ . The resin was then suspended in a 0.1M  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  buffer (8.2g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 5.68g  $\text{Na}_2\text{HPO}_4$ ; pH 6.5) containing 10%  $\text{Na}_2\text{EDTA}$ . 2.5 cm X 0.6 cm resin columns were prepared utilizing plastic support columns equipped with plastic sintered filters (Isolabs). Prior to utilization the columns were washed with 5 ml of  $\text{dH}_2\text{O}$ .

2. Column Procedure

The supernatants from neutralized acid-precipitated

or EtOH-precipitated samples were pipetted onto the resin and allowed to drain into 20 ml screw top (scintillation) vials. The columns were then washed with 2 ml of 0.02 M  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$  buffer (1.66 g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 1.14 g  $\text{Na}_2\text{HPO}_4$ ; pH 6.5) followed by 5 ml of  $\text{dH}_2\text{O}$ . The primary elution plus buffer and  $\text{dH}_2\text{O}$  washes contained all acid and amphoteric compounds, with no detectable amine constituents as ascertained by HPLC analysis. The amine compounds were then eluted with 5 ml of 1.0N HCl. Both fractions were frozen in a dry-ice/acetone bath and lyophilized to dryness. Dried sample residues, after dissolving in appropriate solvent, were analyzed directly by HPLC, or were derivatized and analyzed by GC.

E. Gas Chromatographic Analysis of Catecholamines, THIQs and Their Metabolites

Quantitation of the isolated catecholamines, THIQs and respective O-methylated metabolites were carried out by EC/GC employing either capillary or conventional packed columns. A Varian model 3700 gas chromatograph with  $^{63}\text{Ni}$  detector (pulsed mode) was utilized.

Wall coated, open tubular (WCOT), glass capillary columns (10 M X 0.25 mm i.d.; Alltech Assoc.) coated with polyphenylmethylsiloxane (OV-17)

were employed. The splitter at the injection port was adjusted to a ratio of 10/1. Head pressure of the carrier gas,  $N_2$ , oxygen free ( $N_2^{O_2^F}$ ) was  $0.8 \text{ kg/cm}^2$ . Peak areas and retention times were obtained with a Varian CDS-111C computing integrator.

Conventional, packed, stainless steel columns were prepared in our laboratory. The liquid phase of choice was a 3% polydimethylsiloxane (OV-101; applied Sci. Labs.) coated over a Gas Chrom G, H.P. 100/120 mesh solid support (Alltech. Assoc.) by a solvent evaporation technique, utilizing a rotary flash evaporator. Column lengths varied from 1 ft to 5 ft. Prepared columns were conditioned at their maximum operating temperature for 48 hr prior to use. Peak areas and retention times were obtained utilizing a Varian CDS-111C computing integrator.

1. Preparation of Fluoroacyl Derivatives for GC  
a. Fluoroacyl Derivatives of Non-Carboxylated Catecholamines, THIQs and Their O-Methylated Metabolites

Initial studies were undertaken with several types of acylating agents to determine which agent would give maximum EC responses, completeness of reactions, derivatives with good stabilities and optimum separations. To 1 mg of crystalline compound

or lyophilized tissue residue in a 20 ml screw-top vial were added 500  $\mu$ l of sequanal grade acetonitrile (ACN; pierce) and 50  $\mu$ l of either heptafluorobutyric anhydride (HFBA), pentafluoropropionylanhydride (PFPA), or trifluoroacetic anhydride (TFAA) (Pierce). Each vial was sealed with a plastic lined cap and allowed to react at  $R_T^\circ$  for 30 minutes. The solutions were brought to dryness in a hood with a stream of  $N_2^{Ex}$ . The residues were taken up in 1 ml of sequanal grade toluene (Pierce). 0.5 ml of 1 M  $(NH_4)_3PO_4$  buffer (pH 5.8) was added, the mixture vortexed for 60 sec and centrifuged in a clinical centrifuge for 5 min.

b. Formation of Fluoroacyl Derivatives of Carboxylated THIQs and Their O-Methylated Metabolites

To 1 mg of crystalline carboxylated compound or lyophilized tissue residue in a 20 ml screw-top vial was added 0.2 ml of hexafluoroisopropanol (HFIP; Pierce) and 0.05 ml of PFPA. Each vial was sealed with a plastic-lined screw-top cap and allowed to react at  $R_T^\circ$  for 20 min. The solutions were blown dry under a stream of  $N_2^{Ex}$ . The residue was treated with 0.5 ml of ACN and .05 ml of PFPA for 20 min in sealed vials. The samples were again brought to dryness with  $N_2^{Ex}$ , toluene (1 ml) was added, and the solution was washed with  $(NH_4)_3PO_4$  as described above.

Immediately following centrifugation, the toluene phase (1  $\mu$ l) was injected directly into the GC system.

#### F. HPLC Methodology

Lyophilized tissue extracts were brought up in 0.01N HCl (0.5 ml) and chromatographed on BioSil, C<sup>18</sup>, reverse phase; 25 CM columns (Bio-Rad Labs) using electrochemical detection (B.A.S.; 0.79 volts). Various buffer systems were employed for optimum separations and sensitivity of detection. The mobile phase flow rate utilized was generally 1.0 ml/min, as indicated in Results.

##### 1. Paired-Ion Buffer System

Degassed dH<sub>2</sub>O (~850 ml), 1.1 g (8 mM) heptane-sulfonic acid (HSA; waters) and 9.7 ml of glacial HOAc were mixed and adjusted to pH 3.5 with NH<sub>4</sub>OH, if necessary. MeOH (Baker Reagent grade), 100 ml, was then added and the mobile phase was then brought to 1 liter with dH<sub>2</sub>O.

##### 2. Ionic-Suppression Buffer System

The ionic-suppression buffer consisted of 0.1 M NaH<sub>2</sub>PO<sub>4</sub> with 1 mM Na<sub>2</sub> EDTA. The pH was adjusted to precisely yield optimum separation (pHs 4.7 - 5.2).

##### 3. Reverse Phase HPLC Column Regeneration

Repeated injections of tissue samples lead

to loss of separation of isolated compounds even in the presence of a guard pre-column (Altex). Reverse phase columns were cleaned of absorbed tissue components and paired-ion reagent by the technique of Hannigan (202). After disconnecting the electrochemical detector, the column was washed successively with 25 ml of 10 mM oxalic acid (pH 3.5 with concentrated NaOH; Sigma); 25 ml MeOH/dH<sub>2</sub>O 1/1 (V/V); 25 ml methylene chloride/tetrahydrofuran, 1/1 (V/V); 25 ml MeOH and 25 ml MeOH/dH<sub>2</sub>O, 1/1 (V/V). The mobile phase of choice then was run through the column for 30 min, the detector reconnected, and allowed to stabilize before use.

G. Procedure for In Vivo Studies on Stereoselective O-Methylation of Catechol Isoquinolines

A variety of catechol isoquinolines were investigated to represent various combinations of catechol-amine-carbonyl condensation products. Compounds utilized (Fig. 21) were SAL, 1-CSAL, 3-CSAL, 4,6,7-(OH)<sub>3</sub> THIQ and 1-Me-6,7-DHIQ, a compound which could theoretically form from the oxidation of SAL or oxidative decarboxylation of 1-CSAL. A group of rats were pretreated with the MAO inhibitor pargyline (50 mg/Kg, i.p. 2 hr pre-DA), anesthetized and injected bilaterally in the lateral cerebral ventricles

with DA. Separations and quantitations of isoquinolines and their respective 6- and 7-O-methylated metabolites were carried out by EC/GC on a WCOT glass capillary column and reverse phase HPLC with electrochemical detection, as described (vide supra).

Male Sprague-Dawley rats ( $100 \pm 5$  g) were anesthetized and stereotaxically injected bilaterally (ICV) with 50  $\mu\text{g}$  (25  $\mu\text{g}/\text{ventricle}$ ; 12.5  $\mu\text{g}/\mu\text{l}$ ; free base) in isotonic saline. Control rats were given equal volumes of isotonic saline. 50 min after the ICV injections, rats were sacrificed by decapitation and the hypothalamus, corpus striatum and hippocampus were removed. They were weighed, homogenized in 75% aqueous EtOH with 200 ng of dihydroxybenzylamine (DHBA, internal standard) and centrifuged as described at 30,000 X g, 4°C for 20 minutes. The supernatants volumes were doubled with  $\text{dH}_2\text{O}$ , were decanted onto Bio Rex-70 cation exchange columns and differentially eluted for acid and amine constituents as described. All fractions were frozen on dry ice and lyophilized to dryness. The residues were taken up into 0.01N HCl and analyzed by HPLC utilizing the ionic suppression buffer system with electrochemical detection. An aliquot of each fraction was taken lyophilized and depending on the isoquinoline to be assayed differentially derivatized



for acids (PFPA/HFIP) or amines (HFBA).

Confirmation of peak identity was determined by comparison of retention times of authentic standards on both HPLC and GC as well as co-chromatography with authentic standards added to derivatized tissue samples. Regional brain parts of saline-injected controls were analyzed for the presence of interfering peaks on both HPLC and GC.

#### H. Procedure for Study of the Uptake of Peripheral Salsolinol Into CNS

Male Sprague-Dawley rats were administered 5 - 20 mg/Kg (i.p.) of SAL (HCl). At 0.5, 2.0, 5.0, and 10 hr post-administration, rats were sacrificed by decapitation. The hypothalamus, corpus striatum, and hippocampus were removed, weighed, homogenized in 0.4N HClO<sub>4</sub> with 200 ng of DHBA (as internal standard) and centrifuged at 30,000 X g, 4°C, for 20 minutes. The pH of the supernatant was adjusted to 5.5. The supernatant then was decanted onto Bio Rex-70 cation exchange columns and differentially eluted for acid and amine constituents. The amine fraction was lyophilized, the residue derivatized with HFBA/ACN, and analyzed by capillary GC with EC detection for the possible presence of SAL, 7-O-Methyl-SAL (7M-SAL) and 6-O-Methyl-SAL (6M-SAL).

I. Acute ICV Administration of Catecholic Isoquinolines for Studies on Effects on Endogenous 5HT and Acid Metabolites of Biogenic Amines

Male Sprague-Dawley rats were anesthetized with Na+ pentobarbital (50 Mg/Kg, i.p.) and stereotaxically injected ICV bilaterally with 50 µg (25 µg/ventricle; free base; 12.5 µg/µl) of SAL, 4,6,7-(OH)<sub>3</sub>THIQ, 1-CSAL, 3-CSAL 1-Me-6,7-DHIQ, or DA in isotonic saline [rats given DA were pretreated with 50 mg/Kg pargyline (i.p.) 2 hr earlier]. Control animals were given equal volumes of isotonic saline ICV. 50 min after central administration the animals were sacrificed by decapitation and the hippocampus, hypothalamus, and corpus striatum were removed, weighed, homogenized in 75% aqueous ethanol (containing 200 ng of DHBA as internal standard), and centrifuged at 30,000 X g, 4°C, for 20 min. The supernatant volumes were diluted with an equal volume of dH<sub>2</sub>O, decanted onto Bio Rex-70 cation exchange columns and differentially eluted for acid and amines constituents. The acid fractions were lyophilized to dryness, taken up in 0.5 ml of 0.01 N HCl and analyzed for DOPAC, 5HIAA, and HVA in the corpus striatum and hypothalamus, and for 5HIAA in the hippocampus utilizing paired-ion, reverse phase HPLC with electrochemical detection. Amine

fractions were lyophilized to dryness. Hippocampal samples were analyzed for 5HT as above. Hypothalamus and corpus striatum samples were derivatized with HFBA/ACN and analyzed for further verification of stereoselective O-methylation of amine isoquinolines, possible decarboxylated products of carboxylated isoquinolines, and DA levels. However, due to contaminated derivatizing reagents from Pierce Chemical Co., these samples were lost.

J. Procedure for Study of the Half-Life of 7 M-SAL  
In the Rat CNS

Male Sprague-Dawley rats were anesthetized with Na+ pentobarbital and stereotaxically injected ICV as described with 5 µg/ventricle (5 µg/µl) bilaterally with 7M-SAL (HCl salt). Control rats were given equivalent volumes of isotonic saline. 1, 3, 6, 12, 24, and 48 hr after central administration, rats were sacrificed by decapitation and the hippocampus, hypothalamus and corpus striatum were removed and weighed. They were homogenized in 75% aqueous EtOH containing 200 ng DHBA as internal standard and centrifuged at 30,000 X g, 4°C for 20 min. The supernatant volumes were doubled with dH<sub>2</sub>O. Aliquots of the hypothalamus and corpus striatum were placed on Bio Rex-70 weak cation exchange columns and differentially eluted for amine

and acid constituents. The amine fraction was lyophilized to dryness, derivatized with HFBA/ACN, and analyzed for the presence of 7M-SAL. The remainder of the supernatants were lyophilized and the dried residues were taken up in 0.5 ml of 0.01 N HCl and analyzed by reverse phase HPLC with electrochemical detection. Utilizing the ionic suppression buffer, simultaneous separations and quantitations of DA, DOPAC, 5HT, 5HIAA, HVA, and 7M-SAL were performed.

K. Procedure for Study of Peripheral 1-CSAL Metabolites

Male Sprague-Dawley rats were injected with 1-CSAL (100 mg/Kg, i.p.) daily for 6 days. On the 7th day, the single dose was increased to 300 mg/Kg i.p. Control rats were given equal i.p. volumes of isotonic saline. Rats were sacrificed by decapitation 3 hr after the last injection. Livers, hippocampi, hypothalami and corpus striata were removed, weighed, homogenized in 0.4N HClO<sub>4</sub> containing 200 ng of DHBA as internal standard, and centrifuged at 30,000 X g, 4°C, for 20 minutes. The supernatants were adjusted to pH 5.5 with NaOH and decanted onto Bio Rex-70 weak cation exchange columns. The columns were differentially eluted for acid and amine constituents. All fractions were frozen on dry ice and

lyophilized to dryness. The residue was taken up in 0.01 N HCl and an aliquot was analyzed by reverse phase HPLC with electrochemical detection for the presence of 1-Me-6,7-DHIQ, SAL and their respective 6- or 7-O-methylated metabolites. Two different HPLC conditions were utilized. A mobile phase consisting of 0.1 M  $\text{Na}_2\text{HPO}_4$ , 1 mM  $\text{Na}_2\text{EDTA}$  titrated to pH 7.4 with 0.1 M citric acid, followed by dilution with MeOH (10% V/V), was employed for DHIQ analysis. Samples were analyzed for SAL and its O-methyl metabolites with the mobile phase consisting of 0.1 M  $\text{NaH}_2\text{PO}_4$ , 1 mM  $\text{Na}_2\text{EDTA}$  pH 5.5, with 5% (V/V) methanol. pH adjustments on the mobile phases were made prior to the addition of methanol to avoid organic solvent-induced perturbations in pH.

The remainder of the 0.01N HCl-suspended sample was lyophilized to dryness. HFB-derivatives were prepared and analyzed by capillary GC with EC detection for the presence of SAL, 1-Me-6,7-DHIQ and their respective O-methylated metabolites.

L. Spectrophotometric Studies of a Series of 1-Methyl-6,7-Substituted 3,4-Dihydroisoquinolines

1-Me-6,7-DHIQ, 1-methyl-6-hydroxy-7-methoxy 3,4 dihydroisoquinoline (1-Me-7M-DHIQ), 1-Me-6M-DHIQ, and 1-Me-6,7-DMDHIQ (HCl salts) were prepared by

dissolving the appropriate compound (1.0 mg/ml) in  $\text{dH}_2\text{O}$  and storing it at  $-20^\circ\text{C}$ . U.V. absorption spectra were obtained on a Perkin-Elmer model 320 spectrophotometer between 220 and 500 nm. Excitation and emission spectra were determined at pH 7.0 utilizing an Aminico-Bowman scanning Spectrophotofluorometer. The spectra were examined in 0.1N HCl, 0.01 N NaOH, 0.1N NaOH and 0.1 M buffers (acetic acid-acetate, pH 3.0 - 5.0; Na+ phosphate, pH 6.0 - 8.0; glycine-NaOH, pH 9.0 - 11.0) over a pH range of 2 - 13. All substances studied exhibited significant fluorescence quenching above  $1 \mu\text{g/ml}$ . Therefore, the solutions examined were at concentrations of  $1 \mu\text{g/ml}$  or less where fluorescence intensity was proportional to concentration. Solutions were examined for fluorescence and absorption immediately after they had been prepared, and fluorescence intensity was always determined at  $R_T^\circ$  within 10 - 20 seconds after exposure to exciting light, to avoid possible photodecomposition and temperature effects.

#### M. Cyclic Voltammetry of DHIQs

Cyclic voltammetry was performed on a direct current voltammetry instrument (B.A.S.) utilizing a three electrode linear sweep between +1.2 and -1.2 volts. The reaction cell was constructed in our

laboratory from a 20 ml screw top vial. A teflon-lined, screw-top cap was modified to accommodate a carbon paste working electrode, reference electrode (B.A.S.) and auxiliary electrode. Cyclic voltammograms were recorded on an x-y recorder at pH 3.0, 5.0, and 7.4 utilizing a Na<sup>+</sup>-phosphate-citrate buffer. The change in voltage was monitored by use of a voltmeter (Westronics). All voltammetric studies were done with concentrations of between 2.0 and 5.0 µg/ml (free base), scan speed 200 mv/sec at a sensitivity of 2 µA/cm. Scans were repeated and verified by Dr. Peter Kissinger at the Bio-Analytical Systems (B.A.S.) Laboratories in West Lafayette, Indiana.

#### N. Attempted Synthesis of Glutathione-DHIQ Adducts

Two different DHIQs, 1 Me-6,7-DHIQ HBr (250 mg, 0.95 mmoles) or 1-Me-7M-DHIQ HCl (250 mg, 1.17 mmoles) were each dissolved in 10 ml of dH<sub>2</sub>O containing glutathione (GSH; 921 mg, 3.00 mmoles). The pH was adjusted to 7.4 with 1.0 M NH<sub>4</sub>OH. The reaction mixtures were placed into a warm water bath (37°C) and gently shaken. After 10 hr the solutions were frozen on dry ice and lyophilized to dryness. 5 µg/µl samples of the reaction mixture was spotted on an 4 cm X 10 cm silica gel plate along with standards of GSH and appropriate DHIQs. The plates were developed in a butanol/

HOAc/dH<sub>2</sub>O, 4/1/1 (V/V/V) and visualized by irradiation under fluorescent light for DHIQs, and ninhydrin (peptide reagent) for GSH. NMR spectra of the reaction mixtures were performed in D<sub>2</sub>O on a varian 360 NMR spectrophotometer (courtesy of Dr. Dr. D. Crumrin, Loyola University, Dept. of Chemistry). HPLC analysis of the reaction mixture was performed utilizing a .1M Na<sub>2</sub>HPO<sub>4</sub> buffer titrated to pH 7.4 with 0.1M citric acid in 20% (V/V) MeOH. Combinations of electrochemical/U V or electrochemical/fluorescence detection were utilized to attempt detection of a reaction product.

#### O. Gas Chromatographic Analysis of Ethanol and AcA

EtOH and AcA were analyzed on a Varian 2400 gas chromatograph equipped with a flame ionization detector. The column utilized was a 1/8 in X 6 ft coiled glass column packed in our laboratory with Porapak QS, 80/100 mesh (Alltech Assoc.). Empty glass columns were cleaned by successively drawing through hot soapy water, acetone, and methanol under vacuum. Columns were then dried with N<sub>2</sub><sup>Ex</sup>. Deactivation of glass surfaces was accomplished by treatment with Glass-Treet (Alltech Assoc.). Excess reagent was removed by rinsing with anhydrous MeOH (25 ml). Columns were stoppered at one end with a



glass wool plug and were packed under vacuum by injection of Porapak QS into the open end. They were vibrated during the packing procedure with an engraving gun. The columns were then conditioned for 48 hr at 200°C.

P. Procedure for the Determination of ACA and EtOH  
in Human Blood During Alcohol Detoxification

Blood samples (3 - 5) ml were drawn from consenting male adults (ages 37 - 54) admitted to the Alcohol Detoxification Unit at Hines Veterans Administration Hospital. Non-alcoholic controls were in the same age range and were psychiatric in-patients at the hospital. Both groups were on the same daily diet and between-meal consumption of foods was restricted. The blood samples were stored in citrated tubes in a freezer (-20°C) until preparation for analysis (6 - 48 hr). Aliquots (0.2 ml) of blood were added to 15 ml vacutainers containing 0.5N HClO<sub>4</sub> (1.0 ml) with 25 mM thiourea and 50 mg/dl N-propanol as an internal standard. Prior to use, the vacutainers were washed with hot soapy water, treated with chromerge (S/P), treated further with hydrogen fluoride (5% solution), rinsed well with dH<sub>2</sub>O and dried under vacuum at 100°C. The blood mixtures (0.2 ml) were immediately mixed and frozen

over liquid  $N_2$ , blown for ~15 sec with  $N_2^{Ex}$ , capped, and equilibrated at  $37^\circ C$  for 20 min. Head space samples (1 - 2 ml) were taken with a gas-tight syringe (Hamilton) and analyzed for EtOH, AcA and N-propanol on a Porapak QS column (prepared as described in section O.;  $T_c = 125^\circ C$ ). Standard curves were prepared with AcA, EtOH, and N-propanol added to normal bloods. Control bloods showed negligible formation of AcA during work up procedure. Standard curves were expressed as concentration of EtOH or AcA versus the ratio of peak areas of EtOH or AcA/N-propanol.

## CHAPTER III

### RESULTS

#### A. Synthesis of THIQs

Selective physical data for synthesized compounds is summarized in Table 1. NMR and IR spectra were consistent with expected structures. GC and HPLC analysis determined purity of all synthesized compounds to be greater than 99%. TLCs of reaction mixtures verified that the reactions had gone to completion.

#### B. Extraction and Recovery Studies of Biogenic Amines, THIQs and Their Acid Metabolites

Dihydroxybenzylamine (DHBA) proved to be a good internal standard, consistently reflecting the recovery of a variety of compounds. This verifies the utility of this compound as an HPLC (203) and GC internal standard. Recoveries of all compounds of interest correlated within 5 - 7% of DHBA recovery. The exception was 5HT, whose recovery varied greatly with the isolation technique employed.

Acid extraction utilizing 0.4 N HClO<sub>4</sub> followed by lyophilization caused almost total loss of isolated compounds as determined by HPLC analysis. This loss could be avoided if the supernatant was adjusted to

pH 5.5 prior to lyophilization. When 1.0 N HCl was utilized as the homogenizing medium, no neutralization was necessary; however, 5HT recovery was only 50% of DHBA recovery. When the 1.0 N HCl was adjusted to pH 5.5, the recovery of DHBA directly reflected 5HT recovery. Overall recovery as determined by HPLC of THIQs, biogenic amines, and their metabolites for the neutralized acid (1.0 N HCl) extraction technique ranged from 80 - 85%. Recoveries were calculated from known standards added to cerebellar homogenates, which were then carried through the extraction procedures and compared to identical neat standards by HPLC.

EtOH extraction of tissue samples had the added advantage of not having to adjust the pH prior to lyophilization or addition to weak cation-exchange columns. Doubling the volume of the EtOH-extracted supernatant with dH<sub>2</sub>O yielded a solution of pH 5.5. Utilization of this technique gave 5HT recoveries by HPLC which were directly reflected by the recovery of DHBA. Overall recoveries of THIQs, biogenic amines and their metabolites after EtOH extraction, lyophilization and direct HPLC analysis ranged between 85 - 89%. Recoveries were calculated, as above, from addition of known standards to cerebellar homogenates.

The aforementioned extraction techniques

utilizing HCl, HClO<sub>4</sub>, or EtOH, homogenization, pH or volume adjustment and lyophilization provided reasonable HPLC chromatograms (see section D). However, GC analyses utilizing these abbreviated isolation techniques were unsuccessful. Attempts to chromatograph the lyophilized residues (following derivatization of samples isolated utilizing any of the three simple extraction techniques) yielded chromatograms with solvent peaks exceeding 25 min. Therefore, a rapid sample "clean-up" technique using ion-exchange columns was sought.

The use of a strong cation-exchange resin, Dowex-50, for the separation of amines, acids, and amphoteric constituents from acid or EtOH extracted tissue samples had limited success. The 4N HCl/MeOH which was necessary for the removal of bound amines apparently caused significant destruction of the biogenic amines prior to analysis, as indicated by low recoveries after (and discoloration during) sample lyophilization. In order to increase recoveries, lower concentrations of acid could be utilized. However, this would become impractical due to the large volume necessary for elution. Overall recoveries of THIQs, biogenic amines and their O-methylated metabolites after Dowex, ranged between 25 and 40%, as determined by both HPLC and GC analysis. These low

recoveries and the wide range discouraged the use of this Dowex ion-exchange technique.

When the samples were passed through columns containing a weak cation-exchange resin (BioRex-70) which allowed for selective isolation of amines, acids, and amphoteric constituents, GC analyses of these fractions were possible. The use of BioRex-70 for these isolations was adapted from procedures by Holman (201) and Barchas (204). Eightyfive % of the carboxylated THIQs and acid metabolites (HVA, 5HIAA, DOPAC) of biogenic amines were detected in the primary eluate. An additional 2 ml of 0.02 M sodium phosphate buffer eluted an additional 10% of the carboxylated compounds with no detectable cross contamination from amine constituents. Non-carboxylated THIQs, 5HT, the CAs, and their O-methylated amine metabolites remained bound to the columns until elution with 1.0 N HCL. Overall recoveries of non-carboxylated THIQs, CAs, and their O-methylated metabolites, directly reflected by the HPLC or GC recovery of DHBA, were within 83 - 85%. 5HT recovery (HPLC analysis) was consistently 75%. A suitable internal standard was not found for the acid fraction. The recoveries for all acid constituents, which were obtained by addition of standards to cerebellar tissue homogenates and carried through the column procedure,

were consistently high, ranging from 93 to 95%.

c. Gas Chromatography of CAs, THIQs, and Their Metabolites: Results of Studies on Derivative Formation and GC Separation Conditions

In general, the HFB-derivatives of non-carboxylated THIQs, CAs, and their respective O-methylated metabolites yielded the higher responses, more symmetrical peaks and better isomer separations, when compared with the TFA-derivatives. PFP-derivatives actually gave EC responses equivalent to HFB-derivatives, but GC separation of the structural amine isomers (O-methylated metabolites of SAL, 4,6,7,-(OH)<sub>3</sub> THIQ, and DA) was not achieved following PFPA derivatization. The O-methylated isomers of carboxylated THIQs were separable, however, when PFPA was used in combination with HFIP.

It was found absolutely necessary to wash the derivatives, taken up in toluene, with an aqueous buffer solution just prior to capillary GC analysis. The aqueous washing apparently removed residue fluoroanhydrides and other derivatized volatile compounds which were contributing significantly to a large tailing solvent front. This novel step was accomplished with a 1M NH<sub>4</sub> H<sub>2</sub> PO<sub>4</sub> / (NH<sub>4</sub>)<sub>2</sub> HPO<sub>4</sub> buffer, pH 5.8 - 5.9. The aqueous buffer presumably

hydrolyzed any unreacted fluoroanhydride, and fluoroacids were extracted by the aqueous ammonia. The fluoroacyl esters of phenols are reportedly stable in  $H_2O$  at  $pH \leq 6.0$  (205), and the results with THIQs and CAs indicated this to be true (vide infra).

The use of aqueous  $NH_4^+$  phosphate to wash the toluene samples is demonstrated in Figure 22. Recovery experiments, in which derivatized standards were washed up to 5 times with the aqueous buffer and injected immediately afterward, showed no significant change in peak heights, areas, symmetries or retention times. However, extended storage (72 hrs) of toluene solutions of derivatized THIQs or CAs in the presence of the aqueous  $NH_4$  phosphate resulted in a 90 - 100% decrease in derivative peak heights.

A comparison of capillary versus conventional column separation of a standard mixture of DHBA, DA, SAL, 3M-DA, 4M-DA, 6M-SAL, and 7M-SAL can be seen in Figure 23. The conventional 6 foot X 1/4 inch i.d. packed column was unable to resolve the isomers of 3-M- and 4-M-DA and 6-M and 7-M SAL, despite various combinations of temperature, pressure, and liquid phase. Packed columns of longer length tended to lose efficiency, as demonstrated by excessive peak widths of long retention time compounds, and



required temperatures which neared or exceeded maximum operating temperatures of the columns. Capillary columns were found to separate easily the 6- and 7-O-Methyl isomers of DA, SAL (Fig. 24), 4,6,7,(OH)<sub>3</sub> THIQ (Fig. 25), 1-CSAL (Fig. 26), 3-CSAL (Fig. 27) and 1-Me-6,7-DHIQ (Fig. 28). A summary of capillary chromatographic conditions, retention times, and minimum detectable quantities is shown in Table 2.

D. High Performance Liquid Chromatography (HPLC)  
of Biogenic Amines, THIQs, and Their Metabo-  
lites

The use of paired-ion HPLC permitted simultaneous quantitation of either the acid components or their corresponding amines in a single injection, but separation of amines and acids in the same mixture was not achieved in these studies. Separation of a standard mixture of DOPAC, 5HIAA and HVA by paired ion chromatography is seen in Figure 29. Figure 30 shows the separation of NE, DHBA, DA, SAL, and 5HT. Although paired-ion HPLC was able to separate the 6- and 7-O-Methyl isomers of THIQs, the separations were not adequate enough for precise quantitation. Small adjustments in paired-ion content, % MeOH and flow rate were necessary to accommodate the column degeneration associated with repeated usage.

The use of the 0.1 M Na<sub>2</sub>PO<sub>4</sub>-1 mM Na<sub>2</sub>EDTA buffer (ionic-suppression buffer) allowed for the simultaneous separation and quantitation of the biogenic amines and their acid metabolites. The pH, now an important parameter, was manipulated to achieve the best separations. Because ionic-suppression buffers have pHs of between 4 and 7, electrochemical oxidation of most biological molecules is easily achieved. Figure 31 demonstrates the simultaneous separation of NE, DHBA, DA, DOPAC, 5HT, 5HIAA, and HVA utilizing the ionic-suppression buffer system. Utilization of this buffer system in combination with a C<sup>18</sup> reverse phase (25 cm) column easily separated the following catechol THIQs and their respective 6-O- and 7-O-methylated isomers: SAL (Fig. 32), 467-(OH)<sub>3</sub>THIQ (Fig. 33), 1-CSAL (Fig. 34), and 3-CSAL (Fig. 35). With a slight modification, 1-Me-6,7-DHIQ and its two O-methylated derivatives were also separated (Fig. 36). A summary of retention times and selected HPLC parameters is found in Table 3.

E. In Vivo Studies of the Stereoselective Brain O-Methylation of a Variety of Catechol Isoquinolines and DA

Electron capture responses for HFB-derivatized samples of SAL, 467-(OH)<sub>3</sub>THIQ, 1-CSAL, 3-CSAL,

1-Me-6,7 DHIQ, DA and their O-methylated isomers were all excellent. Minimum detectable quantities were in the range of 2 - 10 picomoles/ml. GC recoveries of all compounds of interest, after EtOH extraction, Bio Rex-70 isolation, derivatization, and sample clean-up with  $\text{NH}_4^+$  phosphate buffer, ranged from 80 - 85%. Representative chromatograms of HPLC and GC separations of SAL/DA (Fig. 37), 4,6,7-(OH)<sub>3</sub> THIQ (Fig. 38), 1-CSAL (Fig. 39), 3-CSAL (Fig. 40), and their respective O-methyl-metabolites are actual tissue-extracted results. Regional brain chromatograms of saline-injected controls showed no interfering components at the retention times of any compounds of interest.

As can be observed in chromatograms, Figures 37 and 39, SAL and 1-CSAL were almost exclusively O-methylated on the para -7- hydroxyl. Figures 38 and 40 qualitatively show that 4,6,7-(OH)<sub>3</sub> THIQ and 3-CSAL had mixed stereospecificity to O-methylation with peaks for both "meta" -6- and "para" -7- O-methyl products. 1-Me-6,7-DHIQ was not O-methylated to any detectable extent in the 50 minute period following central injection. DA, injected ICV into six pargyline-pretreated rats, was apparently O-methylated exclusively in brain on the meta -3-

hydroxyl (Fig. 37); if 4M-DA was present, levels were less than 4 - 5 ng/g striatum. This result insured that the 7-O-methylation seen with catecholic isoquinolines was not an artifact of administration. The quantitative distribution of brain isoquinoline O-methylation can be seen in Figure 41.

Tables 4, 5, and 6 show the % stereoselective O-methylation, % of O-methylation and levels of catechol isoquinoline precursors present in the corpus striatum, hypothalamus, and hippocampus, respectively. In the corpus striatum (Table 4) the levels of all catechol isoquinoline presursors were statistically the same ( $P > .05$ ). However, the % O-methylated [= total O-methylated products/catechol isoquinoline presursor (+) total O-methylated products] are significantly different. SAL and 4,6,7-(OH)<sub>3</sub>THIQ are statistically O-methylated to the same extent, while 1-CSAL and 3-CSAL are O-methylated 67% ( $P < .01$ ) and 33% ( $P < .01$ ) of SAL, respectively.

In the hypothalamus (Table 5) the levels of the carboxylated THIQs were 2 to 3 fold as high as SAL and 4,6,7-(OH)<sub>3</sub>THIQ. The % O-methylation for 4,6,7-(OH)<sub>3</sub>THIQ, 1-CSAL, and 3-CSAL are statistically the same being an average of 70% of the % SAL O-methylation.

In the hippocampus (Table 6) the levels of SAL,

4,6,7-(OH)<sub>3</sub>THIQ and 3-CSAL (1-CSAL was not assayed in this brain part) were statistically the same as were the % O-methylation for 4,6,7-(OH)<sub>3</sub>THIQ and SAL. However, the % O-methylation for the carboxylated THIQ 3-CSAL was 36% (P<.01) of SAL % O-methylation.

F. Survey of Salsolinol Uptake into the Rat CNS  
After Intraperitoneal Administration

This experiment was undertaken to determine whether the levels of THIQs found in the CNS after EtOH administration could have originated in part from a peripheral source such as liver, heart, or adrenals, via the cerebral circulation. GC/EC provided a means by which the presence of picomole quantities of SAL and O-methylated SALs could be detected in brain (minimum detectable quantities, 15 - 30 pmoles/g tissue). The results indicate that at SAL doses between 5 and 20 mg/kg i.p. SAL and O-methylated SALs were not detectable in the corpus striatum, hypothalamus or hippocampus (data summarized in Table 7). The efficacy of GC/EC assay was checked by the measurements of endogenous DA, which fell within literature values. Dopamine levels in the hypothalamus and corpus striatum were not significantly changed at any SAL dose or at any time point (not shown). These results indicate that at doses

of 20 mg/Kg or less (assumed possible physiological range), SAL, a representative, simple catechol THIQ, does not significantly cross the blood/brain barrier.

G. Effects of Acute ICV Administration of Catechol-Isoquinolines on the Levels of 5HT and Acid Metabolites in Various Rat Brain Regions

In Tables 9, 10, and 11, the actual values for 5HT, 5HIAA, DOPAC, and HVA in the hypothalamus, hippocampus and corpus striatum in control and isoquinoline (or pargyline/DA) treated animals are tabulated.

In the hippocampus (Table 9, summary Table 8 and Figure 42) where only 5HT and 5HIAA were assayed, only 1-CSAL failed to significantly increase the levels of 5HT. The three THIQs, SAL, 3-CSAL, and 4,6,7-(OH) THIQ, and the DHIQ all increased the hippocampal levels of 5HT to 145 - 185% of control ( $P < .01$ ); 5HIAA levels were altered (increased 27%;  $P < .01$ ) only by 4,6,7-(OH)<sub>3</sub> THIQ. Pargyline/DA decreased 5HIAA to 26% ( $P < .01$ ) of control as expected.

In the striatum (Table 10, summary Table 8 and Figure 42), where DOPAC, HVA, and 5HIAA levels were assayed, 1-CSAL significantly increased both DOPAC ( $P < .01$ ) and HVA ( $P < .01$ ) 160 and 116% respectively. 3-CSAL significantly lowered 5HIAA levels to 83% of control ( $P < .01$ ) but did not effect the levels of

DOPAC or HVA. Likewise, 4,6,7-(OH)<sub>3</sub>THIQ significantly lowered 5HIAA to 82% of control ( $P < .05$ ), but had no effect on the other acid metabolite levels. On the other hand, SAL did not alter the steady-state levels of any of the acid metabolites in the corpus striatum.

In the hypothalamic tissue (Table 11, summary Table 8 and Figure 42) DOPAC, HVA, and 5 HIAA were assayed. A number of significant changes occurred due to the isoquinolines. First, levels of the 5HT metabolite, 5HIAA, were observed to be increased 127% - 178% ( $P < .01$ ) of control by four of the five isoquinolines, the exception was SAL. The levels of the catechol acid, DOPAC, were lowered to 67% - 79% ( $P < .01$ ) of control values by each of the four THIQs, and additionally, 3-CSAL also decreased HVA levels to 63% of control ( $P < .01$ ). Finally, 1-Me-6,7-DHIQ significantly reduced HVA levels by 26% ( $P < .01$ ).

Thus, it appears that diverse neurochemical changes occur after large (50  $\mu$ g) ICV injections of catechol isoquinolines, depending on the brain area examined. For example, 5HIAA levels were changed (increased) almost exclusively in the hypothalamus but not in the other two brain areas [slight significant decrease in corpus striatum by 3-CSAL and 4,6,7-(OH)<sub>3</sub>THIQ] by all the isoquinolines except SAL.

Hypothalamic DOPAC levels are decreased by all four THIQs, but in the striatum, only 1-CSAL affected DOPAC levels, and this was a 60% increase above control levels. HVA was only affected by one THIQ in the hypothalamus (a 37% decrease in its levels by 3-CSAL), and by 1-CSAL (16% increase) in the striatum. The DHIQ, 1-Me,6,7-DHIQ had a consistent effect on HVA, decreasing it 20% - 25% in both regions measured.

#### H. Half-Life and Effects of 7-Methoxy-Salsolinol Following Acute ICV Injection

The half life ( $T_{1/2}$ ) of ICV administered 7M-SAL was determined by following its disappearance from selected brain parts. Analysis by HPLC and GC showed its  $T_{1/2}$  to be 133 minutes. This was calculated from  $T_{1/2} = 0.693/R$ ; R is equal to the slope of the line generated from the plot of  $\log$  7MSAL ( $\mu\text{g/g}$  tissue) versus time (hr) (Fig. 43).

In tables 12 and 13 the actual values for DA, 5HT, DOPAC, HVA and 5HIAA in the hypothalamus, hippocampus and corpus striatum in control and 7M-SAL treated rats were tabulated.

In the hypothalamus (Table 13A summarized Figure 44) there is no observed significant change in 5HT levels over the 48 hr period assayed. However, 5HIAA levels were increased significantly at



12 hr ( $P < .05$ ), 24 hr and 48 hr ( $P < .01$ ). The increases in 5HIAA were 120% - 137% of control values. The initial reciprocal changes (1 hr - 6 hr) seen in the 5HT and 5HIAA levels were not significant.

In the hippocampus (Table 13B, summarized in Figure 44), 5HT and 5HIAA levels were assayed. 7M-SAL significantly increased 5HIAA levels 130% - 143% from 6 hr to 48 hr ( $P < .01$ ) while initially (1 hr) lowering 5HIAA 19% ( $P < .05$ ). 5HT levels were significantly affected at 3, 6, 12, and 48 hr after ICV injection. At 3 hr and 48 hr 5HT levels were increased 17% and 29% over control values, respectively ( $P < .01$ ), while at 6 hr and 12 hr the 5HT levels are decreased 17% and 11% respectively ( $P < .02$ )

In the corpus striatum (Table 12, summarized in Figure 44) DA, DOPAC, HVA, 5HT, and 5HIAA were assayed. Striatal 5HIAA was significantly increased 3, 6, and 12 hr (114% - 139%;  $P < .05$ ) post ICV injection, while 5HT levels were significantly decreased only at the 12 hr time point (23%;  $P < .02$ ). On the other hand, Striatal HVA was significantly increased 133% - 192% over control at 1, 3, 12 hr ( $P < .05$ ). DA levels were decreased 24% at the 48 hr time point ( $P < .02$ ) and DOPAC levels were increased 25% at the 3 hr time point ( $P < .05$ ).

Thus, it appears that consistent neurochemical changes occur after moderate (10 µg) ICV injections of 7M-SAL in all three brain parts assayed. For example, 5HIAA was observed to be increased as was HVA and to some extent DOPAC in the hypothalamus, hippocampus, and corpus striatum. The biogenic amines, 5HT and DA, have scattered changes with the hippocampus having the most significant 5HT alterations. Examination of the graphed results (Fig. 44) indicates that the general trend is an increase in the acid metabolites of the biogenic amines (DOPAC, HVA, and 5HIAA) over time with no concomitant, consistent changes in DA or 5HT steady-state levels over time.

I. Results of Chronic Intraperitoneal Administration of l-Carboxy-Salsolinol for Determination of In Vivo Formation of Dihydroisoquinolines from a Simple l-Carboxylated THIQ

After chronic l-CSAL administration, the acid eluates of Bio Rex-70 isolation columns (amine fraction) of liver, corpus striatum, hypothalamus and hippocampus were examined for the presence of l-Me-6,7,-DHIQ, 6- and 7-O-Methyl-l-Me-DHIQ, SAL and the two O-methylated SALs. There was no chromatographic evidence for the presence of these compounds which could, in theory, form from the (oxidative) decarboxylation and/or

O-methylation of l-CSAL. Minimum detectable quantities for DHIQs were 12 - 24 ng/g. Excellent recovery (85 - 87%) of the internal standard and biogenic amines insured the integrity of the assay. Addition of l-Me-6,7 DHIQ and its 6- and 7-O-methylated isomers to cerebellar tissue passed through the BioRex-70 column procedure showed that they were exclusively eluted in the amine fraction. Qualitative examination of the primary elution plus 2 ml of .02M Na<sup>+</sup> phosphate (acid and amphoteric fraction) confirmed the presence of l-CSAL and its 7-O-methylated (carboxylated) metabolite in all regions examined. No endogenous amines were found in this fraction. These results indicate no substantial formation of DHIQs from l-carboxy-SAL in these rat tissues in vivo after chronic i.p. administration of the THIQ.

J. Spectrophotometric Studies on a Series of  
l-Methyl-6,7-Substituted 3,4-Dihydroisoquinolines

The proposed neurotoxicity of dihydroisoquinolines is dependent on the structural similarities between the quinoidamine conformation of the DHIQs and that of the oxidized quinones of 6OHDA and of other related compounds (i.e. 6-amino-dopamine, acetaminophen). pH dependency of DHIQs was studied to determine

the tautomeric form of the DHIQs under physiological conditions (Fig. 45).

1. 1-Methyl-6,7 Dihydroxy-3,4-Dihydroisoquinoline  
(I) and 1-Methyl-6-Hydroxy-7-Methoxy-3,4-Dihydro  
Isoquinoline (II)

The UV absorption spectra of (I) and (II) were practically identical throughout the entire pH range tested (Fig. 46). Between pH 2 and 4 the spectra were unchanged; however above pH 5.0 they went through characteristic and similar changes for both substances. The most prominent change was the appearance of an absorption peak at 384 nm which reached a maximum of about pH 9.0. With a further increase of pH up to 13, the absorption at 384 nm was considerably diminished.

Compound (I) had very weak fluorescence in the pH range of 1 to 5. However, above pH 5.0 a fluorescence maximum at 455 appeared which gradually increased with increasing pH. The maximum activation peak was at 380 nm. The fluorescence intensity reached a maximum at approximately pH 8.0; above this pH, the emission intensity weakened, falling to a small value at higher pH's (Figures 47 and 48). Even at pHs 1 to 5, compound (II) exhibited a fairly strong fluorescence with a main excitation maximum at 380 nm and an emission peak at 465 nm. Maximum fluorescence intensity

was attained at about pH 8.0. The fluorescence decreased with increasing pH above pH 8.0 (Figures 47 and 48).

2. 1-Methyl-6,7-Dimethoxy-3,4-Dihydroisoquinoline (III) and 1-Methyl-6-Methoxy-7-Hydroxy-3,4-Dihydroisoquinoline IV

Compounds III and IV had almost identical U V spectra at all pHs tested (1 to 13). The spectra were practically identical between pHs 1 to 7 and in this range they also resembled the spectra of compounds I and II between pHs 1 and 5 (Fig. 49). Above pH 8.0 they underwent reversible and typical changes. The absorption peak at 350 nm gradually decreased and disappeared (Fig. 49).

Compound III showed a high fluorescence intensity from pH 1.0 to pH 7.0 with these spectral characteristics: the main fluorescence excitation peak was at 350 nm and the main emission maximum was at 450 nm (Fig. 50). Above pH 7.5 the fluorescence intensity decreased, disappearing at higher pH values (Fig. 51).

Compound IV exhibited very weak fluorescence compared to the other DHIQs tested. In order to obtain accurate readings the concentrations had to be increased 10-fold.

K. Cyclic Voltammetry Studies on a Series of 1-Methyl-6,7-Substituted-3,4-Dihydroisoquinolines

Cyclic voltammograms were generated from 1-Me-6,7-DHIQ, 1-Me-7M-DHIQ, and 1-Me-6M-DHIQ at pHs of 3.0, 5.0, and 7.4. The results of the cyclic voltammetry for these three dihydroisoquinolines can be seen in Figures 52, 53, and 54, respectively. The voltammograms are strikingly dissimilar from those generated from CAs, THIQs and 6OHDA-like compounds. The DHIQs exhibit no reversible reduction peak. The voltammograms generated do exhibit characteristic shifts in oxidation potentials from higher oxidation potentials to lower oxidation potentials as the pH is increased from 3.0 to 7.4. These shifts indicated an increase in the ease of oxidation as the pH increased. The lack of the reversible reduction peak would indicate electrochemical oxidation to a compound which is stabilized against reduction. Further interpretation of these results are reserved for the discussion section of this dissertation. A summary of pH versus oxidation potential is shown in Table 14.

L. Attempted Synthesis of Glutathione-Dihydroisoquinoline Adducts

TLC analysis of the reaction mixtures of the dihydroisoquinolines and glutathione indicated that

no adduct formation had occurred. Silica Gel TLC plates, developed with butanol/HOAc/dH<sub>2</sub>O(4/1/1), showed only one fluorescence spot whose R<sub>F</sub> (= distance compound from origin/distance solvent front from origin) was identical to the respective DHIQ utilized as starting material. Staining of the plates with ninhydrin (primary amine)-reagent to visualize the location of the glutathione demonstrated one spot whose R<sub>F</sub> was identical to that of GSH and was substantially different from that of the respective DHIQs. No spot appeared having both fluorescence and ninhydrin staining which would indicate adduct formation. The NMR spectra of the two lyophilized reaction mixtures indicated no loss of the 5 or 7 position aromatic protons [as seen in 6OHDA-glutathione adduct formation (206)] which are the expected sites of sulfhydryl addition. HPLC analysis of the reaction mixture utilizing combinations of electrochemical/UV and electrochemical/fluorescence detection also indicated no adduct formation.

M. GC Analysis of Acetaldehyde (AcA) and Ethanol (EtOH) in Human Blood

Routine separation of the compounds of interest, AcA, EtOH, and the internal standard N-propanol can be seen in Figure 55. Standard curves

prepared with AcA and EtOH were linear for both compounds through their physiological ranges, (EtOH, 2.0 to 500 mg/dl, AcA, 0.04 to 1.0 mg/dl). Recoveries ranged from 60 - 70%. Blood AcA and EtOH levels in blood samples were not changed by storage at  $-20^{\circ}\text{C}$  for at least 72 hours.

Table 15 shows mean concentrations of EtOH and AcA in the blood of thirteen male subjects ages 37 - 54, on admission (day 0) and days 1 - 4. Blood EtOH concentrations ranged from 110 to 420 mg/dl in these subjects, with a mean of 229.7 mg/dl. The mean EtOH concentration dropped to 5.0 mg/dl one day after admission and was undetectable on successive days. Our subjects' blood AcA concentrations on admission had a range of 0.06 to 0.70 mg/dl and averaged 0.350 mg/dl. Blood AcA in our studies were somewhat lower than those previously reported by Magrinat et al. (207), but our subjects' average EtOH concentrations were also less.



## CHAPTER IV

### DISCUSSION

#### A. Introduction

These studies have been concerned with several related aspects of THIQ metabolism. The metabolic aspects were studied in vivo to determine in situ involvement of THIQs with the important CA-metabolizing enzymes, COMT and MAO. First, the cerebral O-methylation pattern of several 1-alkyl catecholisoquinolines related to the CAs and their effects on DA, 5HT and the acid metabolites of biogenic amines were studied. Since 7M-SAL was the predominant O-methylated product of SAL in those studies, and since both of these THIQs form in vivo during alcoholism, the half-life and effects of 7M-SAL (ICV) on endogenous DA, 5HT, DOPAC, HVA and 5HIAA levels in various rat brain regions were determined. Third, preliminary investigations on a possible, novel, oxidative pathway for brain THIQs were initiated. Analytical characterization of the possible oxidative products of this pathway was undertaken. Simple but highly sensitive and specific analytical techniques were developed in order to pursue the above neurochemical

studies. The technical achievements in this dissertation include improved GC/EC and HPLC/ED methods for the simultaneous separation and quantitation of biogenic amines, THIQs and their respective metabolites. Lastly, a procedure for the analysis of EtOH and AcA in human blood was modified and endogenous AcA levels were quantitated in alcoholics during detoxification at Hines V.A. Hospital.

The discussion that follows is organized into sections headed by a statement of principle which describes the results to be discussed in that section.

#### B. Improved Methods

##### 1. Direct Assay of Tissue Supernatants by HPLC with Electrochemical Detection Proves to Be a Rapid, Facile, and Versatile Way to Measure Biogenic Amines and Acids in Small Brain Samples

Simultaneous separation and quantitation of DA, DOPAC, HVA, 5HT and 5HIAA in small brain parts allows for direct assessment of the neurochemical consequences of administered agents. Comparing control values of the biogenic amines and acid metabolites obtained by the HPLC techniques developed in this dissertation with literature values obtained by a variety of analytical methods, the efficacy of our rapid HPLC assay system can be seen (Table 16). All results fall well

within established literature values. The ability to do simple EtOH or HCl extraction and direct analysis of the supernatant allows for a rapid profile of brain regions and constitutes a useful and inexpensive addition to the neurochemists' repertoire of analytical tools.

Reverse phase (RP) is a special case of partition chromatography. In the case of RP-HPLC, the organic phase is a permanently bonded non-polar stationary phase. For our purposes we utilized a 18-carbon alkyl side chain covalently attached to silica beads. The aqueous phase is represented by the buffer system utilized to make the mobile phase.

The separations using this system are achieved because of the different degrees of interaction of a compound with both the non-polar stationary phase and the polar mobile phase. Compounds with a charged ionic moiety partition preferentially in the aqueous phase and move through the column quickly. Compounds which are neutral (uncharged) tend to interact with the hydrophobic phase of the column and to be retained.

The biogenic amines and their acid metabolites represent a group of ionic compounds whose charge species are the amine and carboxylic acid, respectively.

For these ionic species the following equilibrium exists;  $\text{RCOOH} \rightleftharpoons \text{RCOO}^- + \text{H}^+$ ;  $\text{RNH}_3^+ \rightleftharpoons \text{RNH}_2 + \text{H}^+$ . By adjusting the pH of the buffer system in the mobile phase, the equilibrium can be driven to favor a particular species. This technique is called ionic-suppression HPLC chromatography.

Paired ion chromatography (PIC) utilizes a large organic counter-ion added to the mobile phase which forms a reversible ion-pair complex with the ionized sample; this complex behaves as an electronically neutral, non-polar compound. The use of octane sulfonic acid (OSA) or heptane sulfonic acid (HSA) as the paired-ion adds a high degree of hydrophobic character to the sample through the paired-ion complex.

In order to assure a high paired-ion sample interaction, the pH of the mobile phase must be such that the sample is present in its ionic form. The extent to which the ionized sample and the counter-ion form an ion-pair complex affects the degree of retention on the column.

In the separation of biogenic amines by PIC with alkyl sulfonates, the pH of the mobile phase should be around 3.5 for best ionic interactions. Maintaining a pH of around 3.5 ensures that both

strong and weak bases will be in their ionic forms and any weak acids will be primarily in the non-ionic species.

With the use of the electrochemical detection a second important pH-dependent reaction must be considered. The oxidations of the phenolic hydroxyls of the biogenic amines and their metabolites are also pH dependent. The lower the pH the higher the oxidation potential which must be applied. While the CAs and catechol isoquinolines and acid metabolites are sufficiently detectable at low pHs (~3.5) using moderate (+.5-+.8 DC Volts) oxidation potentials, their O-methylated metabolites have a poor response at these potentials. Raising the applied potential to a value which would allow for detection of O-methylated compounds results in increased oxidation of buffer components and a general loss in oxidative specificity. Increasing the pH of a strong paired-ion mobile phase to above pH 4.5 results in a low efficiency of paired-ion-complex formation, thereby frustrating separations.

For our investigations a HPLC system was developed which utilized a combination of ion-suppression/ion-paired chromatography for the simultaneous separations of DA, DOPAC, 5HT, 5HIAA, HVA,

a number of THIQs, and their O-methylated metabolites (208). The buffer system of 0.1 M Na H<sub>2</sub>PO<sub>4</sub> utilized 1 mM Na<sub>2</sub>EDTA as the weak complexing paired-ion. EDTA is an unusual paired-ion because of its four ionizable carboxylates with PKa's of 2.0, 2.67, 6.16, and 10.26 (209). The wide range of PKa's allows for this molecule to be utilized as a paired-ion over a broad spectrum of pHs.

In our studies, the pH which gave maximum separation and detection within the shortest time period was determined by titration with concentrated nitric acid (HNO<sub>3</sub>) and found to be between 4.7 and 5.2. Between these pHs the ability to detect the whole spectrum of biogenic amine NT components was excellent. Separation of these compounds were dependent on pH adjustments as little as 1/10 of a pH unit. pH adjustments were made to accommodate column deterioration and THIQs and their O-methylated metabolites which were present.

Therefore, several significant advantages of the system developed in this research can be noted:

- 1) with small change in pH a wide variety of sample mixtures could be routinely separated and quantitated;
- 2) the moderate pHs allowed for quantitation of O-methylated compounds;
- 3) the expense of costly

paired-ion reagents was eliminated; buffer systems can be made from conventional laboratory reagents.

2. Capillary GC with EC Detection: A Very Selective and Extremely Sensitive Approach to the Estimation of Biogenic Amines and THIQ Stereoisomers in Discrete Brain Regions

The use of a 10 M, WCOT column in combination with EC detection yielded the separation of geometrical isomers with sensitivity as low as 3ng/g tissue. These results are comparable, in terms of sensitivity, with those generated by GC/MS. The use of short glass capillary columns allowed for unequivocal determination of isolated compounds. The quantitation of endogenous brain DA levels and the assessment of in vivo THIQ metabolism had never been exploited by glass capillary GC/EC before this work was initiated. The development, therefore, represents the breaking of new ground in the field of analytical neurochemistry. The DA values generated by this technique compare favorably with established literature values (Table 16). Conversion of a conventional EC/GC to a capillary system is a relatively inexpensive investment (~\$380). However, the result of this modification is the addition of a highly versatile and sensitive instrument, without prohibitive cost, to

the average neurochemistry laboratory.

C. A Peripherally-Circulating DA-Related THIQ, SAL,  
Does Not Enter the Rat CNS

Utilizing the highly sensitive (minimal detectable quantity for SAL and O-methylated products, 3 - 5 ng/g) GC/EC assay developed in this dissertation, it was found that low peripheral doses (5 - 20 mg/Kg) of SAL were not taken up into the CNS. This is not surprising, considering various CAs, including DA, the SAL precursor, also do not readily cross the BBB (217). The results here are additional support that SAL (11) and O-methylated-SAL (13), detected in the CNS of experimental animals after EtOH administration, were derived in situ and were not the result of peripheral formation followed by uptake.

SAL can be rapidly O-methylated by COMT with a Km equal to such accepted physiological substrates as DA and NE (151 - 153). The greatest activity for COMT is found in the liver (218) with activity also found in the spleen, intestines, adrenal gland (219) and blood (220 - 221) as well as other peripheral tissues. Peripherally formed SAL therefore would be rapidly O-methylated. Neither of the O-methylated SAL geometric isomers, Meta-6- or para-7- , were found in the CNS tissue assayed.



Peripheral metabolism of SAL could be envisioned as analogous to that of the THIQ trimetoquinol which like the sympathomimetic isoproterenol, is metabolized by a combination of O-methylation and conjugation with glucuronic acid (222). Because of these results, our general approach was to study the interactions of catechol-isoquinolines on the biogenic amine systems after ICV administration.

D. Centrally-Administered Isoquinolines Significantly Affect the Levels and Turnover of Regional Brain Amines and Acid Metabolites to Differing Degrees, Depending on the Isoquinoline

1. Hippocampal 5HT is Increased with Little Change in 5HIAA Levels

The results of this dissertation indicate that hippocampal 5HT levels are increased significantly when measured 50 min following ICV administration of a catechol isoquinoline (with the exception of 1-CSAL), but 5HIAA levels are unchanged (Fig. 42). These findings agree with those of Hannigan and Collins (166) and Patel and Collins (223) that acutely administered THIQs cause increases in 5HT with no increase in 5HIAA in rat brain.

The experiments in this dissertation show

that a variety of THIQs have this effect indicating that as a class catechol-isoquinolines can interact with 5HT-systems. One plausible explanation for increases in 5HT without immediate and concomitant changes in 5HIAA would be inhibition of 5HT-specific MAO. In light of this, these results can be compared to the in vitro work by Meyerson et al. (161), who demonstrated that MAO type A, the 5HT specific enzyme, was more sensitive to inhibition by isoquinolines than MAO type B.

A second possible interpretation of these results could be blockade of 5HT postsynaptic receptors, thereby increasing steady-state levels of 5HT through increased 5HT reuptake and storage. Evidence for THIQ interactions with 5HT receptors was found by Hamilton et al. (146) who demonstrated that SAL antagonized the effects of 5HT on smooth muscle.

It is quite possible that catechol-isoquinolines exert their effects at both the level of receptor and as a metabolic inhibitor. These effects would be synergistic and could account for increases in 5HT with little or no changes in levels of 5HIAA.

What is apparent, is that the catechol-isoquinolines interact with 5HT neurons. It is particularly relevant and interesting that catechol

derived isoquinolines can influence 5HT-systems. The isoquinolines could thus serve as a neuro-modulatory bridge between CA-systems and 5HT systems. The fact that isoquinolines have effects on 5HT steady-state levels and are known to inhibit 5HT uptake (124) would reflect their ability to be taken up into 5HT neurons in vivo. Once taken up, they could act as "false" 5HT-NTs. The presence of isoquinolines in 5HT-neurons, possible release and receptor interactions and the potential to inhibit 5HT deamination, could lead to aberrant 5HT metabolism.

Abnormal 5HT metabolism was proposed long ago by McIsaac (224) as an etiological factor in some forms of mental disease. One feasible hypothetical situation could occur during chronic EtOH intoxication, where the formation of the THIQ, SAL, within the CNS has been documented. THIQ interactions with 5HT-systems would lead to elevated intracellular 5HT levels. This in turn would lead to increased 5HT and tryptophan condensations with free aldehydes (ACA), resulting in the formation of harmaline-like tetrahydro- $\beta$ -carboline (THBC)-alkaloids. THBCs are themselves potent 5HT antagonists and MAO A inhibitors (225) as well as benzodiazepine receptor blockers (226). Such harmaline compounds have

recently been reported in vivo in untreated and alcohol treated rats (227). Therefore, the initial formation of catechol-isoquinolines within CA neurons speculatively could result in a self-perpetuating stimulation of 5HT dynamics. In this way, small quantities of THIQs generated during chronic EtOH ingestion could augment their pharmacodynamic effect by generating a second (possibly more potent) psycho-active agent. This situation could be described very well by Thudichum, a pioneer in brain chemistry, who wrote with foresight in 1884, "many forms of insanity are unquestionably the external manifestations of the effects upon brain substance of poisons fermented within the body" (228).

2. A 6-Hydroxy-Dihydroisoquinoline Quinoidamine Significantly Decreases Hypothalamic and Striatal HVA Levels Without Changes in DOPAC

1-Me-6,7-DHIQ lowered HVA levels with no concomitant changes in DOPAC in both the corpus striatum and hypothalamus (Fig. 42). These results may be due to in vivo inhibition of COMT which was seen only with 1-Me-6,7-DHIQ. The unique aspect of this molecule which may be responsible for this possible action is its quinoidamine tautomer (vide supra, section G) which appears to be the predominant

conformation at physiological pH (Fig. 45). In the quinoidamine conformation the catechol moiety no longer exists; it is an aromatic keto-enol substituent and closely resembles compounds such as tropolones, the tropolone-like pyrones, and pyridones, which are regarded as isosteric with catechol (Fig. 56). 1-Me-6,7-DHIQ would appear to be isosteric with these inhibitory compounds. The tropolones, pyrones and pyridones represent a class of short lived, but highly potent COMT inhibitors. In vivo, the tropolones exhibit more potency of inhibition than do any catechol or catechol-derivative inhibitors (229). It is then reasonable to assume that the specific affect of 1-Me-6,7-DHIQ on the levels of HVA are due to its tropolone-like activity (230). Further support of this suggestion is presented in section E of this discussion.

### 3. 5HIAA Levels Are Increased and DOPAC Levels Are Decreased in the Hypothalamus by Four of the Five Catechol Isoquinolines Examined

The decreased hypothalamic DOPAC with increased 5HIAA, which was potentiated by all the isoquinolines tested with the exception of SAL, reflects differing effects of catechol isoquinolines within 5HT- and DA-neurons (Fig. 42). The increase in 5HIAA could be

explained by displacement of 5HT from binding vesicles, again with an increase in 5HT turnover (therefore, no change in 5HT levels). Either case would require that the catecholic THIQ would be taken up into 5HT neurons. This is further evidence of a structure-activity relationship between 5HT and isoquinolines.

DOPAC levels in the hypothalamus were decreased by all of the THIQs (but not the DHIQ) examined, with generally no change in HVA levels, the exception being 3-CSAL, which also decreased hypothalamic HVA. One would suspect that these decreases reflect inhibition of DA turnover. Inhibition of TH, the rate limiting step in CA synthesis, by isoquinolines have been demonstrated both in vitro and in vivo (171 - 173). If MAO inhibition were responsible for DOPAC decrease, one would expect to see a concomitant decrease in HVA as seen in the case of pargyline pretreatment (Table 8).

#### 4. Carboxylated Isoquinolines Cause Opposite Effects on DOPAC and HVA in the Hypothalamus and Corpus Striatum

1-CSAL causes a corpus striatum-specific (23) increase in DOPAC and HVA. These concomitant increases may reflect an increase in DA turnover. 3-CSAL on the other hand causes significant decreases in both DOPAC and HVA in the hypothalamus. This, as

stated above, could reasonably be due to inhibition of MAO or a decrease in DA turnover in the hypothalamus.

#### 5. Summary

This study shows that various isoquinolines have remarkably different effects on the levels of 5HIAA, DOPAC and HVA. Moreover, the isoquinolines induce different changes in the three brain regions studied. Further studies of isoquinoline interactions with biogenic amine systems will help to interpret the mechanisms by which these interactions occur.

#### E. The Brain O-Methylation Patterns of Catechol-Isoquinolines Are Remarkably Different from Those of Their Parent Amines

The in vivo stereoselective O-methylation of the catechol isoquinolines differed dramatically from their parent amines, demonstrating significant (para) 7-O-methylation. These studies on the stereoselective enzymatic O-methylation of simple catechol isoquinolines by brain COMT are particularly relevant, because they were performed in vivo. In vitro experimental studies seldom permit the liberty to quantitatively relate the actions of an extracted enzyme to its activity in the intact system. To date, methods used to study the stereoselective

O-methylation of THIQs by COMT have usually involved the enzyme being isolated from cell extracts, "purified," and studied in an artificial medium.

Among the many problems associated with extrapolating in vitro results back to in vivo systems are the differences seen between in vitro and in vivo stereoselective O-methylation patterns of physiological substrates of COMT (DOPA, Epi, NE, DA, DOPAC, DHPG, etc.) (229, 231). In vivo results have demonstrated almost exclusive formation of the meta (3-OCH<sub>3</sub>) derivative (232) while conversely, in vitro results have shown meta/para ratios that varied significantly with minor changes in pH, Mg<sup>++</sup> concentrations, and SAM cofactor concentrations (231 - 236).

The reasons for the anomaly between different isomeric ring substitutions in vivo and in vitro are not known. It has been suggested that the differences could be due to the cellular disruption which could alter the native conformation of COMT. The direct in vivo investigations in this dissertation circumvent these complications by allowing substrate-enzyme interactions to occur while maintaining the microenvironment of the biological system under investigation.

The catecholic THIQs have been shown to interact



with COMT as competitive inhibitors in vitro (151 - 153) and in vivo (153, 237), and, furthermore, undergo O-methylation (148, 151, 153). However, the elucidation of the O-methylated products in vivo had not been accomplished. In vitro studies, utilizing purified rat liver COMT to determine the stereoselective O-methylation of 6,7-dihydroxy THIQ, N-Methyl 6,7 dihydroxy THIQ (154), THP and THPB (156), demonstrated significant para (7-OCH<sub>3</sub>) O-methylation. In light of the known lack of agreement between in vivo and in vitro CA results, these findings with THIQs have to be viewed with caution in regard to their physiological relevance.

To date, the only studies on in vivo O-methylation of THIQs in tissues, particularly brain, was forwarded by Bail et al. (155). Their studies presented chromatographic evidence indicating that the O-methylation of (+) SAL and (+) 6,7(OH)<sub>2</sub> THIQ in the rat CNS were predominantly on the "para" (7-position) hydroxyl. These results were unexpected because, as reviewed, endogenous CAs are primarily or exclusively O-methylated in vivo on the meta (3-OH) hydroxyl. Bail et al. were unable to definitively separate and quantitate the two O-methylated SAL and 6,7-(OH)<sub>2</sub> THIQ products due to lack of chromatographic

resolution.

Our results unequivocally demonstrate that the O-methylation of THIQs in vivo follows discrete patterns that are very different from the CAs. In fact, no catechol substrates have ever shown the high degree of p-O-methylation like SAL and l-CSAL. DA O-methylation was observed to occur exclusively on the meta (3) hydroxyl, thus confirming literature indications and insuring that the "variant" O-methylation of the THIQs was not an artifact of administration.

The reasons for the observed differences in the stereoselective site for O-methylation between precursor CAs and product THIQs in vivo are worth considering at this point. Much evidence suggests that a single enzyme catalyzes the formation of both O-methylated products from catechols (229). Extensive purification of COMT did not affect the meta/para ratio of a variety of substrates, providing further evidence that only one enzyme is involved in both meta and para O-methylation of catechols (238).

The ratio of meta and para O-methylated products in vitro was found to be strongly dependent on the nature of the aromatic substrate and the pH of the reaction medium. Meta-(3)-O-methylated isomers predominated with substrates which contained highly polar

substituents [as is the case with physiological substrates (154)], while substrates with non-polar substituents had ratios close to unity.

There are at least two possible explanations for the negative effects of polar substituents on p-O-methylation by COMT (231): a) Catechols containing substituents with highly polar moieties are bound by means of this highly polar substituent in an orientation that favors transfer of the methyl group to the meta (3) position, or, b) A hydrophobic region is present in the active site of COMT which prevents random binding of polar substrates by repulsive interactions so that binding occurs primarily (as is the case of physiological substrates) in a conformation which favors meta O-methylation. Since the apparent affinity of polar substrates for COMT does not differ markedly from those of non-polar compounds, and since both anionic and cationic polar substituents have the same effect on p-methylation, the latter explanation is favored.

The presence of a non-polar region in the catechol binding site of COMT would then militate the orientation of binding, directly influencing meta/para ratios. The meta/para ratios obtained with amine substrates would be due to the presence of the ionized ammonium function in the side chain

being oriented away from this non-polar region. Similarly, studies utilizing analogues of DA and NE suggest meta/para ratios as a function of side chain interactions with hydrophobic centers on COMT (239). Charged species on the substrate molecules would be steered away from the hydrophobic centers, resulting in binding orientations which would result in a stereospecific O-methylation reaction.

Application of these in vitro findings to the in vivo results on THIQ stereoselective O-methylation can help to provide an explanation for the patterns observed. The THIQs, although structurally very similar to CAs (having several structure-activity relationships), have one major structural dissimilarity. The amine substituent is not flexible but is fixed within a heterocyclic ring in the THIQ. This limits the freedom of rotation of the amino functional group (as well as other side chain substituents) and restricts the number of orientations which the side chain can accommodate. This restricted movement of ionizable groups adds another parameter to the binding orientation of THIQs to COMT.

Figure 57A illustrates the manner in which a hydrophobic region adjacent to the active site of COMT could influence binding orientation of

physiological substrates. The hydrophobic area orients the ionized side chain such that binding of substrate best accommodates the charge species outside of the hydrophobic area. Binding such that the para hydroxyl is O-methylated is possible but involves overcoming hydrophobic forces. The THIQs would be particularly susceptible to hydrophobic steering of their ionizable substituents because of the additional loss of rotational freedom due to the heterocyclic ring. The most favored orientation would, as in the case of CAs, place charged substituents outside this hydrophobic area (Fig. 57b).

Utilizing the above rationale, the observed stereoselective binding of the THIQs assayed in this dissertation can be understood. (+) SAL ( $R_1=R_3=R_4=H$ ,  $R_2=CH_3$ ), which was O-methylated to a major (95%) extent on the para-7- hydroxyl, had to bind in an upside-down orientation in relation to CA binding. Thus, the charged amine was positioned away from the hydrophobic center while the hydrophobic region of SAL's ring was oriented into the hydrophobic center. The 5% meta binding observed may be due to  $R_1(CH_3)$  being able to interact with the hydrophobic region thus deferring some of the hydrophobic repulsion forces.

This is a tenable explanation in light of

the results with (+) 1-CSAL ( $R_1=COOH$ ,  $R_2=CH_3$ ,  $R_3-R_4=H$ ). The presence of carboxylic acid moiety on the  $R_1$  substituent eliminates a "meta-wobble" and militates strict (100%) "para" (7-OH) O-methylation. Binding orientation places both the charge amine and carboxylic acid away from the hydrophobic center, thereby positioning the para-hydroxyl for methylation.

4,6,7-(OH)<sub>3</sub> THIQ ( $R_1=R_2=R_3=H$ ,  $R_4=OH$ ) has polar substituents located in a meta orientation to one another and may therefore represent a class of THIQ substrates with intermediate binding specificity. Binding in either the meta or para orientation positions places a polar moiety within the hydrophobic region. This is reflected in the observed (55% meta/45% para) randomized product ratio. Similarly, (-) 3-CSAL ( $R_1-CH_3$ ,  $R_2R_4=H$ ,  $R_3=COOH$ ) has two (2) polar moieties (located ortho to one another). Binding of the substrate in the para orientation brings the carboxylic moiety in close proximity to the hydrophobic region. Binding in the meta position would bring the amine within proximity of the hydrophobic region but also positions the  $R_1$  methyl group in a favorable position for overlap with the hydrophobic center. The binding is randomized but favors the accommodation of the methyl group overlap and yields 60% meta/40% para

results.

1-Me-6,7-DHIQ represents a novel finding in that it appears not to undergo O-methylation to any detectable extent. As described (vide supra in section D2) this compound resides in a quinoidamine conformation and resembles the tropolone-like inhibitors of COMT. The tropolone-like inhibitors compete with CAs for the active site of COMT, but are not metabolized (233, 240) (Fig. 56). The lack of a catechol moiety at physiological pH is no doubt responsible for the inert nature of these compounds toward enzymatic O-methylation.

An additional set of interesting observations can be gleaned from these results. In Tables 4, 5, and 6 the % O-methylation and the levels of catechol isoquinolines ( $\mu\text{g/g}$  brain region) are given as found in the corpus striatum, hypothalamus and hippocampus, respectively. The levels of the catechol isoquinolines appear to be similar in both the corpus striatum and hippocampus. However, the % O-methylation for the carboxylated-THIQs is consistently lower. In the corpus striatum (Table 4) 1-CSAL is 66% of SAL while 3-CSAL is 33% of SAL. In the hypothalamus (Table 5) the levels of carboxylated THIQs are 2 - 2.5 fold that of the noncarboxylated THIQs

and the % O-methylation is found to be approximately the same. In the hippocampus (Table 6) the levels of 3-CSAL, SAL, and 4,6,7-(OH)<sub>3</sub> THIQ are similar. However, the % O-methylation for the carboxylated THIQ is 37% of the non-carboxylated THIQs. These results may reflect a difference in the Km's between the carboxylated and non-carboxylated THIQs. These results again reflect significant differences between the THIQs and the open chain catecholic substrates which bear acidic, neutral, and basic constituents whose Km's are not markedly different (231). If this is the case, the presence of a carboxylic acid moiety on the side of a THIQ would result in an extended T 1/2. The consequence of this increase in T 1/2 would be a similar increase in the duration of the pharmacological effect.

F. O-Methylation of a Catechol Isoquinoline Increases Its Half-Life in Brain Over 10-Fold and Alters Its Effects on Biogenic Amine Systems

1. Centrally Administered 7M-SAL Significantly Affected the Levels and Turnover of Regional Brain Biogenic Amines and Acid Metabolites

The phenolic isoquinoline 7M-SAL tended to increase 5HIAA without increasing 5HT in hypothalamus and hippocampus over the entire 48 hr period assayed (Fig. 44). Striatal increases in 5HIAA, HVA, and



DOPAC levels were restricted to the first 24 hr. These results, increased acid metabolites with no concomitant increases in biogenic amine levels, indicates that 7M-SAL increased biogenic amine turnover in all three tissues assayed. This represents an obvious change in the pharmacodynamics of SAL upon O-methylation. The most significant effect is on striatal HVA levels within the first 12 hrs after ICV administration. In contrast to SAL whose main effect is on 5HT neurons, 7M-SAL appears to effect both 5HT and DA neurons, not as inhibitor of biogenic amine metabolism, but as an activating agent. These effects indicate that the production of endogenous catechol isoquinolines, followed by metabolism to their phenolic "metabolites," would result in a complex array of metabolic consequences.

## 2. O-Methylation of a Catechol Isoquinoline Increases Its T 1/2 in Brain Over 10-Fold

ICV administered 7M-SAL had a calculated T 1/2 of 133 minutes as measured from its disappearance from the corpus striatum and hypothalamus. This represents a 10.6 fold increase in the T 1/2 of SAL in the rat CNS. SAL rapidly disappears from the CNS with a T 1/2 of 12.5 min (150). The T 1/2 was doubled when pyrogallol, a known inhibitor of COMT, was administered, indicating that the disappearance of SAL

is the result of conversion to an O-methylated metabolite. Hamilton et al. (13) provided the chromatographic evidence for O-methylated SAL (isomer unknown) in the CNS of mice treated with chronic EtOH. In light of the relative inertness of SAL to enzymatic oxidative degradation, O-methylation appears to be a major metabolic pathway. The consequences of O-methylation in the case of SAL do not appear to be deactivation. In fact, a growing body of evidence is challenging the initial premise that O-methylation is totally a deactivation process. Recent findings have demonstrated that 3M-DA, the primary in vivo product of DA O-methylation by COMT, is not pharmacologically-inactive compound. Rats treated with 3M-DA exhibited psychomotor effects which mimic those of DA treatment in combination with MAO inhibitors, conditions resulting in increased 3M-DA formation (241).

G. Catecholic and Phenolic 1-Me-DHIQs: Spectrophotometric, Fluorescence and Voltammetric Investigations Provide Interesting Results

1. Spectrophotometric and Fluorescence Assays Indicate that 6-OH-DHIQs Are in the Quinoldamine Conformation at Physiological PH

At physiological pH (7.4), 1-Me-6,7-DHIQ and 1-Me-7M-DHIQ are totally in the quinoldamine

conformation. The interpretation of U V and fluorescence results on 1-Me-DHIQs obtained in this dissertation were based to a large extent on the work of G. Jonsson with HCHO generated DHIQs (lacking the 1-Methyl group) (242, 243).

Jonsson's U V and fluorescence results utilizing 6-hydroxy-DHIQ are identical to those generated in this dissertation for 1-Me-6,7-DHIQ and 1-Me-7M-DHIQ. Jonsson found that at pH 2.0, 6-hydroxy-3,4-DHIQ showed two absorption peaks, one at 236 nm and the other at 323 nm (242). Above pH 6.0 - 6.5 the spectra showed typical drastic changes with an appearance of a strong absorption peak at 365 nm. This peak increased with increasing pH, reaching a maximum at pH 8.0 - 9.0. Above pH 9.0 the absorption gradually decreased and at pH 13 the absorption characteristics were similar to those of pH 2.0. The 6-hydroxy-3,4-DHIQ exhibited only weak fluorescence at low pH's but fluorescence increased with increasing pH, reaching a maximum at around pH 8.0. Above pH 8.0, the fluorescence intensity fell, becoming very weak above pH 12.0.

The 6-hydroxyl-3,4-DHIQ, lacking a 7-position hydroxyl, by default, must form a tautomeric quinone whose structure resembles form B (Fig. 45). 1-Me-7M-DHIQ,

with a blocked 7-hydroxyl, behaves like its dihydroxy derivative, which in turn behaves like 6-hydroxy-3,4-DHIQ. All three compounds show identical pH dependent spectral changes. The disappearance of long wavelength absorption above pH 8.0 is probably due to the ionization of the hydroxyl group in the 6 position (Fig. 58A).

1-Me-6,7-DM DHIQ and 1-Me-6M-DHIQ both have the 6-hydroxyl group blocked and behave almost identically. Their typical spectral changes prevalent above pH 7.5 are probably due to addition of water across the imine double bond to form a carbinolamine (Fig. 58b) (244).

Therefore, these results demonstrate that 1-Me-6,7-DHIQ and 1-Me-7M-DHIQ at physiological pH and ambient temperature exist in a quinoidamine conformation which bears structural similarities to the oxidized (apparent neurotoxic) form of 60HDA (Fig. 19). Previous results (section E) indicate that 1-Me-6,7-DHIQ does not undergo O-methylation and that its possible THIQ precursors (SAL and 1-CSAL) are both O-methylated predominantly on the 7-position hydroxyl. Therefore, even if the dihydroisoquinolines were to be formed after O-methylation of the precursor THIQs, quinoidamine formation would not be blocked by O-methylation.

2. Cyclic Voltammetry of 1-Me-6,7-DHIQ 1-Me-7M-DHIQ and 1-Me-6M-DHIQ Indicate that They Are Irreversible Oxidized

Cyclic voltammograms of the DHIQs, 1-Me-6,7-DHIQ, 1-Me-7M-DHIQ and 1-Me-6M-DHIQ demonstrated irreversible oxidation throughout a range of pHs. This is strikingly different from the cyclic voltammetry of CAs, THIQs, or 6OHDA-like compounds, and suggests that the electrochemical oxidation of DHIQ leads to the formation of an "atypical" product. In order to facilitate the interpretation of the results of the cyclic voltammetry of the compounds of interest, a brief discussion of cyclic voltammetry follows.

In voltammetry, a varying potential ( $E_{app}$ ) is applied between a working electrode and a reference electrode immersed in a quiet (non-stirred) solution containing an electroactive component suspended in a buffer. The buffer, a supporting (background) electrolyte is simply one which can provide overall high conductance, while not being electroactive in the region of interest. The cut-off potential for any voltammetric analysis is that potential where general electrolysis of solvent begins to occur. The working electrode is an inert surface which serves as a source or sink of electrons. As  $E_{app}$

varied, molecules near the electrode surface can either gain electrons from the source (cathodic reduction) or lose them to the sink (anodic oxidation). Diffusion occurs as a result of the concentration difference developed between the electrode surface and the bulk of the solution as soon as electrolysis is initiated. Diffusion is the predominant form of mass-transport in quiet (standing) solution voltammetry.

When a working electrode is placed in a quiet solution and  $E_{app}$  is varied in a linear fashion (a linear potential sweep) the response is a peak voltammogram (Fig. 59a). Figure 59a represents a model compound whose oxidation occurs at  $E_p$  of 0.42 volts and the magnitude of  $I_p$  is directly proportional to the concentration of the compound in solution. By convention, oxidation currents are plotted downward from the zero current line and the potential,  $E$ , is increasing anodic or oxidizing, from right to left. Potentials in voltammetry are usually referred directly to the experimental reference electrode, in the case of our work, an Ag-AgCl electrode.

When a peak voltammetry is seen and the potential sweep is rapidly and reproducibly reversed, a cyclic voltammogram is generated (Fig. 59b). Since the solution is quiet and the time interval between

sweep reversals is short, the products generated in the oxidation are available near the electrode for reduction as the potential sweep reverses. In a simple, rapid electron transfer, with no chemical complications, one obtains oxidation and reduction peaks whose potentials are separated by a small increment as defined by equation:

$$\left| E_{P_{\text{Ox}}} - E_{P_{\text{Red}}} \right| = \frac{0.57}{N} \text{ volts (245) (Eq. 1)}$$

The reversibility of any electron transfer can be qualitatively judged by the separation of the oxidation and reduction peaks (Fig. 60). Figure 60a illustrates a fast (reversible) electron transfer; B, a less reversible (quasi-reversible) transfer; while C, a slow electron (irreversible) transfer.

In Figure 59b, the theoretical voltammogram for a pure reduced species in solution is shown. As can be observed, it represents a reversible electron transfer system. The potential sweep is initiated in an anodic (oxidizing) direction at some arbitrary point. During the time interval of peak b, the originally fully reduced compound is oxidized. At an arbitrarily chosen point, the potential is reversed and peak a is generated and represents the re-reduction of the compound. With no intervening chemical reactions, the

properly measure ratio of a/b currents is unity (246).

All the adrenergic NTs and their metabolites (247), as well as the THIQ alkaloids, SAL and O-Me-SAL (248), possess electrochemical activity with their voltammograms showing that they are quasi-reversible systems. 60HDA, on the other hand, is a model compound for cyclic voltammetry and displays near perfect reversibility (246). It was unexpected to discover that the cyclic voltammograms of 1-Me-6,7-substituted-3,4-DHIQs resembled neither the THIQs nor CAs, their purported precursors, or 60HDA, to which they bear some structural resemblance, at any of the pHs tested. Rather, the cyclic voltammograms of the DHIQs demonstrated an irreversible electron transfer system which could only undergo oxidation. (The reduction peak is so negative and flat it could not be distinguished over the reduction of the support solvent.) The interpretation of these results possibly would constitute detailed information regarding the possible reactions and metabolic degradation of DHIQs at the molecular level.

Aside from the pH-dependent decrease in oxidation potential (Table 14), the cyclic voltammograms were identical for all three DHIQs tested. This would indicate that upon electrochemical oxidation they are



all being converted to the same product. The primary step of the oxidation was probably conversion to the ortho-quinone similar to what is seen with CA oxidation. One possible explanation is that the orthoquinone is an unstable intermediate which undergoes spontaneous intramolecular oxidation/reduction to yield a fully aromatic, heteroaromatic molecule (Fig. 61). This would be similar to the internal oxidation reduction of cyclized 60HDA to form 5,6-dihydroxyindole (249). Attempts to reduce the fully aromatic molecule would be futile, owing to its internal reduction upon aromatization. Any attempts to reoxidize the "fully aromatic" heteroaromatic species would probably require very high electrochemical oxidation potentials to overcome the loss of aromaticity upon oxidation. This would account for the lack of a new oxidation peak when repetitive sweeps were run at applied potentials between (+) 1.2 and (-) 1.2 volts.

H. Oxidative Decarboxylation of l-CSAL to a DHIQ or THIQ Appears to be Insignificant in the Normal Intact Rat

As reported in the Results, detectable levels of l-methyl-DHIQs were not observed after chronic i.p. administration of l-CSAL. However, recently it has been demonstrated that l-carboxylated THIQs can be

decarboxylated to their corresponding 3,4-DHIQs by anodic oxidation at a low potential (184), prolonged aerial oxidation in basic media (183), and exposure to plant laccases and peroxidases (78). In all the oxidative decarboxylations a free phenolic group at position 6 or 7 of the isoquinoline system is essential. These reactions are considered to be decarboxylations induced by oxidation of the phenolic hydroxyl group. To date, no in vivo or in vitro decarboxylations of 1-carboxylated THIQs have been reported using animal tissue, and initial experiments presented here were unsuccessful. This may be due in part to rapid O-methylation and conjugation which is known to occur with other THIQs.

O-methylation of 1-CSAL, as reported earlier in this work was found to be predominantly on the 7-hydroxyl in brain (4-hydroxyl of the precursor moiety) and was qualitatively observed on the 7-hydroxyl in liver. These results imply that a significant metabolic route for 1-CSAL is O-methylation.

Studies utilizing trimetoquinol (TMQ), a catechol THIQ having potent bronchodilating action, demonstrated that 61% of the excreted TMQ was conjugated to glucuronide, 11.5% the free base, and 27.5% as O-methylated-conjugated TMQ (222). Further studies

determined that sulfate conjugation of biogenic amines and their acid and O-methylated metabolites is an important metabolic pathway in their metabolism in the CNS (250 - 251). These reactions would tieup the free phenolic hydroxyls of THIQs (necessary for oxidative decarboxylation) thereby deactivating the THIQs toward oxidative decarboxylation.

Alternatively, the optimum situation for the formation of DHIQs from THIQs in the rat in vivo may be after chronic EtOH or barbiturates when microsomal oxidizing systems are "induced." In an analogous situation, Dajani and Saheb (252), studying the metabolism of 6-hydroxy- and 6-methoxy-1,2,3,4-tetrahydro- $\beta$ -carboline (THBCs), found that varying metabolic conditions favored the formation of a number of oxidized  $\beta$ -carbolines both in vivo and in vitro. These findings give rise to yet another alternative explanation, that is, formation of other oxidative products such as N-oxides and fully aromatic isoquinolines (Fig. 62). Hamilton and Gause (253) have indicated possible formation of N-oxides from SAL and 6,7-(OH)<sub>2</sub>-THIQ after incubation in mild aqueous alkaline conditions. Results of the cyclic voltammograms generated in this dissertation indicate the possible formation of fully aromatic isoquinolines upon oxidation of DHIQs. These

alternative oxidation products may have formed but would not have been detected because those possible products were not available as standards.

I. Simple 6-Hydroxy-DHIQs Do Not Apparently Form  
Glutathione Adducts

CAs after oxidation to ortho-quinones as well as 6OHDA, 6-amino-DA (6NH<sub>2</sub>DA) and their analogues, after oxidation to para-quinones, are known to undergo irreversible nucleophilic additions of sulfhydryl compounds (190, 249, 254). The addition of thiol to carbon-carbon double bonds of a quinone constitutes a special case of nucleophilic addition to an  $\alpha$ - $\beta$ -unsaturated system (255). The nucleophile in these reactions is R-S-H. The reaction occurs rapidly when the electron density of the carbon-carbon double bond is reduced by electron withdrawing substituents (256). Electron withdrawal can be either by induction or resonance, or both.

We can conclude that simple 1-alkyl-6-hydroxy-7-alkoxy-substituted-3,4-DHIQs, which at physiological pH reside as para quinoidamine tautomers, do not participate to any detectable extent in sulfhydryl adduct formation. The plausible explanation for this lack of reactivity may lie in the low electrophilic character of tautomeric double bonds.

Unlike the para-quinones of 6OHDA and 6NH<sub>2</sub>DA which can rely on the strong electron withdrawing capacities of two electron withdrawing substituents, the DHIQs tested have only the proximal catecholic oxygen which cannot sufficiently reduce the electron density of the double bond participating in adduct formation. (The distal atom in the para-quinone being the 1-carbon; carbon atoms are poor electron withdrawing groups.) However, the electrophilicity of the DHIQ tautomers can be enhanced if the 1-methyl substituent would be replaced with an electron withdrawing group. This is the case when dealing with one of the DHIQ products of oxidative decarboxylation of 1-carboxy-1-benzyl THIQs. These THIQ compounds, as noted in the introduction, are present in various pathological conditions (3, 7), and are known to undergo oxidative decarboxylations under a variety of conditions (78, 183, 184) to form  $\alpha$ -keto-3,4-substituted-1-benzyl-6,7-substituted DHIQs. These compounds may be electrophilic enough to undergo adduct formation through the enhanced electron-withdrawing capabilities of the  $\alpha$ -keto and 1-benzyl substituents. If these complex DHIQs share even a degree of the electrophilic character of 6OHDA quinone, they should be capable of producing neuronal damage via covalent attachments

to cellular nucleophiles.

J. Ethanol and Acetaldehyde in Human Blood

GC methods for the determination of EtOH and AcA are specific, sensitive and rapid. However, an acknowledged problem hindering the precise measurement of AcA and EtOH containing samples (blood and tissues) is artifactual (non-enzymatic) generation of the aldehyde (257). It has been reported that addition of 20 - 40 mM thiourea suppressed AcA production from EtOH in deproteinated rat blood which was incubated at 65°C, but that thiourea was only partially effective when human blood was used (258). We found, however, that artifactual AcA formation was essentially absent of human blood sample in perchloric acid and thiourea were incubated at a lower temperature. As would be expected, incubation at a lower temperature lowered AcA and EtOH recoveries. However, the recoveries were adequate to permit minimum detection of 0.04 mg/dl of AcA.

The significant importance of this portion of the study is that mean blood AcA concentrations did not remain elevated throughout the four day detoxification period. An earlier report by Magrinat et al. (207) stated that blood AcA concentrations in patients admitted for alcohol detoxification

remained at admission day levels over four days as blood EtOH dropped to negligible values. Our findings show that blood AcA levels drop 70% within one day after admission and were undetectable on days 2 - 4. The possible differences in subject population, previous alcohol or drug consumption, or nutritional and hepatic status should not account for this large disparity with regard to blood AcA concentration on days 1 - 4 of detoxification. A possible conclusion for the chronically elevated blood AcA levels reported earlier in detoxification is that the workers failed to account for artifactual AcA formation during analysis.

## CHAPTER V

### SUMMARY

In this dissertation, relatively simple, highly sensitive, and specific techniques were developed or improved for use in neurochemical studies of tetrahydroisoquinoline metabolism. A direct assay of tissue supernatants by high performance liquid chromatography with electrochemical detection was used to measure tetrahydroisoquinolines, biogenic amines and acids in small brain samples. Capillary gas chromatography with electron capture detection proved to be a very selective and extremely sensitive approach to the estimation of biogenic amines and catechol isoquinoline stereoisomers in discrete brain regions.

Utilizing the highly sensitive (minimal detectable quantity for salsolinol and O-methylated products, 3 - 5 ng/g) gas chromatography with electron capture detection assay developed in this dissertation, it was found that salsolinol in low peripheral doses (5 - 20 mg/kg) was not taken up into the central nervous system.

These results are additional support that salsolinol and O-methylated-salsolinol, detected in the central nervous system of experimental animals after



ethanol administration, were derived in situ and were not the result of peripheral formation followed by uptake.

Centrally administered catechol isoquinolines (10 - 50  $\mu\text{g}$ ) were found to alter the steady state levels of biogenic amines and acid metabolites to differing degrees, depending on the isoquinoline and the brain region. Of particular interest was the effect of catechol isoquinolines on the serotonergic system. A variety of catechol isoquinolines raised the steady state levels of serotonin, indicating that as a class these compounds can interact, indirectly and/or directly, with serotonergic systems in vivo. The isoquinolines could thus serve as neuromodulating bridges between catecholaminergic systems and serotonergic systems.

The O-methylation patterns of several centrally-administered catechol isoquinolines were found to be different from those of their parent amines, as reflected by the extent of O-methylation on the 7-position hydroxyl (para position in the parent catecholamine). Such in vivo studies on the stereoselective enzymatic O-methylation of simple catechol isoquinolines are more relevant than in vitro approaches, because substrate-enzyme interactions occur in the

endogenous microenvironment of the biological system under investigation.

The endogenous half-life of centrally administered 7-O-methyl-salsolinol (salsoline; the major methylated metabolite of salsolinol in vivo) was determined to be 133 minutes in two brain regions, the striatum and the hypothalamus. This represents a 10.6 fold increase over the reported half-life of the parent isoquinoline salsolinol. In addition, salsoline was found to effect the steady state levels of biogenic amines and acid metabolites over a 48 hour period. These effects, increase in half-life and pharmacological activity of an O-methylated metabolite, indicate that production of endogenous catechol isoquinolines followed by metabolism to phenolic metabolites would result in a complex array of metabolic consequences.

Spectrophotometric studies of 1-methyl-6,7-substituted 3,4-dihydroisoquinolines (potential products of tetrahydroisoquinoline oxidative metabolism) indicated that, at physiological pH, those with 6-hydroxy-substituents are nearly exclusively in the quinoid-amine conformation (the conformation postulated for nucleophilic attack on cellular ligands). The cyclic voltammetry of these dihydroisoquinolines showed that upon electrochemical oxidation they become irreversibly

oxidized, suggesting transformation to a fully aromatic species. 1-methyl-6-hydroxy-7-alkoxy-substituted-dihydroisoquinolines apparently do not readily form glutathione adducts as such. Also, in vivo synthesis of simple 1-methyl-dihydroisoquinolines, from 1-carboxyl-salsolinol (injected chronically) by oxidative decarboxylation, appeared to be insignificant in the normal intact rat.

These findings represent advancements in our knowledge about the discrete metabolic consequences of catecholamine-related isoquinolines in mammalian systems, advancements which could aid in understanding the underlying meaning of condensation product involvement in mammalian homeostasis and disease.

Table 1  
Selected Physical Data for Synthesized  
Compounds in Section II

<u>Compound</u>	<u>MW</u> <sup>A</sup>	<u>MP</u> <sup>O</sup> <u>C</u> <sup>B</sup>	<u>% Yield</u> <sup>C</sup>
Salsolinol HBr (SAL)	264	183 (lit. 182-184) <sup>208</sup>	85
1-Carboxy-Salsolinol (1-CSAL)	223	233 (lit. 230-235) <sup>208</sup>	94
7-O-Methyl-1-Carboxy-Salsolinol (7M-1-CSAL)	237	205 (lit. 205) <sup>210</sup>	85
6,7-Dihydroxytetrahydroisoquinoline (6,7-(OH) <sub>2</sub> THIQ)HBr	250	268 (lit. 267-268) <sup>209</sup>	80
1-Methyl-6,7-dihydroxy-3,4-dihydroisoquinoline (1-Me-6,7-DHIQ HBr)	263	230 (lit. 230) <sup>259</sup>	90

<sup>A</sup>MW = Molecular weight (salt),

<sup>B</sup>MP<sup>O</sup>C = melting point degrees centigrade.

<sup>C</sup>% Yield =  $\frac{\text{actual yield}}{\text{theoretical yield}} \times 100.$

Table 2  
 Summary of GC Capillary Parameters for  
 Selected Compounds of Interest

<u>Compound</u>	<u>Column Temp °C</u>	<u>Retention time (min)<sup>a</sup></u>	<u>Derivative</u>	<u>MDQ (b) (ng/ml)</u>
DHBA	130	4.00	HFBA	--
DA	130	6.00	HFBA	0.25
3M-DA	130	9.50	HFBA	0.45
4M-DA	130	10.20	HFBA	0.45
SAL	130	8.40	HFBA	0.25
6M-SAL	130	21.55	HFBA	0.45
7M-SAL	130	22.55	HFBA	0.45
4,6,7(OH) <sub>3</sub> THIQ	130	12.05	HFBA	2.00
6M-4,7(OH) <sub>2</sub> THIQ	130	17.64	HFBA	5.00
7M-4,6(OH) <sub>2</sub> THIQ	130	18.80	HFBA	7.00
1-CSAL	125	6.45	PFPA/HFIP	1.00
6M-1-CSAL	125	16.20	PFPA/HFIP	5.00
7M-1-CSAL	125	18.16	PFPA/HFIP	5.00

Table 2 (Cont'd)

Summary of GC Capillary Parameters for  
Selected Compounds of Interest

<u>Compound</u>	<u>Column Temp °C</u>	<u>Retention time (min)<sup>a</sup></u>	<u>Derivative</u>	<u>MDQ (b) (ng/ml)</u>
3-CSAL	155	5.40	PFPA/HFIP	1.00
6M-3-CSAL	155	16.70	PFPA/HFIP	5.00
7M-3-CSAL	155	19.25	PFPA/HFIP	5.00
1-Me-6,7 DHIQ	145	4.55	HFBA	1.00
1-Me-6M,7OH-DHIQ	145	7.10	HFBA	7.00
1-Me-7M,6OH-DHIQ	145	8.50	HFBA	2.00

<sup>a</sup>10M X.25mm i.d. OV-17 glass WCOT capillary column N<sub>2</sub>O<sub>2</sub>F 0.8 kg/cm<sup>2</sup> detector temperature 340, injector temperature 250°C.

<sup>b</sup> Minimum detectable quantity (equivalent to twice the baseline noise).

Table 3

Summary of Retention Times<sup>a</sup> and Selected HPLC Parameters

<u>Compound</u>	Retention Times (min)	
	<u>6mM Heptane Sulfonic Acid, 10% MeOH, 9.7% HOAc, pH 3.5</u>	<u>0.1M NaH<sub>2</sub>PO<sub>4</sub>/ 1.0mM Na<sub>2</sub>EDTA pH 5.0</u>
NE	6.2	1.9
DHBA	8.1	3.9
DA	10.4	5.2
DOPAC	4.9	6.8
5HT	21.8	13.6
5HIAA	8.1	17.6
HVA	11.3	20.1
SAL	12.0 (8.40) <sup>b</sup>	8.4
6M-SAL	(27.0) <sup>b</sup>	22.8
7M-SAL	(23.4) <sup>b</sup>	22.8
4,6,7-(OH) <sub>3</sub> THIQ	--	4.0
6M 4,7-(OH) <sub>2</sub> THIQ	--	9.2
7M 4,6-(OH) <sub>2</sub> THIQ	--	11.6

Table 3 (Cont'd)

Summary of Retention Times<sup>a</sup> and Selected HPLC Parameters

Retention Times (min)

<u>Compound</u>	<u>6mM Heptane Sulfonic Acid, 10% MeOH, 9.7% HOAc, pH 3.5</u>	<u>.1M NaH<sub>2</sub>PO<sub>4</sub>/.1mM Na<sub>2</sub>EDTA, pH 5.0</u>
1-CSAL	--	4.0
6M-1-CSAL	--	12.8
7M-1-CSAL	--	16.2
3-CSAL	--	4.4
6M-3-CSAL	--	14.4
7M-3-CSAL	--	16.0
1-Me-6,7-DHIQ	(12.86) <sup>c</sup>	--
1-Me-7M-DHIQ	(18.60) <sup>c</sup>	--
1-Me-6M-DHIQ	(39.60) <sup>c</sup>	--



Table 3 (Cont'd)

Summary of Retention Times<sup>a</sup> and Selected HPLC Parameters

Retention Times (min)

<sup>a</sup>Biosil C<sup>18</sup> reverse phase 25 cm column, flow rate 1.0 ml/min.

<sup>b</sup>Chromatographed using a 0.1M NaH<sub>2</sub>PO<sub>4</sub>, 1mM Na<sub>2</sub>EDTA buffer pH 5.5 with 5% MeOH.

<sup>c</sup>Chromatographed using a 0.1M Na<sub>2</sub>HPO<sub>4</sub>, 1mM Na<sub>2</sub>EDTA buffer pH 7.4 with 10% MeOH.

Table 4

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Corpus Striatum<sup>a</sup>

<u>Compound</u>	% of Total Methylation + S.E.M. (N = 6)		Levels of catechol isoquinoline precursor ( $\mu\text{g/g} \pm \text{s.e.m.}$ )	§ (b) O-methylation ( $\pm \text{s.e.m.}$ )
	<u>6-O-methylation</u>	<u>7-O-methylation</u>		
SAL	5 $\pm$ 1.5	95 $\pm$ 1.5	18.0 $\pm$ 0.98	18.0 $\pm$ 1.30
4,6,7-(OH) <sub>3</sub> THIQ	55 $\pm$ 2.8	45 $\pm$ 2.8	15.8 $\pm$ 3.60	17.4 $\pm$ 0.50
1-CSAL <sup>e</sup>	N.d. (c)	100 $\pm$ 0.0	19.7 $\pm$ 3.90	12.0 $\pm$ 1.60
3-CSAL <sup>e</sup>	60 $\pm$ 1.3	40 $\pm$ 1.3	28.3 $\pm$ 4.80	6.0 $\pm$ 0.70
1-Me-6,7-DHIQ	N.d.	N.d.	--	--
DA <sup>d</sup>	(3M-DA 100 $\pm$ 0.0)	(4M-DA N.d.)	--	--

Table 4 (Cont'd)

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Corpus Striatum<sup>a</sup>

<sup>a</sup> N = 6.

<sup>b</sup> % O-methylated =  $\frac{\text{Total O-methylated product}}{\text{Total THIQ} + \text{total O-methylated THIQ}} \times 100.$

<sup>c</sup> N.d. = Not detectable.

<sup>d</sup> Pretreated with pargyline.

<sup>e</sup> N = 4

Table 5

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Hypothalamus

<u>Compound</u>	% of Total Methylation + S.F.M. (N = 6)		Levels of catechol isoquinoline precursor $\mu\text{g/g} \pm \text{s.e.m.}$	% <sup>b</sup> O-methylation ( $\pm \text{s.e.m.}$ )
	<u>6-O-methylation</u>	<u>7-O-methylation</u>		
SAL	6 $\pm$ 1.1	94 $\pm$ 1.2	22 $\pm$ 1.4	17 $\pm$ 2.0
4,6,7-(OH) <sub>3</sub> THIQ	57 $\pm$ 2.0	43 $\pm$ 1.0	34 $\pm$ 1.8	13 $\pm$ 3.0
1-CSAL <sup>a</sup>	N.d.	100 $\pm$ 0	60 $\pm$ 7.2	12 $\pm$ 1.1
3-CSAL <sup>a</sup>	61 $\pm$ 2.0	39 $\pm$ 2.0	69 $\pm$ 12.2	9.8 $\pm$ 2.5
1-Me-6,7-DHIQ	N.d. <sup>c</sup>	N.d.	--	--
DA <sup>d</sup>	(3M-DA 100 $\pm$ 0)	(4M-DA N.d.)	--	--

Table 5 (Cont'd)

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Hypothalumas

<sup>a</sup> N = 4.

<sup>b</sup> % O-methylated =  $\frac{\text{Total O-methylated product}}{\text{Total THIQ} + \text{total O-methylated THIQ}} \times 100.$

<sup>c</sup> N.d. = Not detectable.

<sup>d</sup> Pretreated with pargyline.

Table 6

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Hippocampus

<u>Compound</u>	% of Total Methylation ± S.E.M. (N = 6)		Levels of catechol isoquinoline precursor µg/g ± s.e.m.	% <sup>b</sup> O-methylation (± s.e.m.)
	<u>6-O-methylation</u>	<u>7-O-methylation</u>		
SAL	3 ± .5	97 ± .5	16 ± 2.6	19.6 ± 2.0
4,6,7-(OH) <sub>3</sub> THIQ	54 ± 1.7	46 ± 1.5	19 ± 2.6	18.6 ± 1.5
3-CSAL <sup>a</sup>	60 ± 1.3	40 ± 1.3	14.5 ± 7.5	7.0 ± 0.42
1-Me-6,7 DHIQ	N.d. <sup>c</sup>	N.d.	--	--
DA <sup>d</sup>	3M-DA 100 ± 0	4M-DA N.d.	--	--

<sup>a</sup> N = 3.

Table 6 (Cont'd)

Stereoselective In Vivo O-methylation of Catechol  
Isoquinolines in Rat Hippocampus

$$^b \text{ \% O-methylation} = \frac{\text{Total O-methylated}}{\text{Total THIQ} + \text{total O-methylated THIQ}} \times 100.$$

<sup>c</sup> N.d. = Not detectable.

<sup>d</sup> Pargyline pretreated.

Table 7

Survey Study of SAL Uptake into the Corpus Striatum,  
Hypothalamus and Hippocampus after Intraperitoneal Administration (5-20mg/kg)<sup>a</sup>

<u>Brain Region</u>	<u>Salsolinol</u> <sup>b</sup>	<u>6M-Salsolinol</u> <sup>c</sup>	<u>7M-Salsolinol</u> <sup>d</sup>	<u>Dopamine</u> <u>µg/g ± s.e.m.</u>
Corpus striatum	N.d. <sup>e</sup>	N.d.	N.d.	9.50 ± 0.78
Hypothalamus	N.d.	N.d.	N.d.	1.90 ± 0.31
Hippocampus	N.d.	N.d.	N.d.	N.d.

<sup>a</sup> N = 12.

<sup>b</sup> Minimum detectable quantity 3 ng/g (17.3 pmoles/g).

<sup>c</sup> Minimum detectable quantity 5 ng/g (28.6 pmoles/g).

<sup>d</sup> Minimum detectable quantity 5 ng/g (28.6 pmoles/g).

<sup>e</sup> N.d. = Not detectable.



Table 8

Levels of 5-HT and Acid Metabolites (Percent of Control)  
 in Brain Areas of Rats 50 Min after (ICV) Treatment  
 with Various Isoquinolines (50 µg)

<u>Compound</u>	<u>HIPPOCAMPUS</u>		<u>STRIATUM</u>			<u>HYPOTHALAMUS</u>		
	<u>5-HT</u>	<u>5-HIAA</u>	<u>5-HIAA</u>	<u>DOPAC</u>	<u>HVA</u>	<u>5-HIAA</u>	<u>DOPAC</u>	<u>HVA</u>
SAL	186*	110	97	99	93	111	73*	94
4,6,7-TIQ	145*	127*	82*	91	94	178*	77*	109
1-CSAL	112	94	103	160*	116*	150*	79*	103
3-CSAL	160*	92	83*	98	93	127*	67*	63*
1-Me-6,7-DHIQ	176*	99	89	111	81*	148*	110	74*
Pargyline/DA	--	26*	18*	29*	55*	39*	39*	90

\* Significantly different from control (p<0.05)

Table 9

Effects of Catechol Isoquinolines on the Levels of 5-HT and 5-HIAA in the Rat Hippocampus<sup>a</sup>

µg/g tissue ± s.e.m. (N = 6)

<u>Compound</u>	<u>5HT</u>	<u>% C<sup>c</sup></u>	<u>p</u>	<u>5HIAA</u>	<u>%C</u>	<u>p</u>
Control (saline)	0.352 ± .040	--	--	0.230 ± .020	--	--
SAL	0.656 ± .090	186	.01*	0.254 ± .040	110	NSD
4,6,7-(OH) <sub>3</sub> THIQ	0.510 ± .020	145	.01*	0.292 ± .040	127	.01*
1-CSAL	0.359 ± .060	112	NSD <sup>b</sup>	0.217 ± .020	94	NSD
3-CSAL	0.569 ± .080	160	.01*	0.211 ± .020	92	NSD
Pargyline/DA	--	--	--	0.061 ± .010	26	.01*
1-Me-6,7-DHIQ	0.618 ± .080	176	.01*	0.229 ± .020	99	NSD

<sup>a</sup> Male Sprague-Dawley rats were given one dose of catechol isoquinoline or dopamine (50 µg/animal) bilaterally into the cerebral ventricles. All animals were anesthetized with 50 mg/kg of pentobarbital i.p.

<sup>b</sup> NSD = Not significantly different.

<sup>c</sup> % C = % of control.

Table 10

Effects of Catechol Isoquinolines on the Levels of Acid Metabolites of the  
Biogenic Amines in Rat Striatum<sup>a</sup>

µg/g tissue ± s.e.m.

<u>Compound</u>	<u>DOPAC</u>			<u>5HIAA</u>			<u>HVA</u>		
	<u>% C</u>	<u>p</u>		<u>%C</u>	<u>p</u>		<u>%C</u>	<u>p</u>	
Control (saline)	1.62 ± .15	--	--	0.72 ± .03	--	--	0.85 ± .05	--	--
SAL	1.61 ± .15	99	NSD <sup>b</sup>	0.70 ± .06	97	NSD	0.79 ± .07	93	NSD
4,6,7-(OH) <sub>3</sub> THIQ	1.48 ± .18	91	NSD	0.59 ± .07	82	.01*	0.80 ± .07	94	NSD
1-CSAL	2.59 ± .13	160	.01*	0.74 ± .04	103	NSD	0.99 ± .04	116	.01*
3-CSAL	1.59 ± .09	98	NSD	0.60 ± .08	83	.02*	0.79 ± .08	93	NSD
Pargyline/DA	0.47 ± .10	29	.01*	0.13 ± .02	18	.01*	0.47 ± .07	55	.01*
1-Me-6,7-DHIQ	1.81 ± .36	111	NSD	0.64 ± .09	89	NSD	0.69 ± .12	81	.05*

<sup>a</sup> Male Sprague-Dawley rats were given one dose of catechol isoquinoline or dopamine (50 µg/animal) bilaterally into the cerebral ventricles. All animals anesthetized with 50 mg/kg of pentobarbital i.p.

<sup>b</sup> NSD = Not significantly different.

<sup>c</sup> % C = % control.

Table 11

Effects of Catechol Isoquinolines on the Levels of Acid Metabolites of  
the Biogenic Amine in Rat Hypothalamus<sup>a</sup>

µg/g tissue ± s.e.m. (N = 6)

Compound	DOPAC			5HIAA			HVA		
	µg/g	% C <sup>c</sup>	p	µg/g	% C	p	µg/g	% C	p
Control (saline)	0.114 ± .009	-- --		0.323 ± .010	-- --		0.305 ± .020	-- --	
SAL	0.083 ± .008	73 .01*		0.358 ± .030	111 NSD		0.287 ± .006	94 NSD	
4,6,7-(OH) <sub>3</sub> THIQ	0.088 ± .008	77 .01*		0.575 ± .040	178 .01*		0.333 ± .020	109 NSD	
1-CSAL	0.090 ± .004	79 .01*		0.486 ± .030	150 .01*		0.314 ± .020	103 NSD	
3-CSAL	0.076 ± .009	67 .01*		0.410 ± .010	127 .01*		0.192 ± .040	63 .01*	
Pargyline/DA	0.045 ± .007	39 .01*		0.121 ± .010	37 .01*		0.276 ± .050	90 NSD	
1-Me-6,7-DHIQ	0.125 ± .061	110 NSD		0.471 ± .050	148 .01*		0.226 ± .022	74 .01*	

<sup>a</sup> Male Sprague-Dawley rats were given one dose of catechol isoquinoline or dopamine (50 µg/animal) bilaterally into the cerebral ventricles. All animals were anesthetized with 50 mg/kg of pentobarbital i.p.

<sup>b</sup> NSD = Not significantly different

<sup>c</sup> %C = % of control

Table 12

Effect of 7-O-Methyl-Salsolinol (10ug/rat) on the Levels of Biogenic  
Amines and Acid Metabolites in the Rat Striatum

µg/g tissue ± s.e.m. (N = 4)

<u>Time of Sacrifice (hr)</u>	<u>Dopamine</u>			<u>DOPAC</u>			<u>HVA</u>		
	<u>% C</u>	<u>a</u>	<u>p</u>	<u>% C</u>	<u>C</u>	<u>p</u>	<u>% C</u>	<u>C</u>	<u>p</u>
Control	8.45 ± 1.04	--	NSD <sup>b</sup>	1.30 ± .20	--	--	.626 ± .036	--	--
1	8.60 ± 0.49	101	NSD	1.43 ± .17	109	NSD	1.202 ± .032	192	.01*
3	8.80 ± 0.64	104	NSD	1.64 ± .07	125	.05*	1.016 ± .088	162	.01*
6	8.40 ± 1.24	99	NSD	1.45 ± .09	111	NSD	0.814 ± .172	130	NSD
12	8.20 ± 0.23	97	NSD	1.51 ± .02	115	NSD	0.836 ± .058	133	.05*
24	9.30 ± 0.67	110	NSD	1.18 ± .12	90	NSD	0.640 ± .134	102	NSD
48	6.42 ± 0.53	76	.02*	1.17 ± .08	90	NSD	0.504 ± .074	80	NSD

---

<u>Time of Sacrifice (hr)</u>	<u>5HT</u>			<u>5HIAA</u>		
	<u>% C</u>	<u>C</u>	<u>p</u>	<u>% C</u>	<u>C</u>	<u>p</u>
Control	0.518 ± .052	--	--	0.366 ± .027	--	--
1	0.482 ± .060	93	NSD	0.351 ± .019	96	NSD
3	0.544 ± .048	105	NSD	0.509 ± 0.25	139	.02*

Table 12 (Cont'd)

Effect of 7-O-Methyl-Salsolinol (10 ug/rat) on the Levels of Biogenic Amines and Acid Metabolites in the Rat Striatum

ug/g tissue  $\pm$  s.e.m. (N = 4)

<u>Time of Sacrifice (hr)</u>	<u>5HT</u>	<u>% C</u>	<u>p</u>	<u>5HIAA</u>	<u>% C</u>	<u>p</u>
6	0.516 $\pm$ .046	99	NSD	0.454 $\pm$ .042	124	.05*
12	0.400 $\pm$ .034	77	.02*	0.418 $\pm$ .017	114	.05*
24	0.458 $\pm$ .050	88	NSD	0.344 $\pm$ .042	94	NSD
48	0.454 $\pm$ .032	88	NSD	0.344 $\pm$ .046	91	NSD

a % C = % control.

b NSD = Not significantly different

Table 13

Effect of 7-O-Methyl-Salsolinol (10 ug/rat) on the Levels of 5-HT  
and 5-HIAA in Rat Hypothalamus (A) and Hippocampus (B)

µg/g tissue ± s.e.m. (N = 4)

## Hypothalamus (A)

<u>Time of Sacrifice</u> (hr)	<u>5-HT</u>	<u>% C<sup>c</sup></u>	<u>p*</u>	<u>5-HIAA</u>	<u>% C</u>	<u>p*</u>
Control	0.606 ± .034	--	--	0.448 ± .024	--	--
1	0.644 ± .090	106	NSD <sup>b</sup>	0.444 ± .027	99	NSD
3	0.672 ± .076	111	NSD	0.410 ± .038	92	NSD
6	0.548 ± .054	90	NSD	0.416 ± .028	94	NSD
12	0.612 ± .050	101	NSD	0.538 ± 0.57	120	.05*
24	0.634 ± .036	105	NSD	0.613 ± .007	137	.01*
48	0.584 ± .058	96	NSD	0.561 ± .035	125	.01*

## Hippocampus (B)

<u>Time of Sacrifice</u>	<u>5-HT</u>	<u>% c</u>	<u>p*</u>	<u>5-HIAA</u>	<u>% c</u>	<u>p*</u>
Control	0.295 ± .011	--	--	0.299 ± .025	--	--

Table 13 (Cont'd)

Effect of 7-O-Methyl-Salsolinol (10 ug/rat) on the Levels of 5-HT  
and 5-HIAA in Rat Hypothalamus (A) and Hippocampus (B)

µg/g tissue ± s.e.m. (N = 4)

## Hippocampus (B)

<u>Time of Sacrifice (hr)</u>	<u>5-HT</u>	<u>% C</u>	<u>p*</u>	<u>5-HIAA</u>	<u>% C</u>	<u>p*</u>
1	0.277 ± .045	94	NSD	0.242 ± .017	81	NSD
3	0.344 ± .009	117	NSD*	0.375 ± .091	125	NSD
6	0.245 ± .006	83	.02*	0.425 ± .045	142	.01*
12	0.263 ± .005	89	NSD*	0.428 ± .018	143	.01*
24	0.318 ± .037	108	NSD	0.466 ± .043	156	.01*
48	0.381 ± .022	129	.01	0.388 ± .025	130	.01

<sup>a</sup> % C = % control.

<sup>b</sup> NSD = Not significantly different.



Table 14

Summary of Oxidation Potential vs pH of Three  
1-Methyl-6,7-Substituted-3,4-Dihydroisoquinolines

<u>Compound</u>	<u>pH</u>	Oxidation Potential E(+) volts
1-Me-6,7-dihydroxy	3.0	+0.64
3,4-dihydroisoquinoline	5.0	+0.49
(1-Me-6,7-DHIQ)	7.4	+0.33
1-Me-6-hydroxy-7-methoxy	3.0	+0.88
3,4-dihydroisoquinoline	5.0	+0.65
(1-Me-7M-DHIQ)	7.4	+0.50
1-Me-6-methoxy-7-hydroxy	3.0	+0.90
3,4-dihydroisoquinoline	5.0	+0.79
(1-Me-6M-DHIQ)	7.4	+0.62

Table 15

Blood Acetaldehyde and Ethanol Concentrations (mg/dl  $\pm$  s.e.m.)  
in Alcoholic Patients on Admission (Day 0) and During Early Detoxification

	DAY				
	0	1	2	3	4
Acetaldehyde (13)	0.350 $\pm$ .071	0.104 $\pm$ 0.42	<0.04	<0.04	<0.04
Ethanol (13)	229.7 $\pm$ 29.8	5.0 $\pm$ 2.0	N.d.	N.d.	N.d.

Number of individual subjects in parentheses.

N.d. = Not detectable.

Table 16

Comparison of Regional Brain Levels of DA, 5HT and Acid  
Metabolites Obtained in These Studies with Published Values

<u>Compound</u>	<u>Tissue</u>	<u><math>\mu\text{g/g} \pm \text{s.e.m.}</math></u>	<u>Method</u>	<u>Reference</u>
DA	corpus striatum	$8.45 \pm 1.04$ (a)	HPLC/ED <sup>d</sup>	--
		$9.50 \pm 0.78$ (c)	GC/ED	--
		$8.43 \pm 0.73$	Fluorometric	166
		$7.93 \pm 0.73$	Fluorometric	165
		$8.78 \pm 0.89$	Fluorometric	201
-----				
DOPAC	corpus striatum	$1.30 \pm 0.20$ (a)	HPLC/ED	--
		$1.62 \pm 0.15$ (b)	HPLC/ED	--
		$0.79 \pm 0.45$	Fluorometric	210
		$0.90 \pm 0.21$	GC/EC	211
		$2.25 \pm 0.37$	GC/EC	212
		$1.95 \pm 0.13$	HPLC/ED	213
-----				

Table 16 (Cont'd)

Comparison of Regional Brain Levels of DA, 5HT and Acid  
Metabolites Obtained in These Studies with Published Values

<u>Compound</u>	<u>Tissue</u>	<u>µg/g ± s.e.m.</u>	<u>Method</u>	<u>Reference</u>
HVA	corpus striatum	0.63 ± .03 (a)	HPLC/ED	--
		0.85 ± .05 (b)	HPLC/ED	--
		0.45 ± .01	Fluorometric	210
		0.38 ± 0.06	Fluorometric	165
		0.69 ± 0.06	HPLC/ED	213
		0.66 ± 0.16	GC/EC	211
		0.58 ± 0.06	GC/EC	212
-----				
5HT	hypothalamus	0.606 ± .034 (a)	HPLC/ED	--
		0.707 ± .050	GC/EC	214
		0.841 ± .059	HPLC/ED	215
		0.786 ± .049	Fluorometric	201
-----				

Table 16 (Cont'd)

Comparison of Regional Brain Levels of DA, 5HT and Acid  
Metabolites Obtained in These Studies with Published Values

<u>Compound</u>	<u>Tissue</u>	<u>µg/g ± s.e.m.</u>	<u>Method</u>	<u>Reference</u>
HIAA	hypothalamus	0.448 ± .024 (a)	HPLC/ED	--
		0.323 ± .010 (b)	HPLC/ED	--
		0.771 ± 0.35	Fluorometric	166
		0.514 ± .057	HPLC/ED	215
		0.400 ± .028	HPLC/ED	216

---

<sup>a</sup> HPLC/ED combination paired-ion ionic suppression system developed in this dissertation.

<sup>b</sup> HPLC/ED paired-ion system utilized in this dissertation.

<sup>c</sup> GC/EC with capillary column developed in this system.

<sup>d</sup> ED electrochemical detection.

Figure 1. CA condensing with a carbonyl to form a Schiff base intermediate followed by an irreversible intramolecular cyclization to form a THIQ alkaloid.

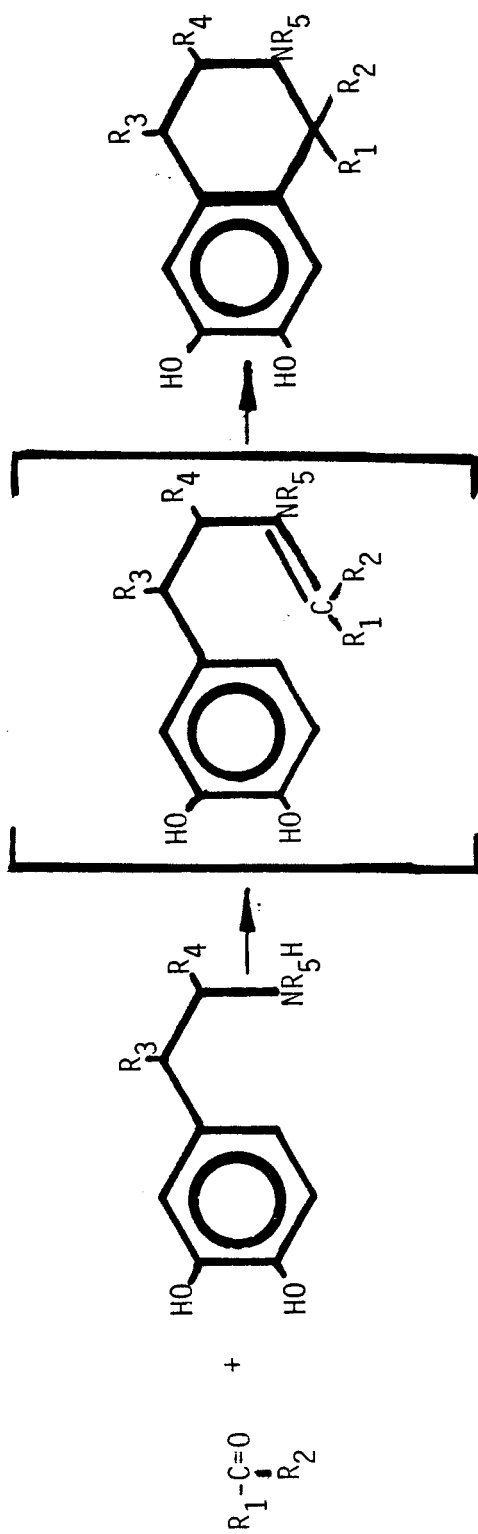
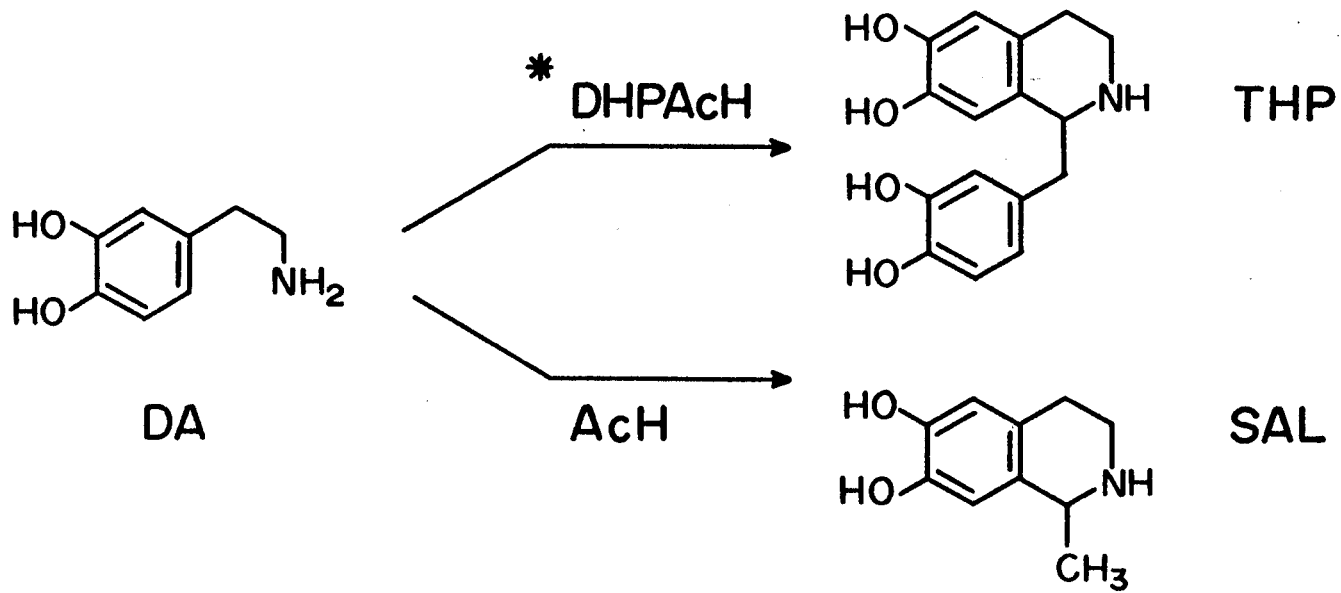


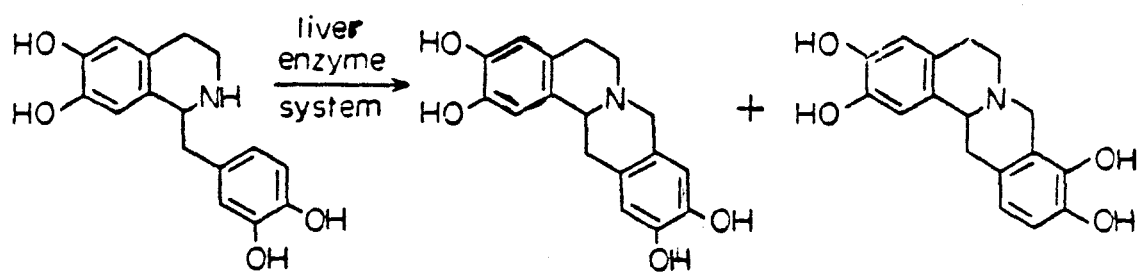
Figure 2. Condensation products of DA found in the urines of Parkinsonian patients undergoing L-Dopa treatment.





\* 3,4 - Dihydroxyphenyl acetaldehyde

Figure 3. Suggested pathway for the formation of THPBs found in the urines of Parkinson's patients undergoing L-DOPA treatment.



THP

Tetrahydroprotoberberines (THPBs)

Figure 4. Condensation products of DA and phenylpyruvic acids found in the urine of Parkinson's patients undergoing L-DOPA treatment.

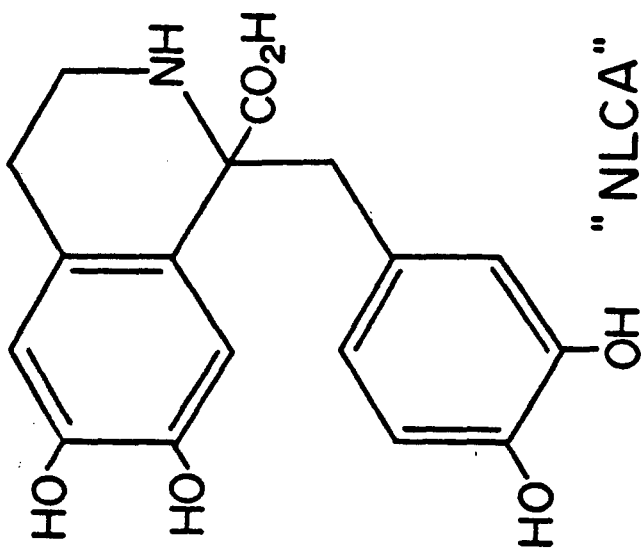
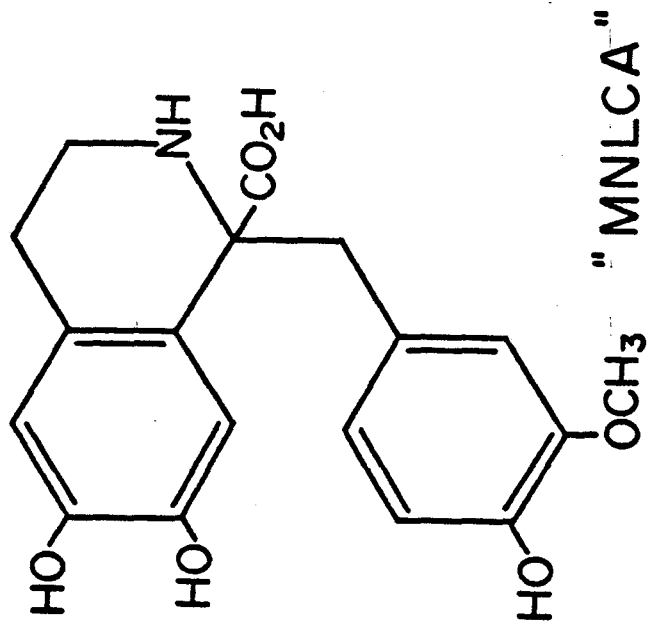


Figure 5. Phenylalanine and phenethylamine (PEA) condensation products found in the urines of PKU individuals and in the brain and urines of rats made "phenylketonuric".

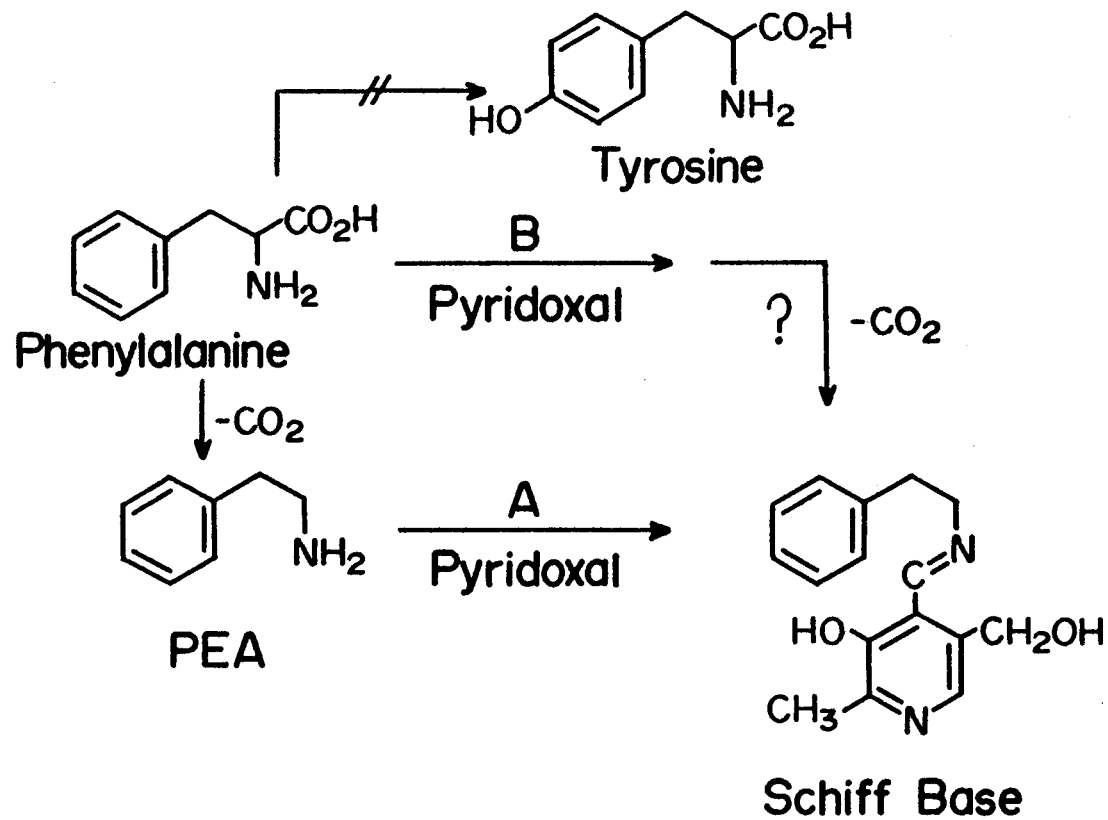


Figure 6. DA/phenylpyruvic acid condensation product found in the urines of PKU children and in the brain and urine of rats with experimentally induced hyperphenylalaninemia.



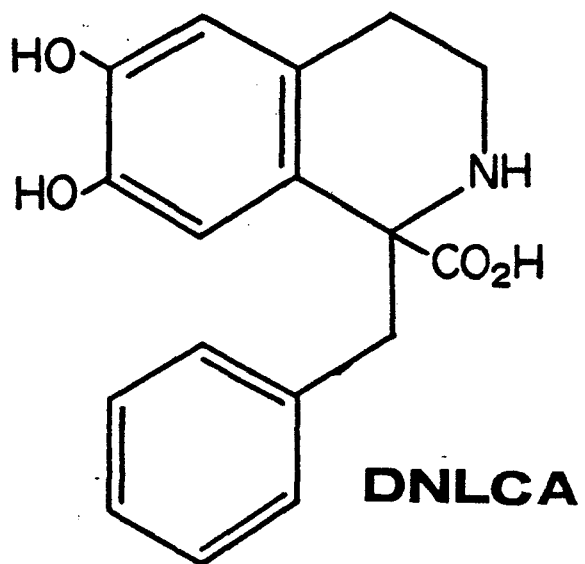
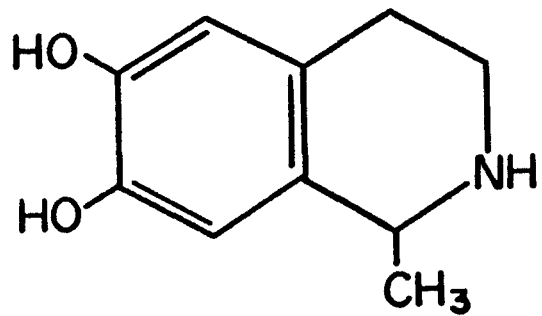
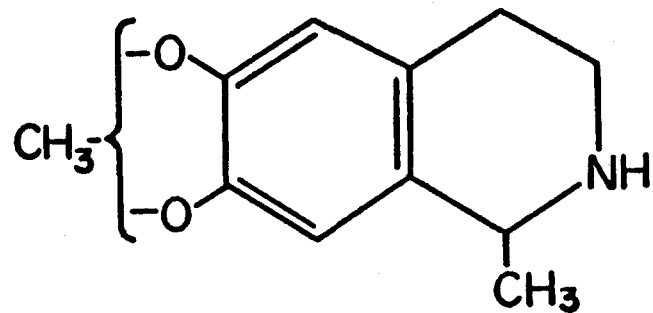


Figure 7. Simple THIQs found in the urines and lumbar spinal fluid of human patients during and after ethanol intoxication.



SALSOLINOL



ISOSALSOLINE (6-OCH<sub>3</sub>)  
OR  
SALSOLINE (7-OCH<sub>3</sub>)

Figure 8. Representation of liver EtOH oxidation.

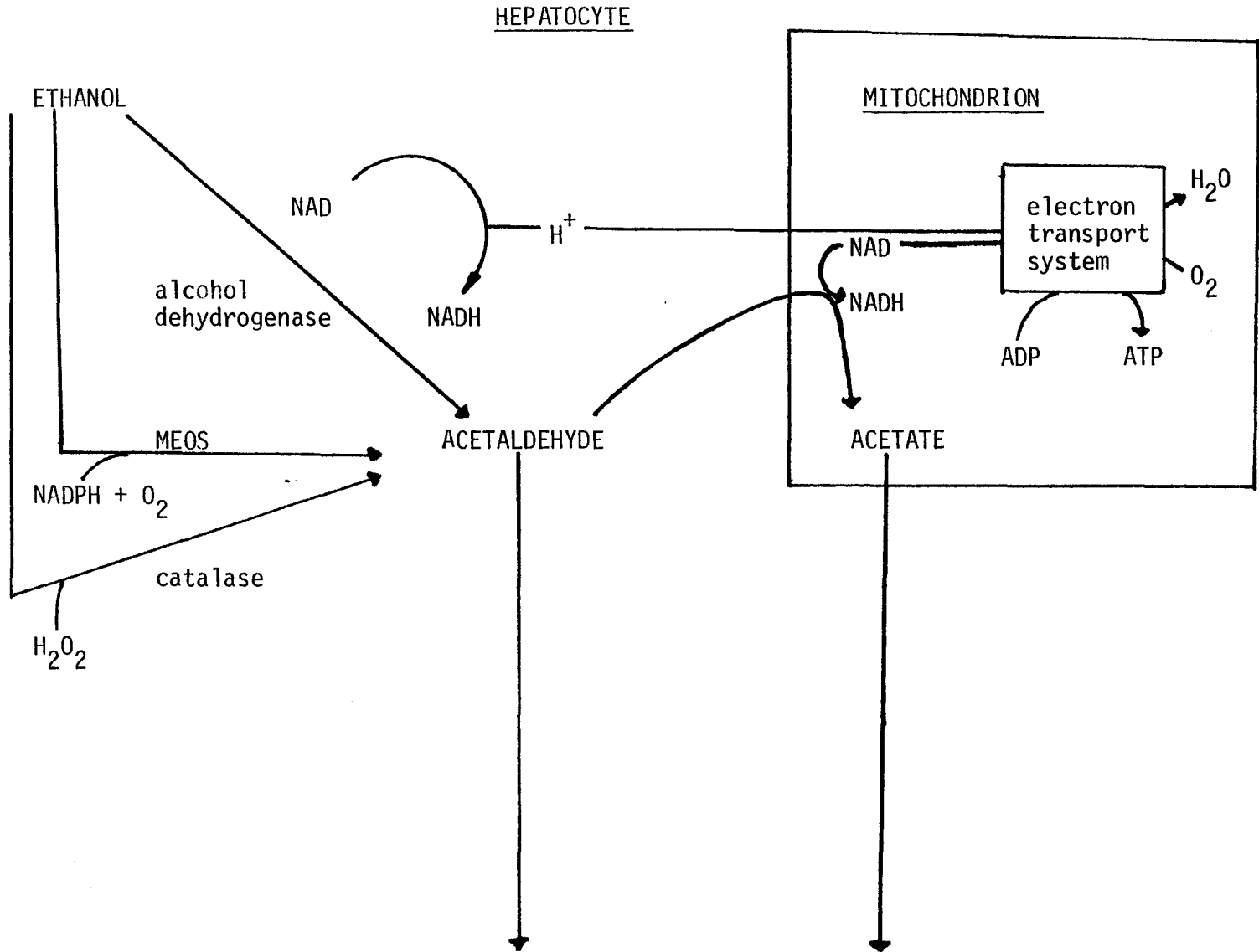


Figure 9. Possible relationship between EtOH consumption, altered AcA levels, and mitochondrial impairment.

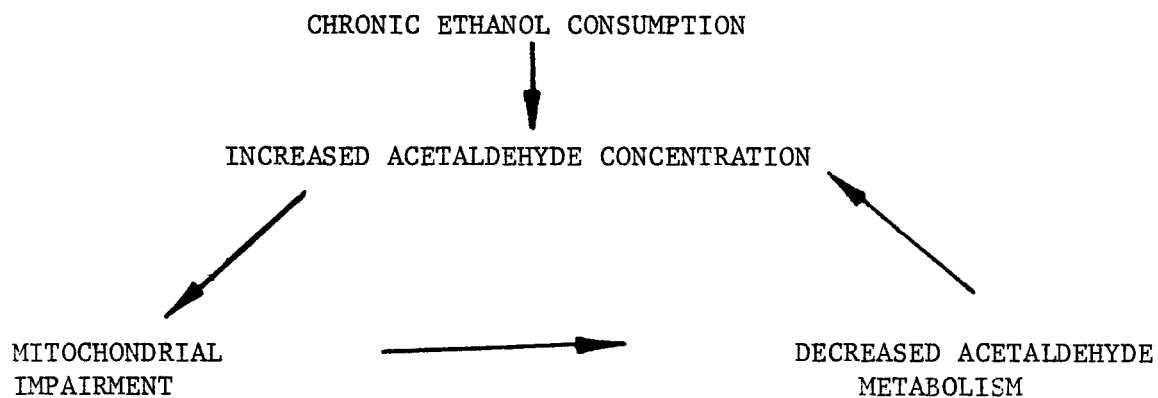


Figure 10. Proposed pathway for the biosynthesis of THP during chronic EtOH ingestion.



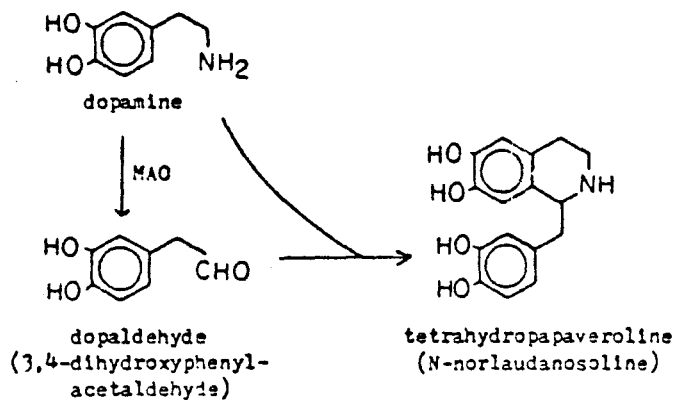


Figure 11. Formation of simple 1-alkyl-THIQs from CAS and ACA.

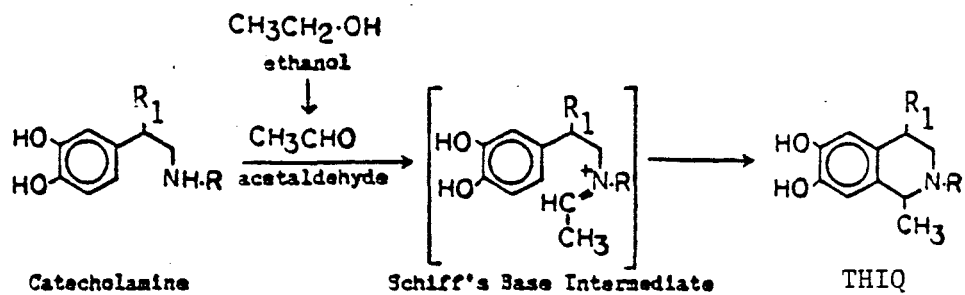
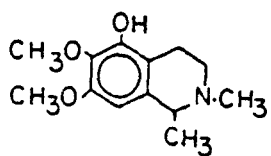
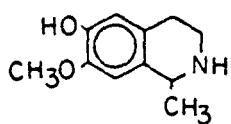


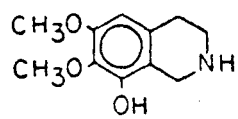
Figure 12. Examples of naturally occurring plant THIQs.



gigantine



salsoline



anhalamine

Figure 13. Proposed pathway for the biosynthesis of THP and related THIQs in plants.

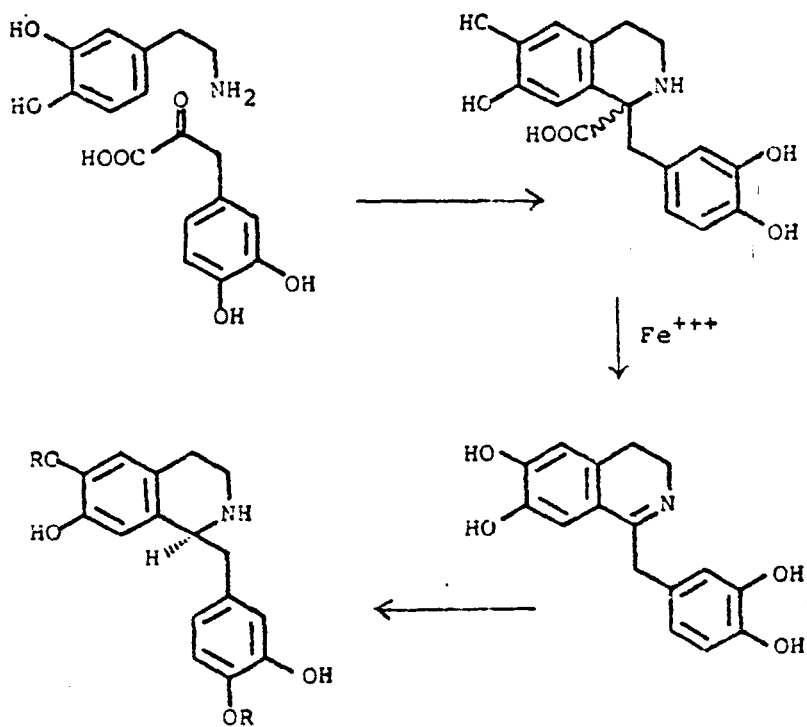


Figure 14. Possible metabolic routes for the metabolism of NE.



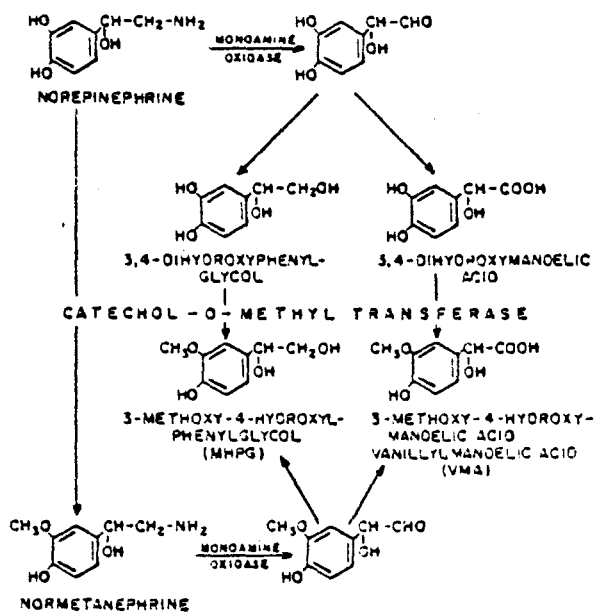


Figure 15. Possible metabolic routes for the metabolism of DA.

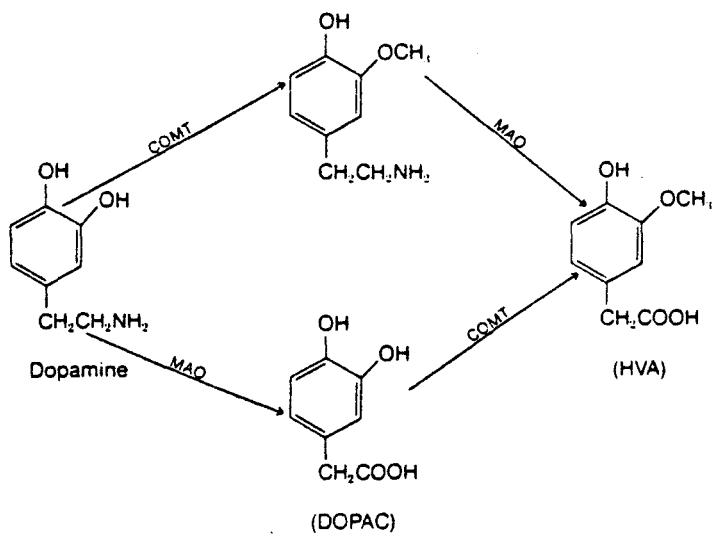


Figure 16. Possible metabolic routes for the metabolism of 5HT.

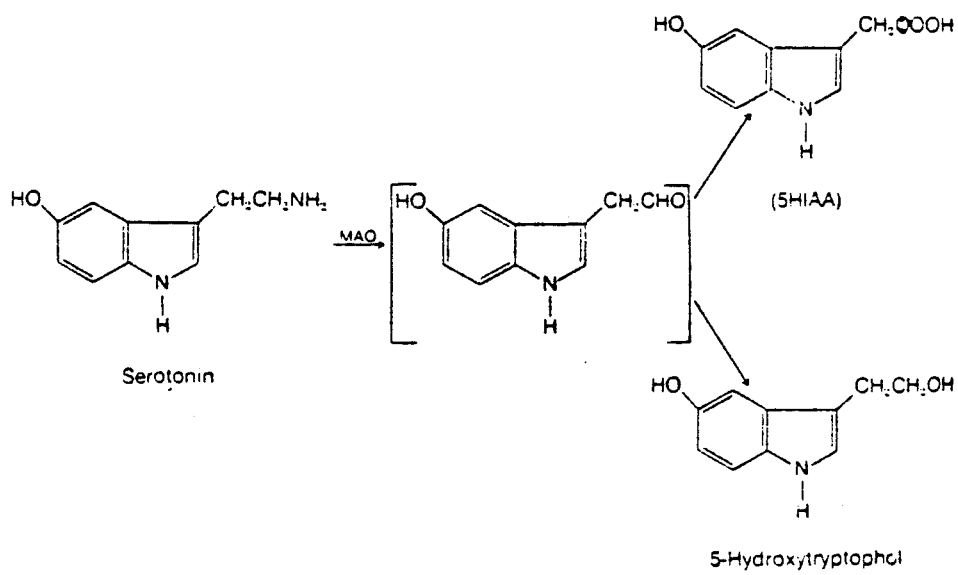
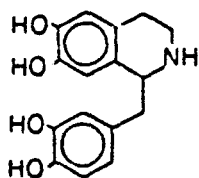
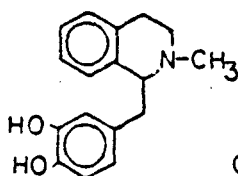


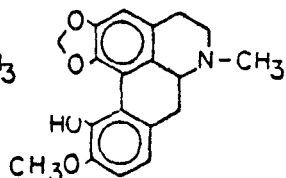
Figure 17. Structural similarities between the DA agonists, apomorphine and bulbocapnine, and the THIQ, THP.



tetrahydro-  
papaveroline



aponorphine



bulbocapnine

Figure 18. Proposed pathways for the formation of DHIQs from THIQs.



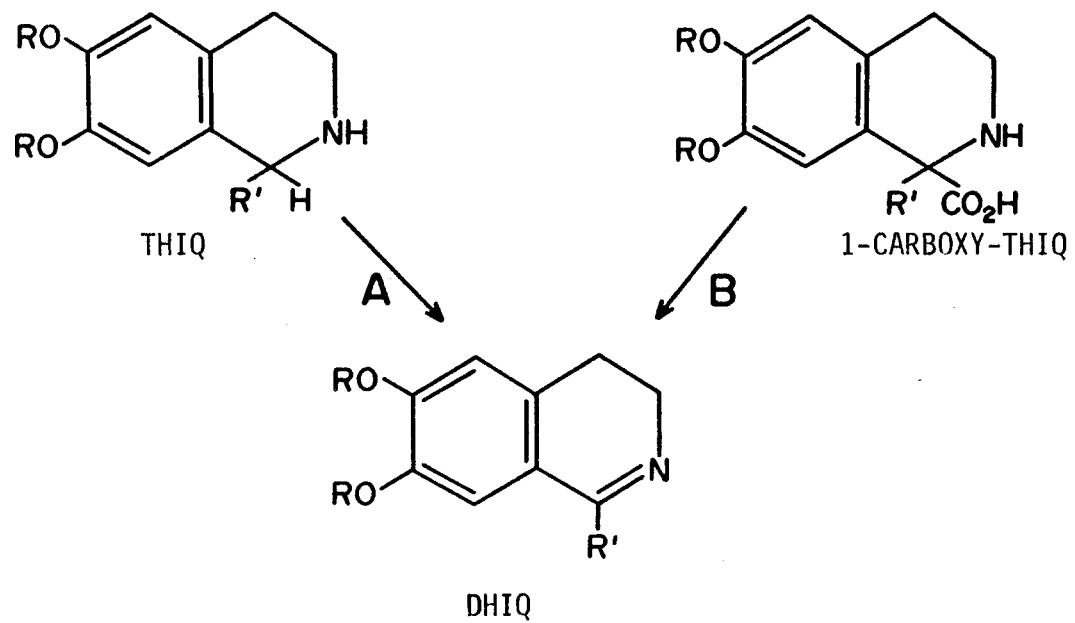
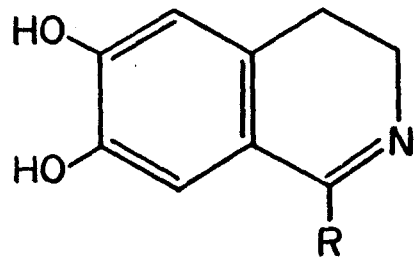
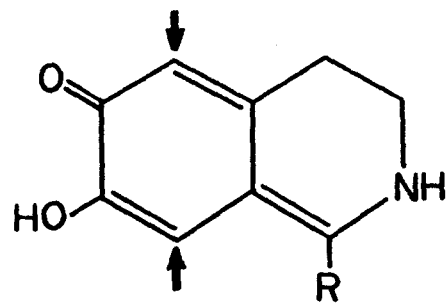
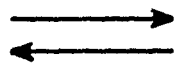


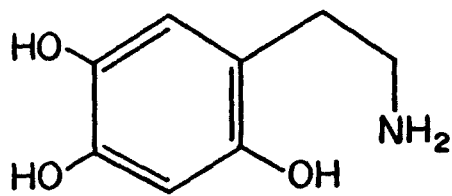
Figure 19. Structural similarities between the quinoidamine tautomer of DHIQs and the oxidized form of 60HDA.



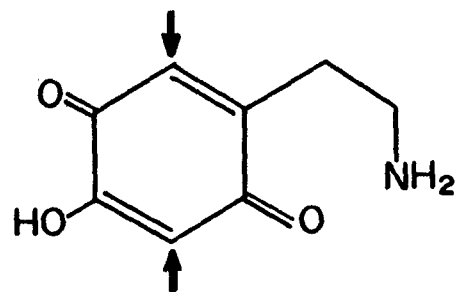
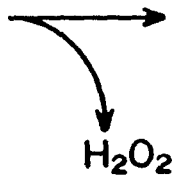
DIQ



DIQ (QUINOIDAMINE)



6-HO-DA



6-HO-DA QUINONE

Figure 20. Schematic of 6OHDA interacting with cellular nucleophiles.

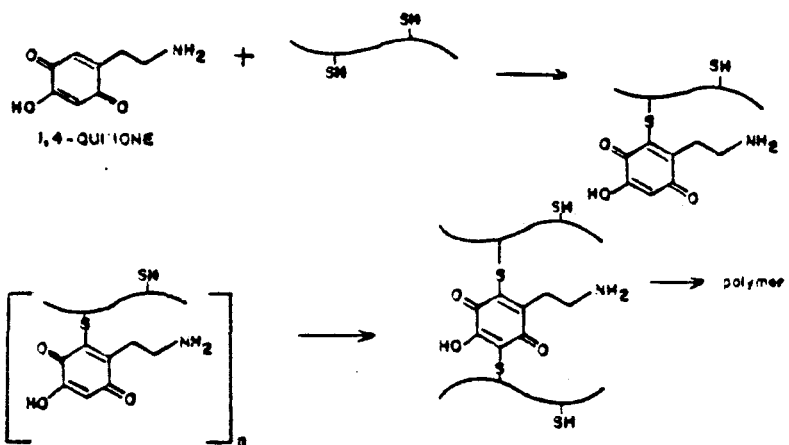


Figure 21. Catechol isoquinolines studied for their stereoselective O-methylation in vivo.

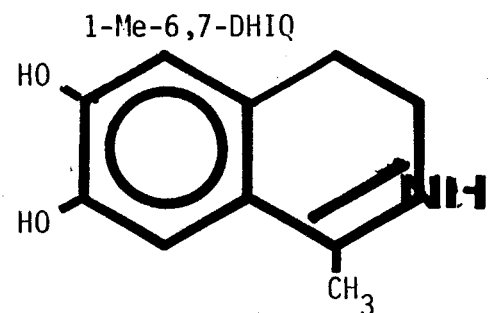
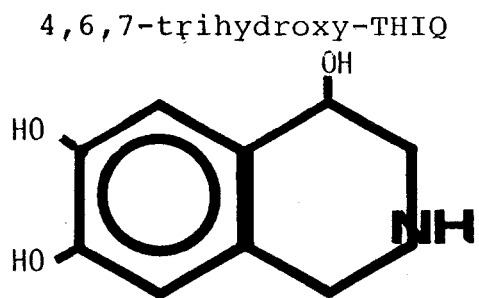
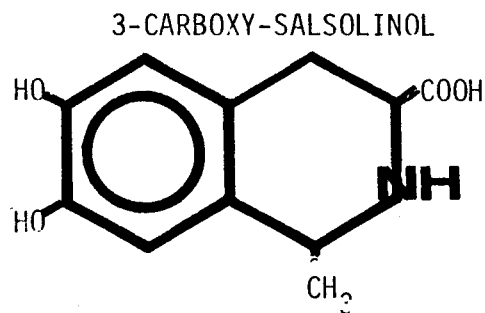
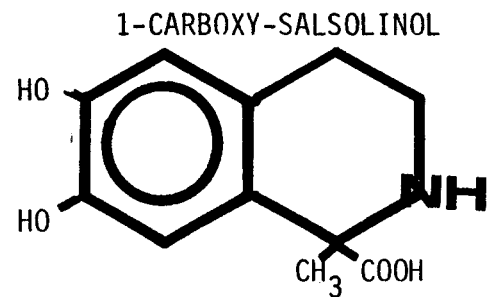
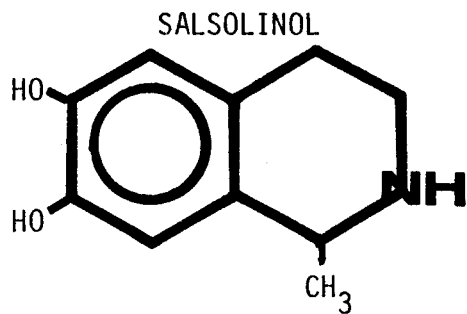
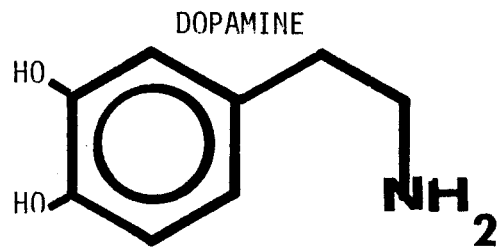
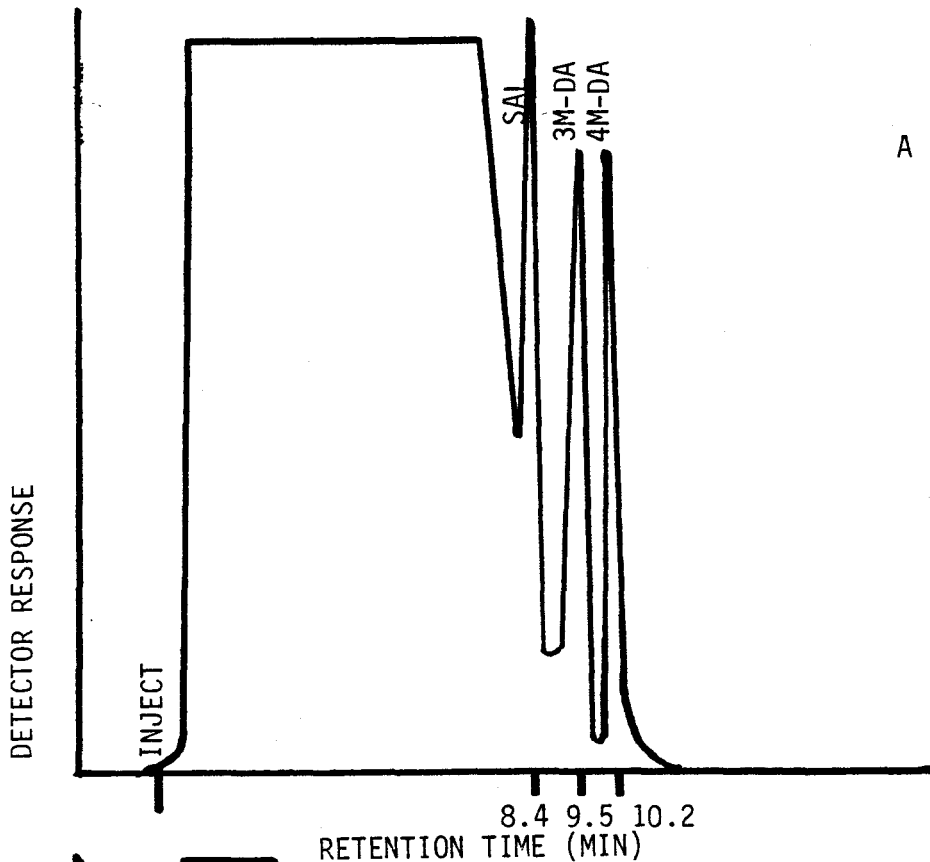
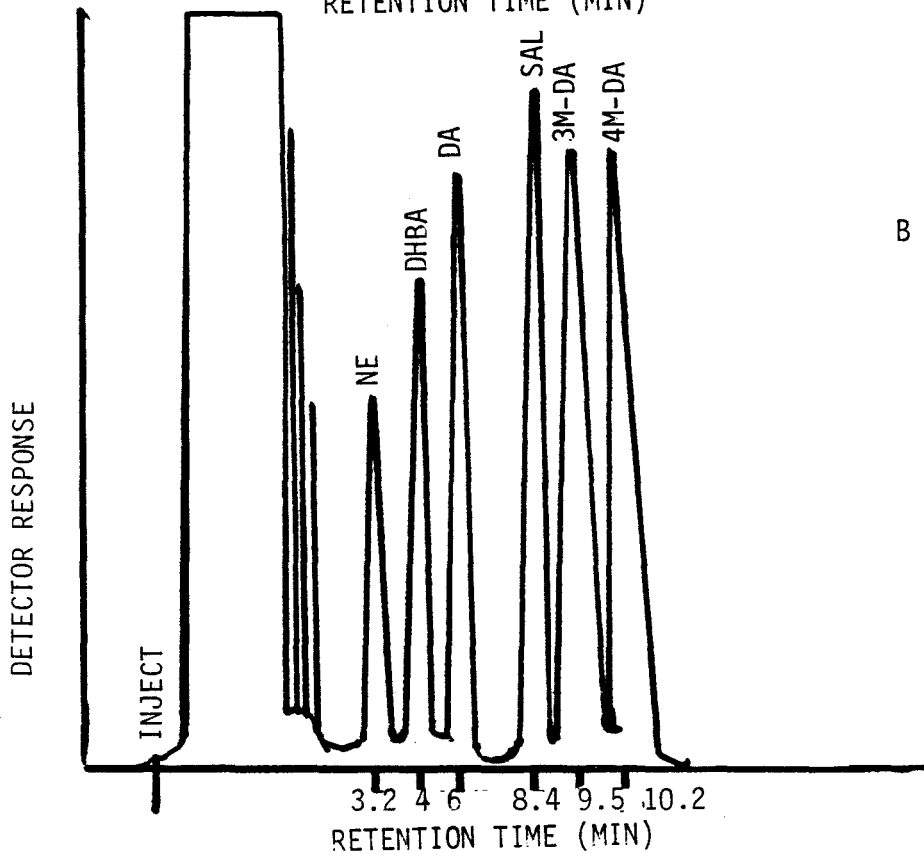


Figure 22. GC results of washing toluene solutions of HFB-derivatives of NE, DA, SAL, DHBA, 3M-DA, 4M-DA with ammonium phosphate buffer, pH 5.9; A, before treatment; B, after treatment. A 0.25 mm i.d. x 10M, OV-17, WCOT glass capillary column with electron-capture detector was utilized. GC conditions: Column temperature 130°C, detector temperature 340°C, injector temperature 250°C, N<sub>2</sub> O<sub>2</sub>F 0.8 kg/cm<sup>2</sup>, splitter set at 10/1.





A



B

Figure 23. Comparison of (A) capillary vs (B) packed column GC separations of HFB-derivatives of DHBA, DA, SAL, 3M-DA, 4M-DA, 6M-SAL and 7M-SAL. A 0.25 mm i.d. x 10M, OV-17 WCOT glass capillary column with electron-capture detection was utilized. Capillary GC conditions: Column temperature 130°C, detector temperature 340°C, injector temperature 250°C, N<sub>2</sub> O<sub>2</sub><sup>F</sup> .8kg/cm<sup>2</sup>, splitter setting 10:1. The conventional packed column (1/4" x 6' stainless steel, 3% OV-101 on Gas Chrom G-HP, 100/120 mesh) conditions: Column temperature 165°C, detector temperature 340°C, injector temperature 250°C and N<sub>2</sub> O<sub>2</sub><sup>F</sup> 25 psi.

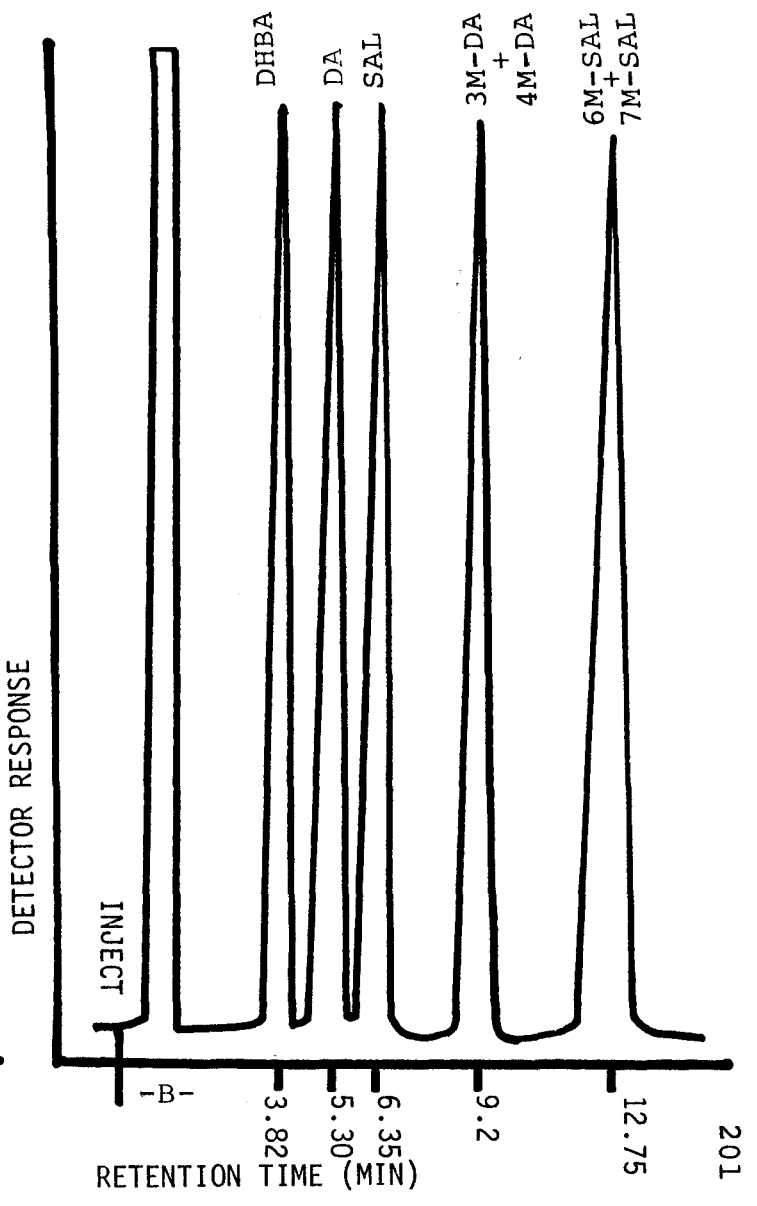
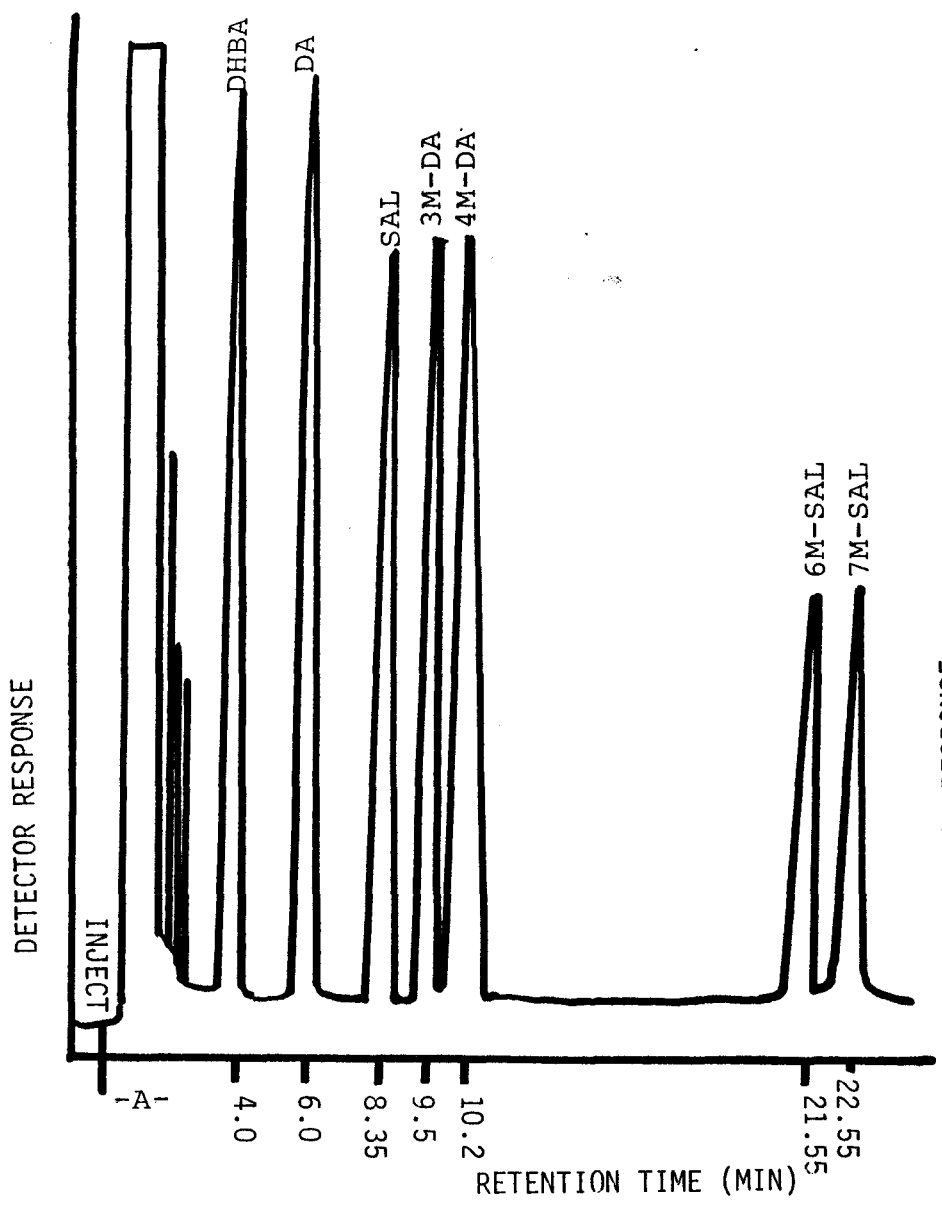


Figure 24. GC chromatogram of a standard mixture of HFB-derivatives of DHBA, DA, SAL, 3M-DA, 4M-DA, 6M-SAL and 7M-SAL. Analysis was performed on a 10M x 0.25 mm i.d. WCOT OV-17 glass capillary column with electron-capture detection. Chromatographic conditions: Column temperature 130°C, detector temperature 340°C, injector temperature 250°C, N<sub>2</sub> <sup>O<sub>2</sub>F</sup> 0.8 kg/cm<sup>2</sup> and splitter set at 10/1.

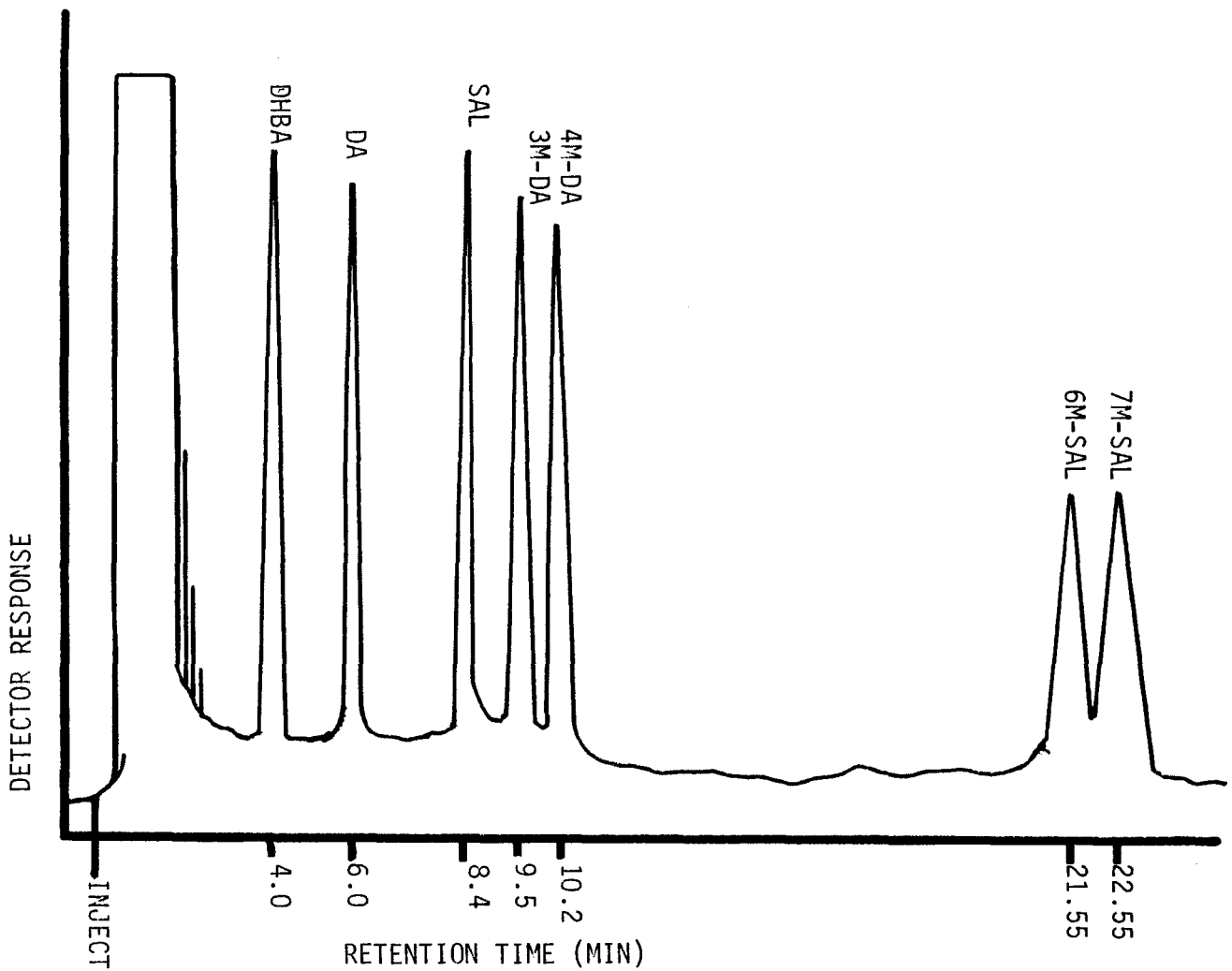


Figure 25. GC chromatogram of a standard mixture of HFB-derivatives of DHBA, 4,6,7-(OH)<sub>3</sub>THIQ, 6M-4,7-(OH)<sub>2</sub>THIQ and 7M-4,6-(OH)<sub>2</sub>THIQ. Analysis was performed on a 10M x 0.25 mm i.d., WCOT, OV-17, glass capillary column with electron-capture detection. Chromatographic conditions: Column temperature 130°C, detector temperature 340°C, injector temperature 250°C, N<sub>2</sub><sup>O<sub>2</sub>F</sup> 0.8 kg/cm<sup>2</sup> and splitter set at 10/1.

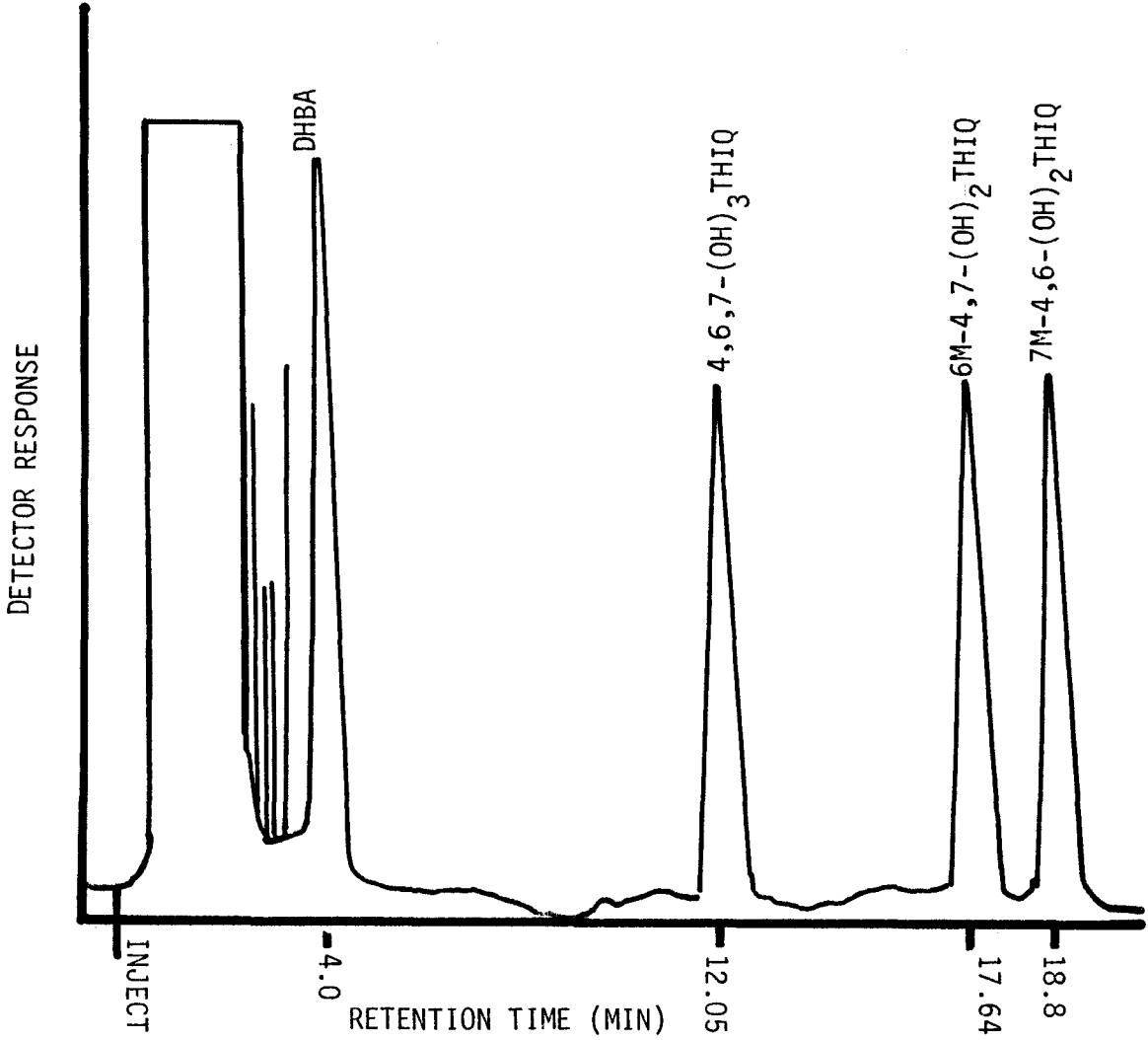


Figure 26. GC chromatogram of a standard mixture of PFP/HFIP-derivatives of 1-CSAL, 6M-1-CSAL and 7M-1-CSAL. Analysis was performed on a 10M x 0.25 mm i.d., WCOT, OV-17, glass capillary column with electron capture detection. Chromatographic conditions: Column temperature 125°C, detector temperature 340°C, injector temperature 250°C,  $N_2$   $^{O_2^F}$  0.8 kg/cm<sup>2</sup> and splitter set at 10/1.



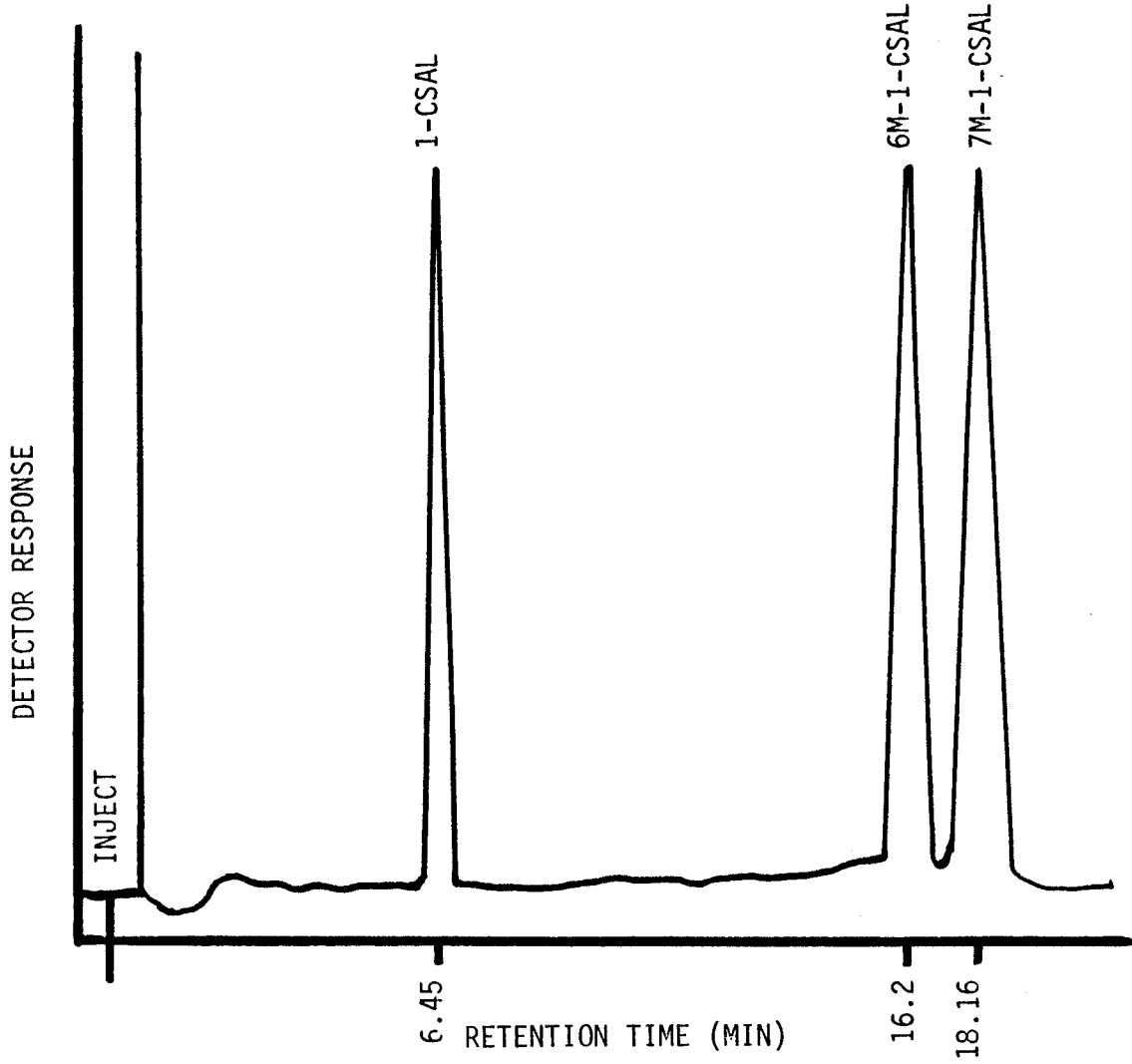


Figure 27. GC chromatogram of a standard mixture of PFP/HFIP-derivatives of 3-CSAL, 6M-3-CSAL and 7M-3-CSAL. Analysis was performed on a 10M x 0.25 mm i.d., WCOT, OV-17, glass capillary column with electron-capture detection. Chromatographic conditions: Column temperature 155°C, detector temperature 340°C injector temperature 250°C,  $N_2O_2^F$  0.8 kg/cm<sup>2</sup>, splitter setting 10/1.

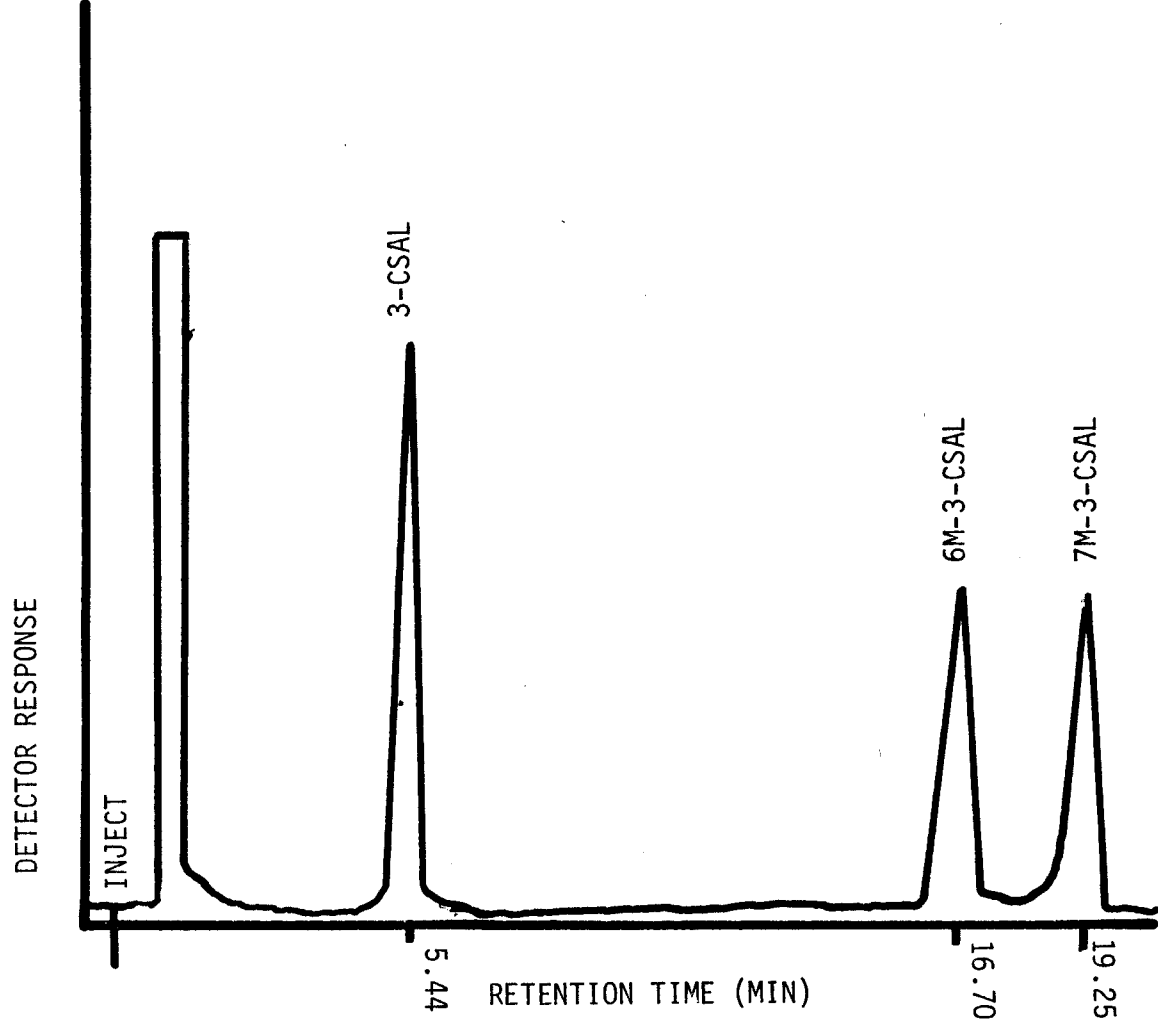


Figure 28. GC chromatogram of a standard mixture of HFB-derivatives of 1-Me-6,7-DHIQ, 1-Me-6M-DHIQ and 1-Me-7M-DHIQ. Analysis was performed on a 10M x 0.25 mm i.d., WCOT, OV-17, glass capillary column with electron-capture detection. Chromatographic conditions: Column temperature 145°C, detector temperature 340°C, injector temperature 250°C,  $N_2O_2^F$  0.8 kg/cm<sup>2</sup> and splitter setting 10/1.

DETECTOR RESPONSE

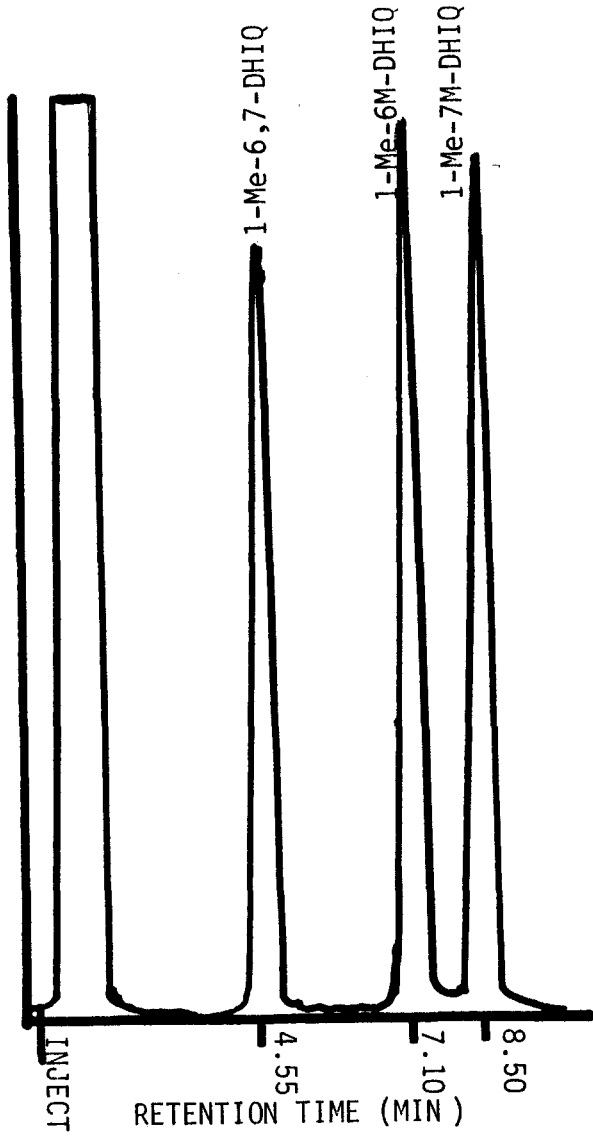


Figure 29. HPLC chromatogram of a standard mixture of DOPAC, 5HIAA and HVA. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse-phase 25 cm column with electrochemical detection. HPLC conditions: Paired-ion buffer (8mM HSA, 10% MeOH, 1% HOAc, pH 3.5) with a flow rate of 1.0 ml/min.

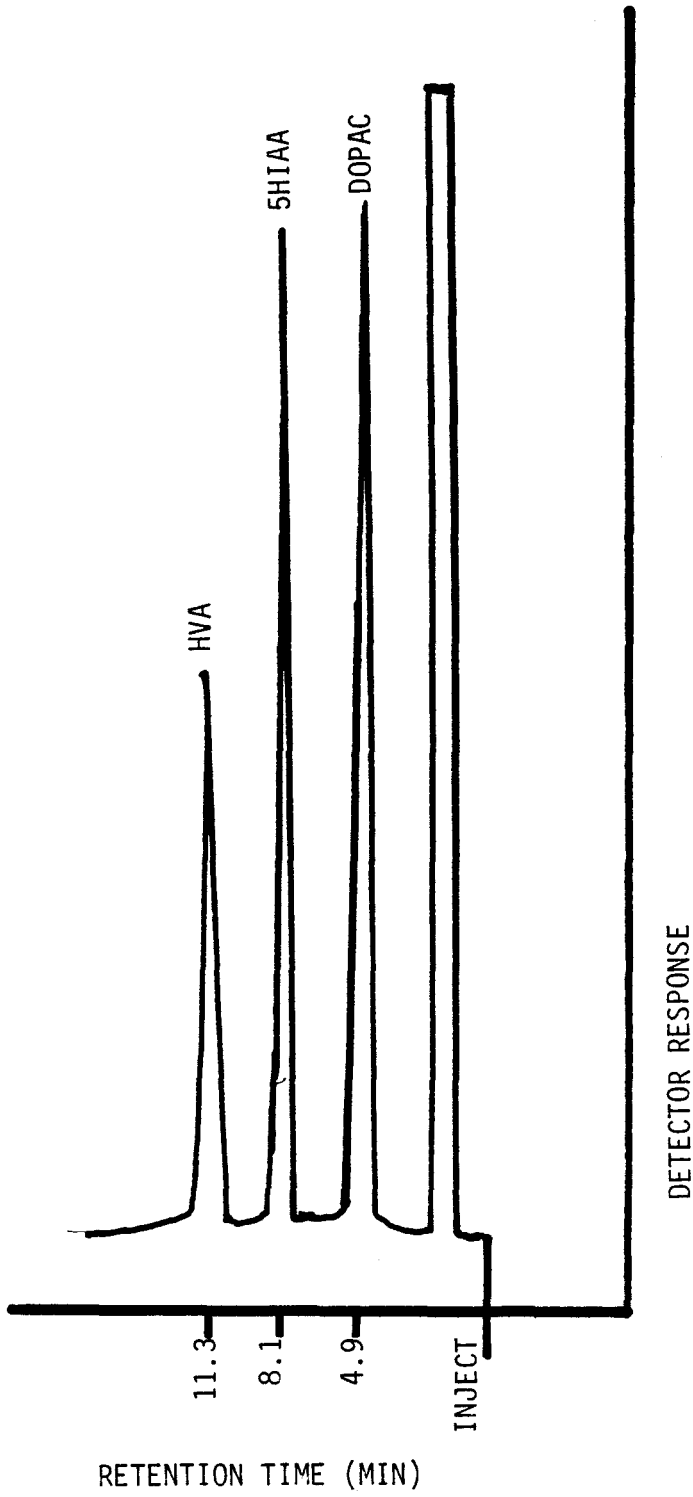


Figure 30. HPLC chromatogram of a standard mixture of NE, DHBA, DA, SAL, and 5HT. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse phase 25 cm column with electrochemical detection. HPLC conditions: Paired-ion buffer (8mM HSA, 10% MeOH, 1% HOAc, pH 3.5) with a flow rate of 1.0 ml/min.



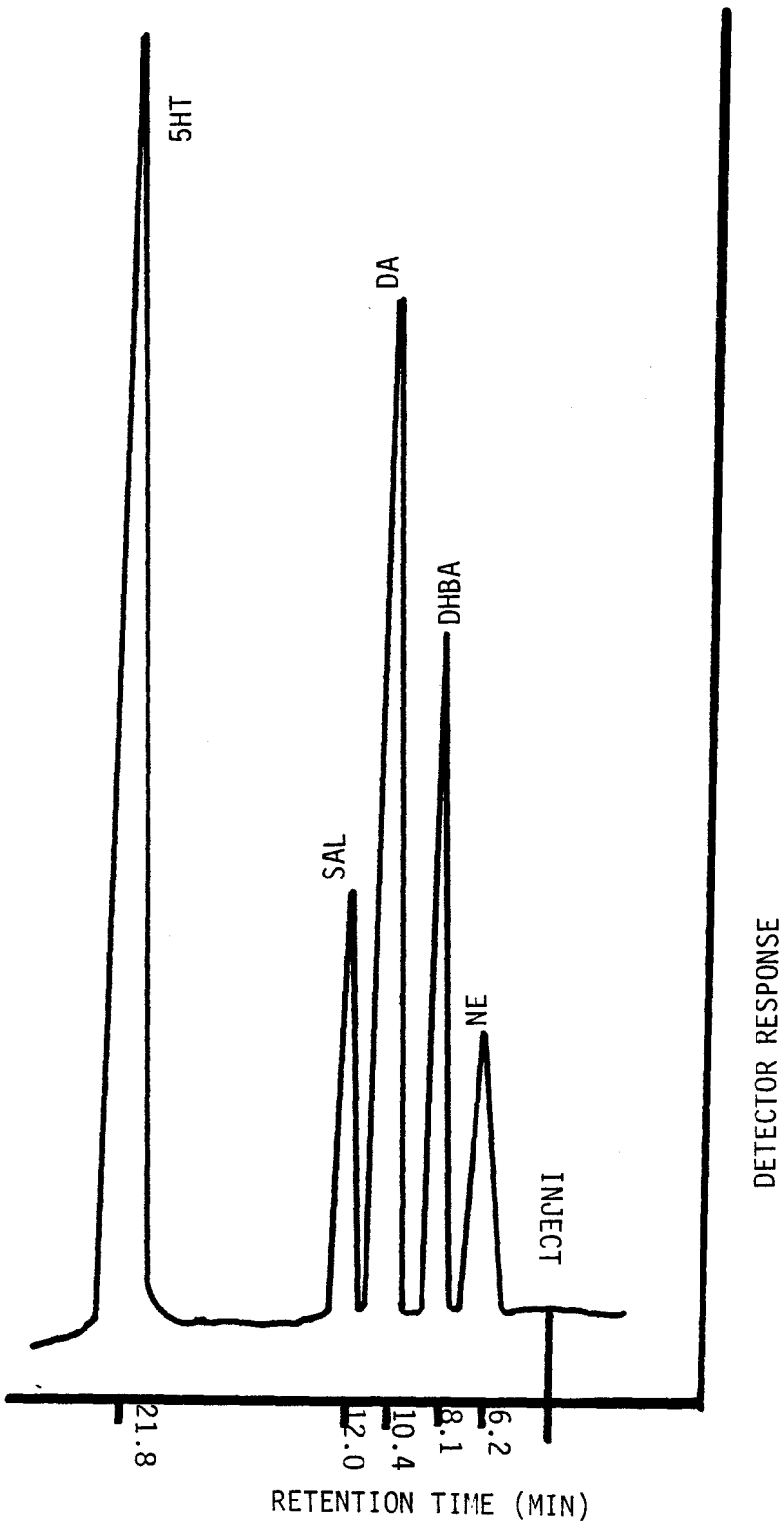


Figure 31. HPLC chromatogram of a standard mixture of NE, DHBA, DOPAC, 5HT, 5HIAA and HVA. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse phase 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 NaH<sub>2</sub>PO<sub>4</sub>/1mM Na<sub>2</sub>EDTA pH 5.0) with a flow rate of 1.0 ml/min.

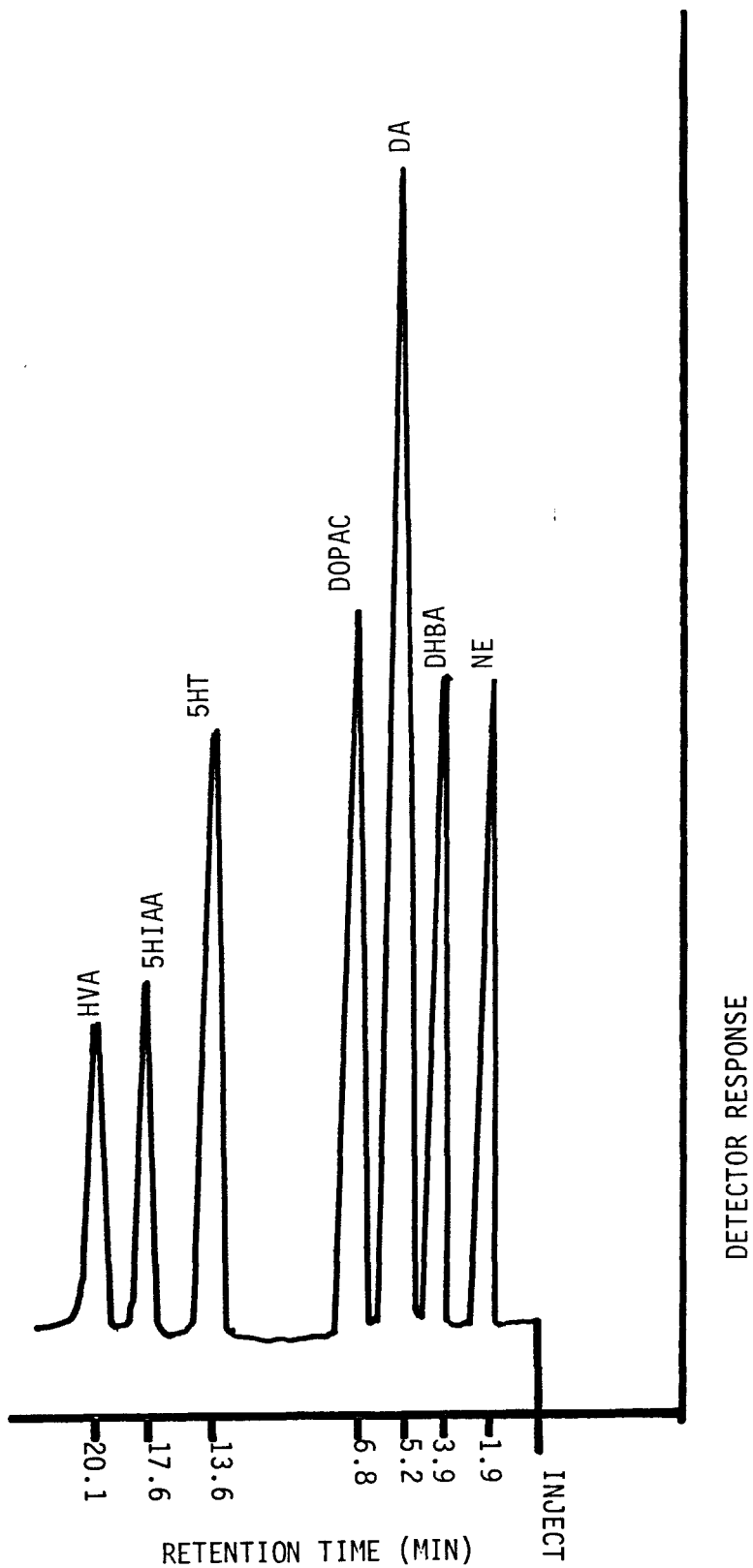


Figure 32. HPLC chromatogram of a standard mixture of DA, DOPAC, SAL, 5HT, 5HIAA, HVA, 7M-SAL and 6M-SAL. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase, 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1mM Na<sub>2</sub>EDTA, pH 5.0) with a flow rate of 1.0 ml/min.

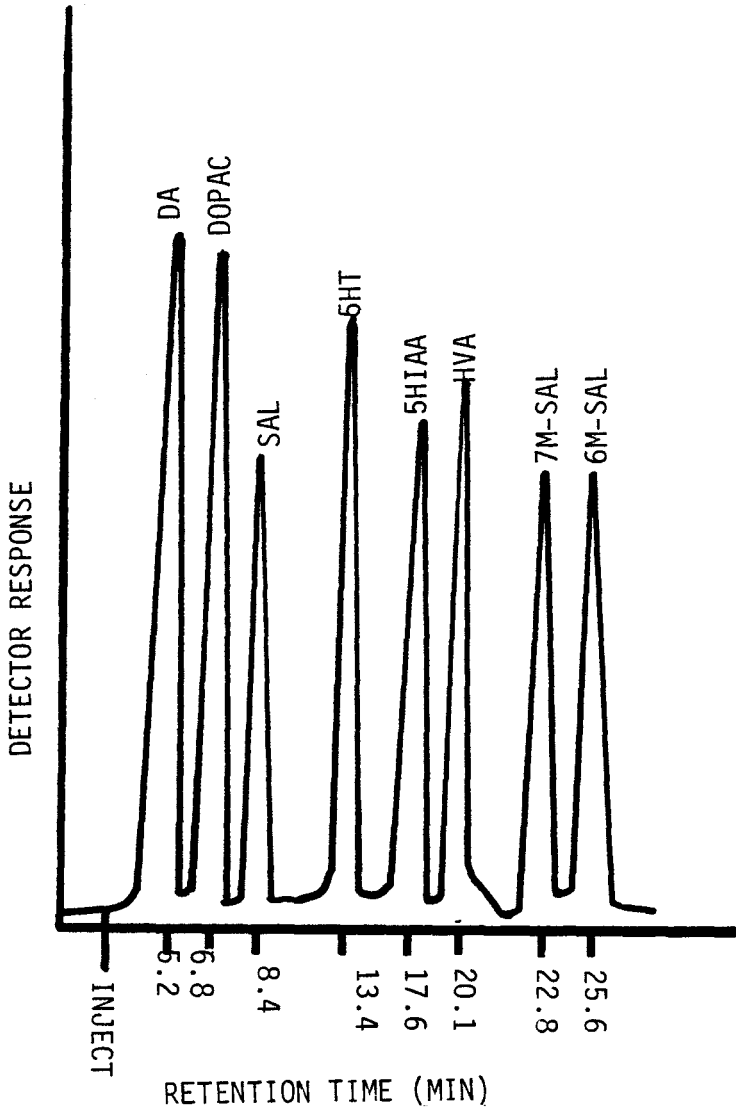


Figure 33. HPLC chromatogram of a standard mixture of 4,6,7-(OH)<sub>3</sub>-THIQ, 6M-4,7(OH)<sub>2</sub>THIQ and 7M-4,6(OH)<sub>2</sub>THIQ. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase, 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1 mM Na<sub>2</sub>EDTA, pH 5.0) with a flow rate of 1.0 ml/min.

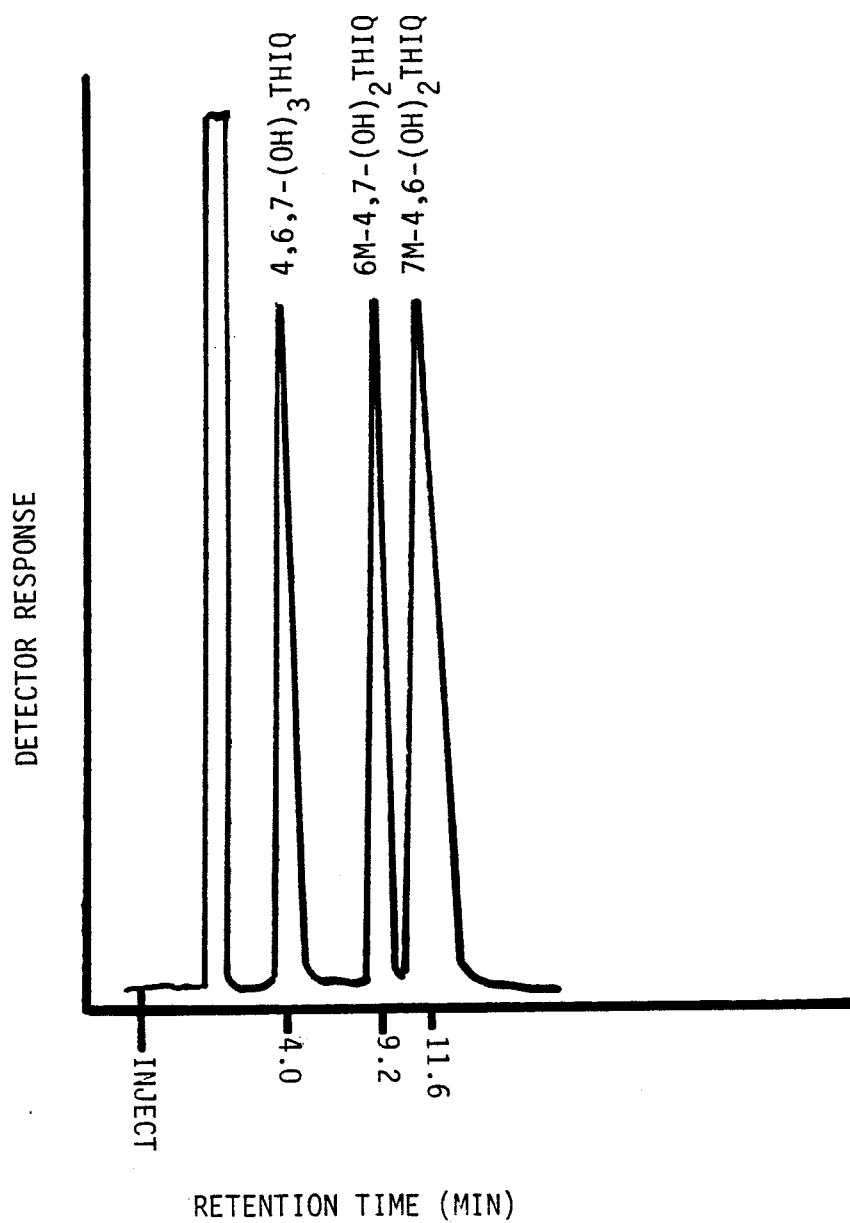


Figure 34. HPLC chromatogram of a standard mixture of 1-CSAL, 6M-1-CSAL and 7M-1-CSAL. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase, 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1 mM Na<sub>2</sub>EDTA, pH 5.0) with a flow rate of 1.0 ml/min.



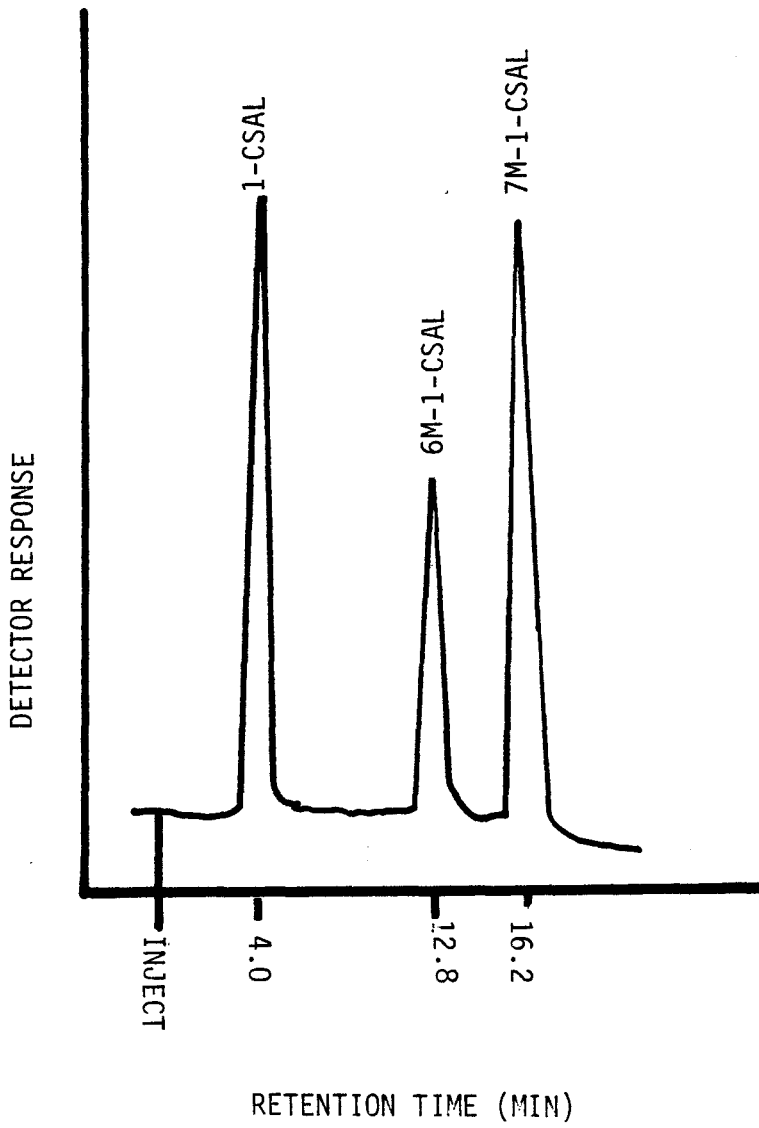


Figure 35. HPLC chromatogram of a standard mixture of 3-CSAL, 6M-3-CSAL and 7M-3-CSAL. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase, 25 cm column with electrochemical detection. HPLC conditions: Ion suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1mM Na<sub>2</sub>EDTA pH 5.0) with a flow rate of 1.0 ml/min.

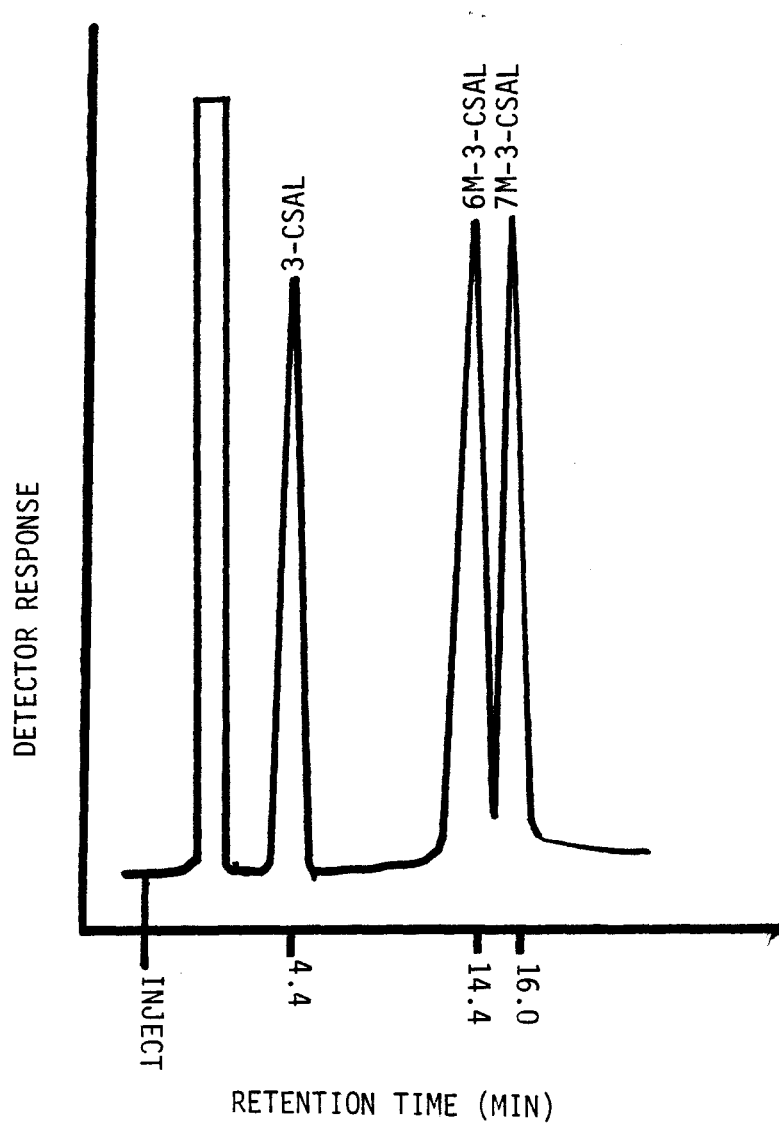


Figure 36. HPLC chromatogram of a standard mixture of 1-Me-6,7-DHIQ, 1-Me-6M-DHIQ and 1-Me-7M-DHIQ. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase, 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M Na<sub>2</sub>HPO<sub>4</sub>/1 mM Na<sub>2</sub>EDTA adjusted to pH 7.4 with 0.1 M citric acid, 10% MeOH) with a flow rate of 1.0 ml/min.

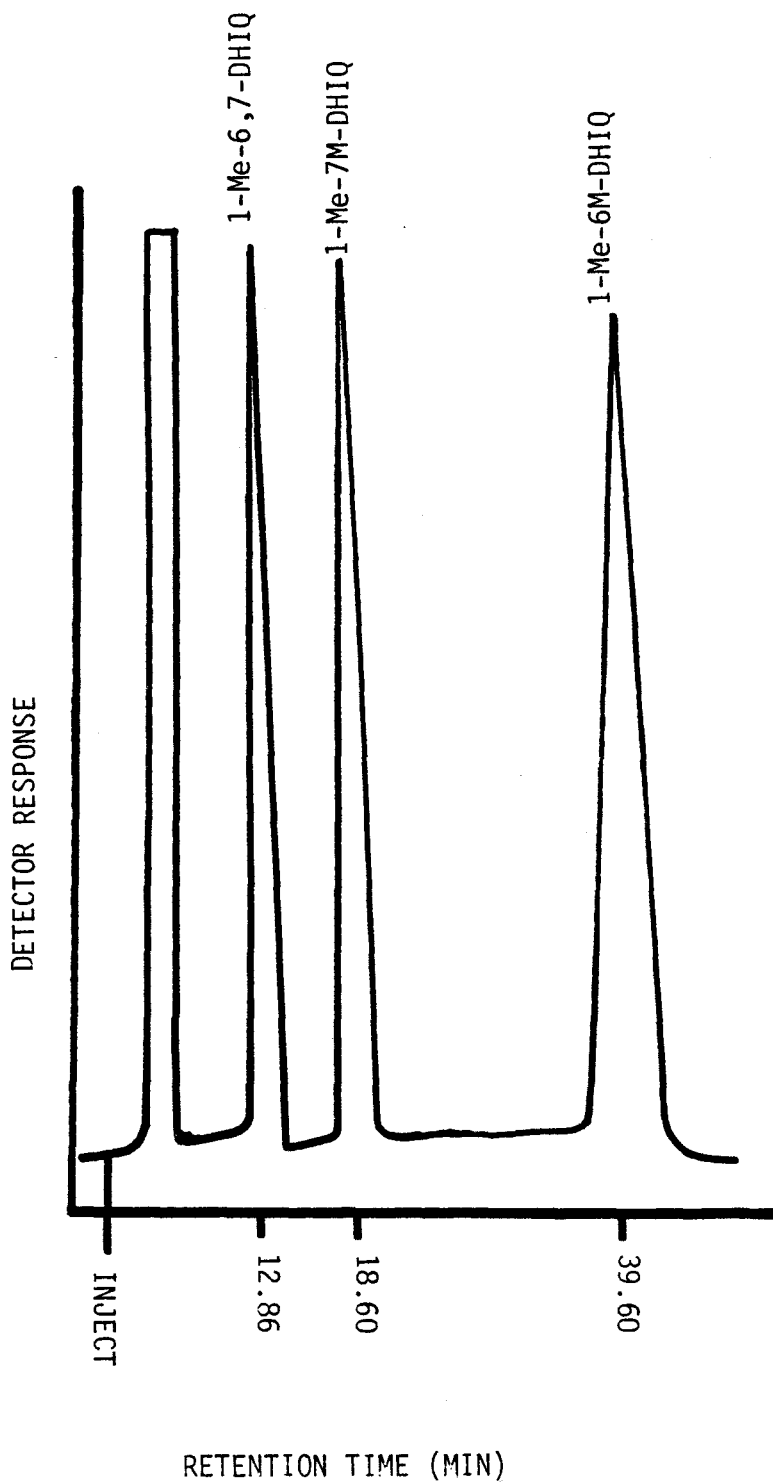


Figure 37. (A) Representative GC chromatogram of a corpus striatum extracted with 70% aqueous EtOH and isolation on BioRex-70 from a rat injected ICV with SAL or DA (---). After lyophilization samples were derivatized with HFBA and analyzed on a 10M x 0.25 i.d., OV-17, WCOT, glass capillary column with electron-capture detection. GC conditions: Column temperature 130°C, detector temperature 340, injector temperature 250°C,  $N_2O_2^F$  0.8 kg/cm<sup>2</sup> splitter set at 10/1.

(B) Representative HPLC chromatogram of a corpus striatum from a rat injected ICV with SAL. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70 lyophilized and taken up in 0.1 N HCl. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse phase, 25 cm, column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1 mM Na<sub>2</sub>EDTA, pH 5.0) with a flow rate of 1.0 ml/min.

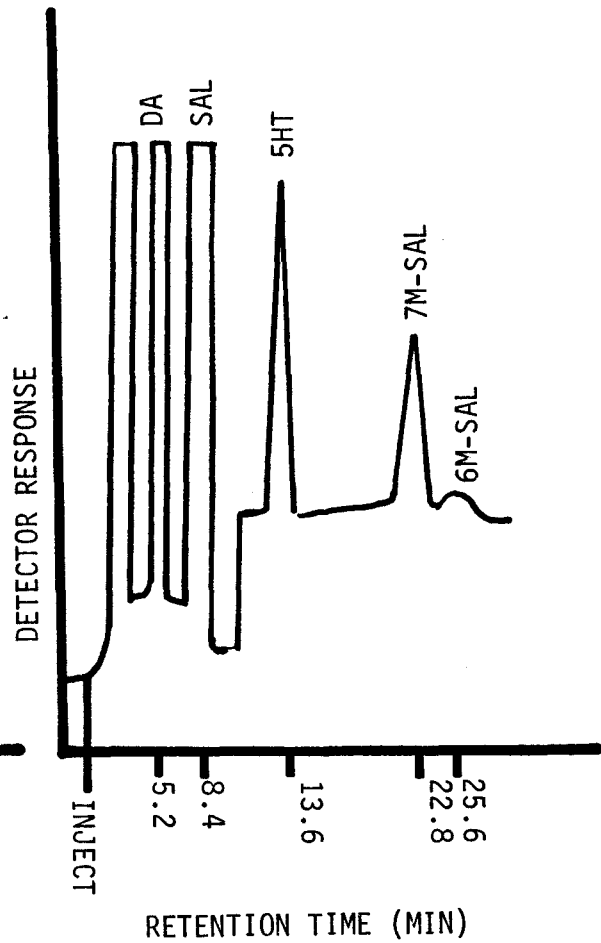
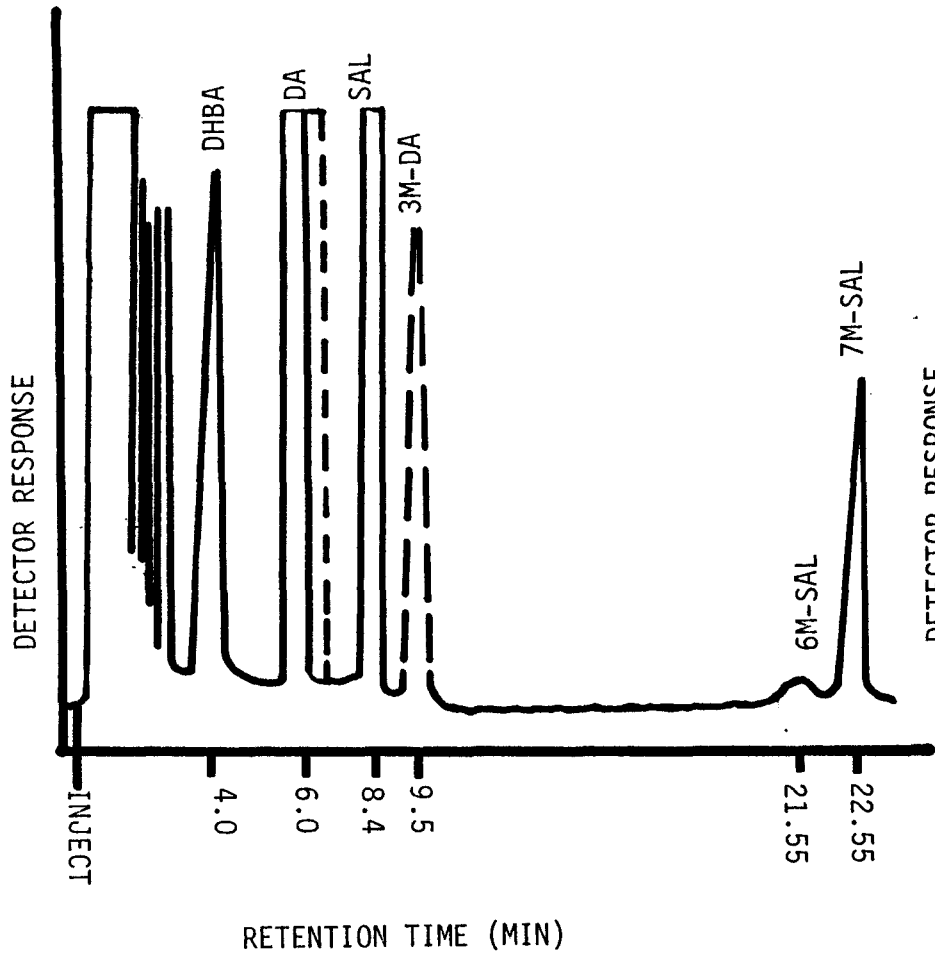
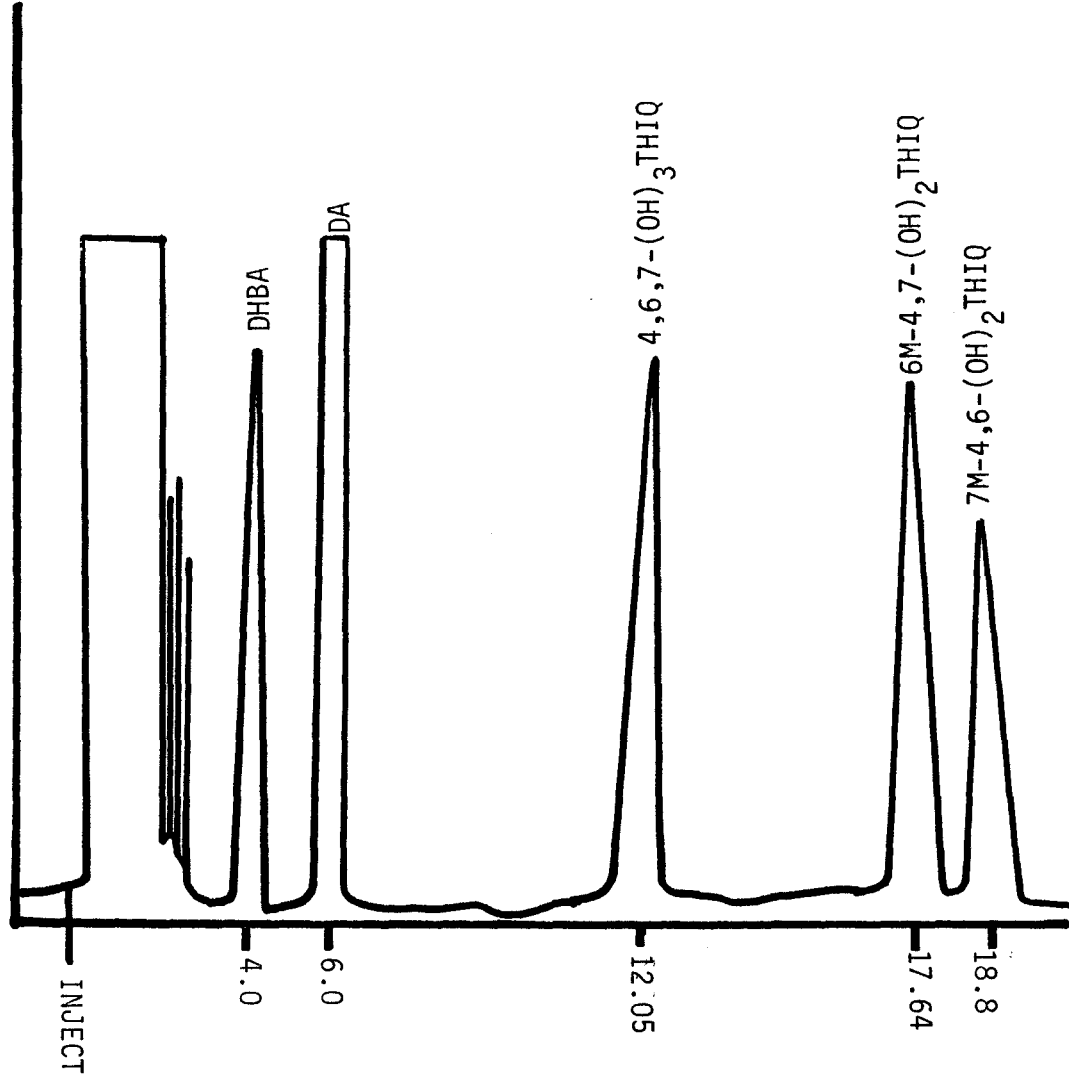


Figure 38. (A) Representative GC chromatogram of a corpus striatum from a rat injected ICV with 4,6,7-(OH)<sub>3</sub>THIQ. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70, lyophilized and derivatized with HFBA. Analysis was performed on a 10M x 0.25 mm i.d. OV-17 WCOT glass capillary column with electron-capture detection. GC conditions column temperature 130°C, detector temperature 340°C, injector temperature 250, N<sub>2</sub>O<sub>2</sub>F 0.8 kg/cm<sup>2</sup> and splitter set at 10/1.

(B) Representative HPLC chromatogram of a corpus striatum from a rat injected ICV with 4,6,7-(OH)<sub>3</sub>THIQ. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70, lyophilized and taken up in 0.01N HCL. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse phase column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>/1 mM Na<sub>2</sub>EDTA pH 5.0) with a flow rate of 1.0 ml/min.



DETECTOR RESPONSE



DETECTOR RESPONSE

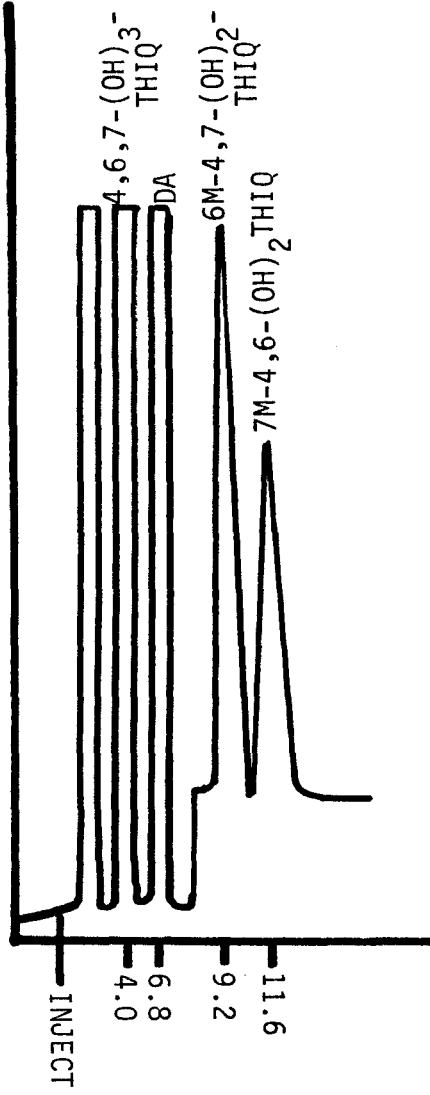


Figure 39. (A) Representative GC chromatogram of a hypothalamus from a rat injected ICV with l-CSAL. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70 lyophilized and derivatized with PFPA/HFIP. Analysis was performed on a 10M x 0.25 mm i.d. OV-17, WCOT, glass capillary column with electrochemical detection. GC conditions: Column temperature 125°C, detector temperature 340°C, injector temperature 250°C,  $N_2O_2^F$  0.8 kg/cm<sup>2</sup> and splitter set at 10/1.

(B) Representative HPLC chromatogram of a hypothalamus from a rat injected ICV with l-CSAL. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70, lyophilized and taken up in 0.01 N HCl. Analysis was performed on a Bio-Sil C<sup>18</sup>, reverse phase 25 cm column with electrochemical detector. HPLC conditions: Ion-suppression buffer (0.1 M  $NaH_2PO_4$ /1 mM  $Na_2EDTA$ , pH 5.0) with a flow rate of 1.0 ml/min.

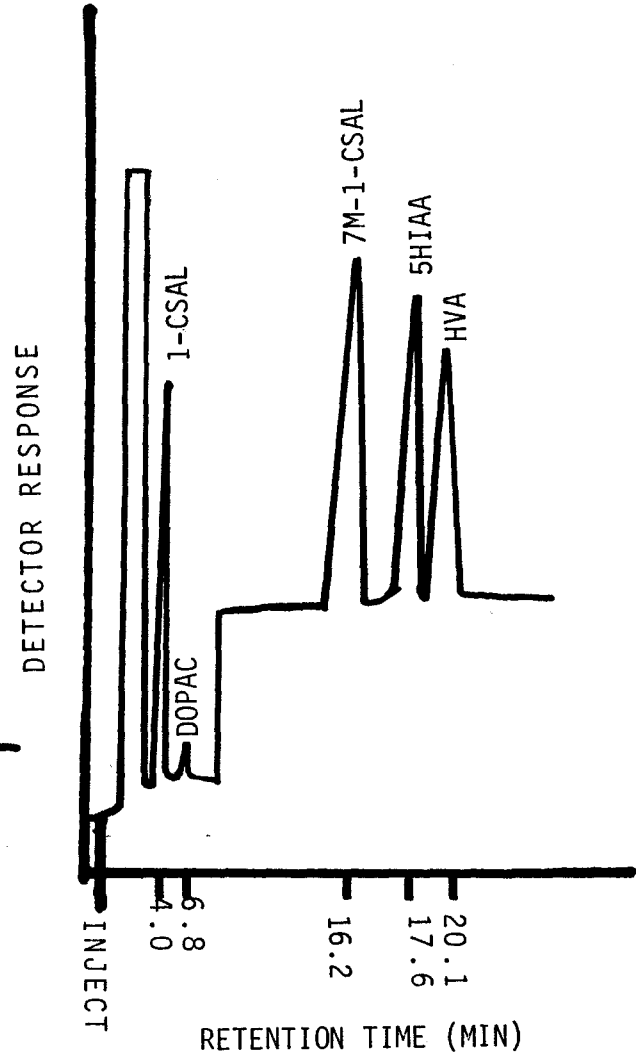
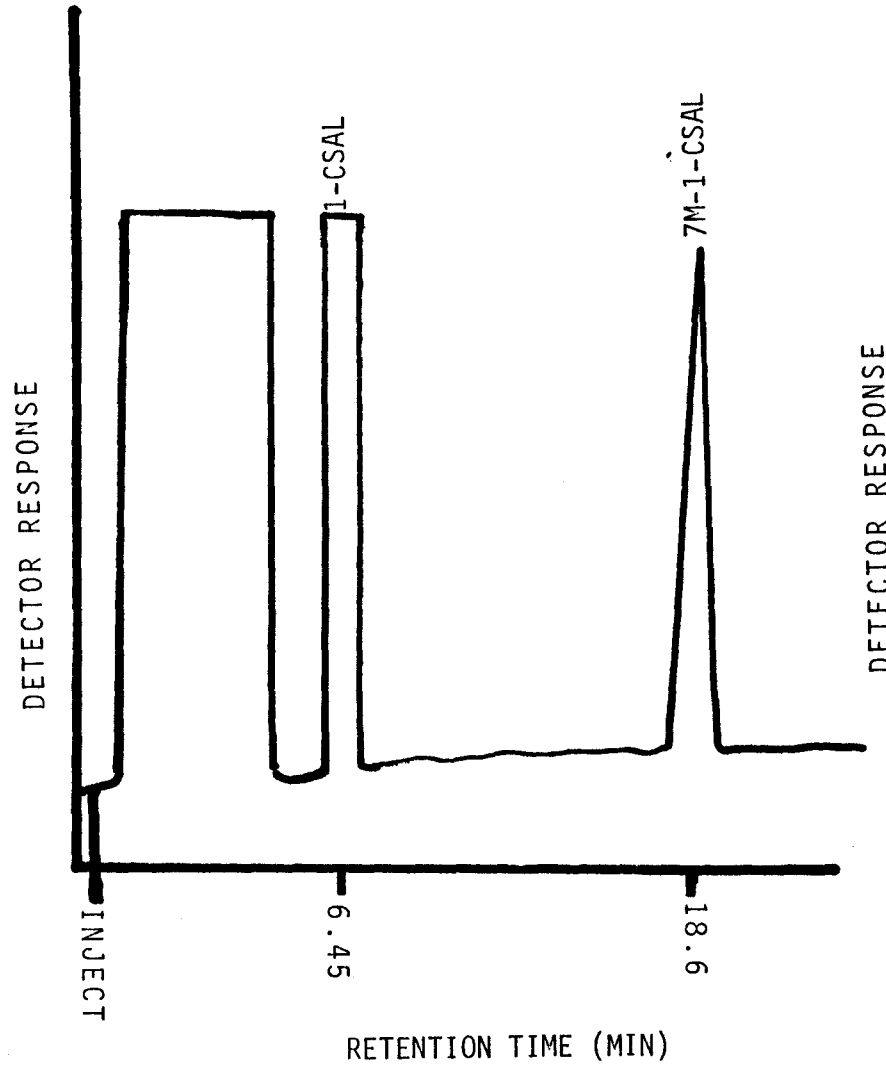


Figure 40. (A) Representative GC chromatogram of hypothalamus from a rat injected ICV with 3-CSAL. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70 lyophilized and derivatized with PFPA/HFIP. Analysis was performed on a 10M x 0.25 mm i.d., OV-17, WCOT, glass capillary column with electron-capture detection. GC conditions: Column temperature 155°C, detector temperature 340°C, injector temperature 250°C,  $N_2O_2^F$  0.8 kg/cm<sup>2</sup> and splitter set at 10/1.

(B) Representative HPLC chromatogram of a hypothalamus from a rat injected ICV with 3-CSAL. Tissues were extracted with 70% aqueous EtOH, isolated on BioRex-70 lyophilized and brought up in 0.5 ml of 0.01N HCL. Analysis was performed on a Bio-Sil C<sup>18</sup> reverse phase 25 cm column with electrochemical detection. HPLC conditions: Ion-suppression buffer (0.1 M  $NaH_2PO_4$ /1 mM  $Na_2EDTA$ , pH 5.0) with a flow rate of 1.0 ml/min.

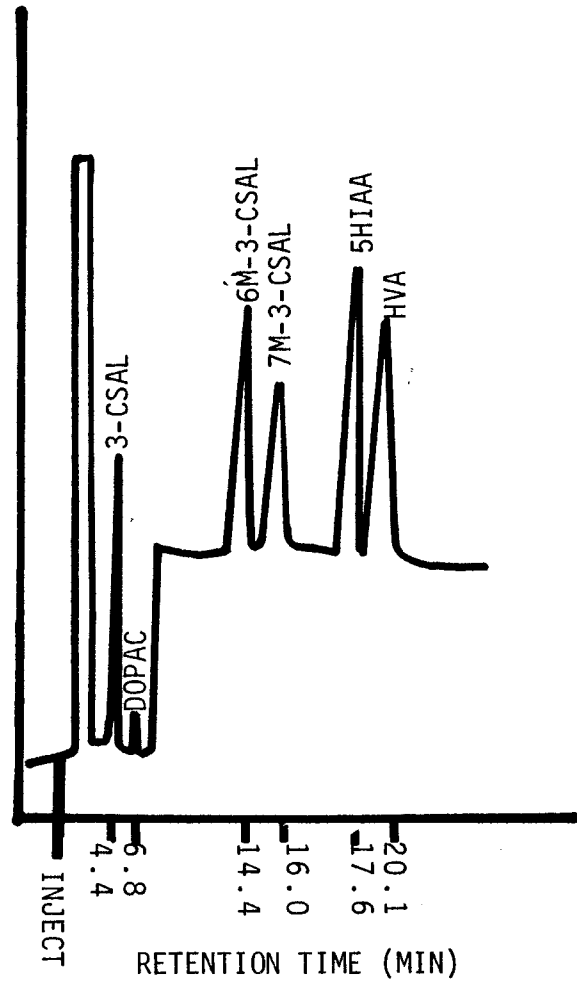
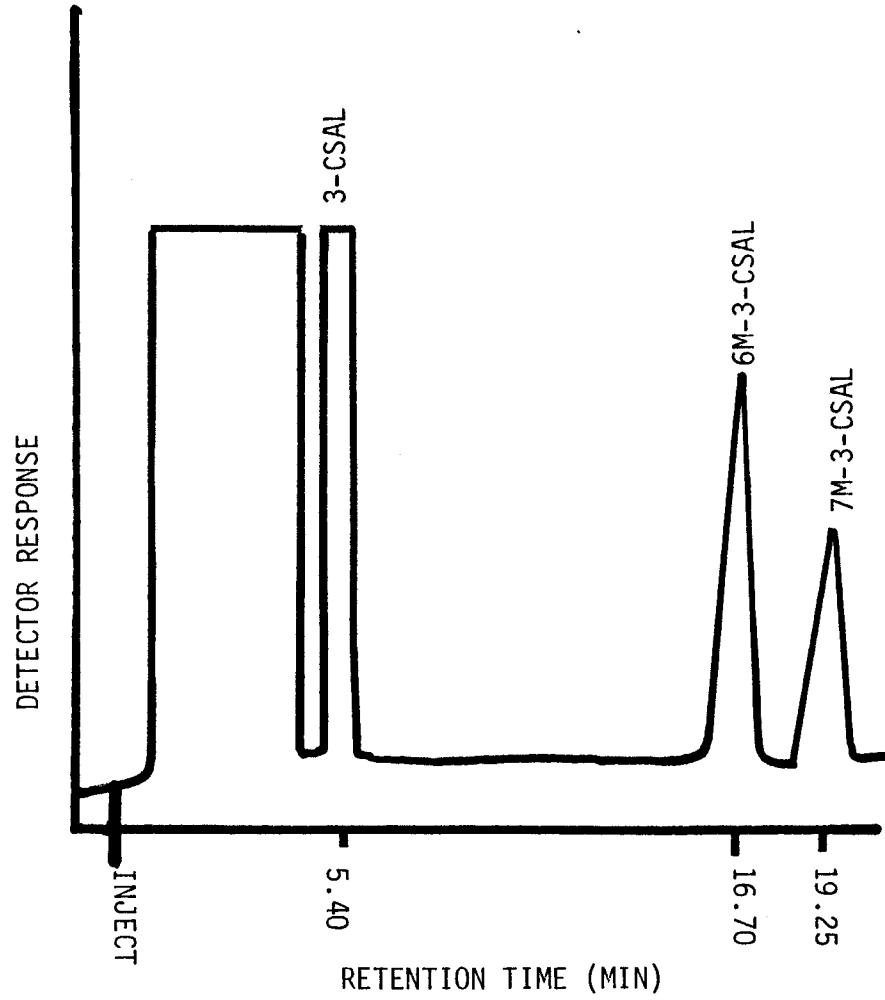
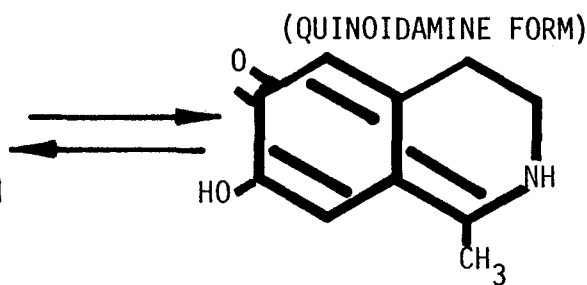
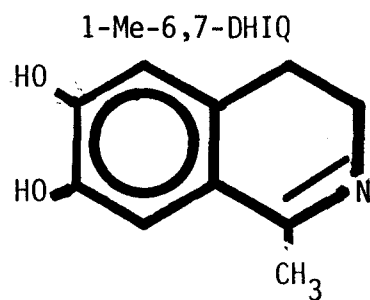
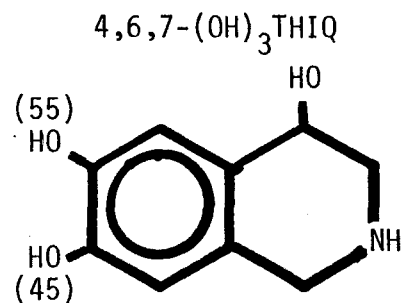
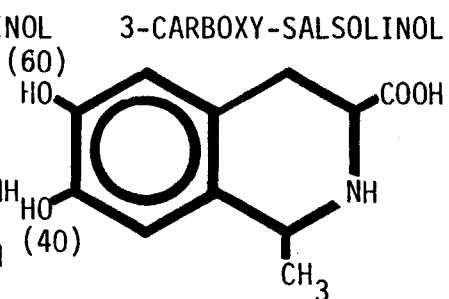
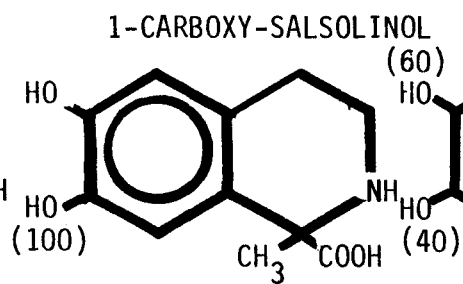
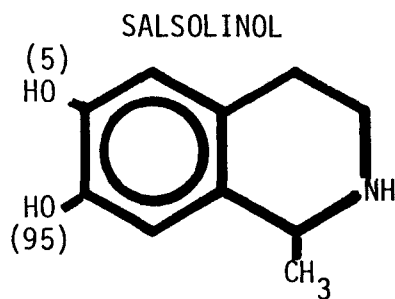
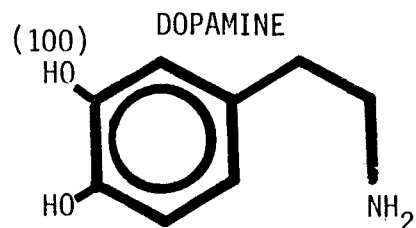


Figure 41. Summary of the site-specific in vivo  
O-methylation of various catechol-isoquinolines in the  
rat CNS, expressed a percent of total O-methylation.



NO DETECTABLE O-METHYLATION

Figure 42. Levels of 5HT, 5HIAA, HVA and DOPAC (percent of control) in three rat brain regions 50 min following ICV administration of catechol isoquinolines (50 ug/animal). \* =  $P \geq .05$ ; \*\* =  $P \geq .02$ ; \*\*\* =  $P \geq .01$ ; corpus striatum ▣ ; hippocampus ■ ; hypothalamus ▤ .



PERCENT CONTROL

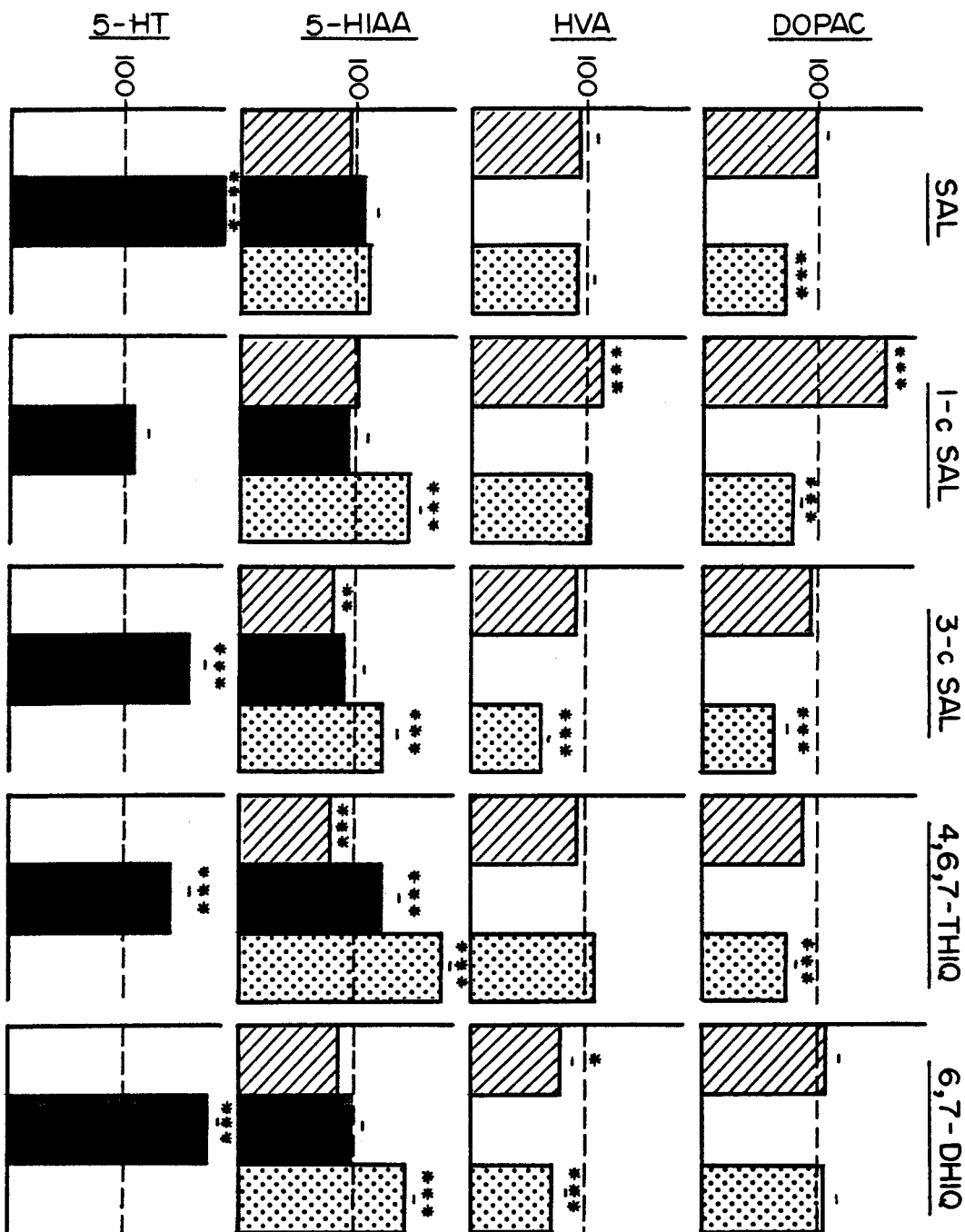


Figure 43. Log of the tissue levels of 7M-SAL at different time points after ICV injection ( 5 ug/ventricle ); ■ hypothalamus; ● corpus striatum.

LOG 7M-SAL UG/G TISSUE

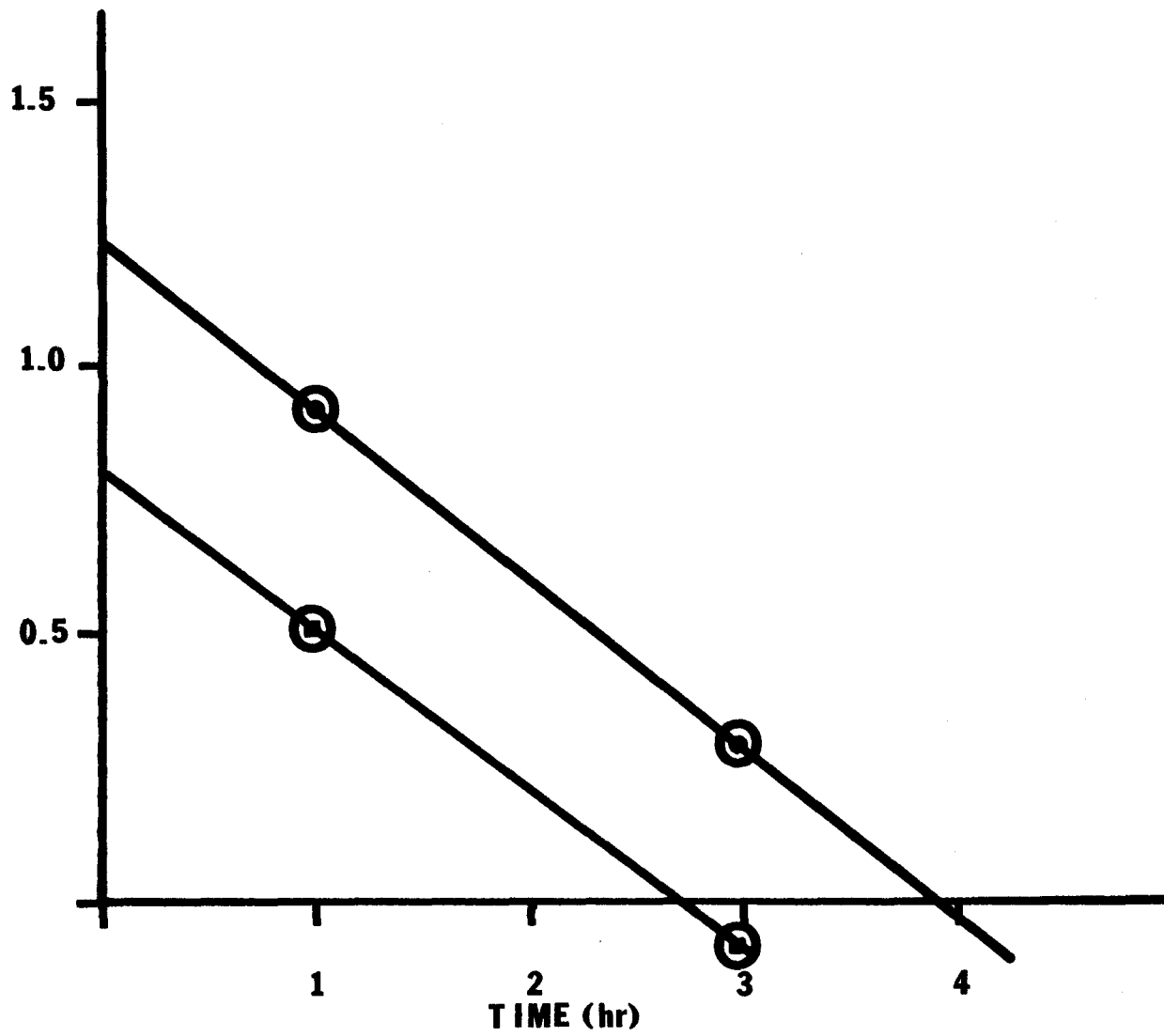


Figure 44. Central effects of 7M-SAL on the levels of biogenic amines and acids. \* =  $P \geq .05$ .

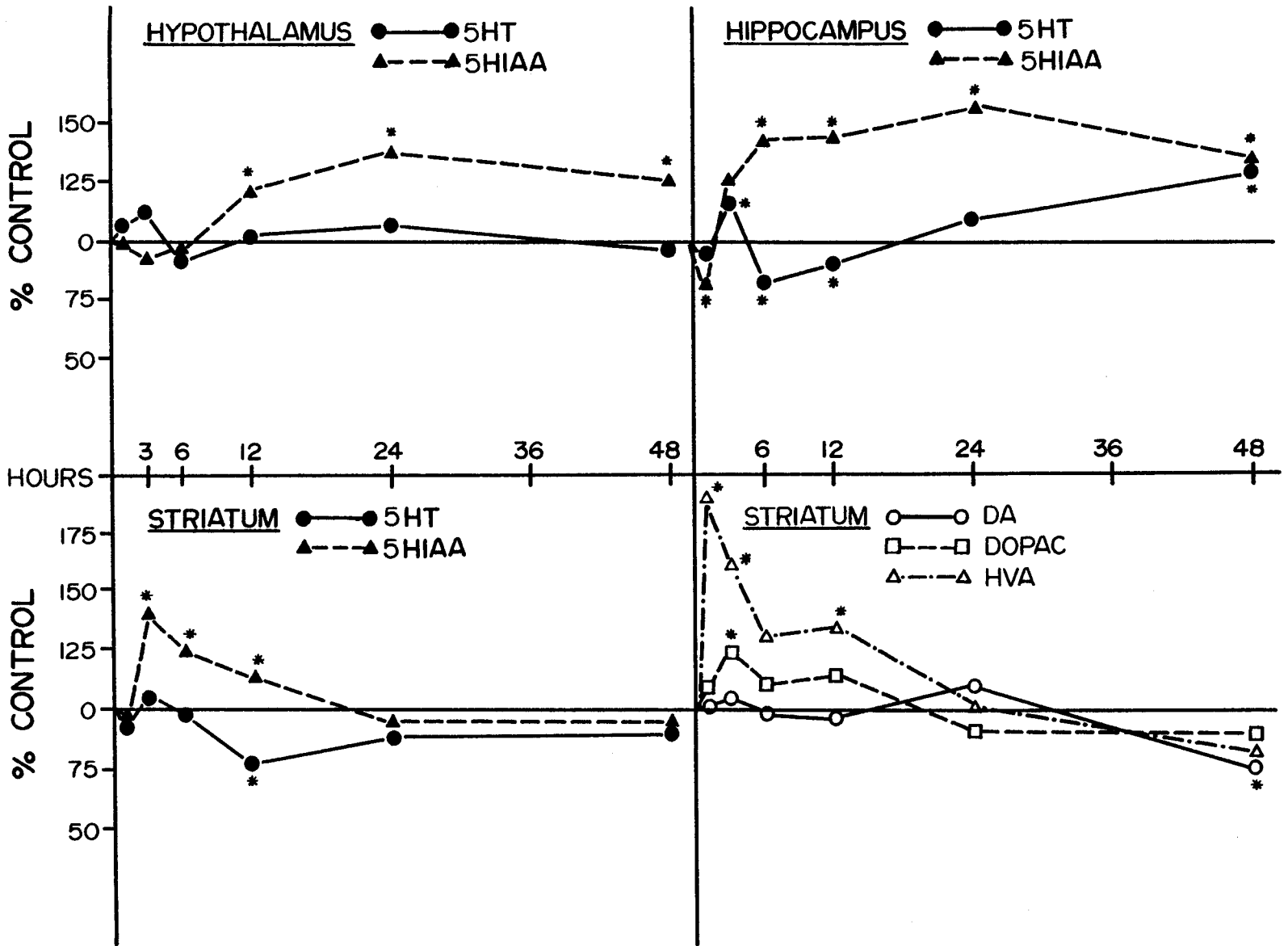
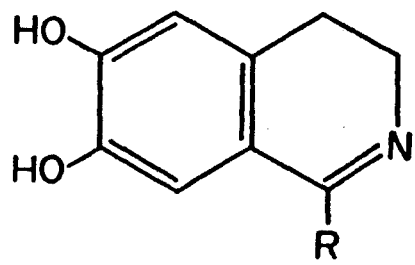
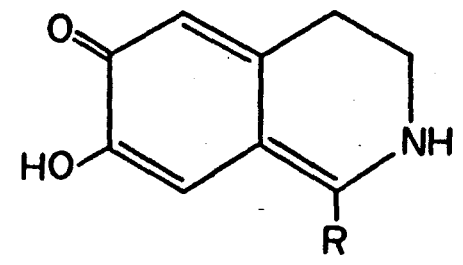
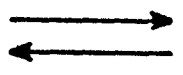


Figure 45. Possible tautomeric forms of DHIQs which can exist under physiological conditions.



**A**



**B**

Figure 46. UV absorption spectra of A, 1-Me-6,7 DHIQ and B, 1-Me-7M-DHIQ; pH 2-4----; pH 7.0-13——.



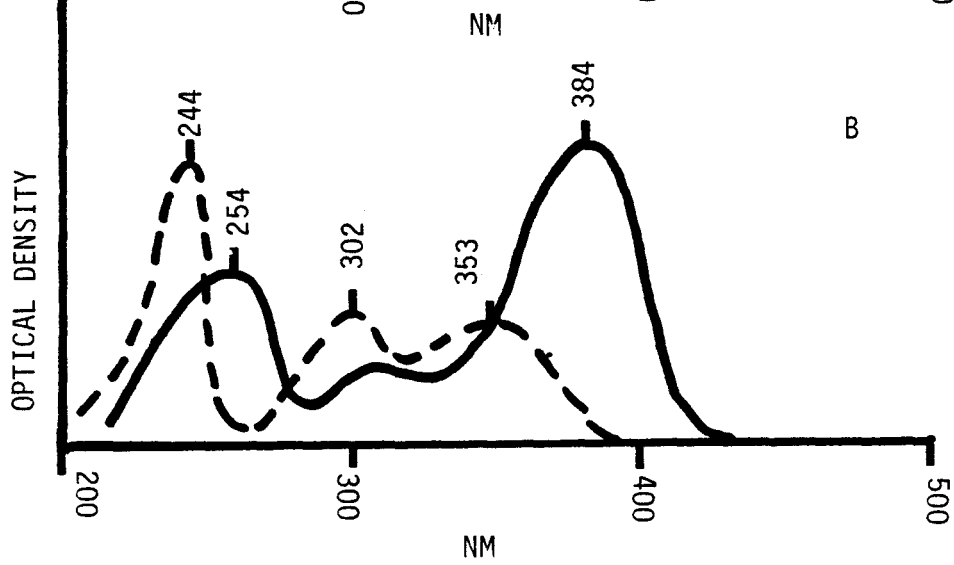
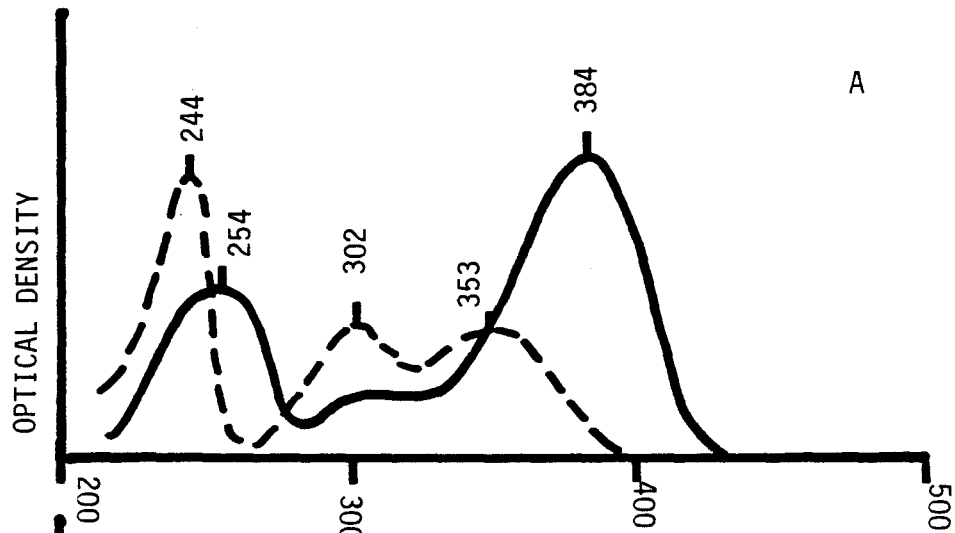


Figure 47. Excitation and emission spectra of A, 1-Me-6,7-DHIQ and B, 1-Me-7M-DHIQ.

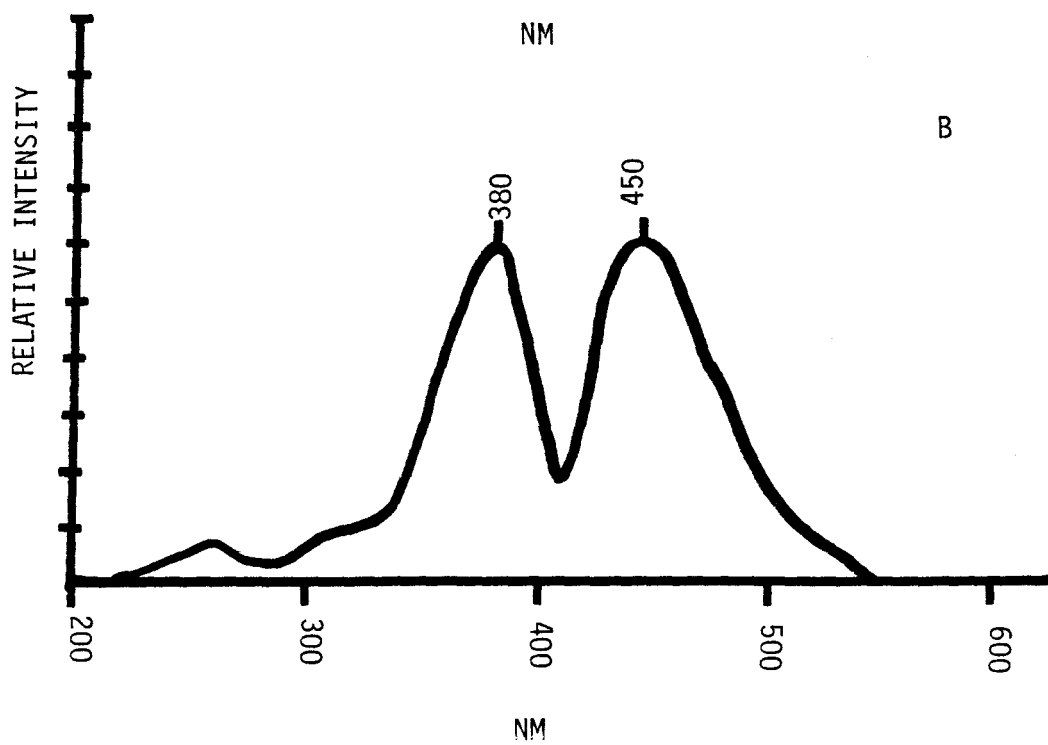
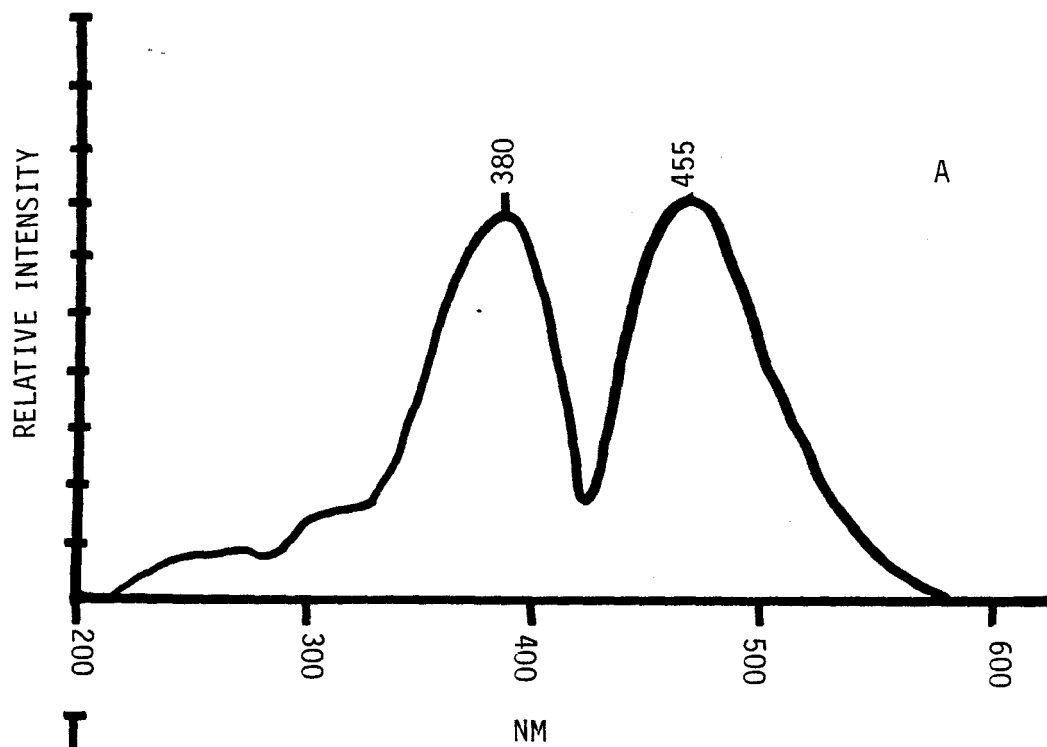


Figure 48. Variation of fluorescence intensity with pH of 1-Me-6,7-DHIQ (O) and 1-Me-7M-DHIQ (□).

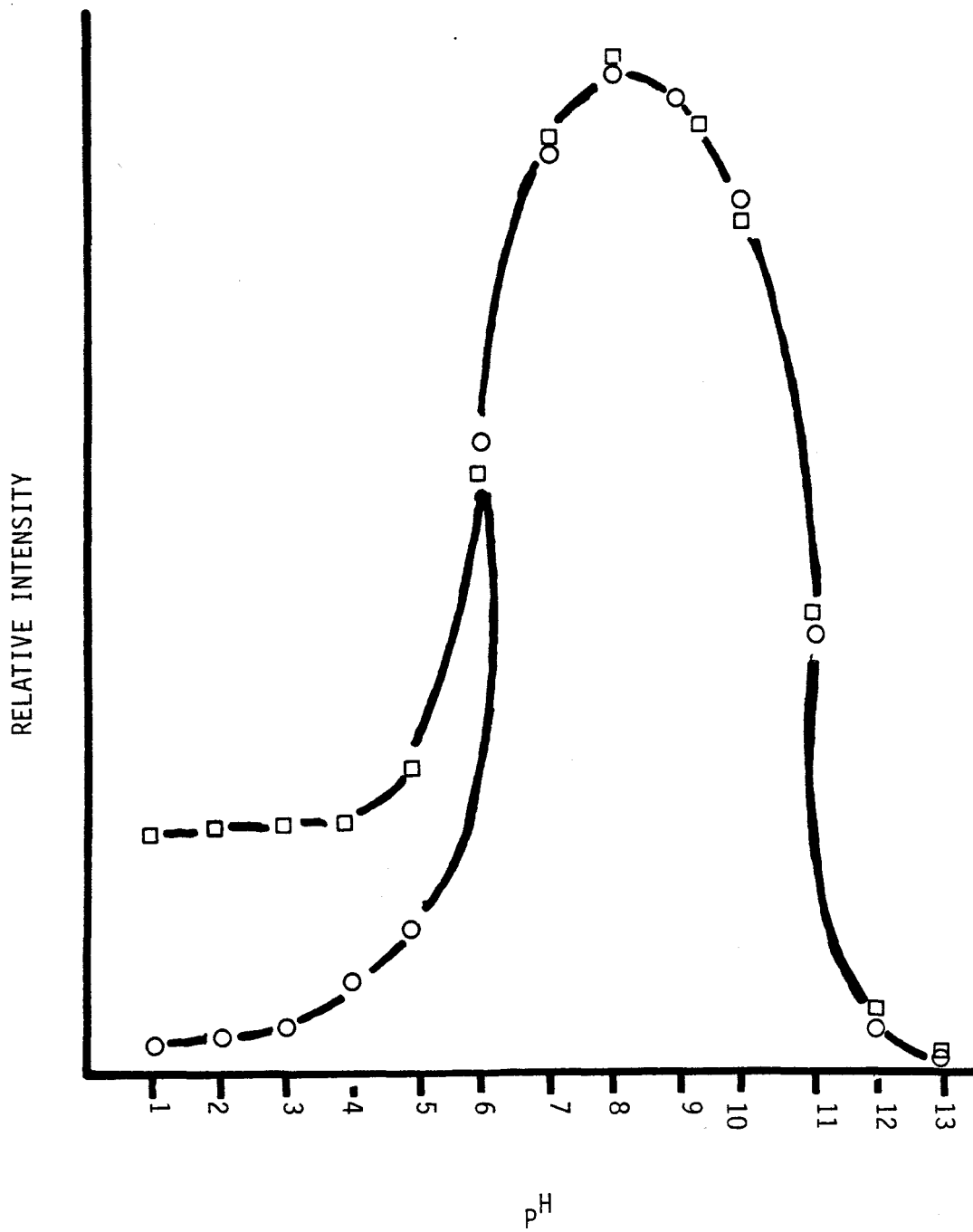


Figure 49. UV absorption spectra of A, 1-Me-6,7-DM DHIQ  
and B, 1-Me-6M-DHIQ; pH 1-9-----; pH 10-13——.

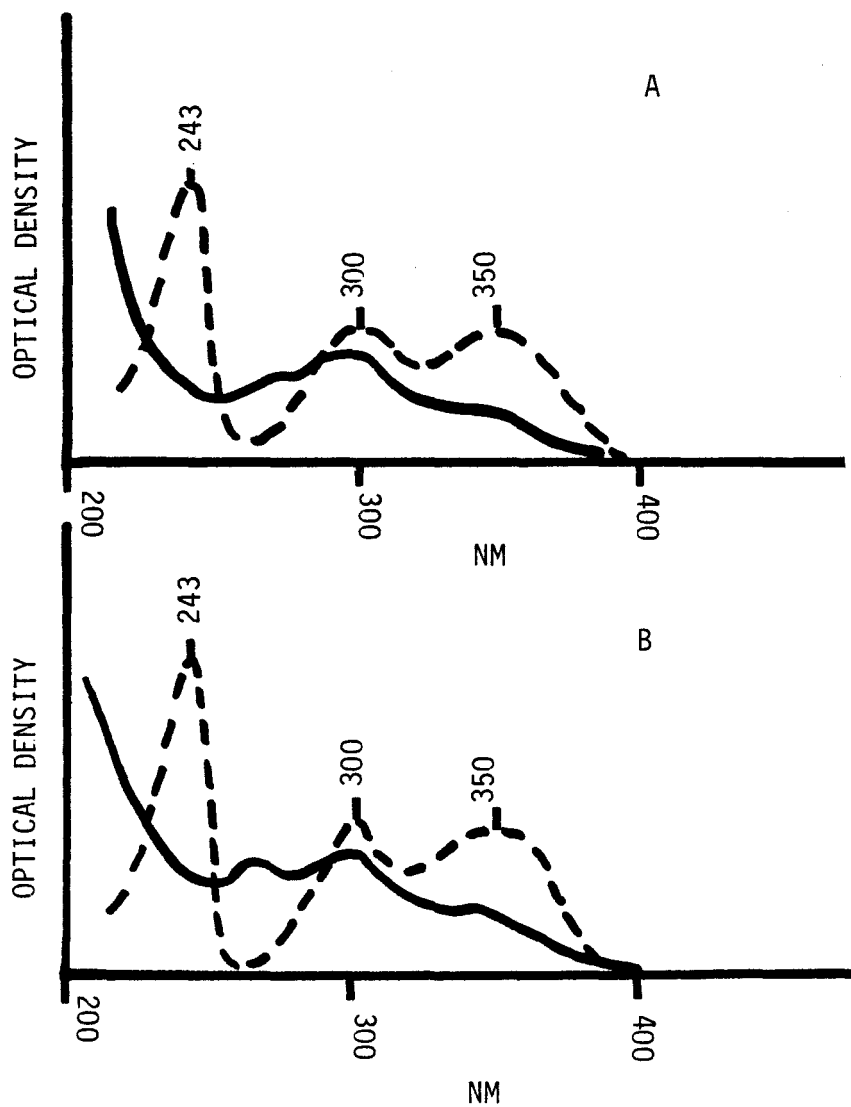


Figure 50. Excitation and emission spectra of 1-Me-6,7-  
DM DHIQ.



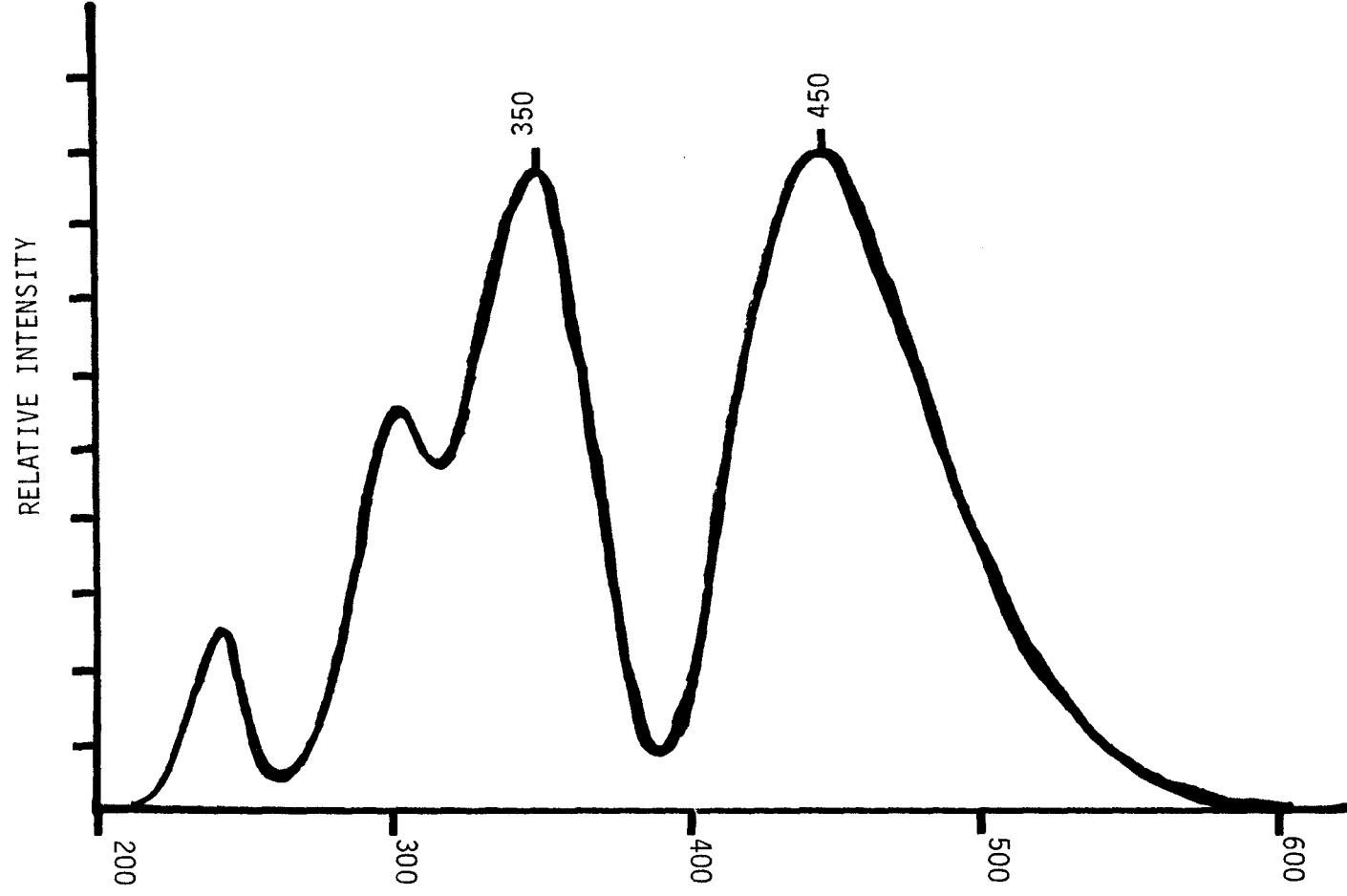


Figure 51. Variation of fluorescence intensity with pH of 1-Me-6,7-DM DHIQ.

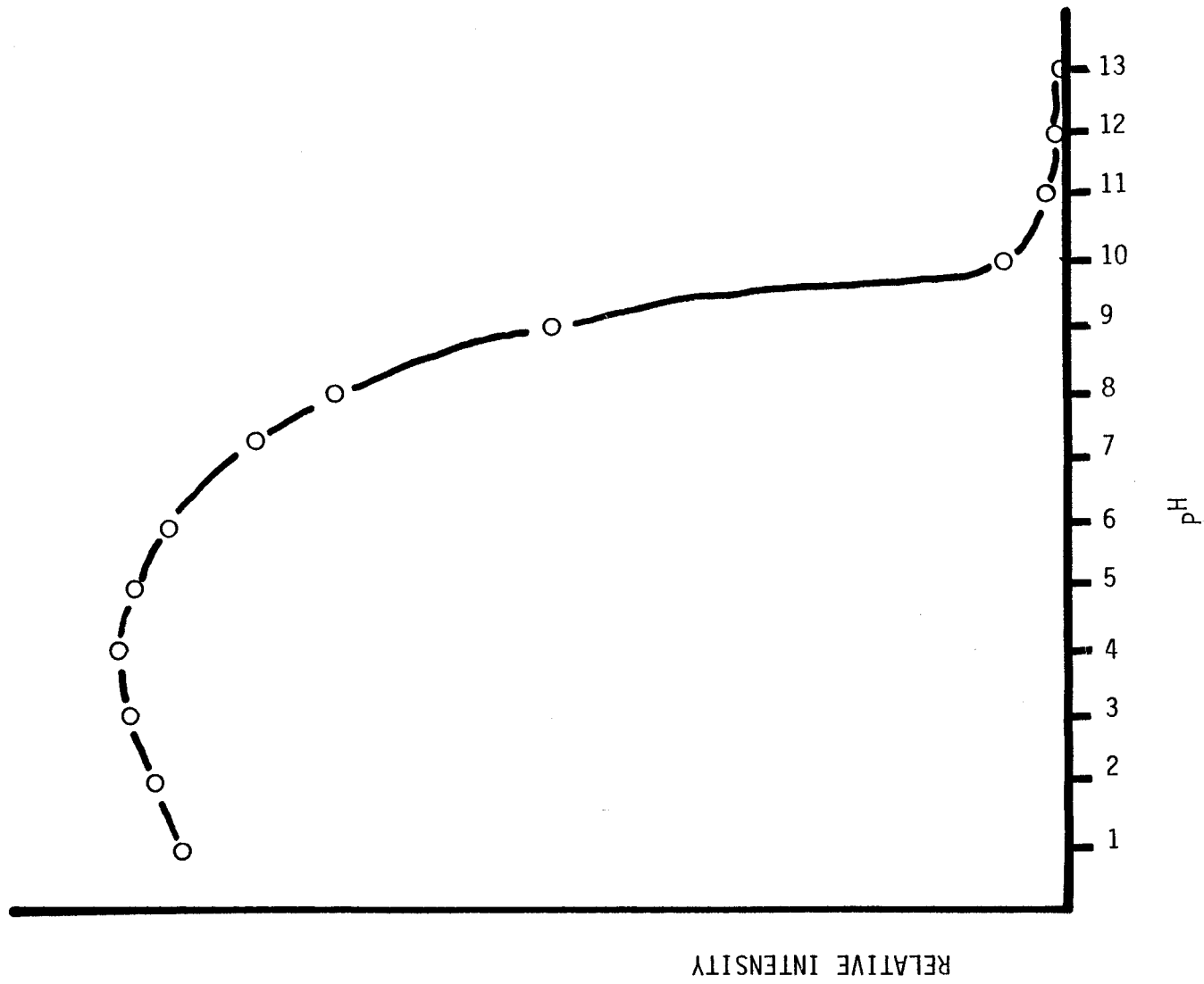


Figure 52. Cyclic voltammograms of 1-Me-6,7-DHIQ; A, pH 3.0 (---); B, pH 5.0 (—); C, pH 7.4 (●—●); scan rate 200 mv/sec sensitivity 2 $\mu$ A/cm.

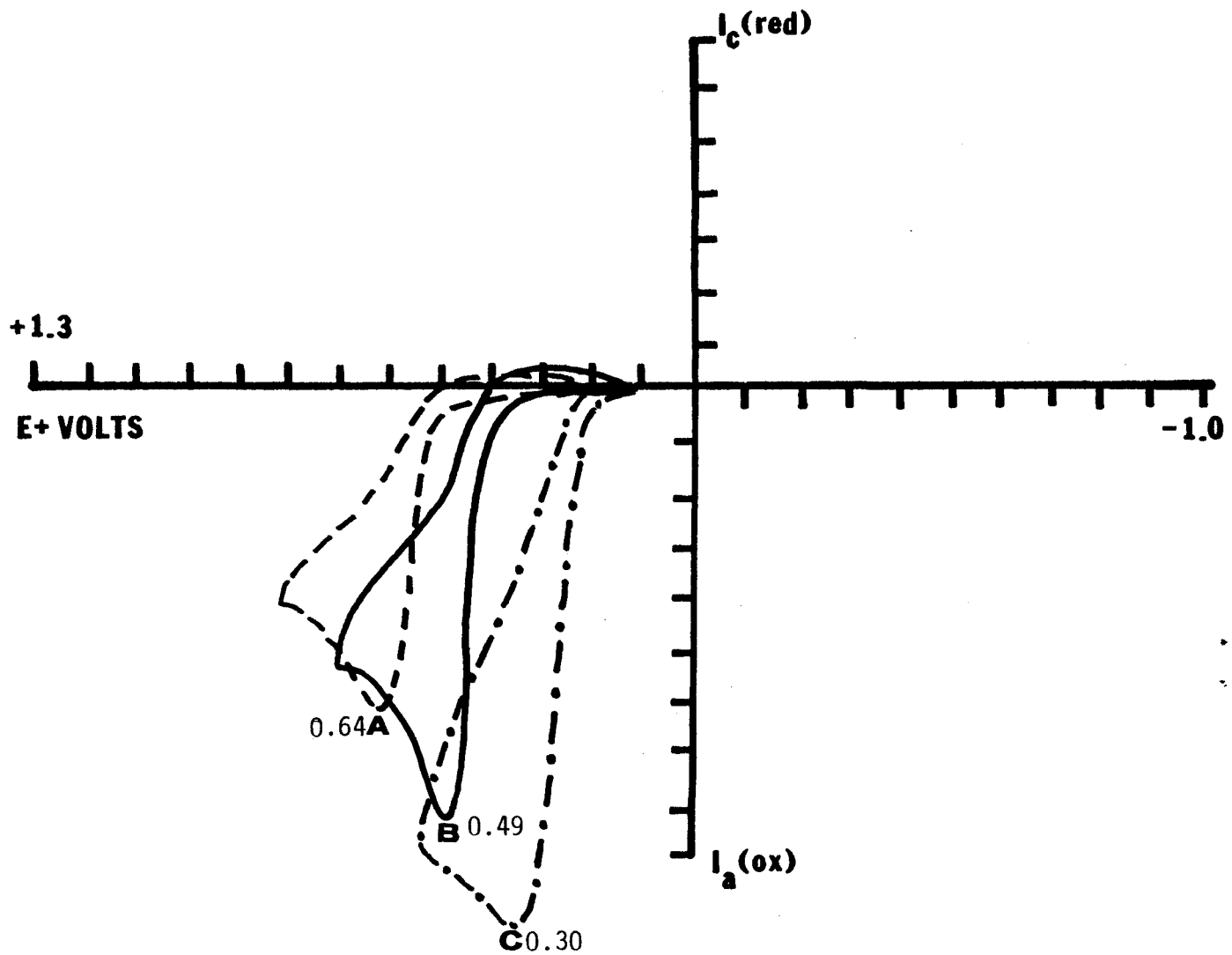


Figure 53. Cyclic voltammograms of 1-Me-7M-DHIQ; A, pH 3.0 (---); B, pH 5.0 (—); C, pH 7.4 (\*-°); scan speed 200 mv/sec, sensitivity 2uA/cm.

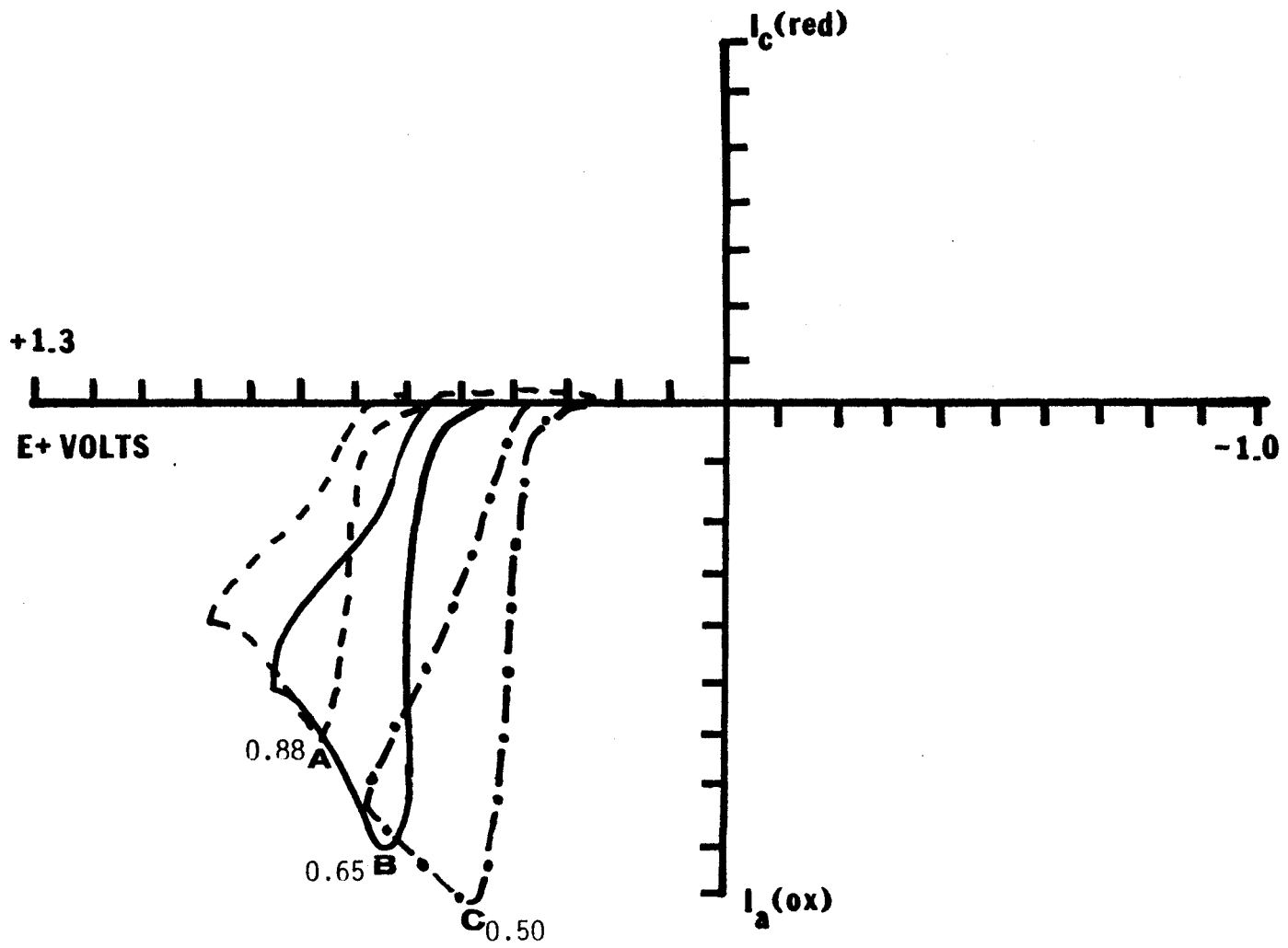


Figure 54. Cyclic voltammograms of 1-Me-6M-DHIQ; A, pH 3.0 (---); B, pH 5.0 (—); C, pH 7.4 (•—•); scan speed 200 mv/sec, sensitivity  $2\mu\text{A}/\text{cm}$ .



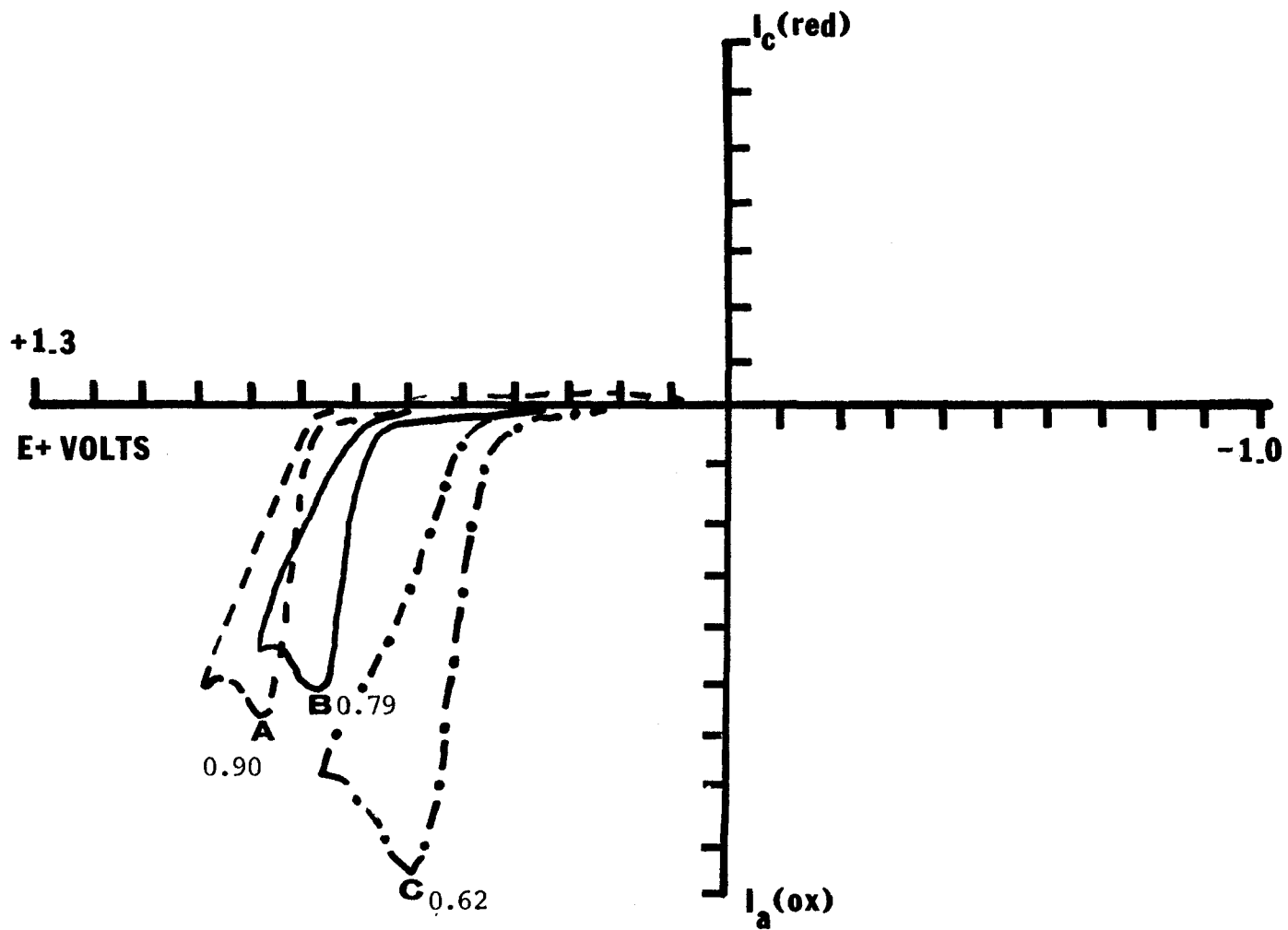


Figure 55. Representative GC chromatogram of head-space of control blood spiked with ACA, EtOH and N-propanol (internal standard) incubated at 37°C, using Porapak QS column with flame ionization detection.

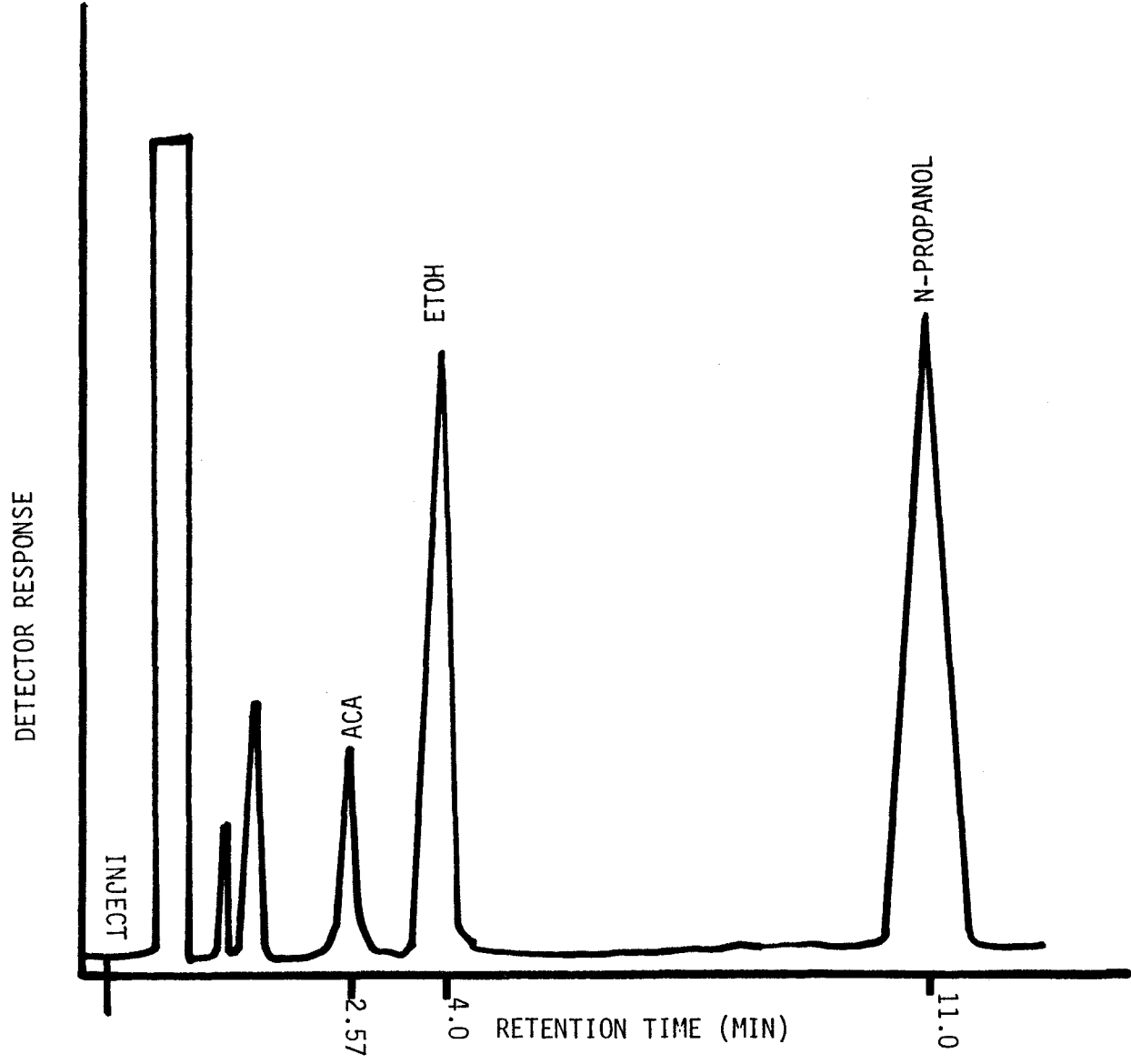
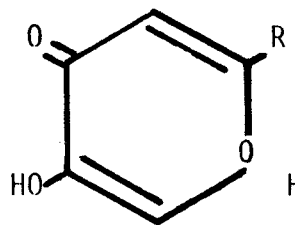
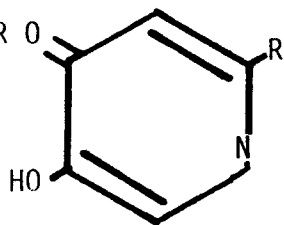


Figure 56. Comparison of structural similarities between DHIQs, pyrones, pyridones and tropolones.

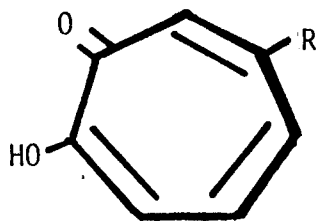
PYRONES



PYRIDONES



TROPOLONES



DIHYDROISQUINOLINES

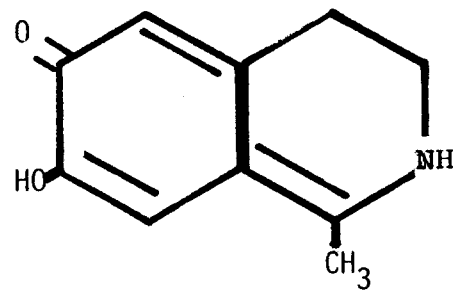


Figure 57. (A) Proposed alternative binding orientations of CA substrates at the catechol binding site of COMT, (—) meta-binding orientation and para binding orientation (---).

(B) Proposed alternative binding orientations of catechol-isoquinoline substrates at the catechol-binding site COMT, para (7) binding orientation (—) and meta (6) binding orientation (---). H = proposed hydrophobic center; CA= CA binding center; SAM = SAM binding site.

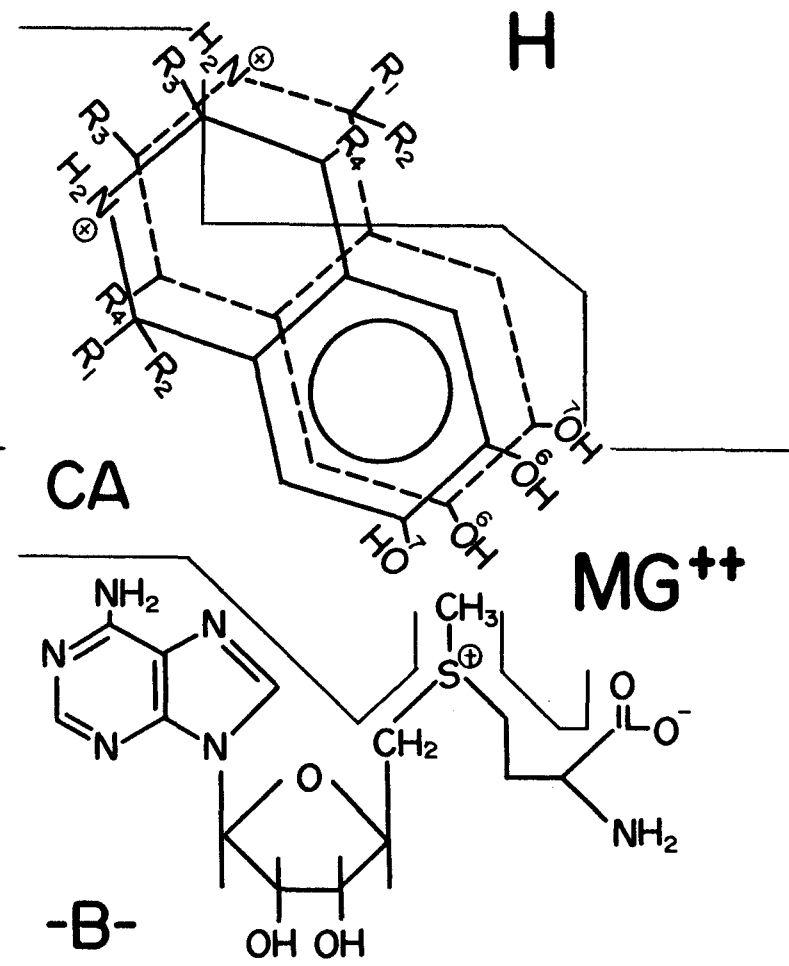
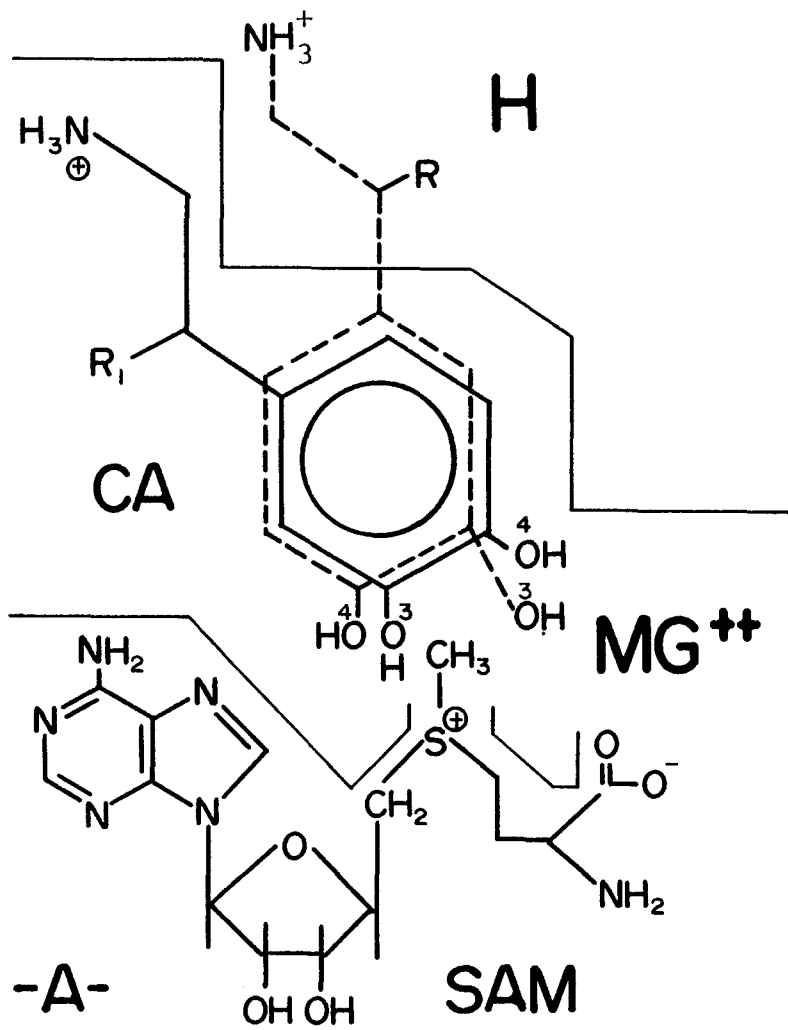


Figure 58. (A) Transition of a 1-Me-6-hydroxy-DHIQ with pH.  
(B) Transition of 1-Me-6-substituted-DHIQ with  
pH.



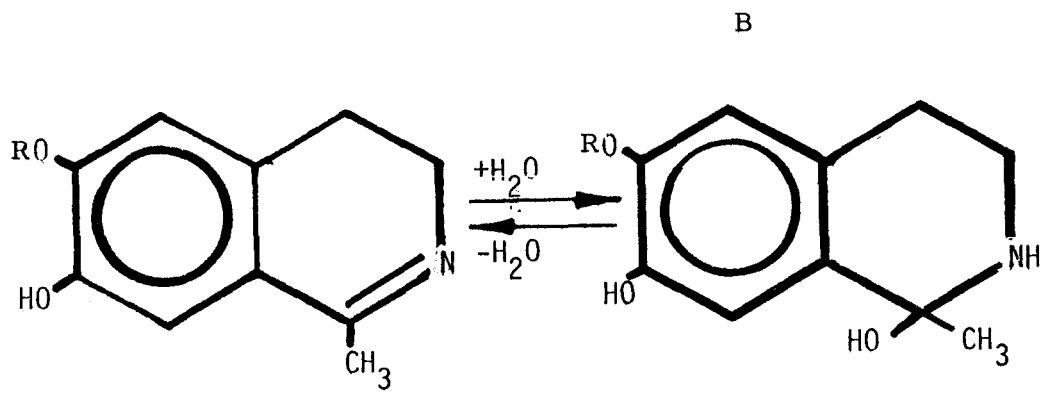
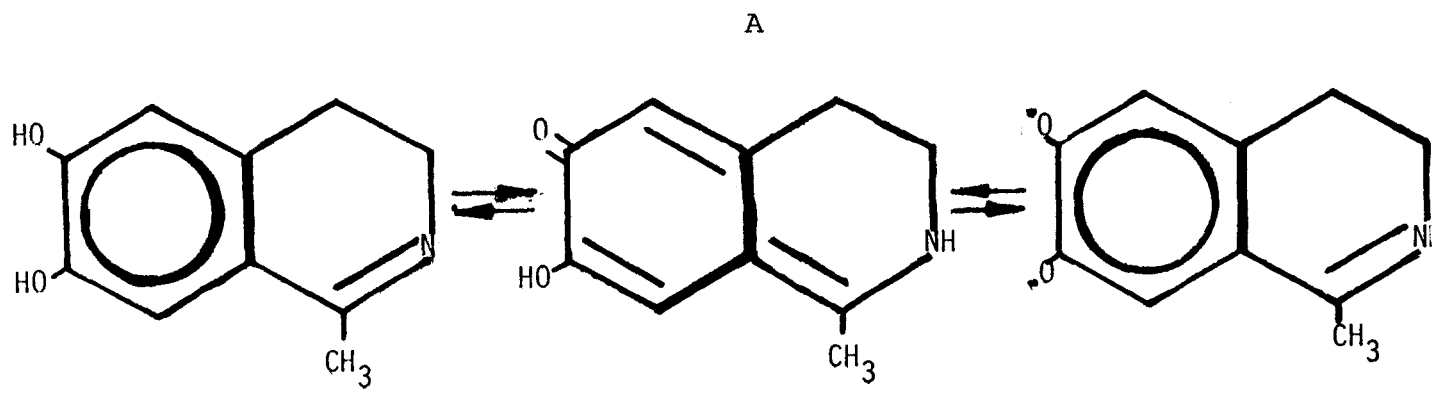


Figure 59. (A) Theoretical single peak voltammogram of a model oxidizable compound.

(B) Theoretical cyclic voltammogram of a model oxidizable/reducible compound.

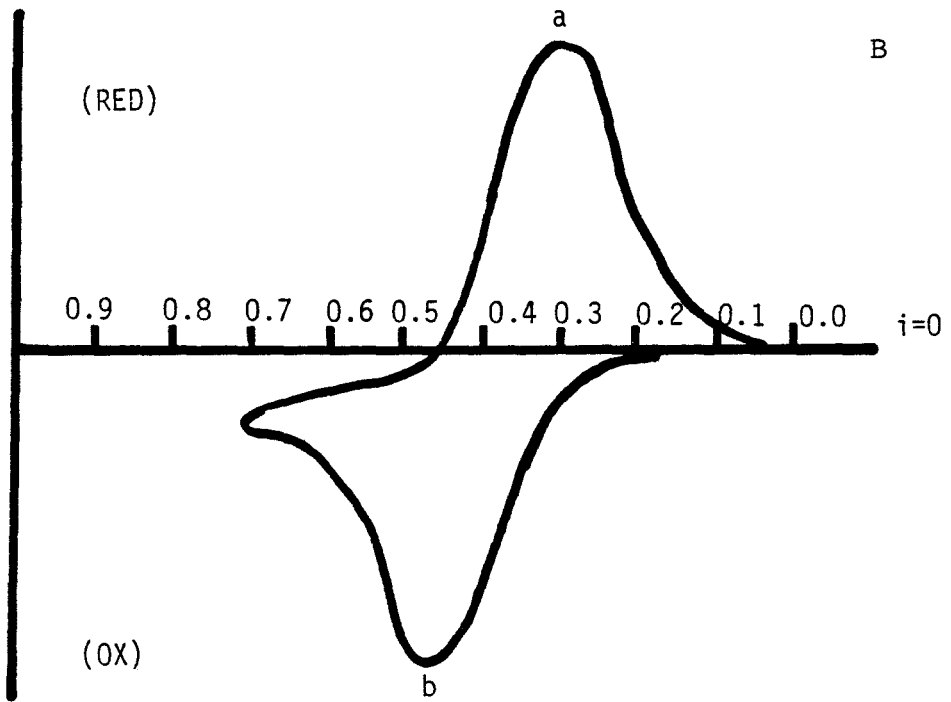
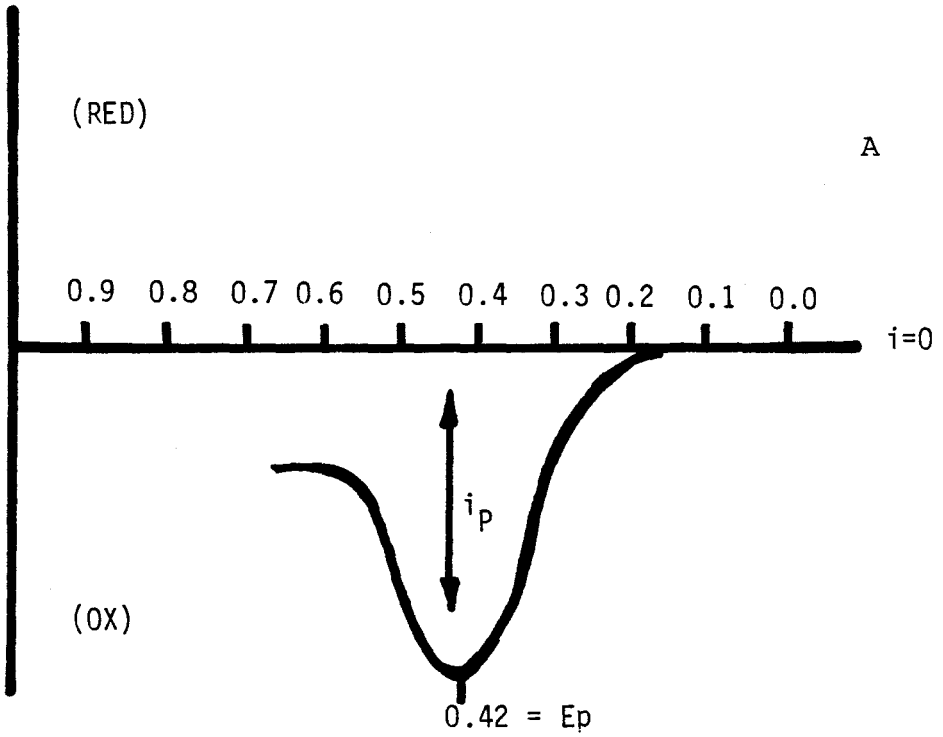


Figure 60. Cyclic voltammograms of model compounds show redox reactions which are; A, fast (reversible); B, intermediate (quasi-reversible); C, slow (irreversible).

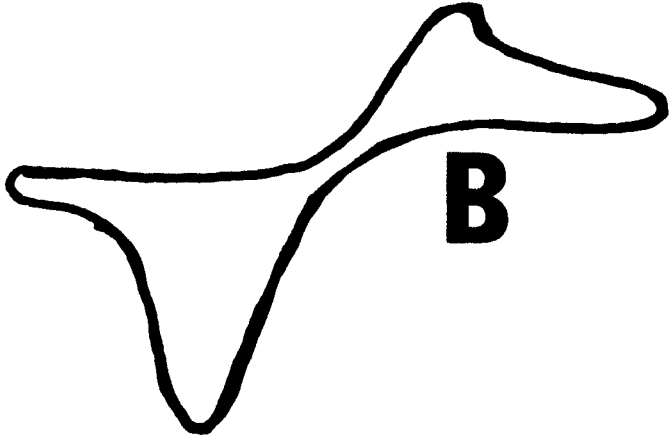
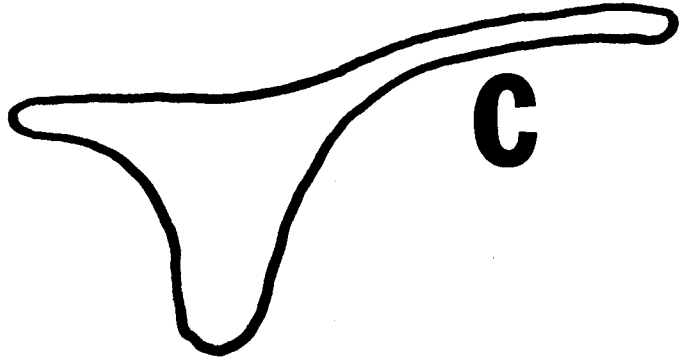
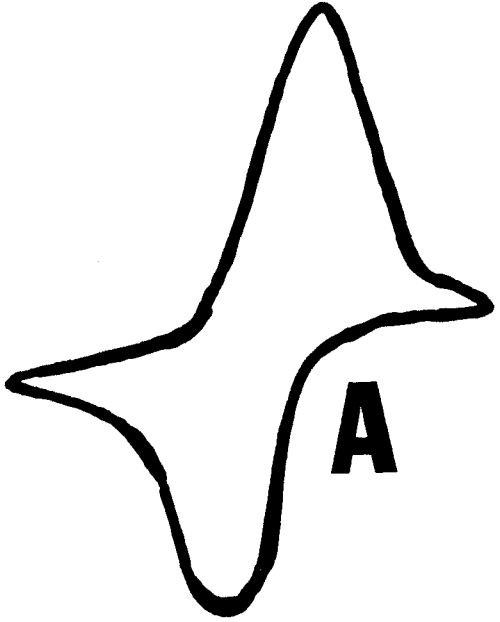


Figure 61. Possible reaction pathways for the electrochemical oxidation of DHIQs.

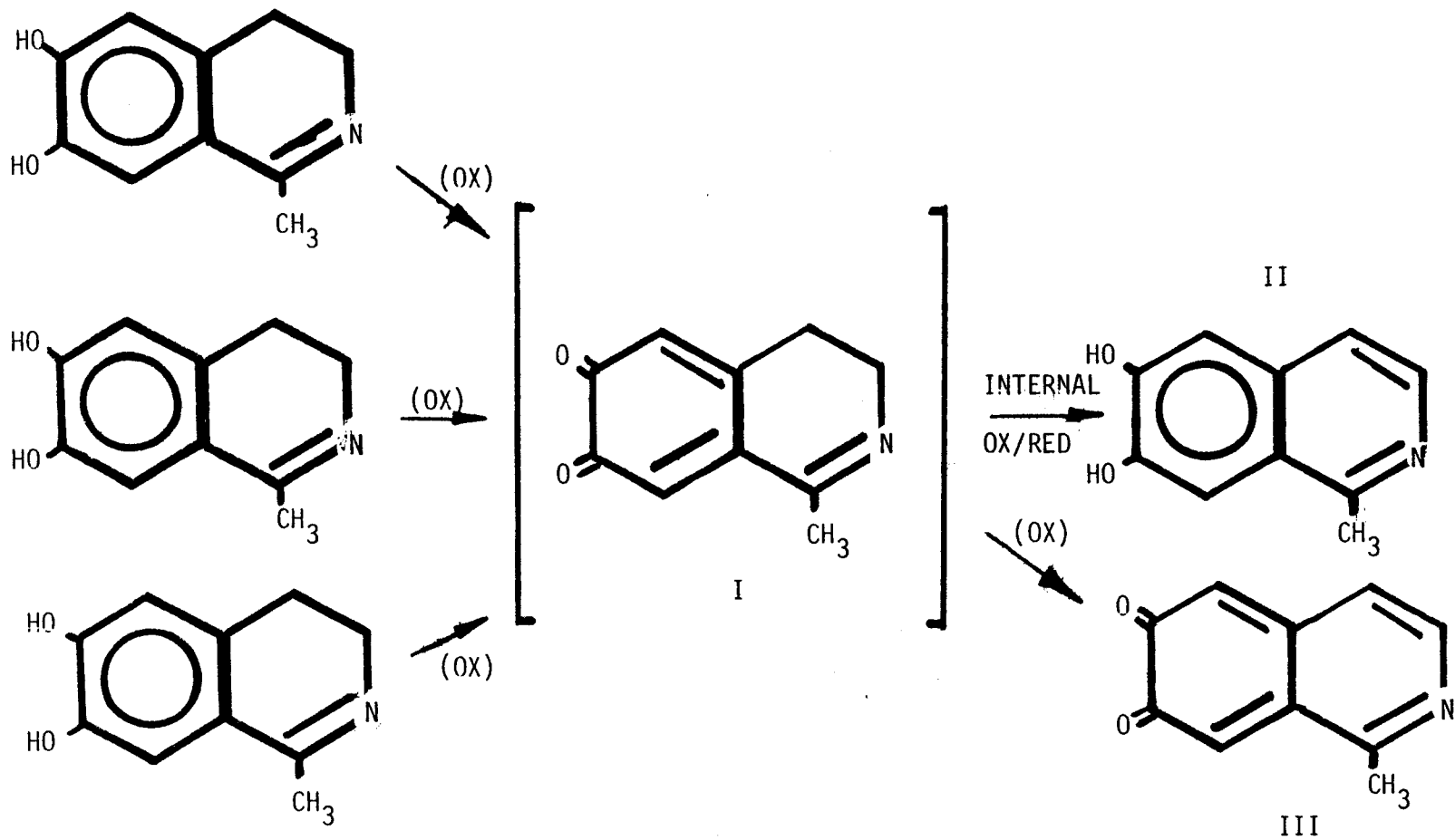
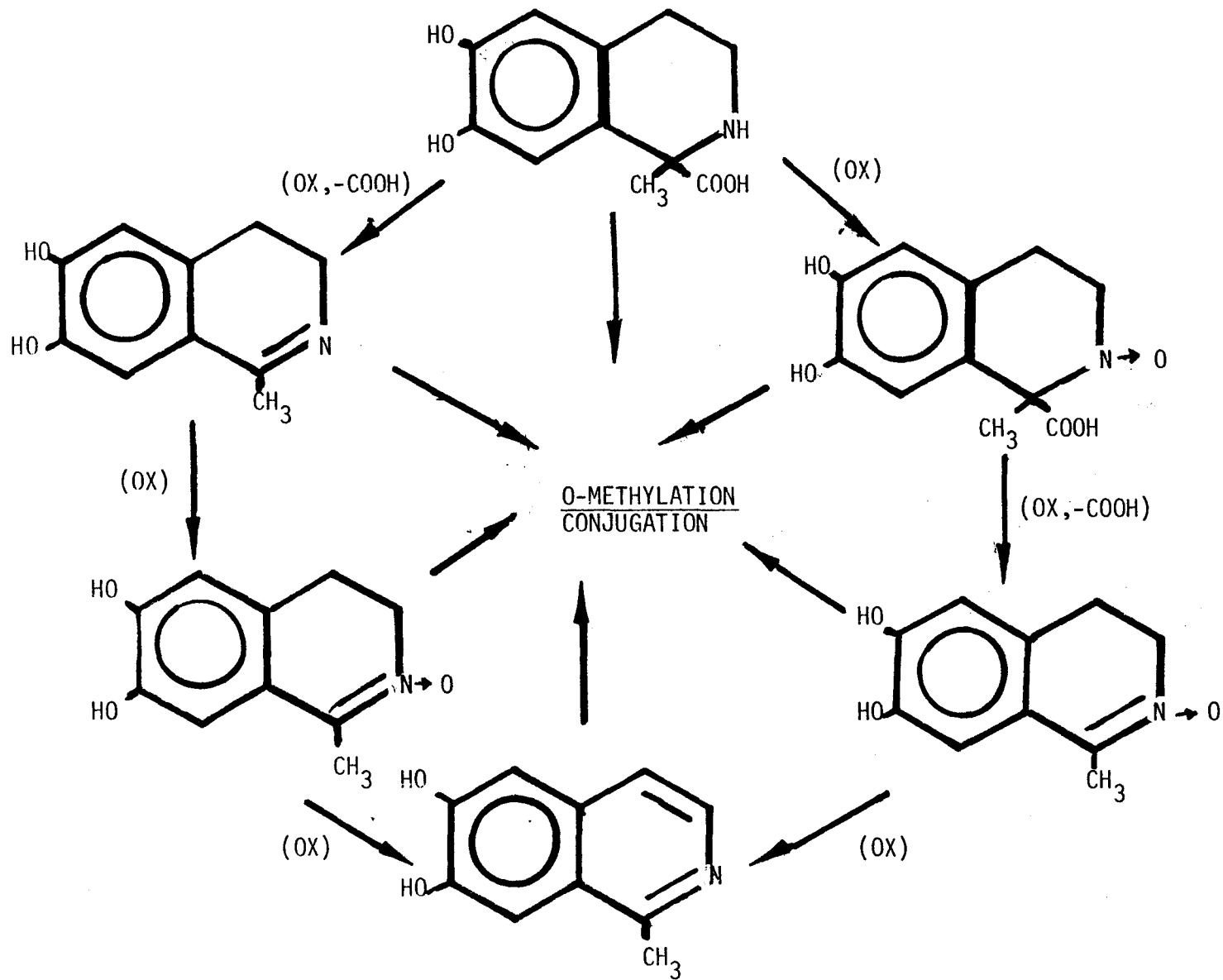


Figure 62. Possible metabolic routes for 1-CSAL in vivo.





## LIST OF ABBREVIATIONS

AAD	Aromatic acid decarboxylase
AcA	Acetaldehyde
ACN	Acetonitrile
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
AL <sub>2</sub> O <sub>3</sub>	Aluminum oxide
BBB	Blood-brain-barrier
BBr <sub>3</sub>	Borontribromide
CAs	Catecholamines
CNS	Central nervous system
CO <sub>2</sub>	Carbon dioxide
1-CSAL	1-carboxysalsolinol
3-CSAL	3-carboxysalsolinol
COMT	Catechol-O-methyl-transferase
DA	Dopamine
DBH	Dopamine-β-hydroxylase
DDC	L-Dopa decarboxylase
DHBA	Dihydroxybenzylamine
DHIQ	3,4-Dihydroisoquinoline
dH <sub>2</sub> O	Deionized-distilled water
DHPAcA	3,4-Dihydroxyphenylacetaldehyde
DNLCA	3,4-Deoxynorlaudanosoline carboxylic acid
D <sub>2</sub> O	Deuterium oxide

DOPAC	3,4-Dihydroxyphenylacetic acid
DOPEG	3,4-Dihydroxyphenylethylene glycol
EC	Election capture
EPI	Epinephrine
EtOAc	Ethyl acetate
EtOH	Ethanol
GC	Gas chromatograph
GSH	Glutathione
HBR	Hydrogen bromide
HCHO	Formaldehyde
HFBA	Heptafluorobutyric anhydride
HFIP	Heptafluoroisopropanol
5HIAA	5-Hydroxyindoleacetic acid
HoAc	Acetic acid
HSA	Heptane sulfonic acid
5HT	Serotonin
5HTOL	5-hydroxytryptophol
5HTP	5-hydroxytryphophan
HVA	Homovanillic acid
I <sub>2</sub>	Iodine
ICV	Intracerebroventricular
IR	Infra red
K <sup>+</sup>	Potassium ion
KBr	Potassium bromide
L-DOPA	3,4-Dihydroxyphenylalanine
MAO	Monoamine oxidase

6M-1-CSAL	6-O-methyl-1-carboxysalsolinol
7M-1-CSAL	7-O-methyl-1-carboxysalsolinol
6M-3-CSAL	6-O-methyl-3-carboxysalsolinol
7M-1-CSAL	7-O-methyl-3-carboxysalsolinol
3M-DA	3-O-methyl-dopamine
4M-DA	4-O-methyl-dopamine
1-Me-6,7-DHIQ	1-methyl-6,7-dihydroxy 3,4-dihydroisoquinoline
1-Me-6,7-DMDHIQ	1-methyl-6,7-dimethoxy 3,4-dihydroisoquinoline
1-Me-6M-DHIQ	1-methyl-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline
1-Me-7M-DHIQ	1-methyl-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline
MeOH	Methanol
MEOS	Microsomal ethanol oxidizing system
MET	Metanephrine
Mg <sup>++</sup>	Magnesium ion
MOPEG	3-methoxy-4-hydroxyphenylethylene glycol
NMLCA	3-O-methylnorlaudanosolinecarboxylic acid
MS	Mass spectrum
6M-SAL	6-O-methyl-salsolinol
7M-SAL	7-O-methyl-salsolinol
6M-4,7(OH) <sub>2</sub> THIQ	6-methoxy-4,7-dihydroxy-tetrahydroisoquinoline
7M-4,6(OH) <sub>2</sub> THIQ	7-methoxy-4,6-dihydroxy-tetrahydroisoquinoline
N <sub>2</sub> <sup>O<sub>2</sub>F</sup>	Nitrogen gas oxygen free
N <sub>2</sub> Ex	Nitrogen gas extra dry

Na <sup>+</sup>	Sodium ion
NAD	Nicotinamide-adenine-dinucleotide oxidized form
NADH	Nicotinamide-adenine-dinucleotide reduced form
NE	Norepinephrine
6NH <sub>2</sub> DA	6-Amino-dopamine
NLCA	Norlaudanosalinecarboxylic acid
NMR	Nuclear magnetic resonance
NORMET	Normetanephrine
NTs	Neurotransmitters
6OHDA	6-Hydroxy-dopamine
OSA	Octane-sulfonic acid
OV-17	Polyphenylmethylsiloxane
OV-101	Polydimethoxysiloxane
PEA	Phenethylamine
PFPA	Pentafluoropropionyl anhydride
PIC	Paired-ion chromatography
PKU	Phenylketonuria
PNMT	Phenethanolamine-N-methyl-transferase
P <sub>2</sub> O <sub>5</sub>	Phosphorous pentoxide
RP	Reverse phase
R <sub>T</sub> <sup>0</sup>	Room temperature
SAL	Salsolinol
SAM	S-adenosyl-L-methionine
SCOT	Support coated open tubular
TCA	Citric acid cycle

TFAA	Trifluoroacetic anhydride
TH	Tyrosine hydroxylase
THBC	Tetrahydro-B-carboline
THIQ	Tetrahydroisoquinoline
4,6,7-(OH) <sub>3</sub> -THIQ	4,6,7-Trihydroxy-tetrahydroisoquinoline
6,7-(OH) <sub>2</sub> -THIQ	6,7-Dihydroxy-tetrahydroisoquinoline
THP	Tetrahydropapaveroline
THPB	Tetrahydroprotoberberine
TLC	Thin layer chromatography
TMQ	Trimetoquinol
VMA	Vanillylmandelic acid
WCOT	Wall coated open tubular
HPLC	High performance liquid chromatography

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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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