Title
Study of deuterium isotope effects in metabolic C-hydroxylation

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Publication Date
1978

Peer reviewed|Thesis/dissertation

STUDY OF DEUTERIUM ISOTOPE EFFECTS IN METABOLIC C-HYDROXYLATION

by

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B.S., Jacksonville State University 1968
M.S., Georgia State University 1971

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA

[redacted]

Date Librarian

Degree Conferred: JUN 25 1978 . . . . . .

To my first mentor and constant advisor,

David W. Boykin, Jr.

and

in memory of my late Grandfather,

Levi Dunn

STUDY OF DEUTERIUM ISOTOPE EFFECTS IN METABOLIC C-HYDROXYLATION

by

Ronald Wesley Woodard

University of California, San Francisco

ABSTRACT

Chapter I: The absolute configurations of the alkaloids cryptopleurine and (-)-tylocrebrine are established to be R and S respectively by comparison of their ord and cd spectra with that of tylophorine.

Chapter II: The absolute configuration of the asymmetric center adjacent to the benzene ring in debromoaplysiatoxin is determined by use of cd.

Chapter III: The absolute configuration of (-)- calipamine [(-)-N-methyl-3,4-dimethoxy-β-methoxyphenethylamine hydrochloride] from Coryphantha calipensis is assigned by the use of cd.

Chapter IV: The synthesis of 10,11-dihydro-5(3,3-dimethylaminopropyl)-5H-dibenz[b,f]azepine (imipramine) and its 3-methylamino analogue (desipramine) labelled with deuterium in either the 1- or the 3-position of the side-

chain in high isotopic purity is described. The 3,3-d_2 compounds are obtained from the common precursor 5-(2-cyanoethyl)-10,11-dihydro-5H-dibenz[b,f]azepine by reduction and alkylation, while the 1,1-d_2 products are accessible from the 5-(3-chloropropionyl) derivative by amination and reduction. These compounds are required for use as non-exchangeable mass spectrometric stable isotope internal standards for the simultaneous determination of imipramine and desipramine in biological fluids.

Chapter V: Section A: Five derivatives of 2-(4-biphenylyl)propane labelled with deuterium in high isotopic purity in the 2 position, the methyl groups and the distal phenyl group in various combinations were synthesized from their common precursory tertiary alcohols. The compounds 2-(4-biphenylyl)propane, 2-(4-biphenylyl)propane-2-d_1, 2-(4-(pentadeuterophenyl)phenyl)propane, 2-(4-pentadeuterophenyl)phenyl)propane-2-d_1 and 2-(4-biphenylyl)-propane-1,1,1,3,3,3-d_6 were required for the investigation of the mechanisn of C-hydroxylation in the metabolism of the anti-inflammatory IPBP by gc-ms techniques.

Section B: The synthesis of several optically active derivatives of IPBP are described. These compounds were needed to investigate the stereospecificity in the metabolism of IPBP. The compounds also allow the many possible mechanisms to be explored individually and/or in concert.

ACKNOWLEDGMENTS

This author has found four requisites to be essential for the successful completion of graduate study.

Firstly, the major professor must provide guidance, encouragement, understanding and occasionally special tutelage during the tenure of the student's training and research. These benefits were amply supplied by the author's major professor, Dr. John Cymerman Craig whose care and concern were deeply appreciated. I wish Dr. Craig continued success, he and his family good health and hope that someday our paths will again cross.

Secondly, a smattering of good fortune must prevail during certain periods of his career. The author has been endowed with a fair apportioned quantity.

Thirdly, but most importantly, a sympathetic, encouraging, loving and always understanding wife must be at his side. The author is everlastingly indebted to his best friend - companion, counsel, psychoanalyst, lover-wife and gives her his love and heartfelt thanks. Because of her, he is.

Fourthly, but hardly of small dimension, a large group of Professors, friends, relatives and concerns who are always present in any successful endeavor but receive all too small amount of praise.

The author extends special thanks to the University

of California School of Pharmacy, the National Institute of Health, the Eli Lilly Corporation and Carol Woodard for financial support over the course of time.

Sincere thanks to Drs. Roger Ketcham, George Kenyon, Paul Ortiz de Montellano, Mary Vore, Eugene C. Jorgensen, Neal Castagnoli for serving on Orals Committees, Reading Committee and just plain being there when they were needed for both personal and scientific discussion.

Thanks to Dr. Larry Gruenke for the Mass Spectra and for technical assistance.

To his parents for their unfaltering encouragement, faith and support, goes the author's deepest gratitude.

To the entering class of 1974 for providing the competition, exchange of ideals and philosophy and deep friendship which has meant so much in the author's development as a scientist and human. I sincerely believe that no such group of individuals will again in the future ever be assembled. To those people -- salute.

The author acknowledges that he got by with a little help from his many other friends.

Finally, I thank myself for those times when I wanted to quit but did not, those times I wanted to settle for less but did not, and the inner person that allows the outer person to survive.

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ORD AND CD STUDIES OF PHENANTHROINDOLIZIDINE ALKALOIDS.

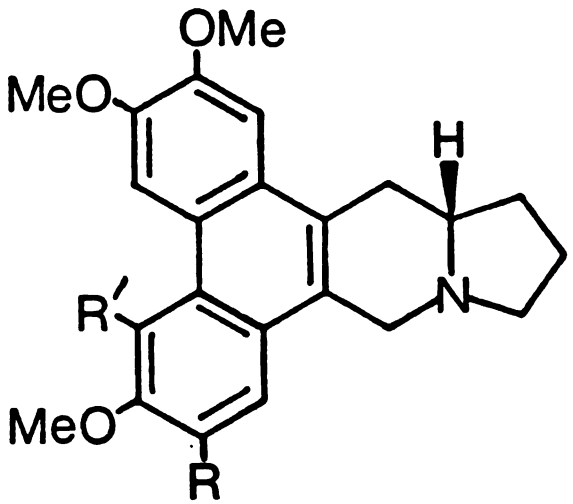
INTRODUCTION

The family Asclepiadaceae is comprised of over 320 genera and 1700 species.[1] Only a few plants of the many studied in this family contain alkaloids. Cryptolepine was isolated from *Cryptolepis triangularis*[2] and *Cryptolepis sanquinolenta*.[3] This chapter will examine the stereochemistry of alkaloids isolated from plants belonging to the genus *Tylophora*. Two of the alkaloids, tylophorine (1) and tylocrebrine (3), have been isolated from *Ficus septica*, a plant belonging to the Moraceae.[4] An additional alkaloid antofine (2) has been isolated from *Vincetoxicum officinale*[5] and *Antitoxicum funebre*.[6] Antofine (2) has the same phenanthroindolizidine skeleton as do the *Tylophora* alkaloids. The alkaloid cryptopleurine (4) isolated from a plant in the Lauraceae family has the phenanthroquinolizidine ring system. This closely similar ring system is interesting in view of the vast botanical differences in the families.

Tylophorine (1) and tylocrebrine (3) have also been obtained from *Tylophora asthmatica* Wight et Arn.,[8] a perennial branch climber of the family Asclepiadaceae which grows wild in the plains forests in eastern and southern India, and *Tylophora crebriflora* S. T. Blake,[9] a plant which grows in North Queensland.

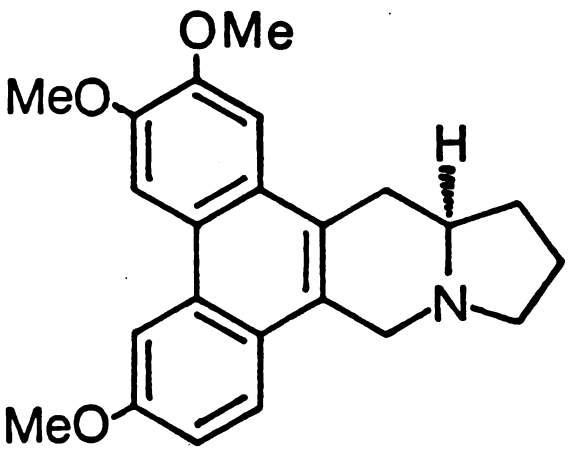
The related alkaloid antofine (2) has been isolated

from *Antitoxicum funebre* Boiss. and Kotschy[6] and *Vincetoxicum officinale*[5] along with tylophorine (1), tylocrebrine (3) and septicine (5).

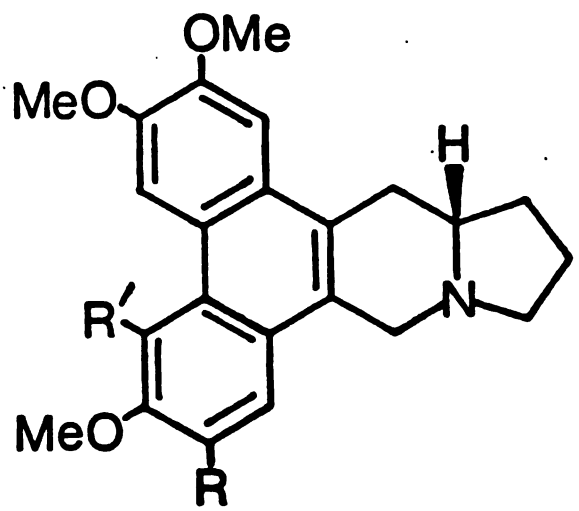


1 R = OMe, R' = H

Tylophorine



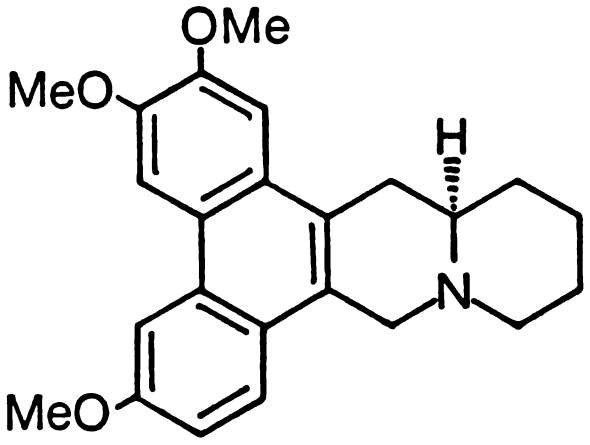
2 Antofine



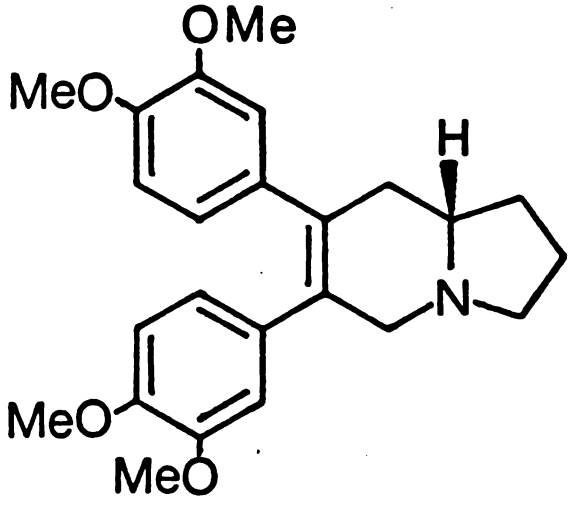
3 R = H, R' = OMe

Tylocrebrine

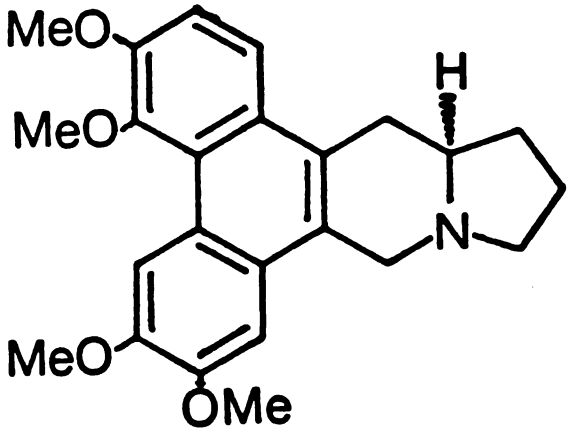
Figure 1



4 Cryptopleurine



5 Septicine



6 Isotylocrebrine

Figure 2

The structures of these alkaloids have all been determined by classical chemical and physical techniques.[10,11] The final structure proof in each case was the synthesis of the usually racemic compound which was identical to the natural product.[12,13,9]

The presence of (1), (2), (3), (5) in the same plants suggests a possible biogenetic[14,5,10] scheme in which a condensation between two molecules of dihydroxyphenylalanine or one molecule of dihydroxyphenylalanine and one of tyrosine (or their equivalents, the corresponding benzoylacetic and phenylpyruvic acids) and ornithine (or its equivalent, γ-aminobutyraldehyde) occur (see Fig. 3).

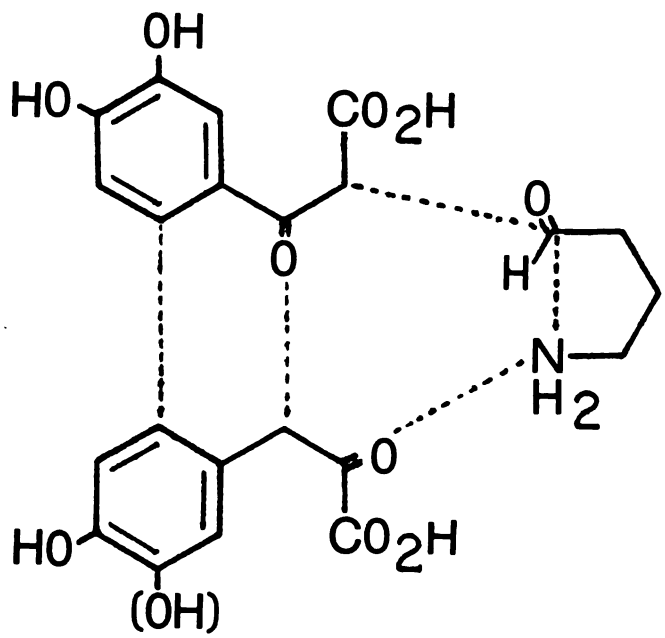


Figure 3

The secophenanthridine alkaloid L-septicine (5) has been chemically photo-oxidized[4] to tylophorine (1) (some tylocrebrine (3) was also isolated) lending support to the suggested biogenetic pathway (septicine could be the biological precursor to tylophorine (1) or tylocrebrine (3) via some natural photochemical or enzymic process).

The pharmacology of these alkaloids is very similar. They all appear to be strong vesicants and highly toxic.[9,15,16] Tylophorine (1) has a paralyzing action on the heart but a stimulating action on the muscles of the blood vessels.[17] Tylocrebrine (3) shows high activity against lymphoid leukemia L1210 in mice[18] and interferes with protein and nucleic acid synthesis.[19] Cryptopleurine (4) is a mitotic poison[20,21] and in small concentration, stimulated nerve regeneration.[22]

The stereochemistry of L-septicine (5) has been shown to correspond to the S- configuration at the C-13a by synthesis from (S)- pyrrolidine-2-methanol.[23] The alkaloid antofine (2)(isolated from Cynanchum vincetoxicum L. Pers) upon ozonolysis yielded the amino acid proline which was shown by reaction with D-amino acid oxidase to be D-proline (R- configuration).[24] The absolute configuration of tylophorine (1) has been determined by ozonolysis to give (S)- pyrrolidine-2-acetic acid (identical with a sample synthesized from (S)-proline) and by comparison of the ord's of antofine and tylophorine.[25] The alkaloid (+)-

isotylocrebrine (6) has been reported to have the R-configuration by comparing its ord with (1) and (2).[25] It has also been found that tylocrebrine (3) isolated from Tylophora crebriflora (Asclepiadaceae) is levorotatory[9] while from Ficus septica (Moraceae) it is dextrorotatory.[4]

This chapter will report the absolute configuration of the major phenanthroindolizidine alkaloid from Tylophora crebriflora,[9] tylocrebrine (3) and the closely related phenanthroquinolizidine compound[7,26,27] cryptopleurine (4) as determined by optical rotatory dispersion (ord) and circular dichroism (cd).

RESULTS AND DISCUSSION

The main Cotton effect (ce) of **1**, 3 and 4 is seen (Fig. 4) to be in the 240-260 nm region, in agreement with the uv absorption of the polyoxygenated phenanthrene system present in these alkaloids.[7,9,27,28] The ord curve obtained for the tylophorine base **1** is in good agreement with that reported[25] for the hydrochloride, both showing a negative ce centered around 255 nm.

The ord curve of (-)-tylocrebrine 3 (Fig. 4) shows it to have the S- configuration like tylophorine, the two curves being almost superimposable between 200 and 280 nm. The negative sign of the ce was confirmed by a negative cd maximum at 252 nm. The slight differences shown in the 280-320 nm region of the ord spectrum may be ascribed to the different oxygenation pattern, in agreement with the uv spectra of these alkaloids.[9] It appears that, as in the aporphine series,[29,30] the sign of the ce in the 280-320 region is substitution-dependent[29] whereas the sign of the major aromatic ce in the 240-260 nm region is independent of substitution.[30]

The interpretation of the ord curves for the phenanthroindolizidine alkaloids relies on the indolizidine ring junction being in the stable <u>trans</u> configuration.[31] In the case of cryptopleurine 4, it has already been shown[7,26,27] that the phenanthroquinolizidine system is in

the trans configuration. The ord measurement can therefore be used for assignment of absolute configuration for cryptopleurine 4 and shows (Fig. 4) that this has the opposite configuration from tylocrebrine and tylophorine. It is therefore, like antofine 2, a member of the R- series. The positive ce was confirmed by a positive cd maximum at 233 nm.

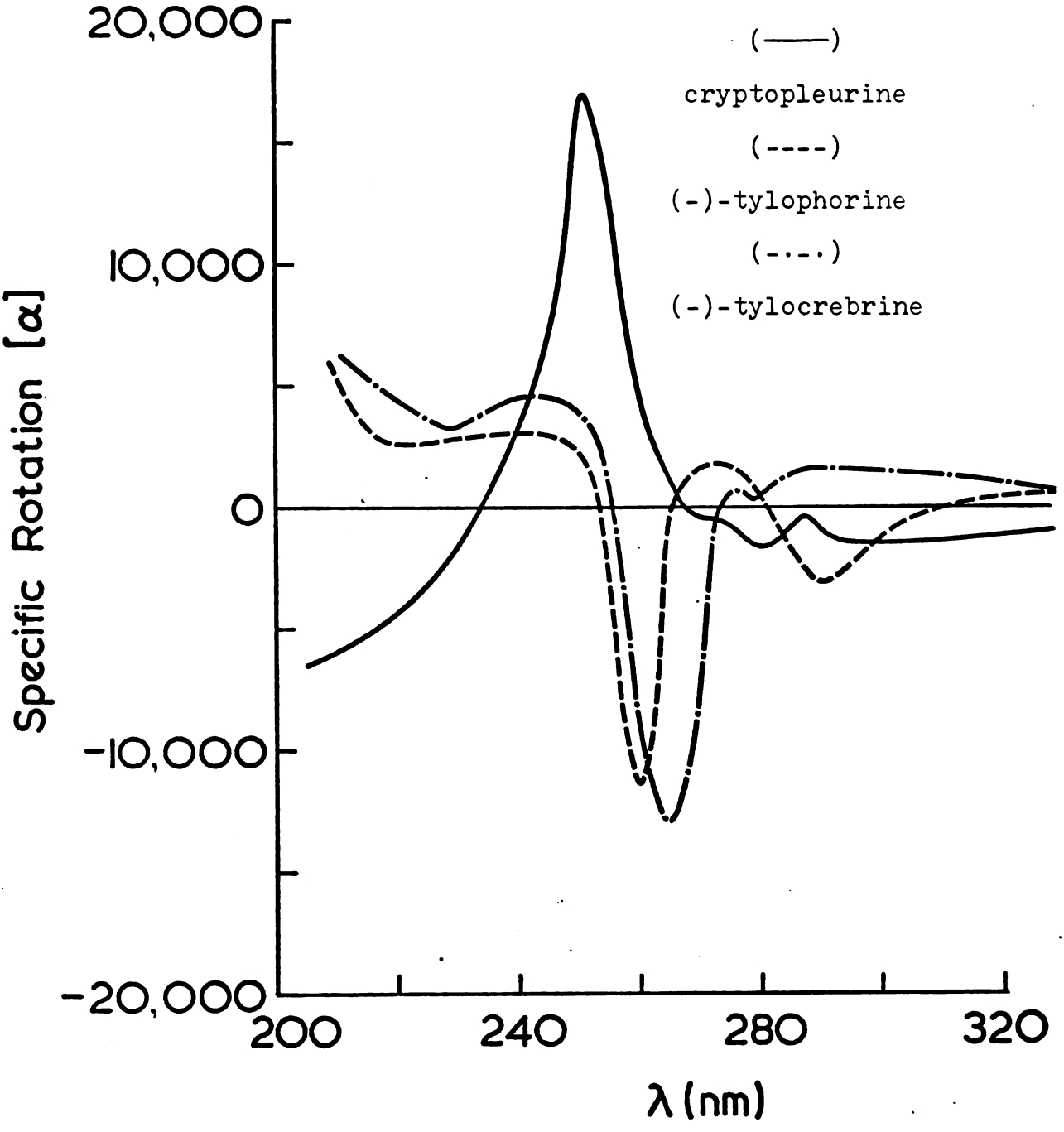

20,000
10,000
0
-10,000
-20,000
Specific Rotation [α]
200
240
280
320
λ (nm)
(——)
cryptopleurine
(----)
(-)-tylophorine
(-·-·)
(-)-tylocrebrine

Figure 4

EXPERIMENTAL

Ord and cd curves were measured with a JASCO ORD/CD5 and a JOUAN Mark II spectropolarimeter at 25° in 95% ethanol. Only cd maxima are given, and are recorded in terms of molecular ellipticity[32] $[\theta]$.

Cryptopleurine had mp 196.5-197°, $[\alpha]_D^{25}$ - 105° (C 1.0, $CHCl_3$); ord (C 0.0043, 95% ethanol); cd (C 0.0085, 95% ethanol) $[\theta]_{233}$ +15,000.

Tylophorine had mp 282-284°, $[\alpha]_D^{25}$ - 12° (C 0.7, $CHCl_3$); ord (C 0.0014, 95% ethanol).

Tylocrebrine had mp 218-220°, $[\alpha]_D^{25}$ - 44° (C 0.7, $CHCl_3$); ord (C 0.0035, 95% ethanol); cd (C 0.014, 95% ethanol) $[\theta]_{252}$ -4,370.

Acknowledgment: The author is extremely grateful to Dr. E. Gellert, Department of Chemistry, University of Wollongong, N. S. W., Australia, for the samples of alkaloids.

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CHAPTER II

THE ABSOLUTE CONFIGURATION OF MARINE TOXIN.

CD OF DEBROMOAPLYSIATOXIN.

INTRODUCTION

Several aplysiatoxins have been isolated from the gastropod molluscan family Aplysiidae or sea hares.[1] They lack the spectacular external shell and their toxicity has been known at least since Roman times.[2]

Watson[3] was the first to link the toxicity of sea hares common to Hawaiian reefs to constituents of their midgut or digestive glands. The constituents in the midgut appear to be reflective of the hare's diet of algae and not metabolites. This concept was strengthened by Faulkner's[4] isolation of identical metabolites from Aplysia californica and from their red algae diet.

The midgut toxins in several species of sea hare from Hawaiian reefs were fractionated into water-soluble and ether-soluble portions which differed in pharmacological properties (Watson).[3]

Scheuer [5,6] et al. were able to isolate two compounds, aplysiatoxin and debromoaplysiatoxin, from the ether-soluble fraction of Stylocheilus longicanda. Considerable efforts[4] have been expended by several workers to prove the midgut constituents of the sea hares are of dietary origin. Moore et al.[7] isolated debromoaplysiatoxin from several species of bluegreen algae collected at Enewetok atoll in the Marshall Islands and showed it was an algae metabolite with antileukemia activity. Debromoaplysiatoxin (1) also produces dermatitis.[8]

The structures of debromoaplysiatoxin and aplysiatoxin were determined by standard chemical and physical methods.[1]

This chapter will report the absolute configuration at C-15, the benzylic position, of debromoaplysiatoxin (1). The absolute configuration of several natural compounds possessing the substituted benzylic alcohol have been studied.[9,10] The appearance of the asymmetric benzylic alcohol center in many different families and species may be some evolutionary common denominator.

RESULTS AND DISCUSSION

The circular dichroism (cd) spectrum of (1) showed a positive Cotton effect (ce) with cd maxima at 286 and 269 nm. An almost identical cd spectrum was given by R-(-)-noradrenaline hydrochloride (2)(Table 1). The cactus alkaloid R-(-)-calipamine hydrochloride[9] (3), with an identical carbon skeleton and nitrogen and oxygen substitution pattern has a similar cd spectrum, showing a positive maxima at 281 nm (Table 1 and Fig. 2). Since the nitrogen $n \rightarrow \sigma^*$ transition in both (2) and (3) has been abolished on protonation, the observed cd maxima in the 260-280 nm region in (2) and (3), like that in (1), correspond to the ^1L_b transition of the benzene chromophore, asymmetrically perturbed by the center of asymmetry adjacent to the aromatic ring. As usual when oxygen substitution is present in positions 3 and/or 4 of the ring, the ^1L_b transition is shifted from 260 to 280 nm, accompanied by a loss of most or all of the fine structure normally present for this transition.[11] This wavelength shift is due to an overlap of the π orbital of benzene with the nonbonding p orbital of the oxygen substituents in the ring.[11] This was confirmed by the cd of S-(-)-1-phenylethanol (5) and its methyl ether S-(-)-1-methoxy-1-phenylethane (6), which showed identical superimposable maxima (positive ce) centered at 268 nm, with the normal

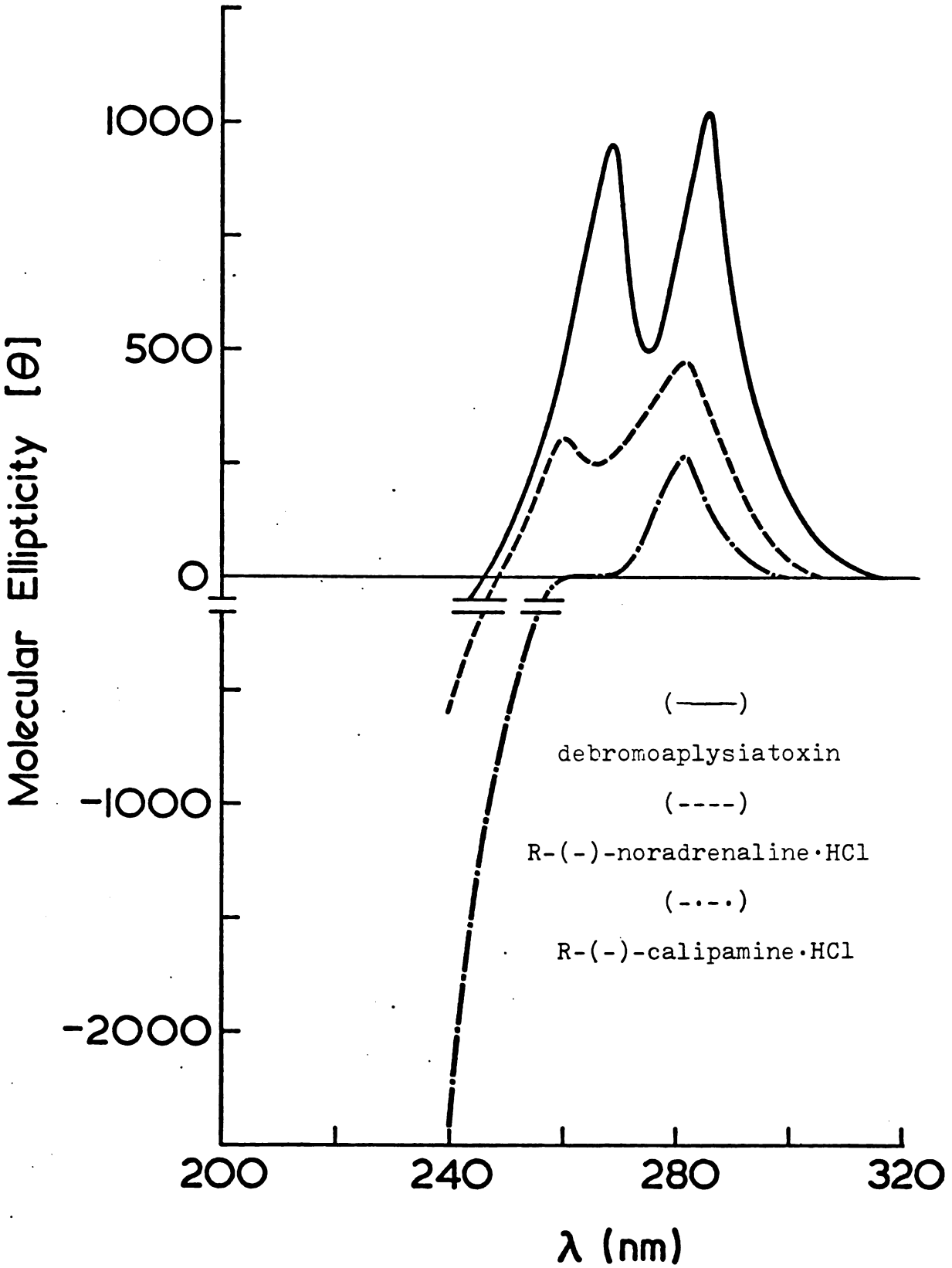

Molecular Ellipticity [θ]
1000
500
0
-1000
-2000
200
240
280
320
λ (nm)
(——)
debromoaplysiatoxin
(----)
R-(-)-noradrenaline·HCl
(-·-·)
R-(-)-calipamine·HCl

Figure 1

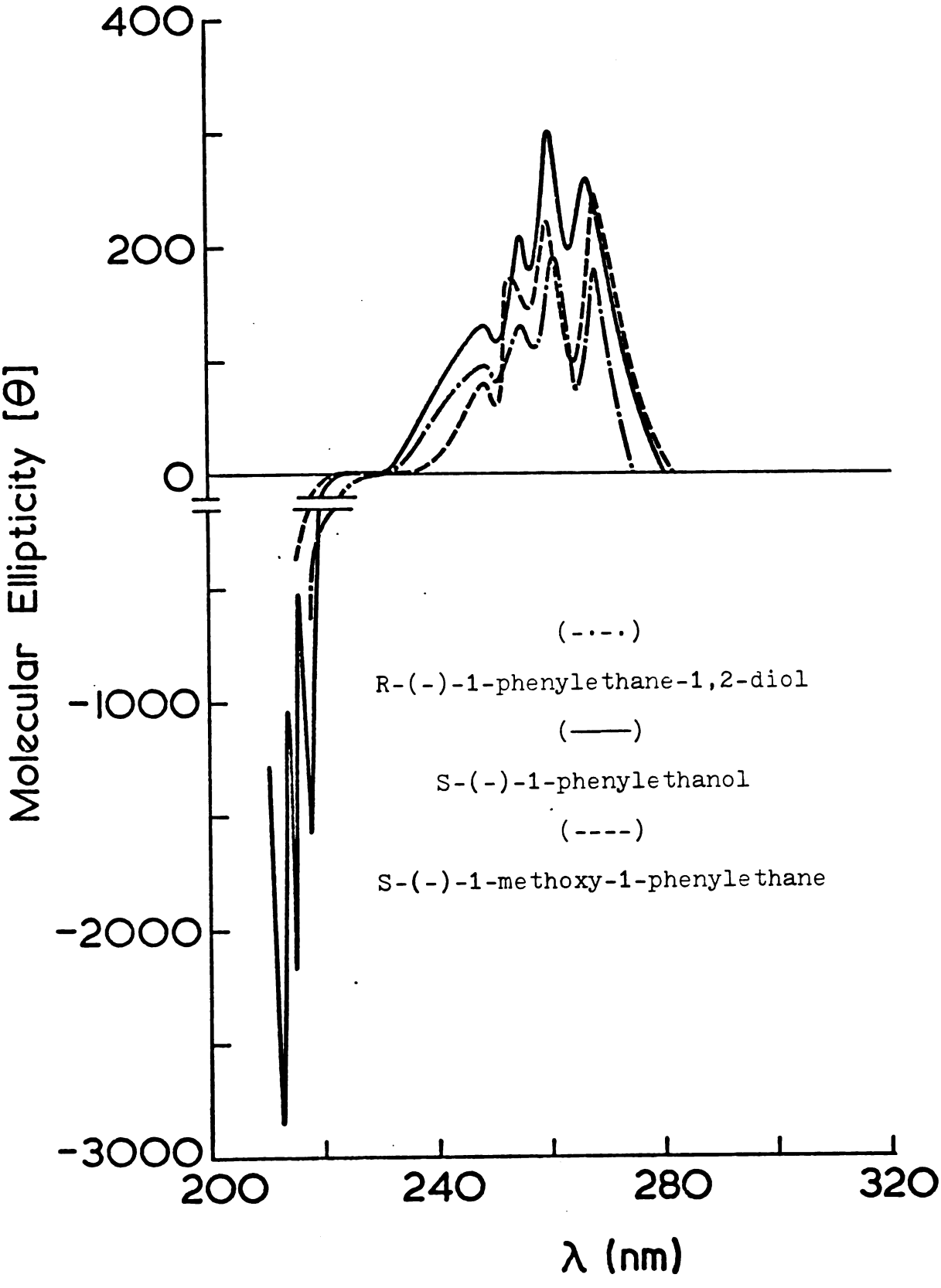
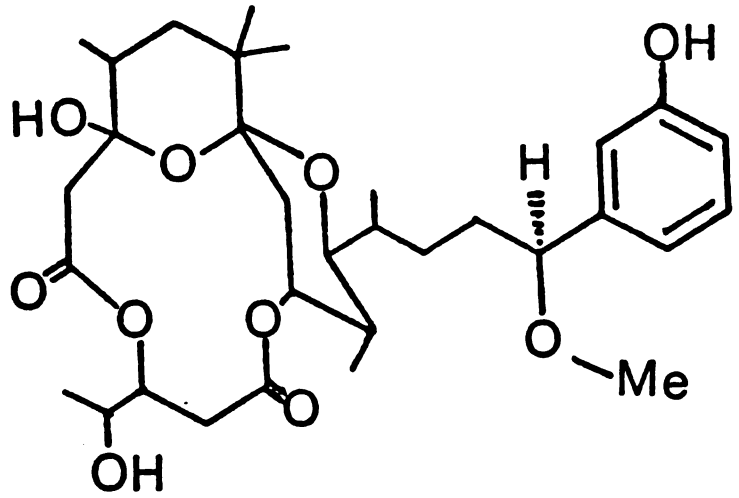
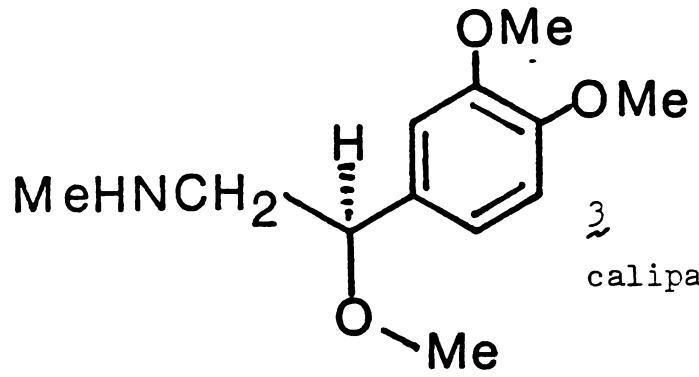

400
200
0
-1000
-2000
-3000
Molecular Ellipticity [θ]
200
240
280
320
λ (nm)
(-·-·-)
R-(-)-1-phenylethane-1,2-diol
(——)
S-(-)-1-phenylethanol
(----)
S-(-)-1-methoxy-1-phenylethane

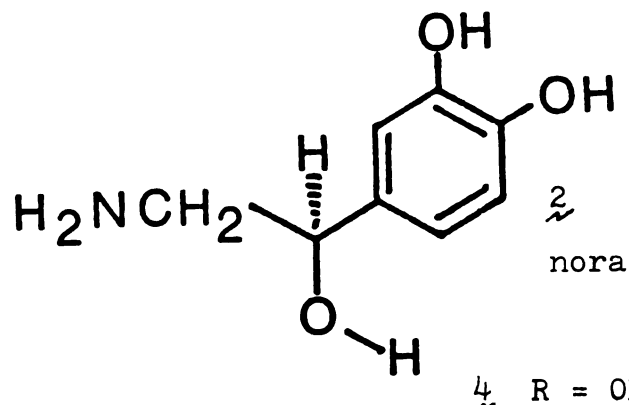
Figure 2



1 debromoaplysiatoxin

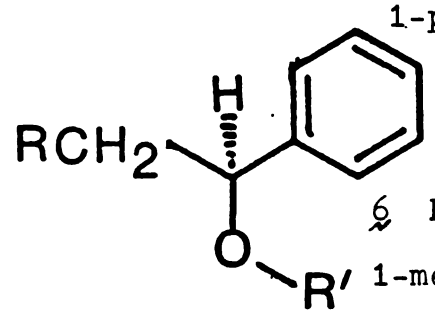


3 calipamine·HCl



2 noradrenaline·HCl

4 R = OH, R' = H
1-phenylethane-1,2-diol



5 R = H, R' = H
1-phenylethanol

6 R = H, R' = CH_3
1-methoxy-1-phenylethane

Figure 3

fine structure for the ^1L_b transition (Table 1) and no effect due to the change OH→ OMe at the asymmetric center (Fig. 3).

The presence of an additional hydroxyl group along the aliphatic chain, as in R-(-)-1-phenylethane-1,2-diol (4), was similarly found not to change the cd spectrum (Table 1) which remained virtually superimposable on that of the configurationally[12] identical (5) and (6) (Fig. 3). The additional hydroxylic centers present in (1) will therefore be similarly without effect on the cd of the compound, indicating the configuration of the asymmetric center adjacent to the benzene ring in (1) to be the same as in compounds (2-6), i.e. (D). Compound (1) thus has the S- configuration.

Table 1: Circular Dichroism Spectra in 95% Ethanol

Compound	CD Maxima [θ] for ^1L_b Band			
S-(-)-1-Phenyl-ethanol (D)	260 (267)	300 (260)	208 (255)	130 (249)
S-(-)-1-Methoxy-1-phenylethane (D)	246 (268)	220 (260)	163 (254)	79 (249)
R-(-)-Phenyl-ethane-1,2-diol (D)	178 (268)	193 (261)	131 (255)	92 (249)
R-(-)-N-Methyl-β-methoxy-3,4-dimethoxyphenethyl-amine·hydrochloride (D)	260 (281)			
R-(-)-noradrenaline·hydrochloride (D)	449 (281)	300 (260)		
Debromoaplysiatoxin	1031 (286)	902 (269)		

EXPERIMENTAL

Cd curves were measured with a JOUAN Mark II spectropolarimeter at 25° in 95% ethanol, and were recorded in terms of molecular ellipticity units[13] [θ]. Only cd maxima are given (Table 1).

Acknowledgment: The author is extremely grateful to Dr. R. E. Moore, Department of Chemistry, University of Hawaii, Honolulu, Hawaii, for the sample of debromoaplysiatoxin.

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CHAPTER III

THE ABSOLUTE CONFIGURATION OF THE CACTUS ALKALOID CALIPAMINE.

INTRODUCTION

Although the cactaceae species have long been known to produce alkaloids, the genus Coryphantha[1] has only recently been thoroughly investigated. From Coryphantha macromeris (Engelm.) Br. and R., Hodgkins et al.[2] isolated the alkaloid macromerine (7), which is physiologically active. Macromerine (7) caused hallucinogenic reactions when tested on squirrel monkeys and cats (20 mg/kilo - I. P.) and showed "anti" adrenaline results in the turtle heart.[2] The structure of macromerine (7) is closely related to epinephrine (the former is the O^3, O^4, N-trimethyl derivative of the latter) and the β -phenethylamine alkaloids found in several cacti.[3,4]

Further investigation on Coryphantha species showed the presence of macromerine in Coryphantha runyonii Br. and R.* as well,[5]

*C. runyonii is sometimes considered a variety of C. macromeris and these two species have also been grouped together in the Lepidocoryphantha.[6,7]

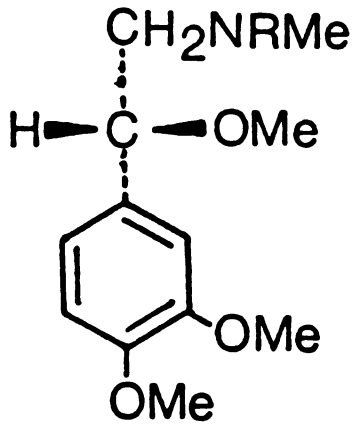
and several simpler phenethylamines. Recently, the β-hydroxylated phenethylamine normacromerine (9),[8] N-formylnormacromerine (10), metanephrine (11), N-methylmetanephrine (12) and synephrine (13)[7] were found in C. runyonii.

The structural relationship of macromerine (7) and

other cactus phenethylamines to adrenaline has prompted investigations into the configurational relationship of these compounds. Exhaustive methylation of natural adrenaline (R- configuration) gave a compound identical to natural macromerine (7) in all physical properties including optical rotation meaning natural macromerine must have the R- configuration. This same relationship is true[9] for naturally occuring synephrine[10] (13) and octopamine (14).[10,11,12]

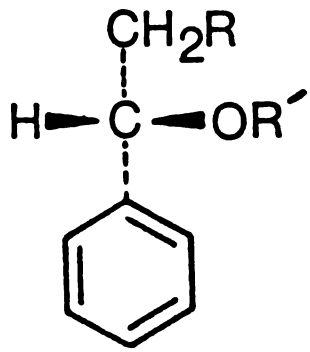
Recently in a study of the Mexican cacti, C. calipensis H. Bravo,[13] two new alkaloids were isolated by Bruhn et al.[14] Based on chemical and spectroscopic data, their structures were determined as (-)-N-methyl-3,4-dimethoxy-β-methoxy-phenethylamine [(-)-β-O-methylnormacromerine] "calipamine" (1) and (-)-N,N-dimethyl-3,4-dimethoxy-β-methoxy-phenethylamine [(-)-β-O-methylmacromerine] (6). Both (-)normacromerine (9) and N-methyl-3,4-dimethoxyphenethylamine were also isolated from this species.

This chapter will report the determination of the absolute configuration of the alkaloid calipamine (1).



1 R=H Calipamine

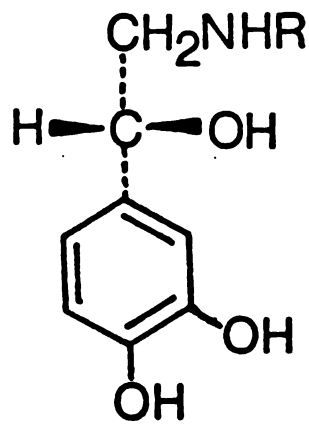
6 R=CH_3 N-Methylcalipamine



3 R=OH, R'=H Phenylethanediol

4 R=H, R'=H Phenylethanol

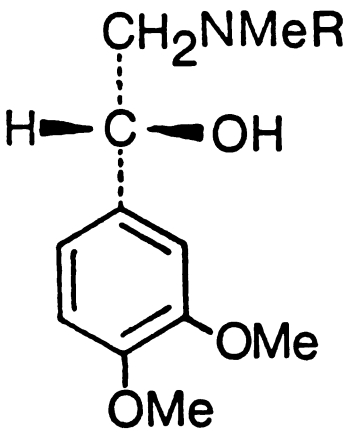
5 R=H, R'=CH_3 Methoxyphenyl-ethane



2 R=H Noradrenaline

8 R=CH_3 Adrenaline

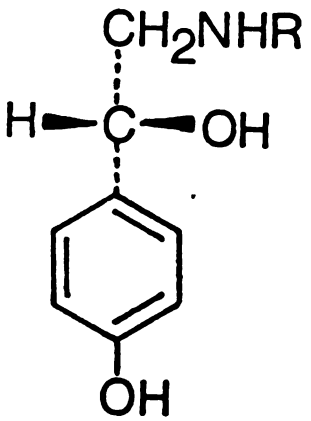
Figure 1



7 R=CH_3 Macromerine

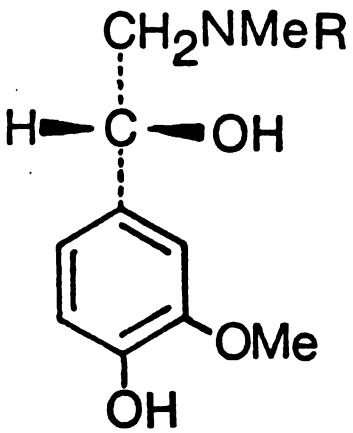
9 R= H Normacromerine

10 R=COH N-Formylnormacromerine



13 R=CH_3 Synephrine

14 R=H Octopamine



11 R=H Metanephrine

12 R=CH_3 N-Methylmetanephrine

Figure 2

RESULTS AND DISCUSSION

The circular dichroism (cd) curve for (-)-calipamine hydrochloride (1) showed a positive Cotton effect centered at 281 nm and a strong negative ce centered at 239 nm. The compound R-(-)-noradrenaline hydrochloride (2), with an identical carbon skeleton and nitrogen and oxygen substitution pattern, had a very similar cd spectrum (Table 1). Since the nitrogen $n \rightarrow \sigma^*$ transition in both (1) and (2) has been abolished by protonation, the observed cd maxima correspond to the ^1L_b and ^1L_a transitions, respectively, of the benzene chromophore, asymmetrically perturbed by the center of asymmetry adjacent to the ring. As is usual when oxygen substitution is present in positions 3 and 4, the ^1L_b transition is shifted from 260 to 280 nm, while the ^1L_a transition is moved to 235 nm from 210 nm, both shifts being accompanied by a loss of fine structure. This wavelength shift is due to an overlap of the π orbital of benzene with the nonbonding p orbital of the oxygen substituents in the ring.[15,16]

This was confirmed by the cd spectra of R-(-)-1-phenylethane-1,2-diol (3) and S-(-)-1-phenylethanol (4) (Table 1) which showed superimposable maxima centered at 268 (positive ce) and 216 nm (negative ce), with the normal fine structure for both transitions. The weak $n \rightarrow \sigma^*$ transition of the additional OH group in (3) thus does not change the nature of the cd spectrum compared to (4).

Further, methylation[17] of the OH group in (4) (silver oxide/methyl iodide) gave S-(-)-1-methoxy-1-phenylethane (5) with an almost identical cd spectrum (Table 1), indicating the lack of effect of the change $OH \rightarrow OCH_3$ at the asymmetric center.

Since compounds (2-5) all possess the identical (D) configuration,[18] this also indicated the asymmetric center in (-)-calipamine to have the configuration shown in (1), i.e. (R). As (-)-1 was converted (formaldehyde/sodium borohydride) to (-)N,N-dimethyl-3,4-dimethoxy-β-methoxyphenethylamine identical with the natural product (6) found in the same plant,[14] it follows that the natural N,N-dimethyl derivative (6) also has the (R)- configuration. It is interesting to recall that the closely related cactus alkaloid (-)-macromerine (7) was shown to have the (R)- configuration by chemical correlation with natural (R)- adrenaline (8).

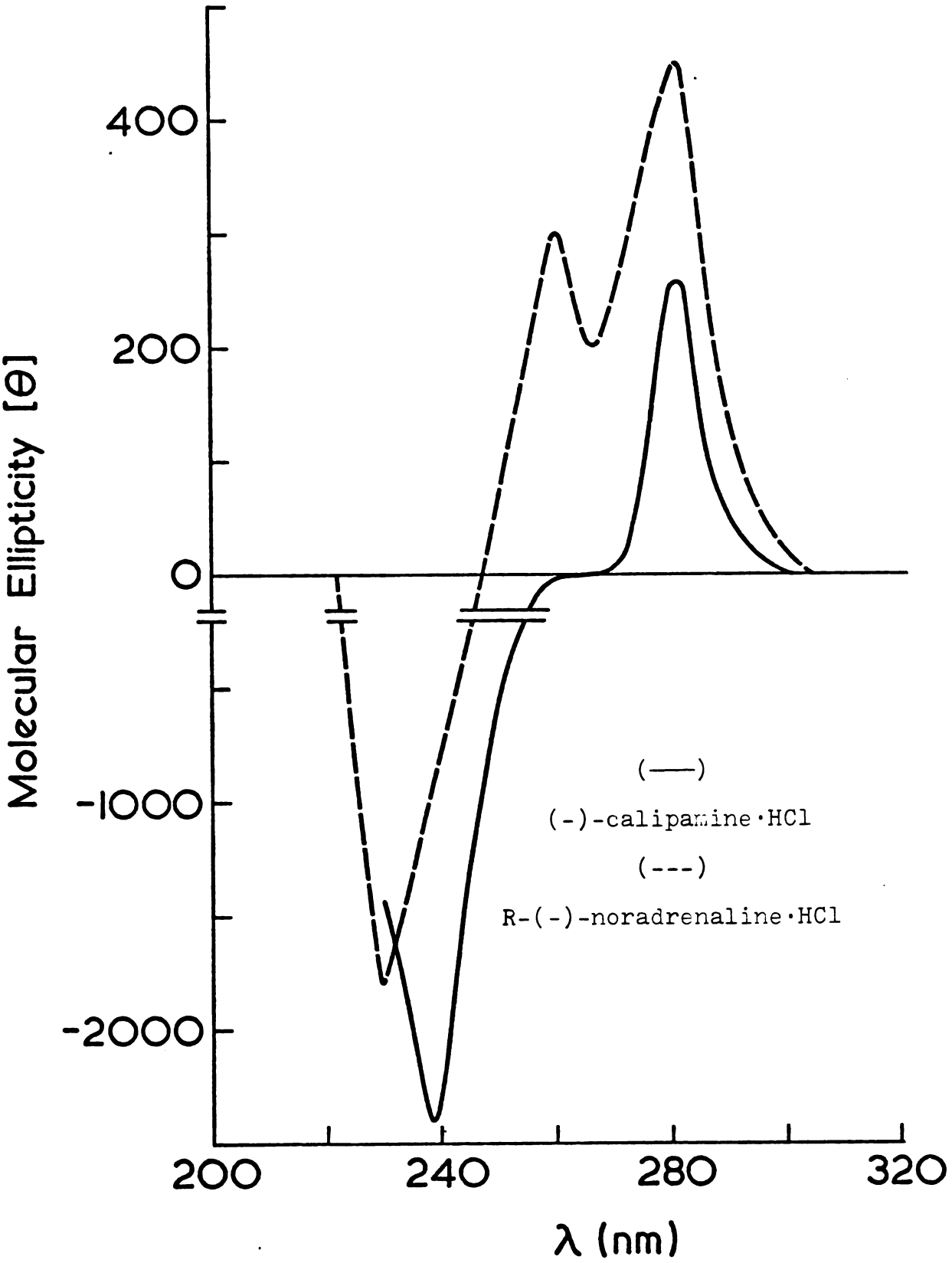

400
200
0
-1000
-2000
Molecular Ellipticity [θ]
200
240
280
320
λ (nm)
(——)
(-)-calipamine·HCl
(---)
R-(-)-noradrenaline·HCl

Figure 3

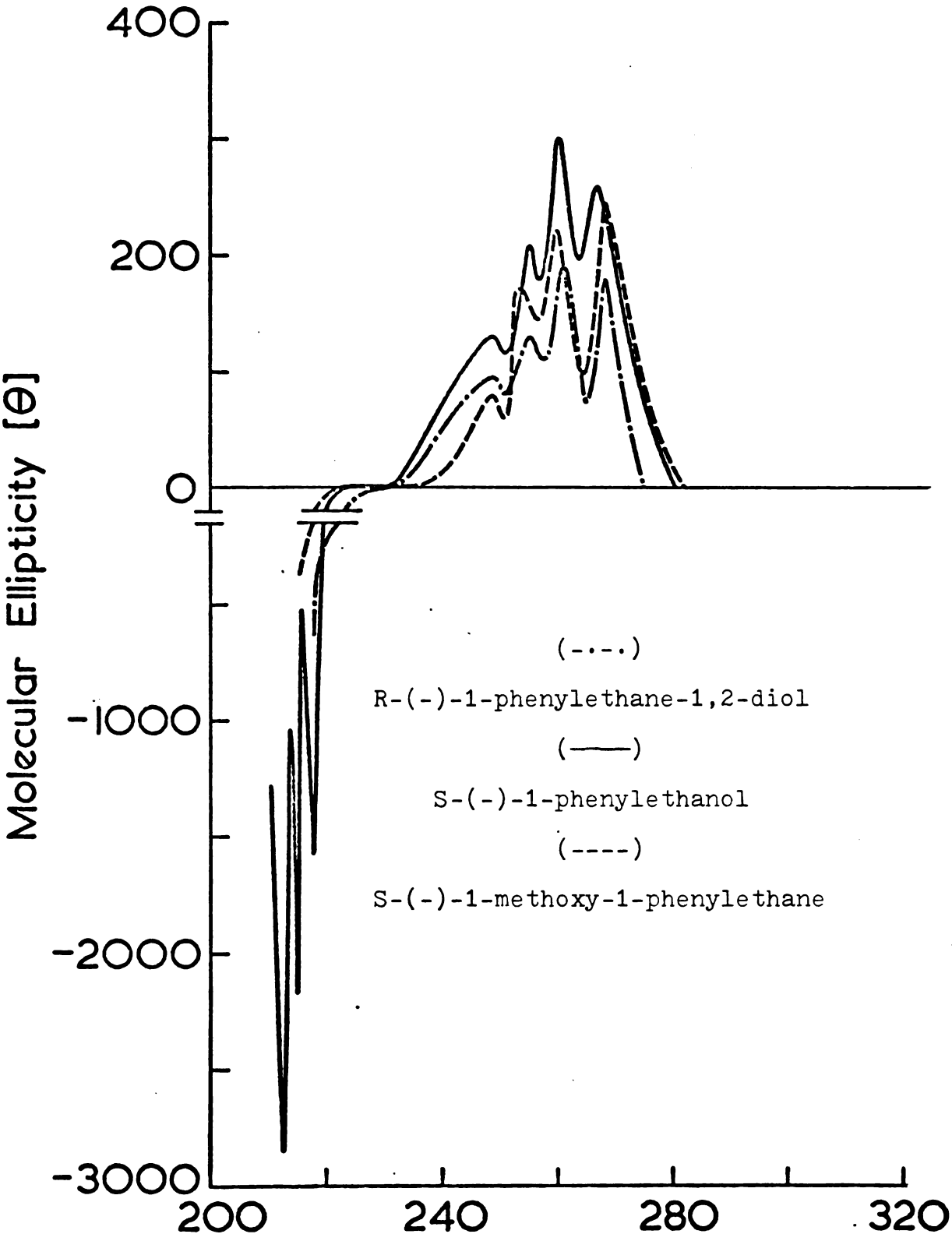

400
200
0
-1000
-2000
-3000
Molecular Ellipticity [θ]
200
240
280
320
λ (nm)
(-·-·-)
R-(-)-1-phenylethane-1,2-diol
(——)
S-(-)-1-phenylethanol
(----)
S-(-)-1-methoxy-1-phenylethane

Figure 4

Table 1: CD Spectra and Rotations

Compound	CD maxima [θ] (nm)[a]							$[\alpha]_D^{25}$ in 95% Ethanol
	^1L_b		BAND		^1L_a		BAND	
S-(-)-Phenylethanol 4 (D)	260 (267)	300 (260)	208 (255)	130 (249)	-1561 (218)	-2342 (215)	-2863 (213)	-39.1° (c 1.92)
S-(-)-1-Methoxy-1-phenylethane 5 (D)	246 (268)	220 (240)	163 (254)	79 (249)		-352 (216!)		-119.2° (c 1.02)
R-(-)-Phenylethane-1,2-diol 3 (D)	178 (268)	193 (261)	131 (255)	92 (249)		-620 (218!)		-40.0° (c 0.294)
R-(-)-Noradrenaline·HCl 2 (D)	449 (281)	300 (260)				-1795 (230)		-40.0° (c 0.034)
(-)-Calipamine·HCl 1	260 (281)					-2392 (239)		-91.7° (c 0.01)[b]

[a] in 95% Ethanol [b] in absolute ethanol ! lowest wavelength reached

EXPERIMENTAL

Cd curves were measured with a JOUAN Mark II spectropolarimeter at 25° in 95% ethanol, and were recorded in terms of molecular ellipticity units $[\theta]$.[19] Only cd maxima are given.

Acknowledgment: The author is extremely grateful to Dr. Jan Bruhn, Department of Pharmacognosy, Faculty of Pharmacy, Biomedicum, Uppsala, Sweden, for the sample of calipamine.

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18. Note, however, that the changing priority requirements of the sequence rule cause a change from the (R)- to the (S)- designation.

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CHAPTER IV

THE SIMULTANEOUS DETERMINATION OF IMIPRAMINE AND DESIPRAMINE IN PLASMA.

SYNTHESIS.

INTRODUCTION

Although the pharmacology and metabolism of the widely-used tricyclic antidepressant imipramine (10,11-dihydro-5(3,3-dimethylaminopropyl)-5H-dibenz[b,f]azepine) (1a) has been extensively studied,[1,2,3] there is little information on its pharmacokinetics, and only a few attempts have been made to follow steady state levels in man after single doses.[4] Plasma levels achieved in man and animals are rather low, and large inter-individual differences in plasma levels among subjects receiving identical dosages appear to be due to genetic factors.[5] Also the rate of demethylation of the drug to its major metabolite desipramine (10,11-dihydro-5-(3-methylaminopropyl)-5H-dibenz[b,f]azepine) (1b) appears to depend on the route of administration.[6] The simultaneous determination of both imipramine and desipramine in biological fluids demand methods combining complete specificity and sensitivity in the ng/ml region.

Numerous analytical methods for the determination of imipramine and its metabolites in urine and biological tissues have been described. Thin layer and paper chromatographic methods have been used extensively for studies concerned with the broad spectrum of metabolites[7,8,9,10] and, coupled with the use of radioactive drugs, provide the best quantitative estimates of the amounts of the various metabolites formed.[11,12,13]

Mechanisms to study the possibility that the antidepressant activity of imipramine is mediated through the metabolite desipramine require that highly sensitive and selective analytical methods be developed for plasma determinations. Early fluorometric methods suffered from low sensitivity and were not selective.[14,15] The method of Moody et al.[16] described the fluorometric estimation of both imipramine and desipramine in plasma through the separation of desipramine by acetylation with a sensitivity down to 20 ng/ml. Hammer and Brodie, using an isotope-derivative method based on acetylation of desipramine with tritiated acetic anhydride and scintillation counting, were able to determine desipramine levels with a lower limit of sensitivity of 5 ng/ml.[17] A modification of this procedure described by Harris et al. additionally provides for the determination of imipramine by the conversion to the ^{14}C methiodide quaternary salt with a resulting lower limit of sensitivity to either desipramine or imipramine of 15 to 20 ng/ml.[18] Gillette et al.[19] and Viala et al.[20] have described the use of gas chromatography for the determination of imipramine and desipramine in plasma. Weder and Bickel reported on a gc method for the determination of imipramine and seven of its major metabolites in biological samples using selective extraction and derivation techniques and flame ionization detection.[21] Recently, a highly sensitive gc method for the determination of desipramine has been described by Ervik

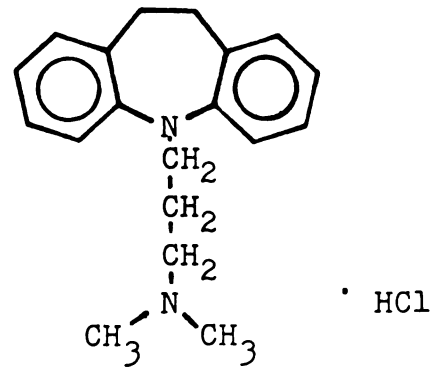
et al.[22] This involves the treatment of desipramine with trifluoroacetic anhydride resulting in the formation of a bis-trifluoroacetyl derivative in which one trifluoroacetyl group is attached to the secondary amine and the second is inserted in the sidechain as a trifluoroacetyl enamine. This allows the use of electron capture detection with resulting sensitivity down to 1 ng/ml. Nagy and Trieber have reported the use of direct densitometry on thin layer chromatograms which provides a method for the estimation of both imipramine and desipramine in the 20 ng/ml sensitivity range. The procedure is specific, relatively simple and rapid.[23] The relatively low sensitivity of some of the methods precludes their use for measurement in cerebrospinal fluid (CSF), where low concentration can be expected.

Due to the interest in pharmacokinetic studies folowing single doses of imipramine and determination of steady-state levels in small plasma and CSF samples, several authors have developed specific and sensitive methods for simultaneous measurement of both imipramine and desipramine using the combined techniques of gas chromatography-mass spectrometry (gc-ms)

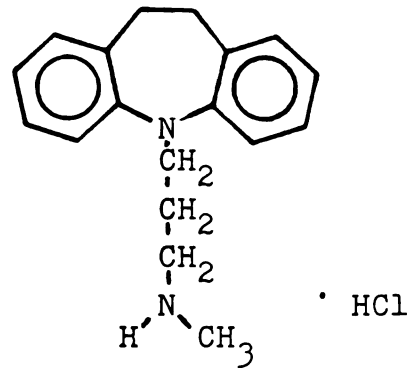
The combination of the techniques of gas chromatography with mass spectrometry makes possible the utilization of the high resolving power of the first method and the precise identification provided by the second.[24] In

addition , the technique of selected ion recording (SIR), by focusing on the mass at which the ion abundance is to be measured, can provide a sensitivity two or three magnitudes greater than that of gas chromatography alone.[24,25] Such a gc-ms method for the simultaneous measurement of imipramine and desipramine using promazine as internal standard has been reported.[26] However, it is well known that an ideal internal standard (added at the beginning of an extraction procedure) should have identical chemical and physical behavior to the compound being determined, and that this can best be achieved by the use of a stable isotope labelled variant of the compound itself.[24,27,28] The ideal internal standard should be specifically and completely labelled in a non-exchangeable position where back-exchange cannot occur during extraction procedures at acid or alkaline pH values, and should not contain any unlabelled drug so that the labelled material may be used in large excess as a carrier in an inverse isotope dilution method for maximum recovery of trace amounts of drug or metabolite, e.g. in CSF.

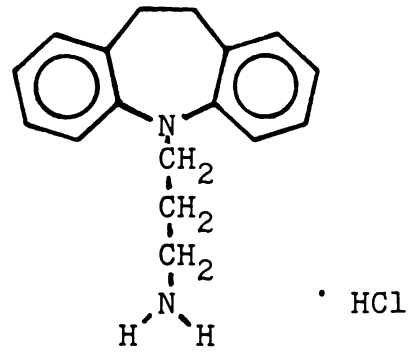
The present chapter reports the synthesis of imipramine, desipramine, and the primary amine metabolite (10,11-dihydro-5-(3-aminopropyl)-5H-dibenz[b,f]azepine) (1c) (Fig. 1), isolated as a urinary metabolite in man and animals,[29] containing two deuterium atoms at the 1- or 3-position of the sidechain.



1a



1b



1c

Figure 1

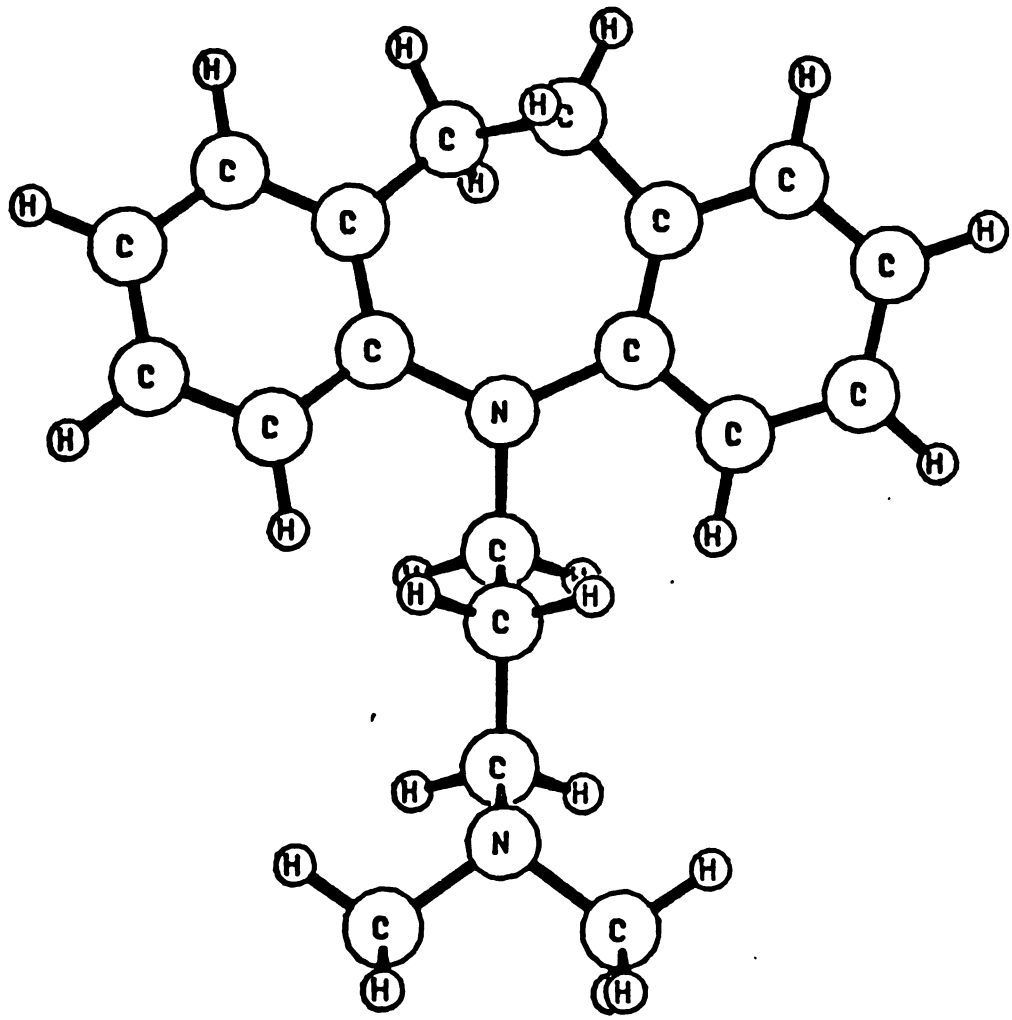


Figure 2

RESULTS AND DISCUSSION

A recent publication[30] reports the preparation of d_4-imipramine and d_4-desipramine by introduction of label into the 2, 4, 6 and 8-positions of the aromatic system using a multiple acid-catalyzed exchange. Isotopic distributions (by cims) were approximately d_4 55%, d_3 35%, and d_2 7%. However, there remains a risk of back-exchange during acid or alkaline work-up conditions if the label is in the 2, 4, 6, 8, 10 or 11 positions, i.e. the aromatic moiety or at a benzylic position. For these reasons our synthesis aimed at carbons 1 or 3 of the side-chain as the ideal sites for a non-exchangeable label.

In order to synthesize the desired amines (8), (10) and (11) of the same high isotopic purity, it was desirable to use, if possible, a common intermediate which already contained the deuterium. The intermediate was the primary amine (8) which possibly by conventional means could be converted into the secondary amine (10) and the tertiary amine (11). It was decided to synthesize the nitrile (5a) which could be reduced to the primary amine (8). The nitrile (5a) had previously been synthesized[36] by a laborious method in low yield (see Fig. 3). In our hands, however, each step gave a multitude of products which frequently proved inseparable. Therefore a new route was sought.

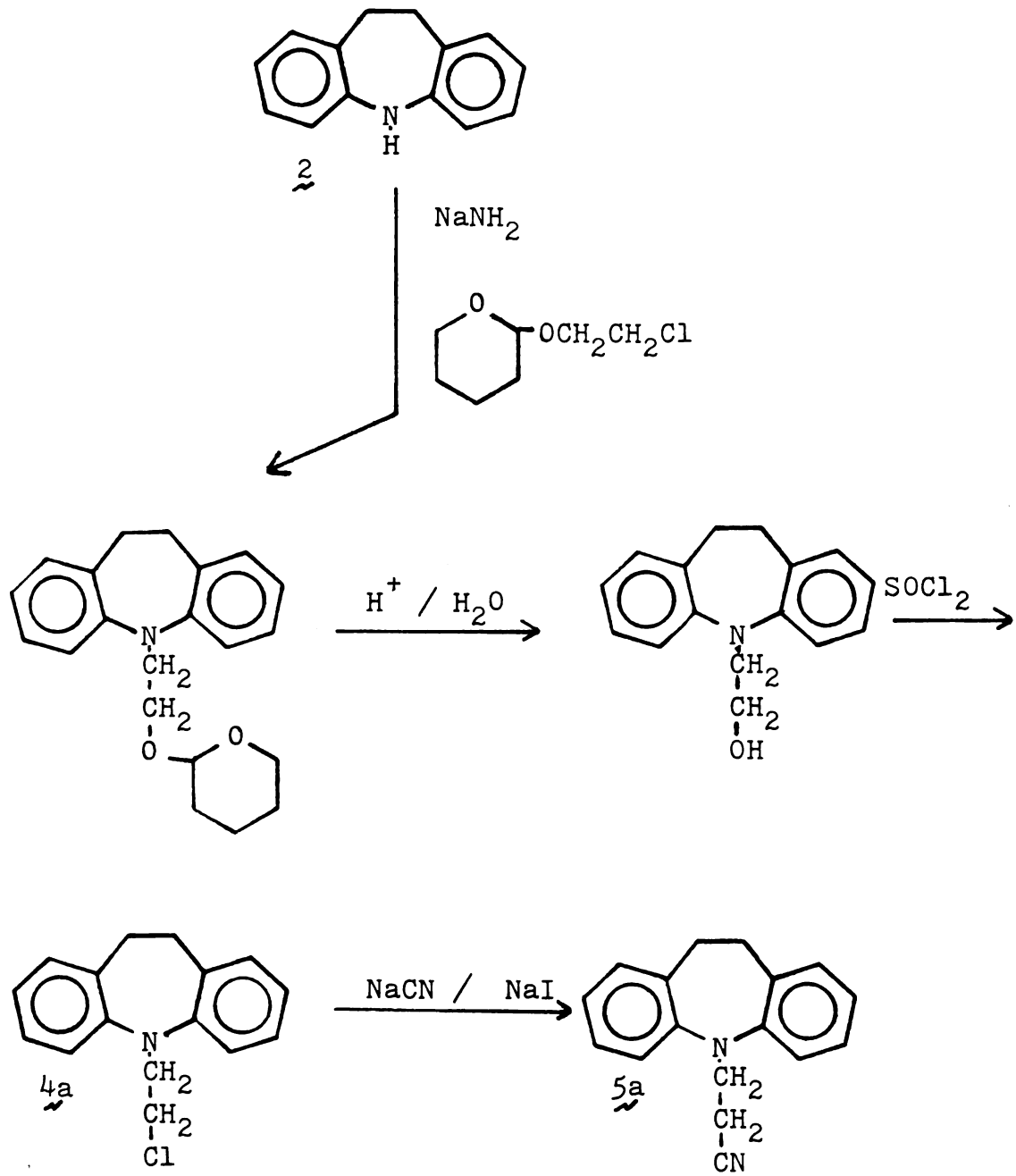


Figure 3

It has been shown in the synthesis of chlorpromazine[32] that the Michael condensation of acrylonitrile with 2-chlorophenothiazine in the presence of a weak base (see Fig. 4) gave excellent yields of the cyanoethyl derivative.

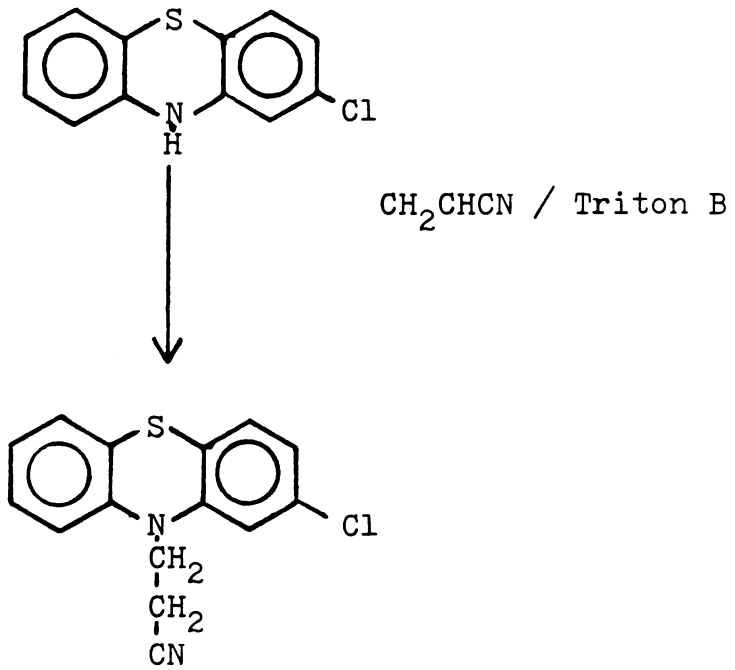


Figure 4

Attempts to condense iminodibenzyl with acrylonitrile under a variety of conditions failed to give anything but acrylonitrile polymer. Several bases were tried and each gave the same negative results. A number of alkali-metal derivatives of iminodibenzyl were treated with acrylonitrile under various thermal conditions as well as sol-

vent changes. The same alkali metal derivatives reacted with β-chloro and β-bromopropanonitrile to give only acrylonitrile. Several investigators[33] have shown an improvement in the yields of the condensation of a number of N-heteroaromatics by using the thallium (I) derivative. The thallium salt is generated by the reaction of thallium (I) ethoxide under very mild conditions and the ensuing alkylation conditions are also mild (Fig. 5).

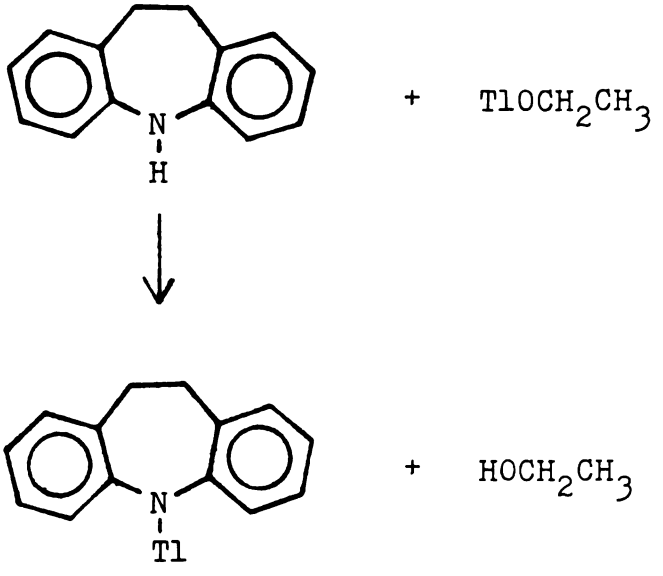
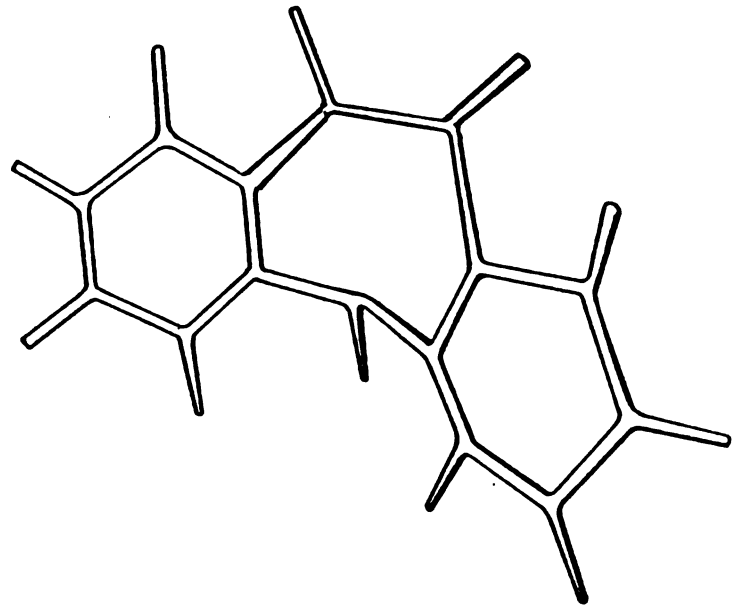
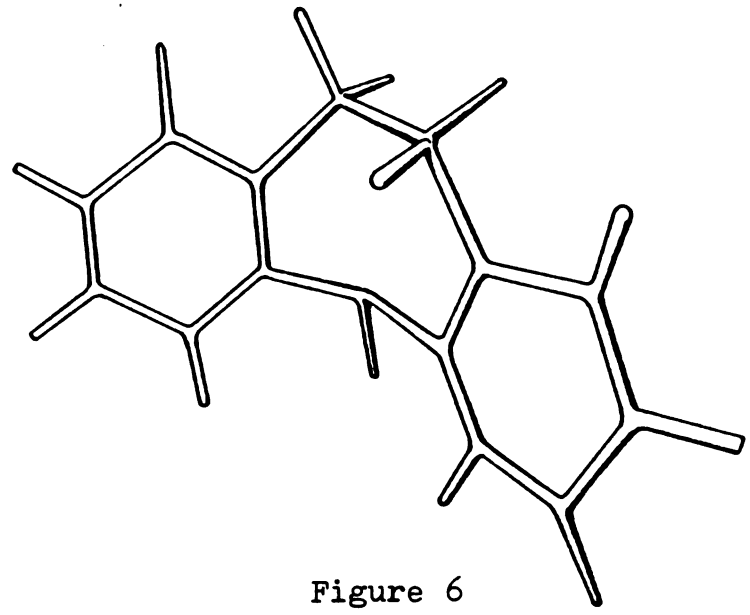


Figure 5

Extending the thallium work to dibenz[b,f]azepine (14) (iminostilbene) showed that only the small alkyl can overcome the non-planar effect of the etheno bridge and the 10,11-dihydrodibenz[b.f]azepine (iminodibenzyl) (2) ethanobridge puckers the ring to an extent that not even methyl iodide or methyl bromide could react (see Fig. 6).



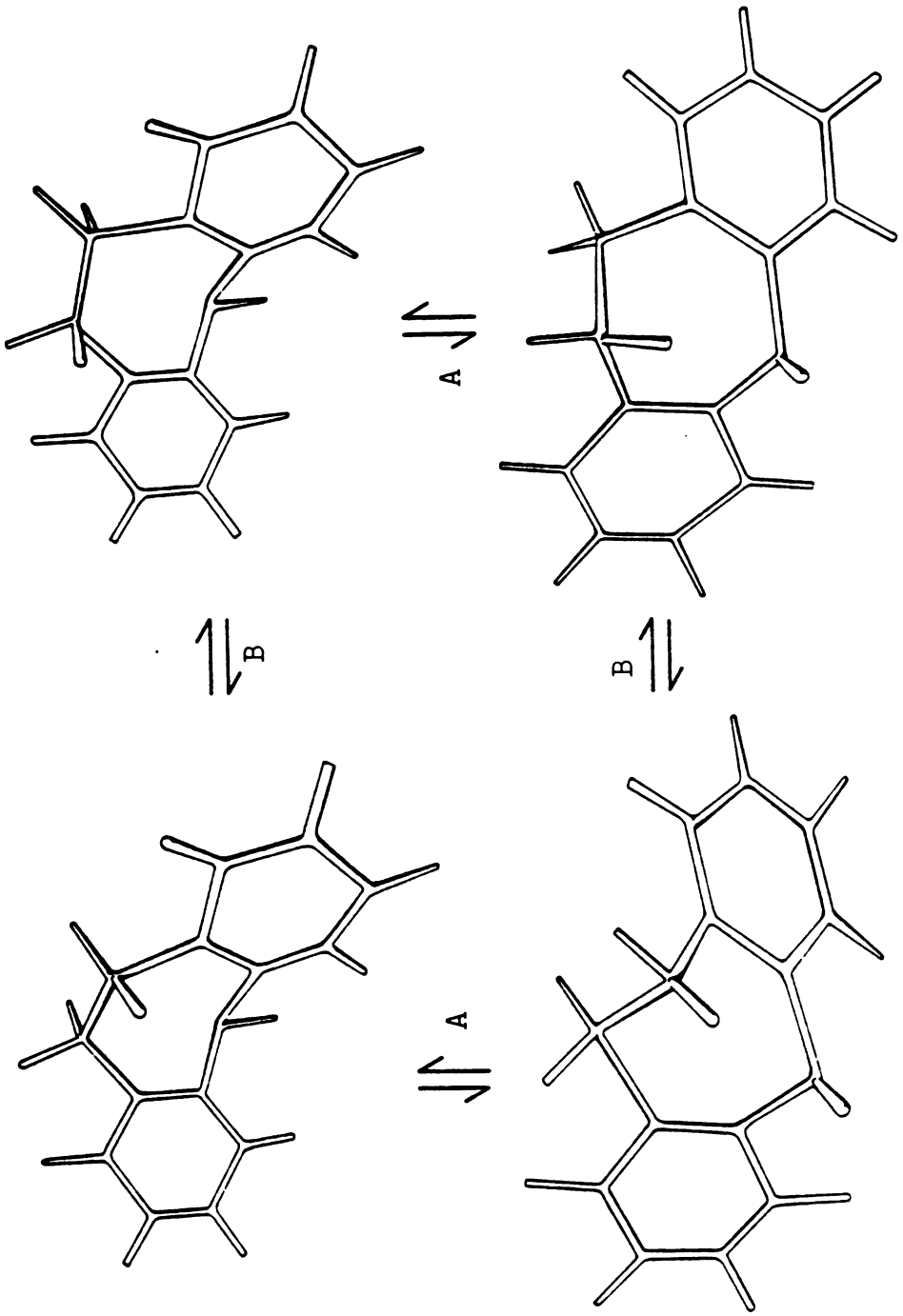
Iminostilbene



Iminodibenzyl

Figure 6

These steric effects were better demonstrated in an nmr study[34] on the solution conformations of various iminodibenzyl derivatives. There are several modes of inversion and rotation: one the inversion about the nitrogen (sp^3) and the other rotation about the ethano bridge (see Fig. 7). This leads to magnetic non-equivalence in the aromatic protons and ethanobridge protons.



A- Nitrogen inversion, B- Ethano rotation

Figure 7

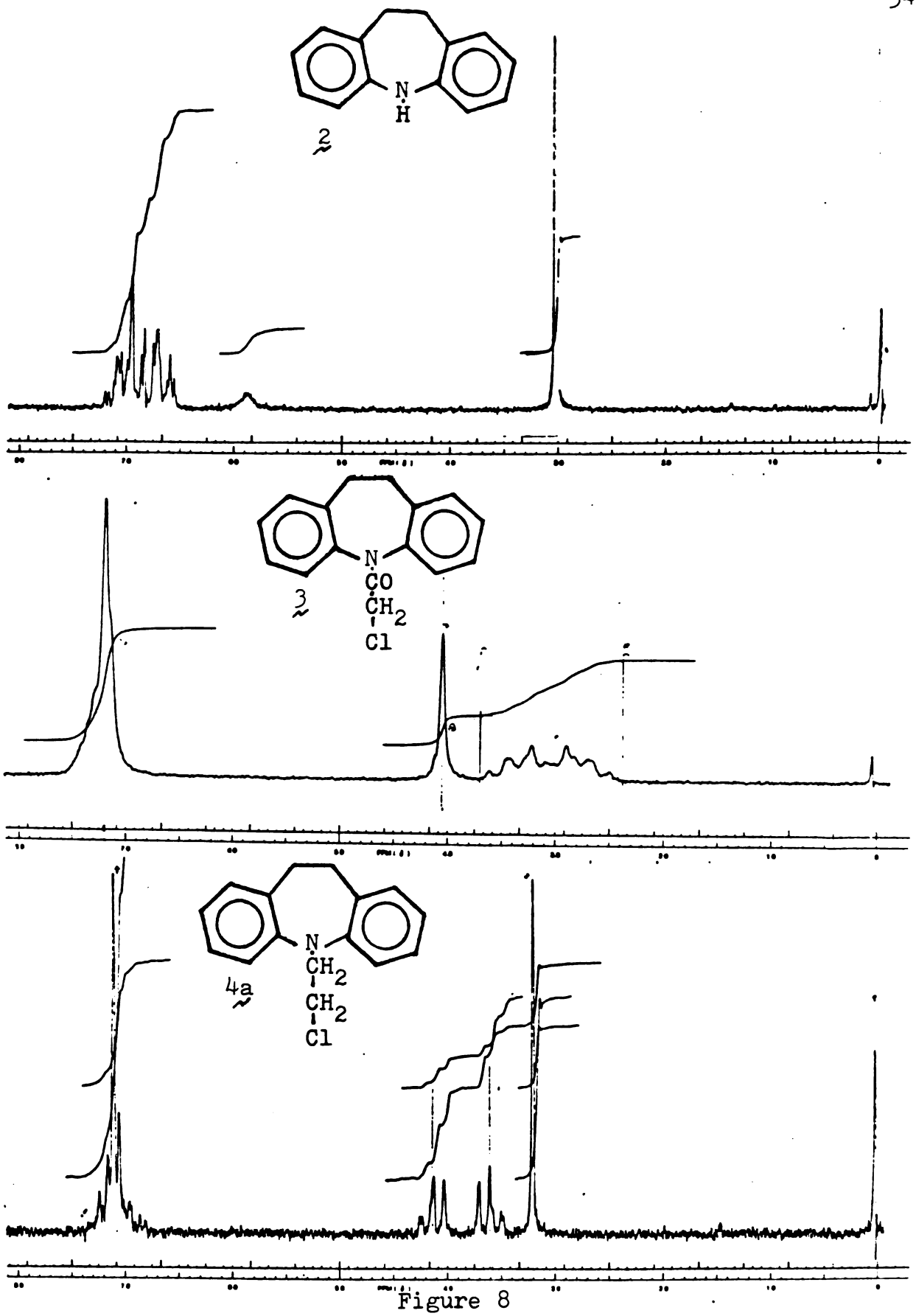


Figure 8

It can also be seen that if the ring nitrogen is attached to an sp^3 carbon the four ethano (benzylic) protons are chemical shift equivalent due to the fast (nmr time scale) flip-flopping of the carbons, thus placing both carbons in somewhat identical magnetic environments. If, however, the ring nitrogen is attached to an sp^2 carbon, the resonance form in which the nitrogen has delocalized its electron to the carbon has a high enough population to restrict the inversion modes, causing the ethano protons to be split into a complex 12-line multiplet (see Fig. 8). This constant puckering and flip-flopping of the seven-membered azepine ring would probably affect the generally accepted four-centered transition state of thallium alkylations[33] (see Fig. 9).

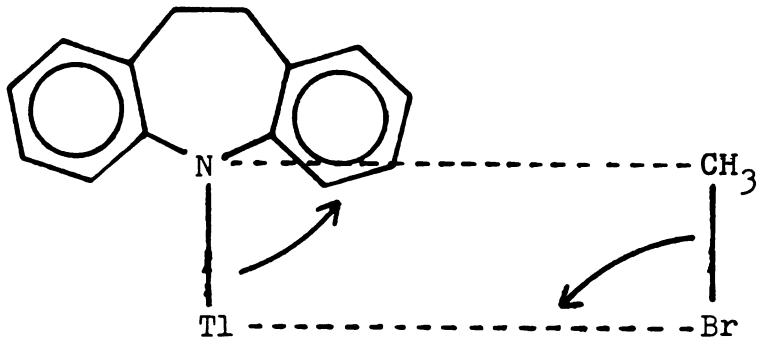


Figure 9

An additional geometric problem is that the nitrogen's lone pair of electrons in iminodibenzyl are probably not delocalized in the adjacent aromatic ring as they are in carbazole, phenothiazine or iminostilbene.[35]

A third possible route (see Fig. 10) involves the reaction of chloroacetyl chloride with iminodibenzyl[36] to give the chloroamide (3) which could be reduced with diborane[37] to give the chloroethyl derivative (4a). This method offers the added possibility that deuterium could be introduced by deuterodiborane reduction to give a 1', 1'-d_2 substitution in case the nitrile reductions were to fail to give the 3',3'-d_2 compound in good yields or high isotopic purity (see Fig. 10).

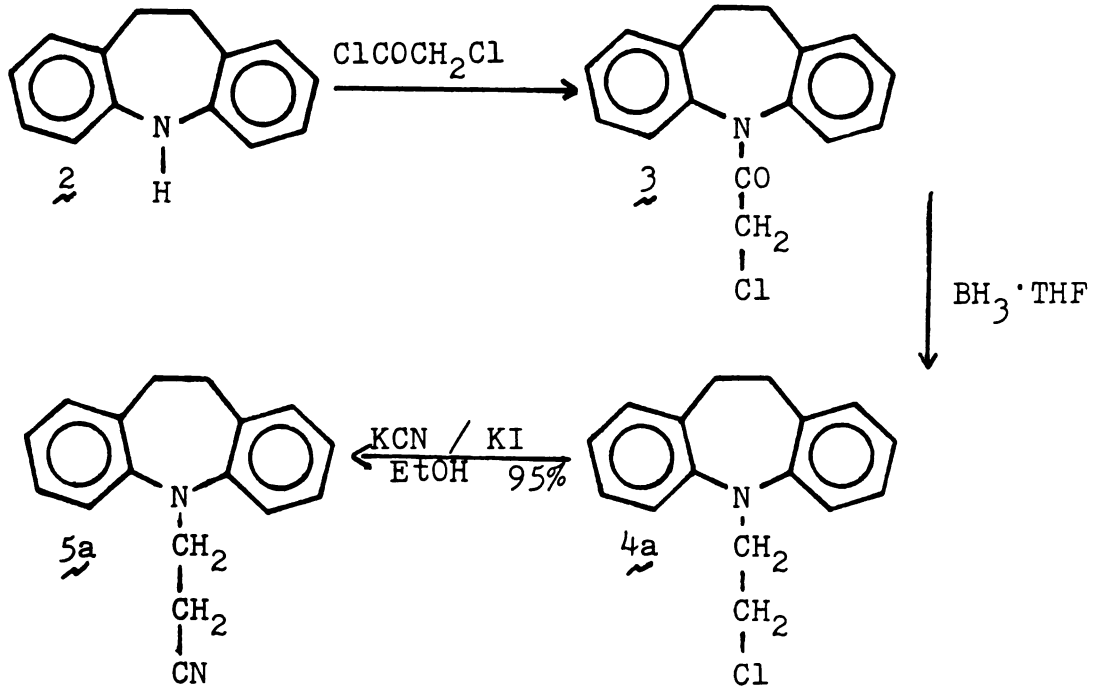


Figure 10

The problem with this route was the Sn_2 displacement of the chloride by a cyanide ion. Carrying out the displacement in various aprotic-polar solvents (dimethyl sulfoxide (DMSO)[38] and N,N-dimethylformamide (DMF)[39]), which are known to promote this type of reaction, failed to give the nitrile (5a). The reaction of the alkali (Na^+, K^+)-cyanide proceeds to 50% completion, then the cyanide in these strongly polar solvents becomes more basic than nucleophilic. The cyanide anion removes the now acidic proton α to the nitrile to give, in a reverse Michael-type addition, iminodibenzyl and an acrylonitrile polymer (Fig. 11). The reaction temperature and time were varied with no differences in results.

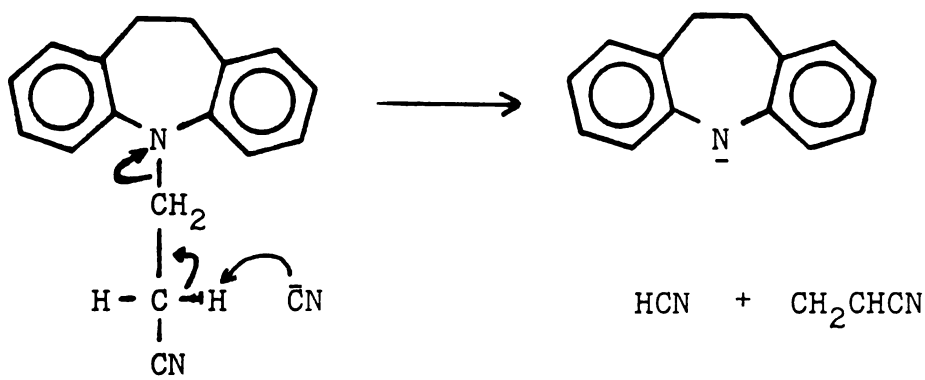


Figure 11

The chloro-compound (4a) was treated with various alkali cyanides in the presence of crown ethers.[41] Since 18-crown-

6[45] is cation-specific for potassium, potassium cyanide was treated with the chloride (4a) in the presence of 18-crown-6 under varying times and temperatures. The reaction either failed to go or went to 50% and gave the reverse Michael addition. The cyanide was successfully prepared from the chloro compound (4a) by reaction with potassium cyanide in the presence of a catalytic amount of potassium iodide by heating in 95% ethanol.* The cyano derivative (5a) crystallized out of solution as white needles as the reaction proceeded. The reaction was almost quantitative after four days. The aqueous ethanol must have provided a buffer system such that the α protons in the cyano compound were no longer the most acidic protons and/or that once 5a was formed, it precipitated.

The nitrile (5a) was reduced with lithium aluminum deuteride with little carbon-nitrogen bond cleavage[48] to give the desired primary amine (8) in good yields. The secondary amine (10) was obtained from the reduction of the formamide (9), which was produced by condensation of the primary amine (8) with 98% formic acid, using diborane.[32] The normal Eschweiler-Clarke method[40] on the primary amine (8) failed to give anything but polymer. A modification of the Eschweiler-Clarke reaction using

*The purpose of the potassium iodide is probably to effect a Finkelstein[42] halide exchange of chloride by iodide since iodide is easier to displace than is chloride.

$NaCNBH_3$[31] as the reducing reagent instead of formic acid gave high yields of the tertiary amine (11) (Fig. 12).

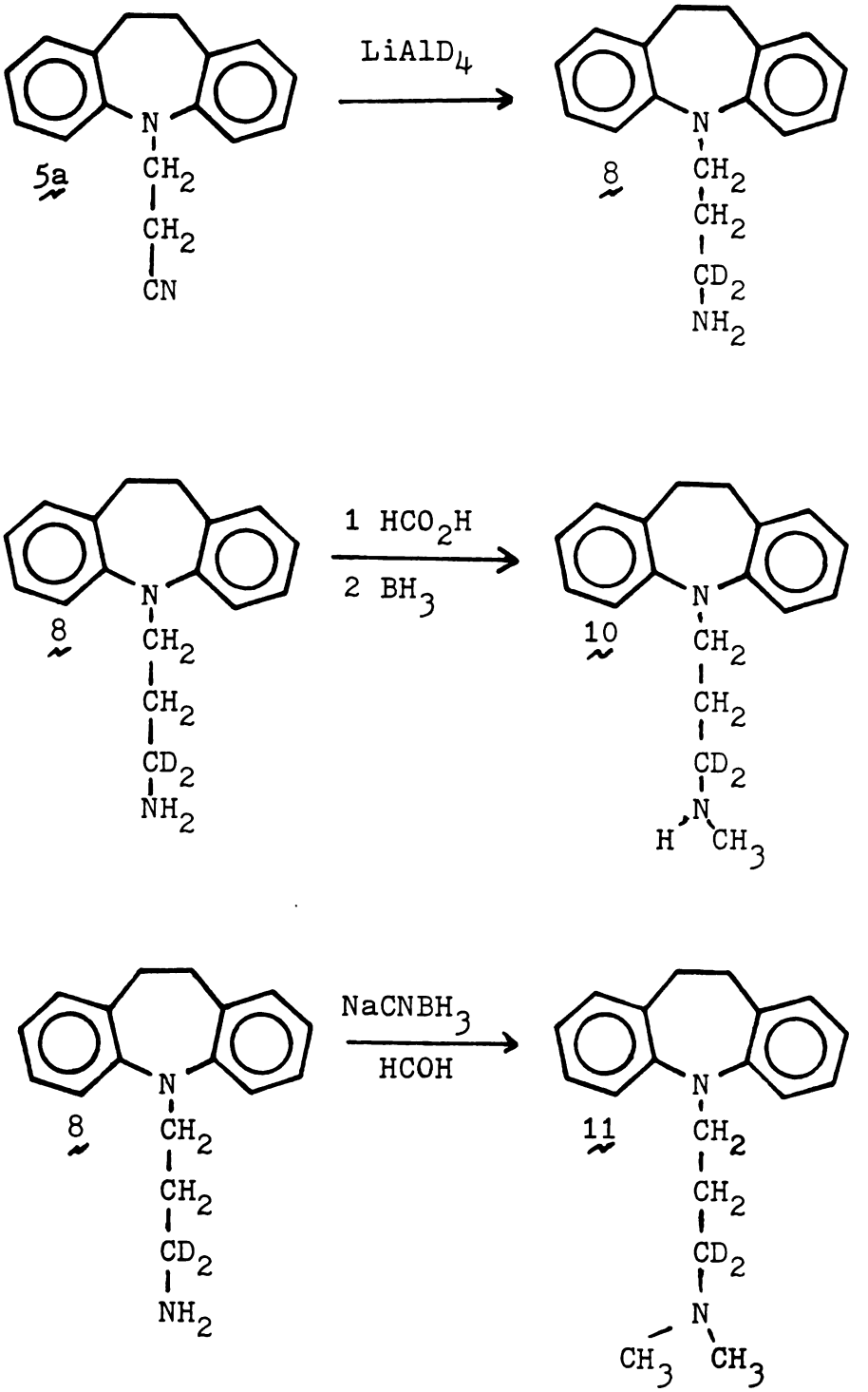


Figure 12

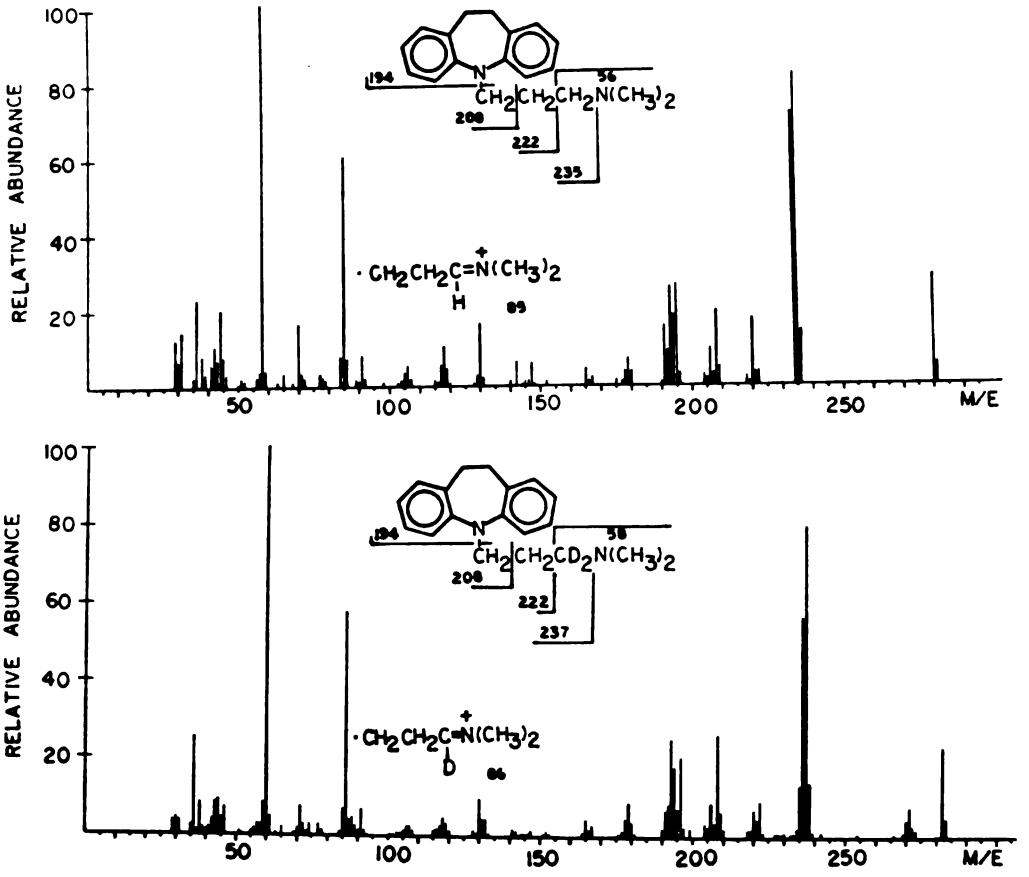

RELATIVE ABUNDANCE
100
80
60
40
20
50
100
150
200
250
M/E
194
208
222
235
56
89
RELATIVE ABUNDANCE
100
80
60
40
20
50
100
150
200
250
M/E
194
208
222
237
58
86

Figure 13

The isotopic purities were 98% d_2 and 2% d_1 (Fig. 13).

The cyanide (5b) was prepared (see Fig. 14) but the isotopic abundance of deuterium in the 1',1'-position was low (87% d_2, 10% d_1 and 3% d_0).

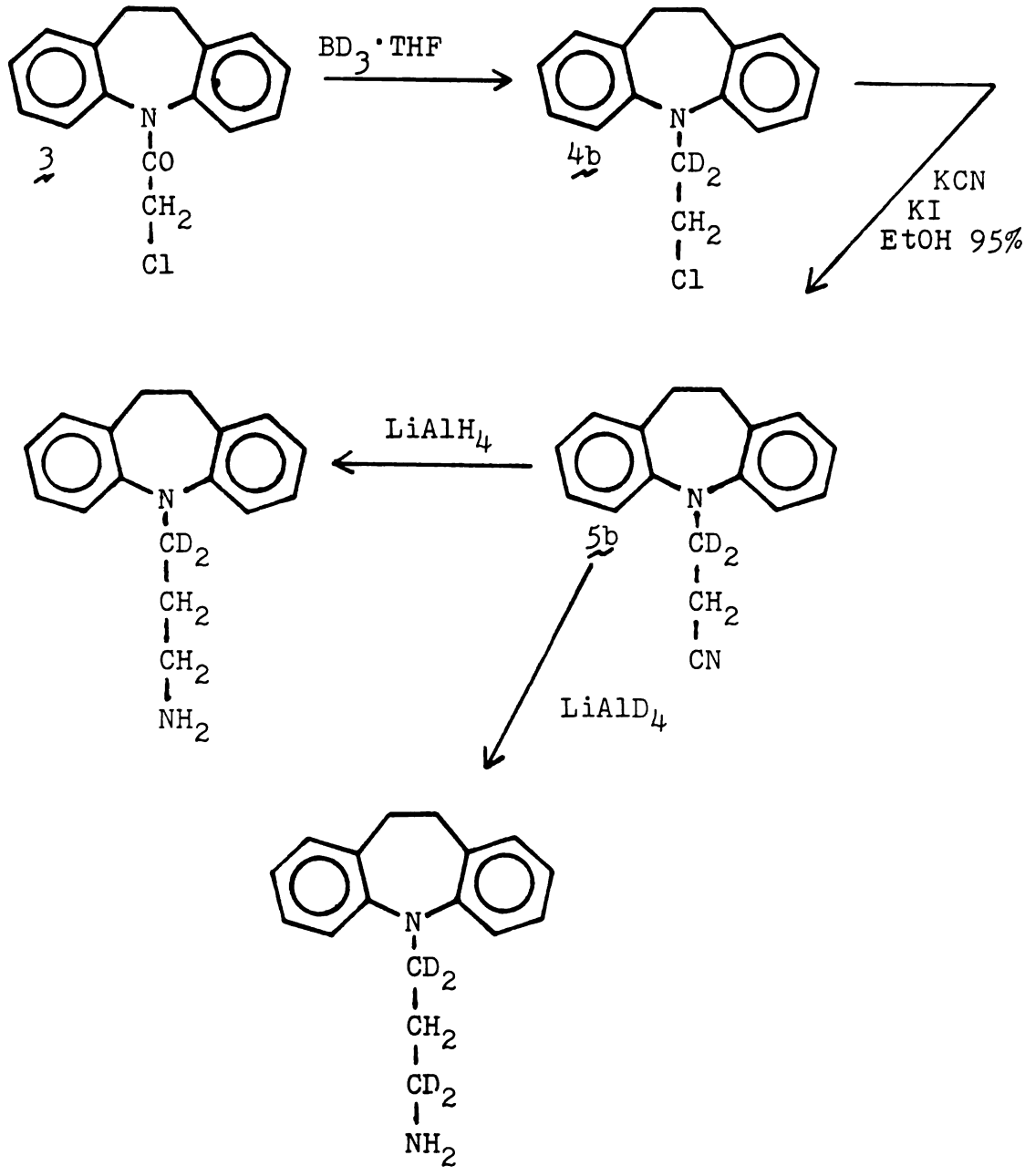


Figure 14

The conversion of (5b) to an amine could be accomplished either by reduction with $LiAlH_4$ to give a 1',1'-d_2 derivative or with $LiAlD_4$ to give a 1',1',3',3'-d_4 derivative; however, due to the low isotopic purity (diborane from Alfa-Ventron was only 95% d_3) of the cyanide, further exploration at this time was not considered. The deuterium in the 1',1'-position of the cyanide caused the expected shift of two mass units (208→210) in the base peak fragment which would open a different modification of SIR.[49] One could look at all drug metabolites (unlabelled) which would have a peak at 208 and use the 1',1'-d_2 as internal standard because its base peak would be at 210.

Although scheme 3 provided the desired amines of high isotopic purity, it gave relatively poor yields. It was hoped to synthesize sufficient quantities for distribution to several laboratories for use as an internal standard for gc-ms plasma analysis. Therefore a less laborious, higher yielding synthetic pathway was sought.

The new method makes simple modifications of reactions already shown to give good yields. The scheme (see Fig. 15) began with the reaction of iminodibenzyl with chloropropionyl chloride[43] and gave the chloroamide (6) in excellent yield.

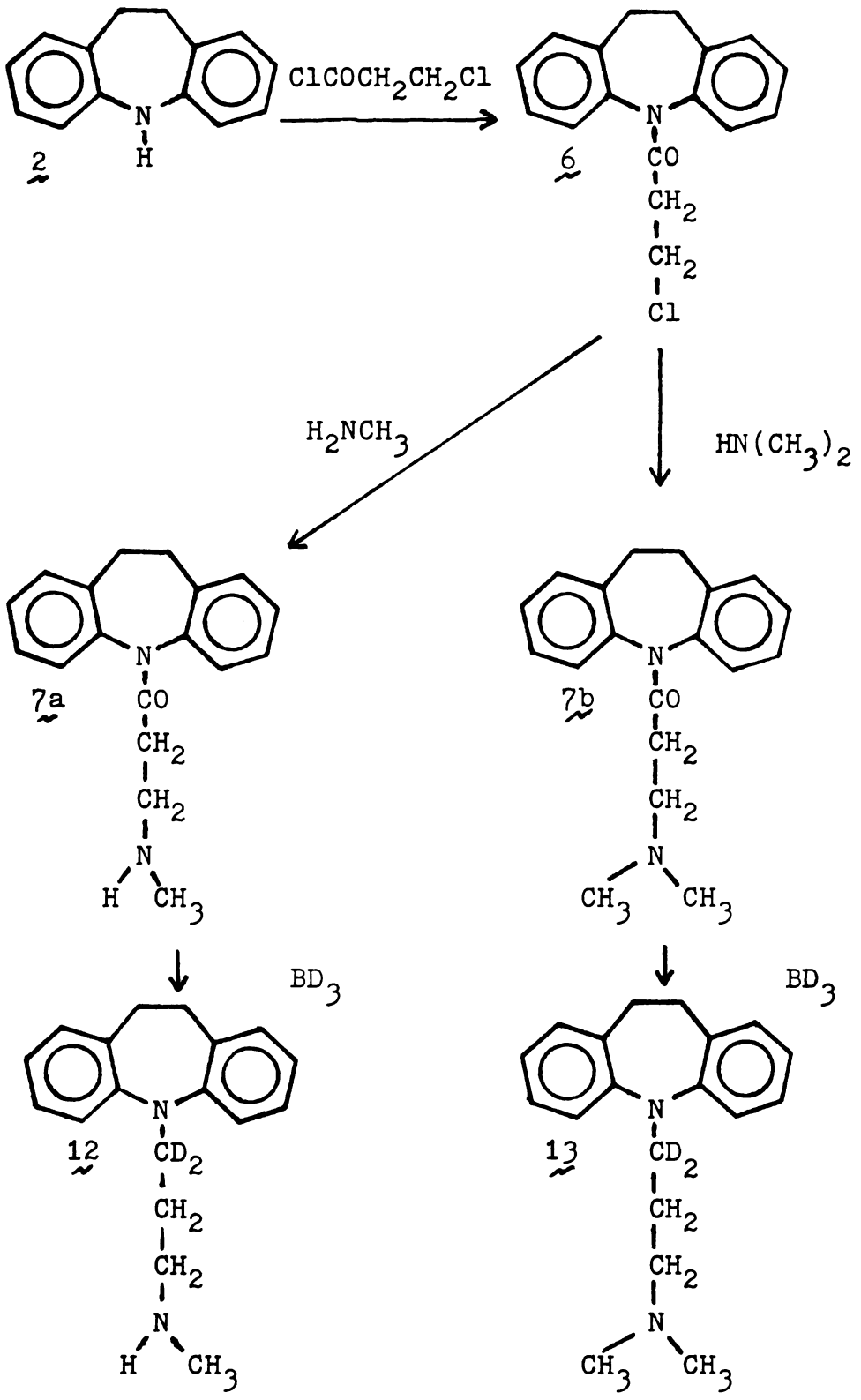

ClCOCH2CH2Cl
2
6
CO
CH2
CH2
Cl
H2NCH3
HN(CH3)2
7a
7b
N
H
CH3
BD3
12
13
CD2

Figure 15

The chloro group could be replaced either by gaseous dimethylamine[43] in toluene to give the imipramine precursor (7b), or by gaseous methylamine in toluene to give the desipramine precursor (7a). The amino amides (7a) and (7b) were both difficult to purify and unstable as hydrochloride salts but were quite stable as picrates. The picrates were easy to purify and easily liberated *via* chromatography on basic alumina by elution with chloroform. Both amides were readily reduced with deuteriodiborane to give the 1',1'-d_2 labelled amines (12 and 13) with 90% d_2 and 10% d_1. This low incorporation could be improved by preparing deuteriodiborane from $NaBD_4$ (99% d_4) (BD_3·THF from Alfa-Ventron is now available ≈95% d_3) and methyl iodide or dimethyl sulfate[44] as shown in Figure 16.

$$NaBD_4 + CH_3I \longrightarrow CH_3D + NaI + BD_3$$

Figure 16

This method has been shown to produce excellent deuteriodiborane with high deuterium content. This three-step synthesis gave the desired amines in an overall 55% yield. The most important advantage was the introduction of deuterium in the last step which gave an 80% yield.

EXPERIMENTAL

Melting points, determined with a Thomas-Hoover Uni-Melt melting point apparatus, are uncorrected. Nmr spectra at 60 MHz were determined with a Varian A-60A instrument unless otherwise stated, and 100-MHz nmr spectra were recorded on a Varian XL-100-15 instrument operating in the Fourier transform mode using an internal deuterium lock. Nmr chemical shift values are expressed in δ units (parts per million) relative to TMS in organic solvents and sodium 2,2-dimethyl-2-silapentane-5-sulfate in D_2O. For the presentation of nmr spectra, the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, compm = complex multiplet. Infrared (ir) spectra were recorded on a Perkin-Elmer 337 spectrometer. The electron-impact mass spectra (eims) were obtained on an AEI MS-12 instrument at 70 eV, and the chemical ionization mass spectra (cims) on an AEI MS-902 instrument modified for chemical ionization using isobutane as the reagent gas. Glc analyses were performed on a Varian Aerograph Model 2100 gas chromatograph with a 6 ft. U-shaped Pyrex column packed with 3% SE-30 on Chromosorb W. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley. Thin-layer chromatography (tlc) was carried out on pre-coated silica gel sheets.

10,11-Dihydro-5-(chloroacetyl)-5H-dibenz[b,f]azepine (3).

To a stirred solution of 10,11-dihydro-5H-dibenz-[b,f]azepine (2) (10 g, 51.2 mmol) in 50 ml of dry benzene was added chloroacetyl chloride (5.78 g, 52.2 mmol) in 10 ml of dry benzene over a period of 10 min, followed by stirring under reflux for an additional 4.5 h or until the evolution of HCl subsided. The benzene was removed by vacuum distillation, and the resulting oil was heated at 50° at 0.1 mm for 1 h to remove unreacted chloroacetyl chloride and chloroacetic acid. Crystallization was effected by adding 25 ml of anhydrous Et_2O. The residue which separated was recrystallized from absolute EtOH. The total yield was 13 g (47.8 mmol, 93%), mp 96-97.5° (lit.[36] 91.5-93.0°); tlc (I_2) R_f (benzene) 0.41; nmr ($CDCl_3$) δ 2.99 (m, 4H, $-CH_2-CH_2-$), 4.00 (s, 2H, $OCCH_2Cl$), 7.15 (m, 8H, aromatic H).

Anal. Calcd for $C_{16}H_{14}NOCl$: C, 70.71; H, 5.19; N, 5.15. Found: C, 70.47; H, 5.17; N, 5.10.

10,11-Dihydro-5-(2-chloroethyl)-5H-dibenz[b,f]azepine (4a).

A 100 ml flask was dried in an oven and cooled in a dry N_2 atmosphere. The flask was equipped with a rubber septum cap, a magnetic stirring bar, and a reflux condenser which was connected to a N_2 bubbler. The flask was immersed in an ice bath at 5° and 20 ml (20 mmol) of 1 M BH_3·THF was introduced into the reaction vessel. Then 2.72 g (10 mmol) of the amide (3) in 10 ml of THF was in-

troduced. The reaction mixture was 1 M in BH_3 and amide (3). The progress of the reaction was monitored *via* tlc. At the end of 4.5 h, the reaction mixture was cooled to 5° and 10 ml of conc HCl in 5 ml of THF was added carefully. The temperature was allowed to come to 27° and the whole mixture subjected to vacuum distillation. The residue was placed in a Soxhlet extractor and extracted with Et_2O. The Et_2O was dried, evaporated, and the residue recrystallized from absolute EtOH. The yield of 4a was 2.05 g (8 mmol, 82%), mp 83.5-85° (lit.[46] 84.5-85.5°); tlc (I_2) R_f (benzene) 0.54; nmr ($CDCl_3$) δ 3.16 (s, 4H, $-CH_2-CH_2-$), 3.5 (t, 2H, J= 6 Hz, $N-CH_2-$), 4.1 (t, 2H, J= 6 Hz, $-CH_2-Cl$), 7.04 (m, 8H, aromatic H).

Anal. Calcd for $C_{16}H_{16}NCl$: C, 74.55; H, 6.26; N, 5.43. Found: C, 74.68; H, 6.16; N, 5.21.

10,11-Dihydro-5-(2-cyanoethyl)-5H-dibenz[b,f]azepine (5a).

To a solution of 1 g (15.7 mmol) potassium cyanide and 0.1 g (0.6 mmol) potassium iodide in 150 ml of 95% EtOH was added 1 g (3.8 mmol) of the chloro compound (4a) in 50 ml of EtOH (95%). The mixture was stirred at 65° for 4 days with the progress of the reaction monitored *via* tlc. The solvent was removed under vacuum and the residue was taken up in cold water and filtered. The product was washed with 100 ml of cold water and recrystallized from EtOH, then benzene to yield 0.8 g (3.22 mmol, 85%), of 5a, mp 149-150° (lit.[46] 150°); ir (KBr): 2250

cm^{-1} (C≡N); tlc (I_2) R_f (benzene) 0.31; nmr ($CDCl_3$) δ 2.49 (t, 2H, J= 6 Hz, -CH_2-C≡N), 3.16 (s, 4H, -CH_2-CH_2), 3.97 (t, 2H, J= 6 Hz, N-CH_2), 7.09 (m, 8H, aromatic H); eims: 248 (42.4), 208 (100), 193 (46.0), 31 (96), 30 (92).

Anal. Calcd for $C_{17}H_{16}N_2$: C, 82.22; H, 6.45; N, 11.30. Found: C, 81.96; H, 6.45; N, 11.07.

10,11-Dihydro-5-(2-chloroethyl)-5H-dibenz[b,f]azepine-1',1'-d_2 (4b).

Using the method described above for 4a, 20 ml (20 mmol) of 1 M borane-d_3 solution in THF and 2.72 g (10 mmol) of the amide (3) gave 2.00 g of 4b (8 mmol, 81%), mp 83.5-85° (lit.[3] 84.5-85.5°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (benzene) 0.54; nmr ($CDCl_3$) δ 3.1 (s, 4H, -CH_2-CH_2-), 4.25 (broad s, 2H, -CH_2-Cl) 7.06 (m, 8H, aromatic H).

10,11-Dihydro-5-(2-cyanoethyl)-5H-dibenz[b,f]azepine-1',1'-d_2 (5b).

Prepared from 4b by the method described above for 5a, the product 5b had mp 145-150° (lit.[19] 150°). A mixed melting point with the authentic undeuterated sample was not depressed; ir (KBr): 2250 cm^{-1} (C≡N); tlc (I_2) R_f (benzene) 0.31; nmr ($CDCl_3$) 2.49 (broad s, 2H, -CH_2-C≡N), 3.16 (s, 4H, -CH_2-CH_2-), 7.10 (8H, aromatic H); eims: 250 (42.8), 249 (7.6), 248 (1.9), 210 (100), 193 (54.2).

10,11-Dihydro-5-(3-aminopropyl)-5H-dibenz[b,f]azepine-3',3'-d_2 (8·HCl).

The cyanide (5a) (100 mg, 0.427 mmol) was placed in a predried, flamed 25 ml Soxhlet extractor and lithium tetradeuteroaluminate (99% d_4) 100 mg (2.43 mmol) in 50 ml of anhydrous Et_2O was placed in a predried, flamed 50 ml flask cooled in a stream of N_2. The reaction mixture was heated at reflux for 86 h. The excess deuteride was destroyed by the addition of 2 ml D_2O. To the cloudy solution was added 1 g of NaOH in 5 ml of water, and the whole mixture was extracted with Et_2O. The ether layers were combined, washed with sat NaCl and dried over Na_2SO_4. The drying agent was removed and ethereal HCl was added until further addition produced no precipitation. The solution was cooled overnight and the filtrate recrystallized from EtOH to yield 8·HCl (100 mg, 0.35 mmol, 81%), mp 275-276° (lit.[36] 275°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (15 AcOH: 65 BuOH: 20 H_2O) 0.80; nmr ($CDCl_3$) δ 1.26 (s, 2H, $-NH_2$), 1.62 (broad t, $-CH_2-CH_2-CD_2$), 3.08 (s, 4H, $-CH_2-CH_2-$), 3.70 (t, 2H, J= 6 Hz, $N-CH_2$), 6.99 (m, 8H, aromatic H); eims: 254 (33.8), 253 (0.6), 237 (38.2), 208 (100), 194 (37.7), 193 (67).

10,11-Dihydro-5-(3-formamidopropyl)-5H-dibenz[b,f]-azepine-3',3'-d_2 (9).

To a solution of the amine (8) (50 mg, 0.1968 mmol) in 30 ml of dry benzene was added 2 ml of 98% formic acid all at once. The flask was equipped with a Dean-Stark separator for the removal of water. The reaction was heated at reflux for 6 h, 2 ml of 98% formic acid was added and the mixture was heated at reflux overnight. After 24 h of refluxing, the Dean-Stark apparatus was replaced with a Soxhlet extractor containing molecular sieve (3A) and refluxing was continued for 24h. The solvent was removed under vacuum to leave a brown residue which was dissolved in Et_2O and washed with 50 ml of 10% HCl. The ether was dried over $MgSO_4$, filtered, and evaporated leaving a tan solid which recrystallized from acetonitrile to yield 39 mg (0.1 mmol, 70%) of 9, mp 142-143°, nmr ($CDCl_3$) δ 2.04 (broad t, 2H, $-CH_2-C\underline{H}_2-CD_2-$), 3.1 (s, 4H, $-C\underline{H}_2-C\underline{H}_2-$), 3.81 (t, 2H, $N-C\underline{H}_2-$), 6.40 (broad s, 1H, N-H), 7.05 (m, 8H, aromatic H), 8.10 (d, 1H, NOC-H); eims: 282 (42.3), 281 (1.5), 208 (100), 193 (43.1); cims: exact mass 282.1696 (calcd for $C_{18}H_{18}N_2OD_2$ 282.172).

10,11-Dihydro-5-(3-methylaminopropyl)-5H-dibenz[b,f]-azepine-3',3'-d_2 (10·HCl).

A 50 ml flask was dried in an oven, flamed and cooled in a dry N_2 atmosphere. The flask was equipped with a

rubber septum, a magnetic stirring bar, and a reflux condenser which was connected to a N_2 bubbler. The flask was immersed in an ice bath and 5 ml (5 mmol) of 1 M borane solution in THF was introduced into the reaction flask. Then 0.0425 g (0.15 mmol) of the amide (9) in 12 ml of THF was introduced. The reaction was heated at reflux for 6 h, cooled to 5°, and 5 ml of conc HCl in 5 ml of THF was added carefully. The whole mixture was subjected to vacuum distillation. The residue was washed with Et_2O, dissolved in 0.1 N NaOH and extracted with Et_2O. The basic ether extract was washed with 10% $NaHCO_3$ and sat NaCl, dried over Na_2SO_4 and filtered. Freshly prepared ethereal HCl was added to the filtrate until further addition produced no cloudiness. The solution was cooled overnight and the precipitate recrystallized from EtOH to yield 0.0334 mg (0.11 mmol, 75%) of the hydrochloride of 10, mp 210-212° (lit.[36] 212-214°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (15 AcOH: 65 BuOH: 20 H_2O) 0.75; nmr ($CDCl_3/D_2O$) 2.02 (broad t, 2H, CH_2-$C\underline{H}_2$-CD_2), 2.75 (s, 3H, N-$C\underline{H}_3$), 3.10 (s, 4H, -$C\underline{H}_2$-$C\underline{H}_2$-), 3.8 (t, 2H, N-$C\underline{H}_2$-), 7.00 (m, 8H, aromatic H); eims: 268 (38), 267 (3), 237 (100), 236 (80), 208 (65), 195 (60), 193 (66), 46 (80).

10,11-Dihydro-5-(3-dimethylaminopropyl)-5H-dibenz[b,f]-azepine-3',3'-d_2 (11·HCl).

To a stirred solution of 500 mg (2 mmol) of the amine (8) and 2 ml (25 mmol) of 37% aqueous formaldehyde in 15 ml of acetonitrile was added 500 mg (8 mmol) of sodium cyanoborohydride. A vigorous exothermic reaction ensued, and a dark residue separated. The reaction mixture was stirred for 15 min, and then 0.3 ml glacial HOAc was added dropwise over a period of 7 min. Stirring was continued for 2 h, and an additional 0.3 ml of glacial HOAc added. After stirring for an additional 30 min, the solution was diluted with 100 ml of Et_2O, washed with 20 ml of 0.5 N NaOH, and then extracted with three 10 ml portions of 1 N HCl. The acid extracts were combined, neutralized with solid NaOH, extracted with Et_2O, and the combined ether extracts dried (Na_2SO_4) and filtered. Ethereal HCl was added to the filtrate and the solid which separated was recrystallized from EtOH to yield 545 mg (1.7 mmol, 86%) of the hydrochloride of 11 mp 171-173° (lit.[47] mp 173-174°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (15 AcOH: 65 BuOH: 20 H_2O) 0.70; nmr ($CDCl_3$) δ 2.10 (broad t, 2H, -C$\underline{H}_2$-CD_2-N), 2.68 (s, 6H, -N-(C$\underline{H}_3$)$_2$), 3.15 (s, 4H, -C$\underline{H}_2$-C$\underline{H}_2$-), 3.75 (t, 2H, N-C$\underline{H}_2$), 6.94 (m, 8H, aromatic $\underline{H}$); eims: 283 (5), 282 (23.4), 281 (0.9), 237 (80.4), 236 (57), 208 (26.3),86 (58), 60 (100), 36 (25).

10,11-Dihydro-5-(3-chloropropionyl)-5H-dibenz[b,f]-azepine (6).

To a stirred solution of 19.53 g (100 mmol), 10,11-dihydro-5H-dibenz[b,f]azepine (2) in 150 ml of anhydrous benzene was added dropwise a solution of 14.0 g (110 mmol) 3-chloropropionyl chloride (freshly distilled) in 50 ml of dry benzene. The mixture was stirred at reflux for 3 h or until HCl was no longer evolved. The benzene was removed under vacuum to give an oil. The oil was heated to 70° under vacuum (ca 0.1 mm) for 1 h to remove excess propionyl chloride, then cooled to room temperature, and Et_2O was added. The white solid was recrystallized from EtOH to yield 25 g (87.5 mmol) of the amide (6), mp 103-104° (lit.[43] 105-106°); ir (KBr) 1645 cm^{-1} (amide C=O); tlc (I_2) R_f (benzene) 0.38; nmr ($CDCl_3$) δ 2.46-3.98 (m A_2B_2 + AA'BB', 8H, $-C\underline{H}_2-C\underline{H}_2-$, $OC-C\underline{H}_2-C\underline{H}_2-Cl$), 7.12 (s, 8H, aromatic H).

Anal. Calcd for $C_{17}H_{16}ClNO$: C, 71.45; H, 5.64; N, 4.90. Found: C, 71.31; H, 5.67; N, 4.67.

10,11-Dihydro-5-(3-methylaminopropionyl)-5H-dibenz[b,f]-azepine (7a).

A 250-ml two-necked flask was oven-dried and equipped with a dry-ice condenser and a rubber septum cap. The flask was immersed in an ice bath (0°) and 100 ml of dry toluene were introduced via syringe. Gaseous methylamine

was condensed into the toluene until 4 ml were collected. To this solution was added 3 g (10.5 mmol) of the chloro compound (6) in 25 ml dry toluene. The reaction was stirred with cooling for 72 h and finally heated at reflux for 2 h.

The solution was filtered to remove the $(CH_3)NH_2 \cdot HCl$ and the resulting solution was concentrated under vacuum to yield 7a as an oil. The oil was dissolved in Et_2O and $Et_2O \cdot HCl$ was added until further addition produced no cloudiness. The hydrochloride salt of 7a was hygroscopic and could not be crystallized; it was dissolved in H_2O and treated with aqueous lithium picrate to yield the picrate of 7a. Recrystallization from EtOH afforded an analytical sample, mp 140-141° (dec), nmr ($CDCl_3$) δ 2.5-3.6 (compm, 12H, methylenes H and $-NCH_3$), 7.3 (m, 8H, aromatic H), 8.8 (s, 2H, picric acid H).

Anal. Calcd for $C_{24}H_{23}N_5O_8$: C, 56.58; H, 4.55; N, 13.75. Found: C, 56.31; H, 4.56; N, 13.63.

10,11-Dihydro-5-(3-methylaminopropyl)-5H-dibenz[b,f]-azepine-1',1'-d_2 (12·HCl).

A 100 ml flask was dried in an oven, flash-flamed and cooled down in a dry N_2 atmosphere. The flask was equipped with a rubber septum cap and a magnetic stirring bar, and a reflux condenser connected to an N_2 source. The flask was immersed in an ice bath (ca 5°) and 11.4 ml (10 mmol) of 0.88 M $BD_3 \cdot THF$ was introduced into the reac-

tion flask. Then 2.7 g (10 mmol) of the amide (7a) in 8.6 ml of THF was introduced over a period of 0.5 h. The reaction mixture was 0.5 M in BD_3 and amide. After the addition was completed, the resulting mixture was heated at reflux for 3 h. The reaction flask was cooled and 10 ml of 6 N HCl was added slowly. The THF was removed under vacuum as hydrogen was evolved. The aqueous phase was basified with solid NaOH and extracted with Et_2O. After drying over sodium sulfate, the ether was acidified with ethereal HCl. The hydrochloride of 12 was recrystallized from absolute EtOH to give 2.4 g (8 mmol, 80%), mp 210-212° (lit.[47] 212-214°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (15 AcOH: 65 BuOH: 20 H_2O) 0.75; nmr ($CDCl_3$) δ 2.00 (broad t, 2H, $-CD_2-C\underline{H}_2-CH_2-$), 2.42 (m, 3H, $-NCH_3$), 2.85 (broad t, 2H, $-C\underline{H}_2-CH_2-N$), 3.15 (s, 4H, $-C\underline{H}_2-C\underline{H}_2-$), 7.09 (m, 8H, aromatic H); eims: 268 (35.7), 267 (4.5), 266 (0.4), 235 (76.7), 195 (100), 193 (79.1), 44 (93.0).

10, 11-Dihydro-5-(3-dimethylaminopropionyl)-5H-dibenz-[b,f]azepine (7b).

A 250-ml two-necked flask was oven-dried and equipped with a dry ice condenser and a rubber septum cap. The flask was immersed in an ice bath (0°) and 100 ml of dry toluene were introduced via syringe. Gaseous dimethylamine was condensed into the toluene until 4 ml were collected. To this solution was added 3 g (10.5 mmol) of

the chloro compound (6) in 25 ml dry toluene. The reaction was stirred with cooling for 72 h and finally heated at reflux for 2 h.

The solution was filtered to remove the $(CH_3)_2NH \cdot HCl$ and the resulting solution was concentrated under vacuum to yield 2.50 g (8.4 mmol, 81%) of 7b as an oil $bp_{0.1}$ 180-182° (lit.[43] $bp_{0.2}$ 195-197°). The oil was dissolved in Et_2O and $Et_2O \cdot HCl$ was added until further addition produced no cloudiness. The hydrochloride salt of 7b was hygroscopic and was dissolved in H_2O and treated with aqueous lithium picrate to yield the picrate of 7b. Recrystallization from EtOH afforded an analytical sample, mp 179-181° (dec), nmr ($CDCl_3$) δ 2.4-3.7 (compm, 14H, methylenes H, and $-N(CH_3)_2$), 7.3 (m, 8H, aromatic H), 8.8 (s, 2H, picric acid H).

Anal. Calcd for $C_{25}H_{25}N_5O_8$: C, 57.36; H, 4.18; N, 13.38. Found: C, 57.25; H, 4.83; N, 13.29.

10,11-Dihydro-5-(3-dimethylaminopropyl)-5H-dibenz[b,f]-azepine-1',1'-d_2 (13·HCl).

A 100 ml flask was dried in an oven, flash-flamed, and cooled down in a dry N_2 atmosphere. The flask was equipped with a rubber septum cap and a magnetic stirring bar, and a reflux condenser connected to an N_2 source. The flask was immersed in an ice bath and 11.4 ml (10 mmol) of 0.88 M $BD_3 \cdot THF$ was introduced in the reaction

flask. Then 2.8 g (10 mmol) of the amide (7b) in 8.6 ml of THF was introduced over a period of 0.5 h. The reaction was then carried out as for 1h above, and the amorphous hydrochloride was dissolved in water and treated with a neutral solution of lithium picrate. The picrate of 1i crystallized from EtOH in 87% yield, mp 144-146° (dec); eims: 282 (15.6), 281 (1.5), 236 (60.2), 235 (39.1), 87 (46.9), 58 (100).

The free base was liberated from the picrate by elution on a basic alumina column with chloroform. The chloroform was evaporated and ether added. Addition of $Et_2O \cdot HCl$ yielded the hydrochloride salt of 13, 1.8 g (6.4 mmol, 64%), mp 171-173° (lit.[47] 173-174°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (I_2) R_f (15 AcOH: 65 BuOH: 20 H_2O) 0.70; nmr ($CDCl_3$) δ 2.08 (broad t, 2H, $-CD_2-C\underline{H}_2-C\underline{H}_2-$), 2.57 (s, 6H, $-N-(C\underline{H}_3)_2$), 2.94 (broad t, 2H, $-CH_2-C\underline{H}_2-N$), 3.10 (s, 4H, $-C\underline{H}_2-C\underline{H}_2-$), 7.04 (m, 8H, aromatic $\underline{H}$); eims: 282 (19.5), 281 (2.3), 236 (72.2), 235 (42.9), 234 (15.7), 210 (16.8), 87 (45.6), 58 (100), 36 (21.4).

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CHAPTER V

SECTION A

BENZYLIC MICROSOMAL HYDROXYLATION OF 2-(4-BIPHENYLYL)PROPANE (AN ANTI-INFLAMMATORY).

SYNTHESIS.

HISTORICAL BACKGROUND

An important class of non-steroidal anti-inflammatory agents currently available to the medical practice for the relief of the symptomatic pain of arthritis are 2-arylpropionic acids and the arylacetic acids[1] (Fig. 1).

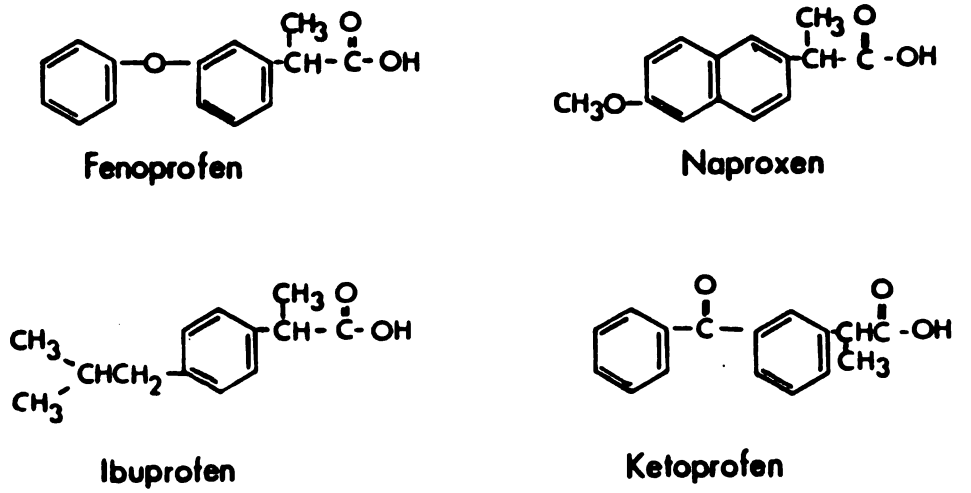


Figure 1

In a search for new agents with superior therapeutic indices or with unique properties, investigators at the Lilly Research Laboratories[2,3] synthesized compounds that were structurally analogous to the aromatic acids but were not, in themselves, acids. One of the most interesting compounds they found was the hydrocarbon isopropylbiphenyl (Fig. 2).

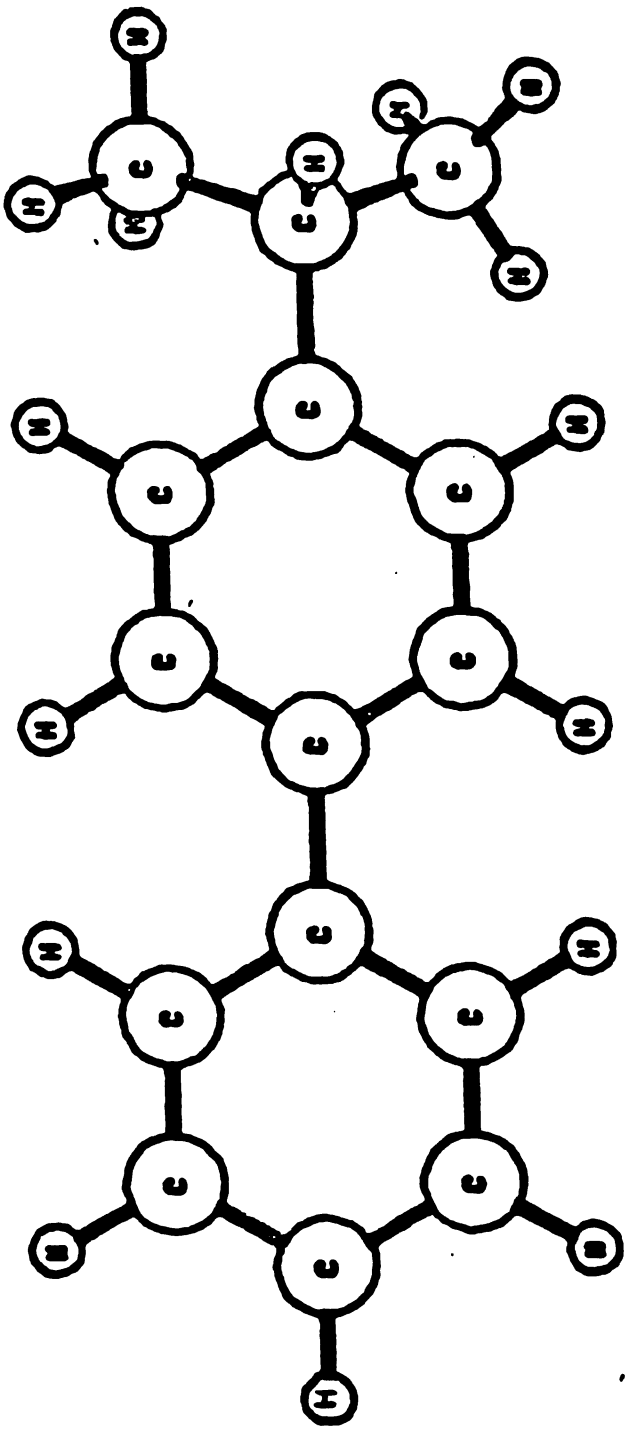
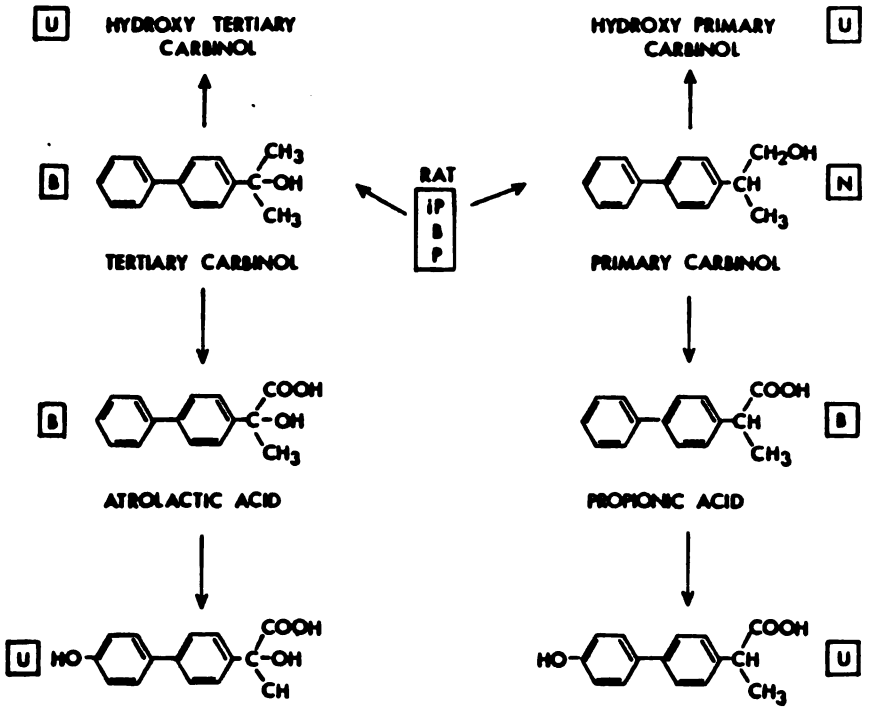


Figure 2

The hydrocarbon isopropylbiphenyl (IPBP) possessed pronounced anti-inflammatory activity. IPBP was orally active when evaluated in assays such as erythema blocking tests with rats,[4] guinea pigs and to a lesser extent, dogs, and had a low toxicity potential.

The fact that IPBP showed such species variation suggested that metabolism must play a role in activating or inactivating the IPBP. Since hydrocarbons also represent a unique class of drugs, McMahon [3] et al. investigated the metabolism of IPBP in several species by use of gc-ms. The metabolites of IPBP in rats are shown in Fig. 3.



Circulating and eliminated metabolites of IPBP in the rat. (U) - eliminated metabolites, (B) - circulating metabolites, (N) - postulated intermediate metabolites.

Figure 3

The results obtained from their metabolic studies suggested that two distinct pathways were involved in the biotransformation of IPBP in the rats (Fig. 4).

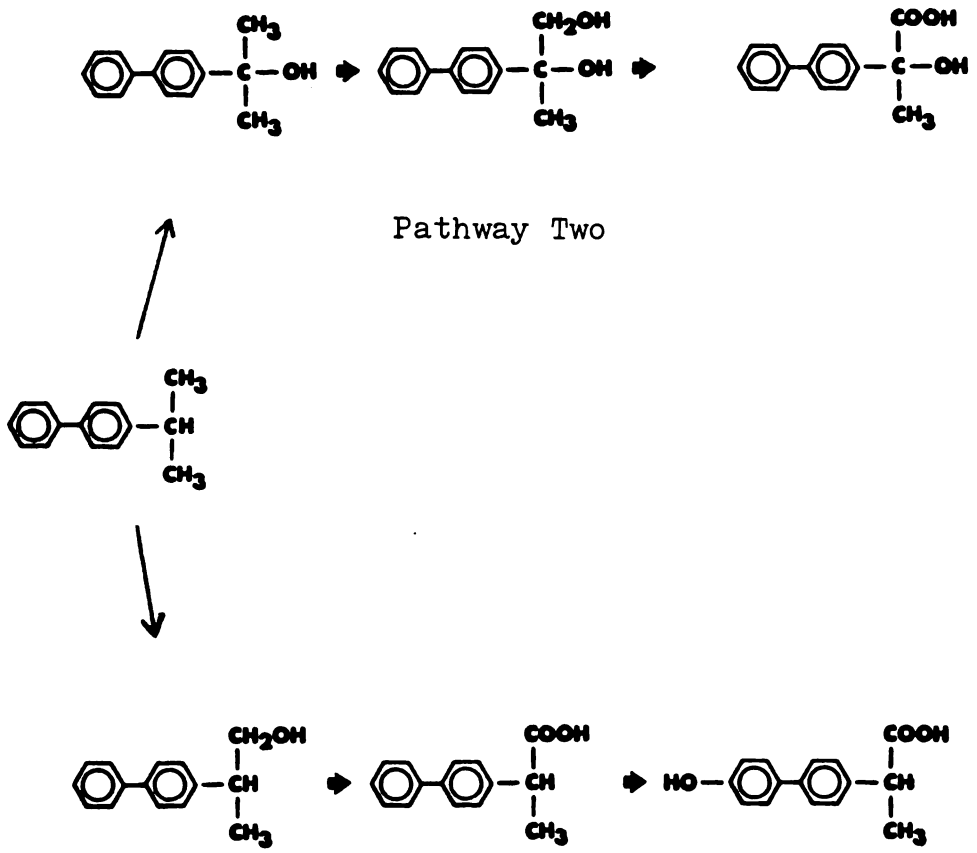


Figure 4

The initial reaction in both pathways involves aliphatic hydroxylation. One, the propionic acid pathway, which will be discussed more extensively in Section B, has as one of its products the pharmacologically active metabolite, 2-(4-biphenylyl) propionic acid (11a) (Table 1).

Table 1: Effect of Isopropylbiphenyl and Related Compounds on Ultraviolet Induced Erythema on Guinea Pig Skin

Compound	Estimated ED_{50} (mg/kg)*
Isopropylbiphenyl	2.5
Biphenylpropionic acid	0.3
Biphenyl-1-propanol	0.2
Biphenyl-2-propanol	40
Biphenyl-α-methyl glycolic acid	17
2-Biphenylpropene	38

*The oral dose at which the UV-induced erythema produced on the shaved guinea pig back is blocked to the extent of 50%.

The second pathway, leading to the formation of p-phenylatrolactic acid, has as its initiating step hydroxylation by oxygen insertion at the benzylic carbon to yield the tertiary carbinol which was then enzymatically oxidized to the p-phenylatrolactic acid. None of the metabolites in the second pathway possessed anti-inflammatory activity (Table 1). Investigation of several other species showed the same basic pathways except that in man and dog pathway two (hydroxylation of the tertiary carbon) was the major pathway while in rat and monkey, pathway one

(hydroxylation of the primary carbon) was the predominant route. This difference was significant since the major portion of the pharmacological activity ascribed to IPBP was due to the aryl acid.

In vitro studies using a 10,000 g rat liver supernatant with appropriate co-factors showed the production of both the tertiary carbinol and the acid indicating, again, two pathways (Fig. 5).

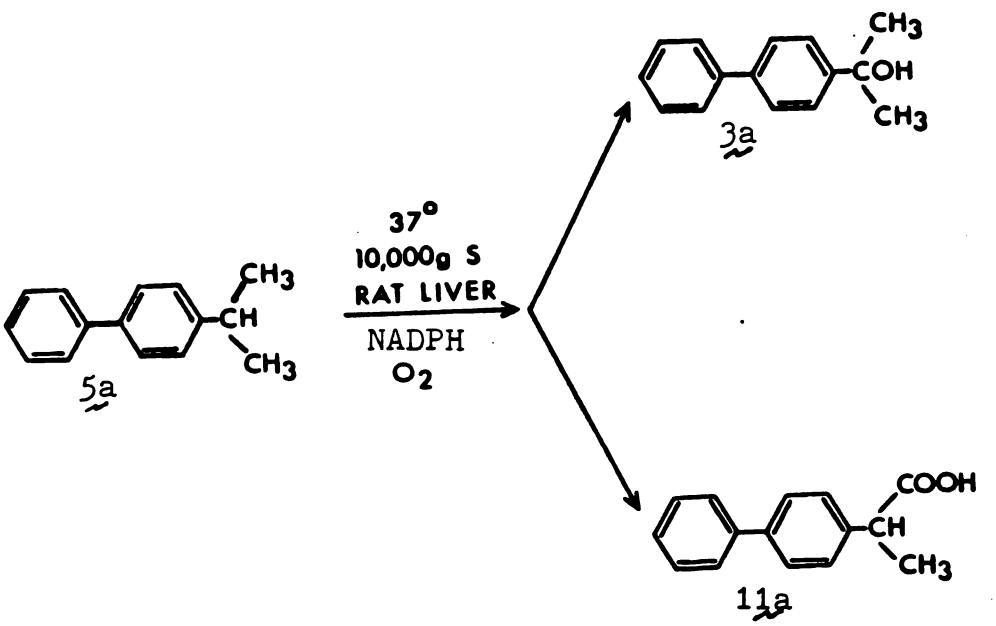


Figure 5

INTRODUCTION

Our laboratory has been involved in the investigation of the mechanism and stereochemistry of microsomal monooxygenase systems.[5] The *in vitro* hydroxylation of the tertiary carbon of IPBP (Pathway Two) provides a new system to study further the nature of microsomal oxygenation.

Oxygenases are frequently involved in the initial metabolism of drugs, especially in the liver.[6] There are two oxygenase systems.[7] Each activates molecular oxygen and releases "oxene" (formal equivalent of atomic oxygen)[8] to a nucleophilic center in the molecule adsorbed by the oxygenase complex. Oxene is isoelectronic with carbenes and its reactions appear to be analogous. Oxene inserts into σ bonds (especially C-H and N-H bonds to give the corresponding hydroxy derivatives) and/or adds to π bonds (especially C=C bonds to give epoxides). Oxene also reacts with the lone pair of electrons of tertiary amines[9] to give N-oxides.

If oxene inserts directly into an R-H bond to give R-OH, then an isotope effect* with a consequently slower reaction rate should occur for the corresponding R-D compound. The isotope effects reported to date involve the conversion of C-H(D) to C-OH and had a magnitude of

*The isotope effect throughout this chapter refers to the hydrogen-deuterium system.

≈ 2, as for example, the demethylation of O-nitroanisole[10] and the hydroxylation of ethylbenzene.[5] A value of 7 has been reported for the oxidative metabolism of cotinine[11] and a value of 10 has been encountered for the demethylation of p-trideuteromethoxy-anisole.[12] The maximum theoretical isotope effect ($^1H/^2H$) is 18.[13]

Mass spectrometry not only allows the metabolism of nanogram amounts of a drug and its deuterated analogue (separately or in admixture) to be studied but also permits determination of isotope effects of compounds which give molecular ions or appropriate fragment ions of reasonable intensity. The technique of determining isotope effects using mass spectrometry has been greatly expanded with the advent of field ionization,[14] chemical ionization,[15] and field desorption[16] mass spectrometry because molecular ions may be obtained from a much wider range of compounds.

The kinetics measurements may be carried out on the ^1H form of the molecule using the ^2H form as an internal standard or vice versa. If the kinetics of the reaction are measured independently, the microsomal system must be saturated with respect to substrates to insure a true isotope effect. The best way to determine isotope effects accurately, however, is to mix the ^1H and ^2H compounds to insure identical experimental parameters. Since Km's for deuterated and non-deuterated substrates may vary, the

calculated isotope effect may vary slightly with variation in substrate concentration.[12]

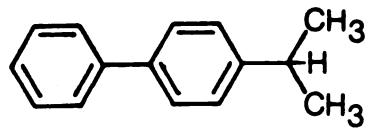
The two compounds necessary to measure the isotope effect of the <u>in vitro</u> hydroxylation of IPBP are 2-(4-biphenylyl) propane (5a) and 2-(4-biphenylyl)propane-2-d_1 (5b) (Fig. 6).

Figure 6

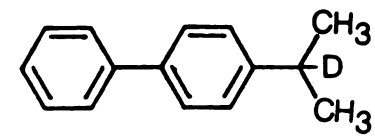
The use of these two compounds, however, presents two basic problems. One is that the enzymic product of both the ^{1}H and ^{2}H substrates is the same carbinol (tertiary alcohol). Second, the substrates differ in molecular weight by only one mass unit which results in the molecular ion of the ^{2}H compound overlapping with the m + 1 ion (due to the natural abundance of ^{13}C) of the ^{1}H compound. In order to avoid the $^{2}H(m)$, $^{1}H(m + 1)$ overlap, it was decided to synthesize substrates differing by several mass units in order to determine the <u>in vitro</u> isotope effect of the microsomal hydroxylation of IPBP. The purpose of this section of Chapter Five is to describe the synthesis of the compounds needed to ascertain the rate of the limiting step of the hydroxylation of the tertiary benzylic carbon of IPBP.

RESULTS AND DISCUSSION

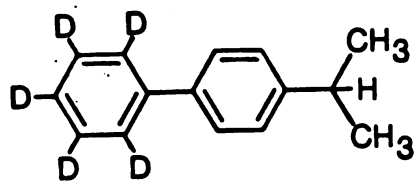
In order to determine accurately the primary and secondary isotope effects in the microsomal oxygenation of IPBP, it was decided to synthesize the five compounds shown in Fig. 7.



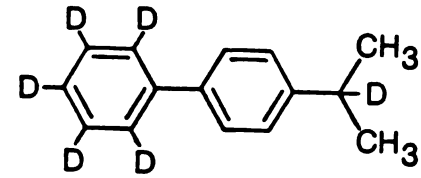
5a



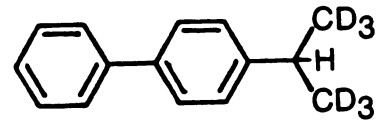
5b



5c



5d



5e

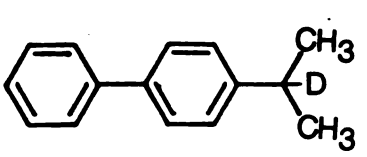
Figure 7

By comparing the reaction velocity of the hydroxylation of compound 5a against 5c (the distal phenyl is totally deuterated), the presence of a secondary isotope effect due to the deuteration of the distal phenyl group may be es - tablished (Fig. 8).

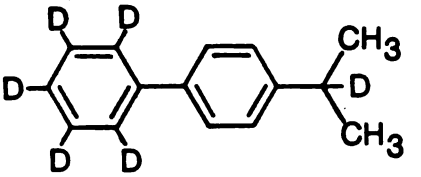
5a 5c

Figure 8

The substrates differ in molecular weight by five mass units (196 vs. 201) and the carbinol products also differ by five mass units (212 vs. 217). There will be no primary isotope effect since both substrates 5a and 5c have hydrogens in the tertiary position. The rate of 5b compared to 5d should provide a check on the results obtained from 5a vs. 5c except there will be a primary isotope effect from both 5b and 5d (Fig. 9).



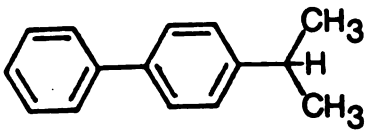
5b



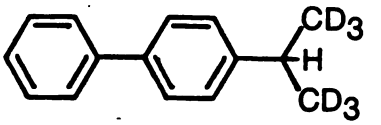
5d

Figure 9

Therefore any difference in rate will be due to the secondary isotope effect. The atomic mass difference between 5b and 5d is again five mass units for both substrate and carbinol (197 vs. 202 and 212 vs. 217 respectively). The difference in rates between 5a and 5e will provide a measure of the influence of the deuterated methyl groups on the kinetics (Fig. 10).



5a



5e

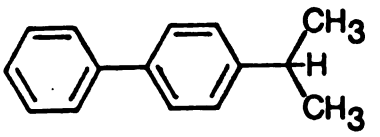
Figure 10

This difference would also be a secondary isotope effect (different from the above secondary isotope effect) since the carbon atoms deuterated are not involved directly in the rate limiting step (oxene insertion). The substrates and products both differ by six mass units (196 vs. 202 and 212 vs. 218 respectively).

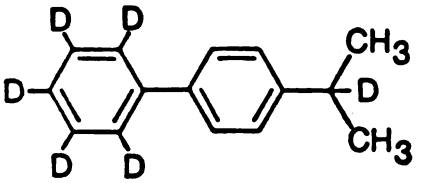
The primary isotope effect may be measured by comparing the rates of any of the following pairs: 5a vs. 5d (substrates 196 vs. 202, carbinol 212 vs. 217), 5b vs. 5c (substrates 197 vs. 201, carbinol 212 vs. 217), and 5b vs. 5e (substrates 197 vs. 202, carbinol 212 vs. 218) (Fig. 11).

The use of the combined techniques of gc-ms and SIR[17] will allow the microsomal enzyme mixture to be extracted (Note: the efficiency need not be 100 % since both compounds are labelled variants of each other and the physical properties will be almost identical)[12] and the isotope effect may be obtained directly by comparing the ion current of the selected ions (either substrates or products) over a period of time points.[12]

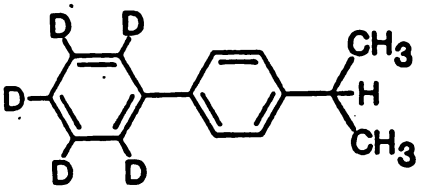
The logistics of the synthesis of the five substrates were as follows: synthesize a common precursor that can be converted in one step to the hydrocarbons by use of either a protio or deutero reagent. The precursor should be easy to synthesize via a route which will allow for the introduction of a perdeuterated phenyl group into the



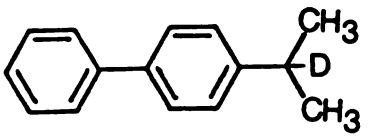
5a



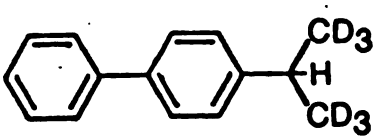
5d



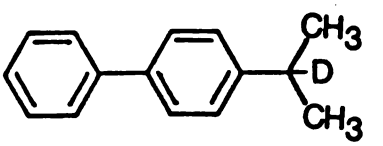
5c



5b



5e



5b

Figure 11

distal ring of IPBP.

The number of deutero reagents of high isotope purity are few.[18] Most of the deutero reagents are deuterides/ which may be nucleophilic ^{2}H(such as $LiAlD_4$) or electrophilic such as borane ($BD_3 \cdot THF$ - the deuterium is still nucleophilic). Therefore, the precursor must possess a leaving group, capable of being displaced by a deutero nucleophile, at the tertiary carbon or an electron-rich center capable of complexing with a deutero-containing electrophile.

Theoretically, the tertiary carbinol 3a appears to be the ideal precursor since the hydroxyl group could easily be converted to a good leaving group such as a tosylate or halide (Fig. 12).

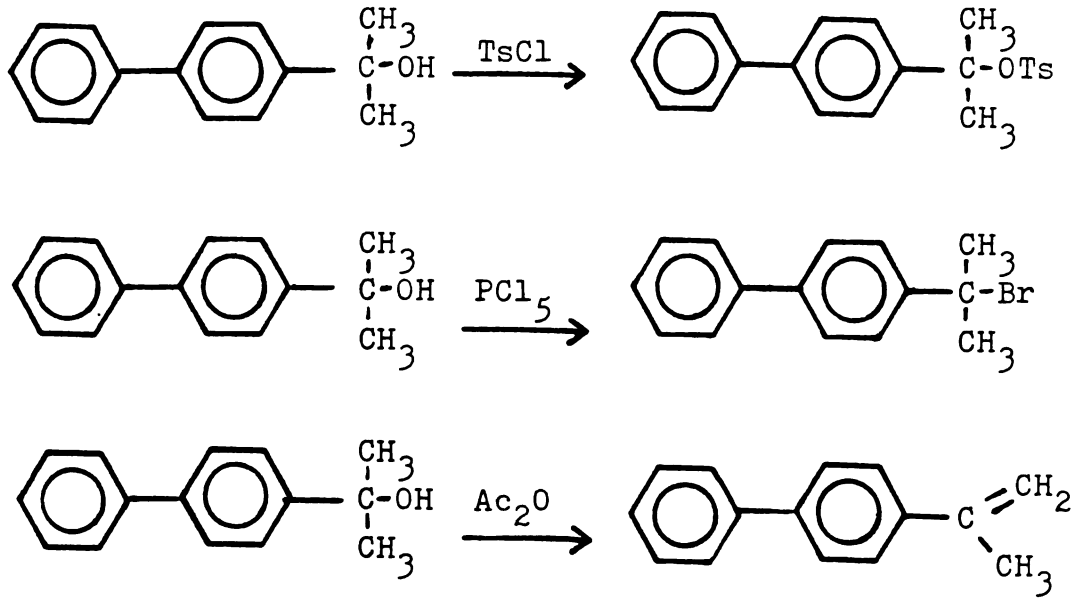


Figure 12

The alcohol could possibly be dehydrated to the styrene (4a). The styrene could be hydroborated if the nucleophilic displacement of the alcohol derivative failed (Fig. 13).

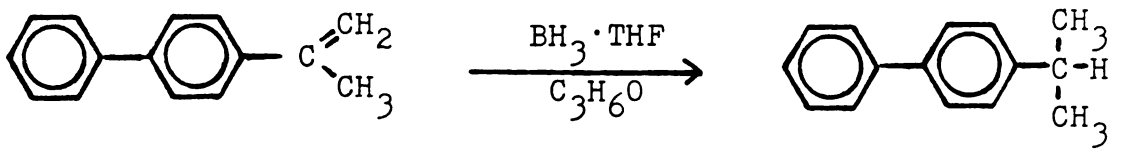


Figure 13

The alcohol (3a) could be prepared by three different methods each involving a Grignard reagent. The most useful was the reaction of acetone with the Grignard prepared from 4-bromobiphenyl (Fig. 14).[19]

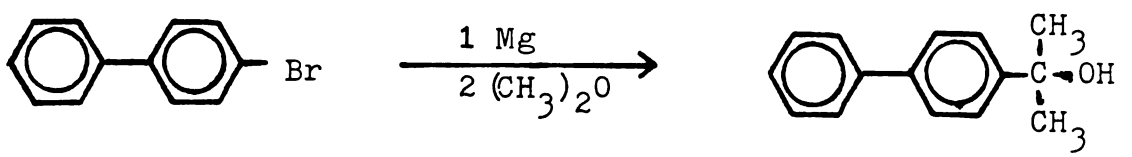
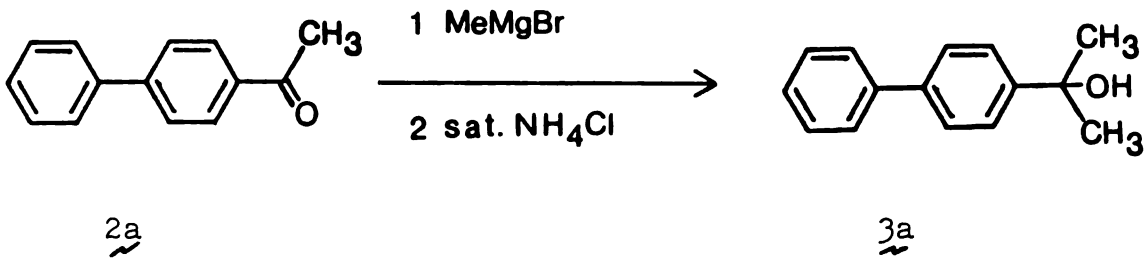


Figure 14

Methylmagnesiumbromide could be added to either 4-phenylacetophenone[20] or ethyl-4-phenylbenzoate[19] to give the tertiary carbinol (3a) (Fig. 15).

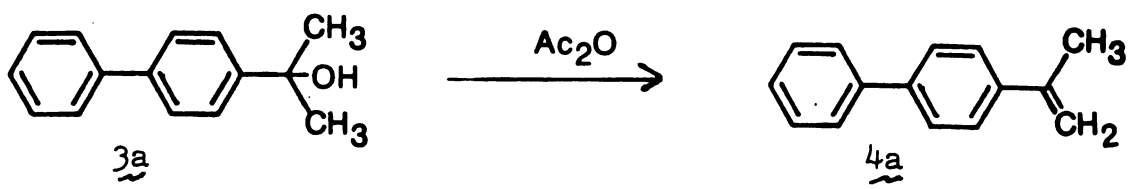


2a 3a

Figure 15

Dehydration of the carbinol during workup was a problem if mineral acid or heat was used. It was necessary to use saturated NH_4Cl to hydrolyze the Grignard complex and remove the solvents at room temperature under reduced pressure. Any styrene formed could be removed by chromatography.

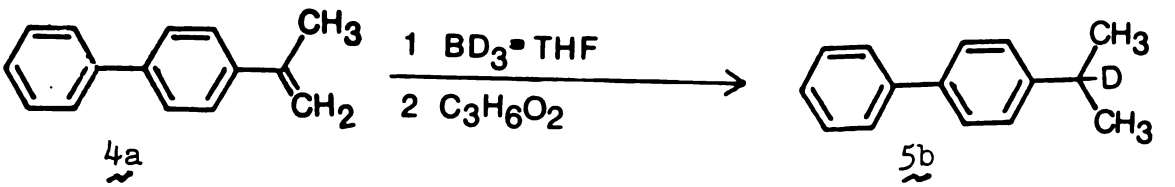
The alcohol could be converted to the styrene by several methods; however, the highest yield and purest product was obtained by dehydration with acetic anhydride (Fig. 16).[21]



3a 4a

Figure 16

The styrene could be easily hydro- or deuteroborated[22] (Fig. 17) but the protonolysis of the borane complex gave poor yields.



4a 5b

Figure 17

The conversion of the alcohol (3a) to a better leaving group was not considered an important pathway due to low yields and problems in displacing tertiary benzylic leaving groups by hydride reagents.[23]

Several investigators[24,25,26] have reported the ability of trialkylsilanes to transfer their hydrogen as hydrides to carbonium ions (Fig. 18) which are at least as stable as a secondary carbonium ion.

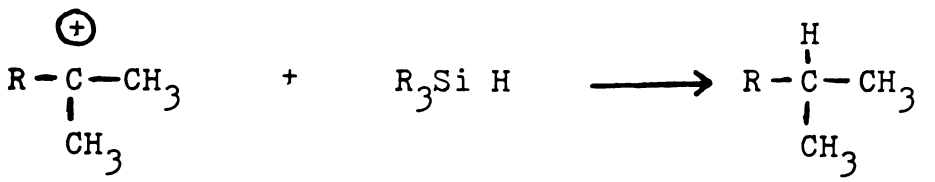


Figure 18

The positive carbon can be created in acidic solvents (trifluoracetic acid) by protonation of an olefin (Fig. 19) or dehydroxylation of an alcohol.[24]

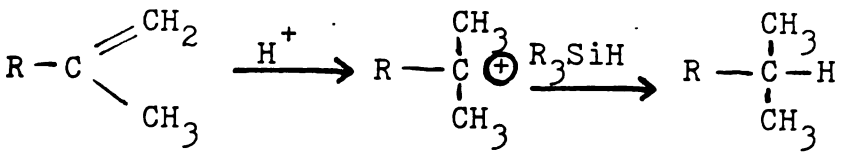


Figure 19

The positive carbon has also been generated by Lewis acids such as $AlCl_3$ reacting with a halide derivative.[26] The thermodynamics are in favor of the transfer of the hydride to the positive carbon by 8 kcal/mole since silicon is less electronegative than carbon.[25] This reaction should work perfectly for the tertiary alcohol since the carbon is benzylic as well as tertiary. The reaction of the carbinol (4a) and triethylsilane in methylene chloride with trifluoroacetic acid (6 N) proceeded smoothly. The best yields were obtained at lower temperatures ($\approx -20^{\circ}$). Triethylsilane-d_1 was synthesized by reduction of triethylchlorosilane by lithium tetradeuteroaluminate[26] and transferred its deuterium as well as the hydrido derivative transferred hydrogen.

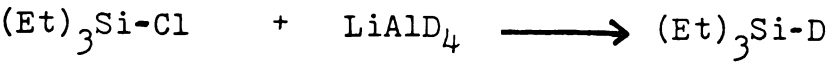


Figure 20

The hydrocarbons 5a, 5b, 5d and 5e were all synthesized (Fig. 21) from the appropriate alcohols by the use of the required silane.

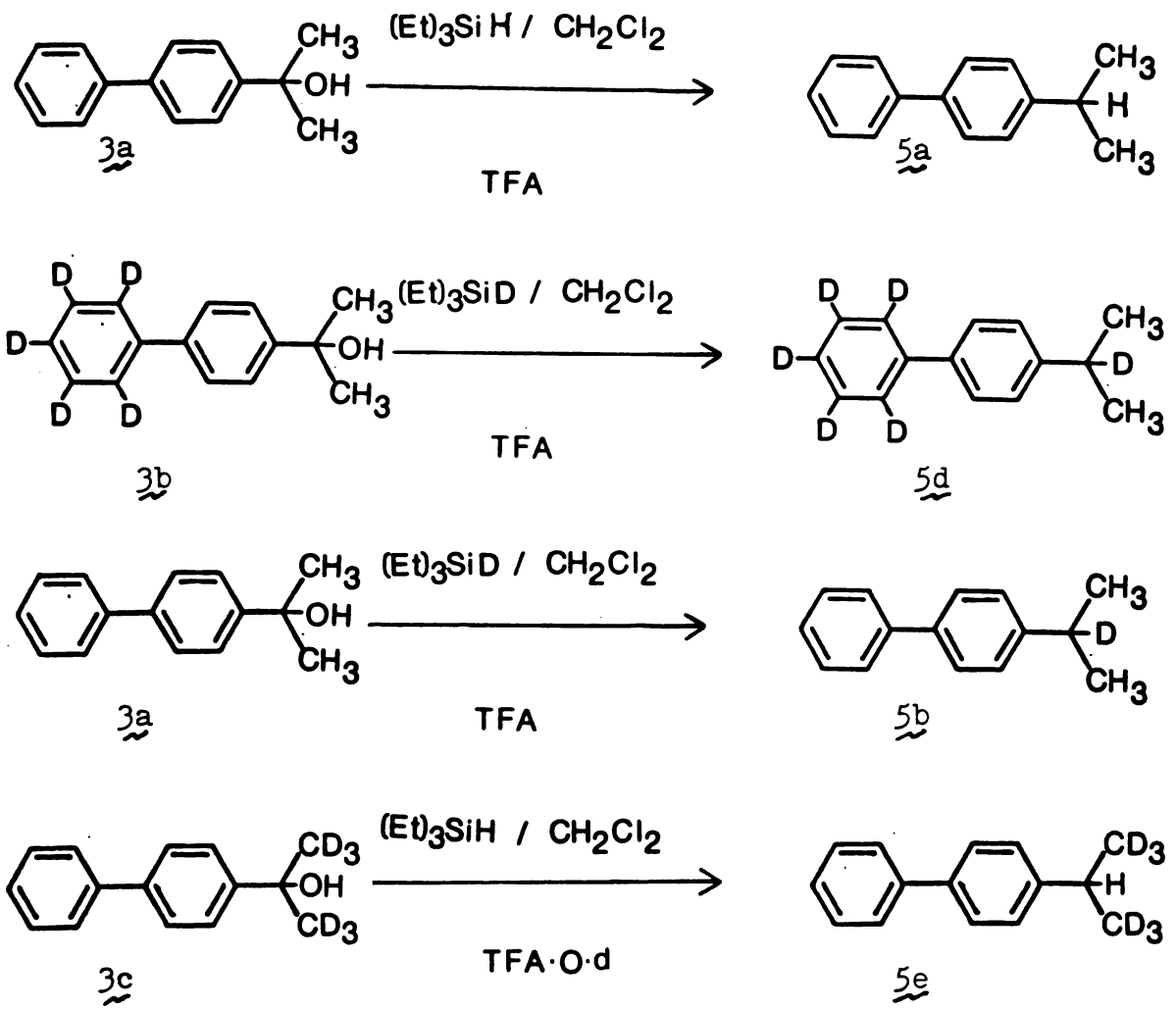
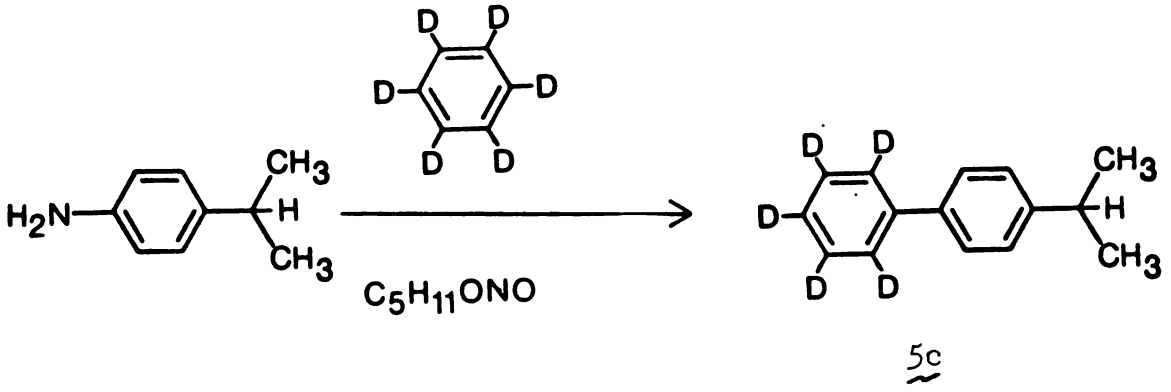


Figure 21

The hydrocarbon 5c was synthesized by the diazotization of 4-isopropylaniline (Fig. 22) by isoamylnitrite in the presence of a 200-fold excess of deuterated benzene (d_6 = 99.5%).[27]



5c

Figure 22

The purification of 5c from the excess benzene (the recovered benzene may be reused after purification) and isoamyl alcohol was fairly simple. The only problem was the formation of some 4,4'-diisopropylbiphenyl which contained no deuterium, but the by-product could be eliminated by use of large excesses of deuterobenzene.

A modification of the above diazotization reaction was used to prepare the bromo compound 7b (Fig. 23) from 4-bromoaniline.[27]

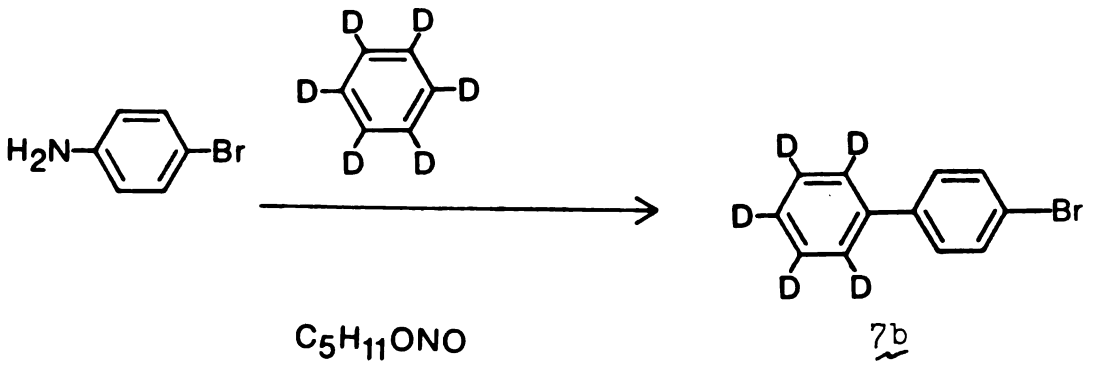


Figure 23

The bromo compound 7b was converted to the alcohol 3b by the reaction of its Grignard complex with acetone[19] (Fig. 24).

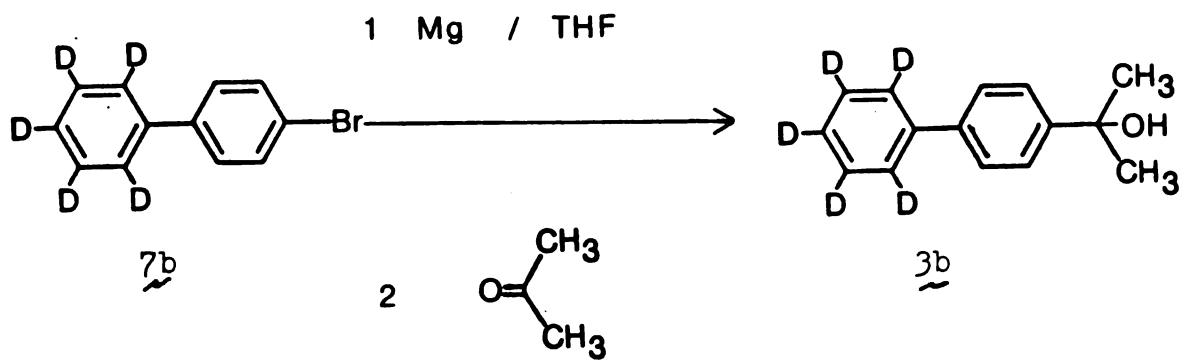


Figure 24

The alcohol 3c used for the synthesis of 5e was prepared by reacting totally deuterated acetone (Fig. 25) with the Grignard complex of 4-bromobiphenyl (7a).[19]

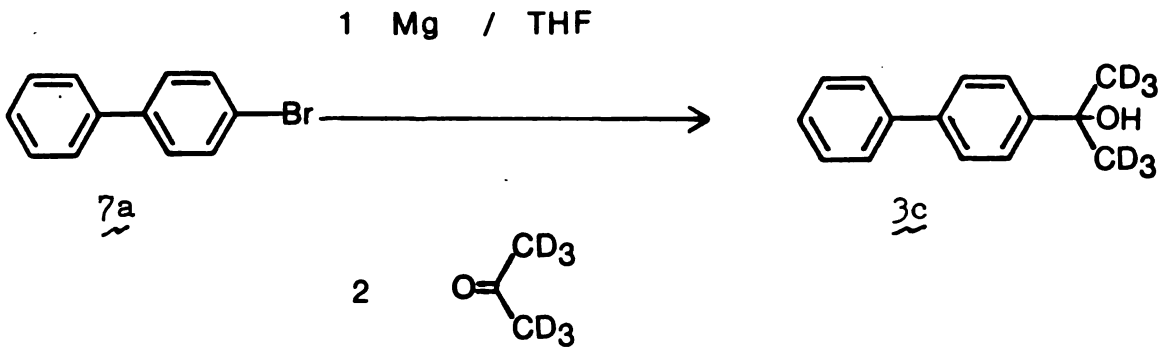


Figure 25

SECTION B

STEREOSPECIFICITY IN THE METABOLIC OXIDATION OF 2-(4-BIPHENYLYL)PROPANE.

SYNTHESIS.

INTRODUCTION

The presence of asymmetry in biological macromolecules causes some of their unique properties. When an optically active macromolecule (e.g. an enzyme) binds with the racemic modification of a chiral molecule, two different complexes are formed. The physical and chemical properties of these complexes will be different due to their diastereoisomeric relationship. This phenomenon explains why stereochemical factors are important in the metabolism of drugs.[28]

Prelog[29] distinguished two different types of enzymatic stereospecificity. He used the concept of substrate stereospecificity when two stereoisomers of an asymmetric substrate are metabolized at different rates. If the asymmetric center is created during the enzymic process, the two possible stereoisomers may be formed at different rates, leading to the concept of product stereospecificity.

Testa and Jenner[28] have introduced two modifications in Prelog's classifications. The term stereoselectivity is used instead of stereospecificity to indicate the preferential but not complete predominance of one stereoisomer over the others in the metabolic process. Therefore stereospecificity means complete stereoselectivity. The second modification deals with the introduction of a second element of chirality in an already chiral molecule.

This allows one to classify those cases where substrate and product stereoselectivity show some degree of interdependence.

Racemic modifications of drug molecules frequently possess only half the activity of the active enantiomers.[30] This difference in activity has been attributed to several factors: differential transport, differential metabolism, differential binding, or the spatial arrangement of substrate in the active site.[30]

In the non-steroidal anti-inflammatory arylpropionic acids, the activity resides in only one stereoisomer (the S antipode) (Fig. 26).

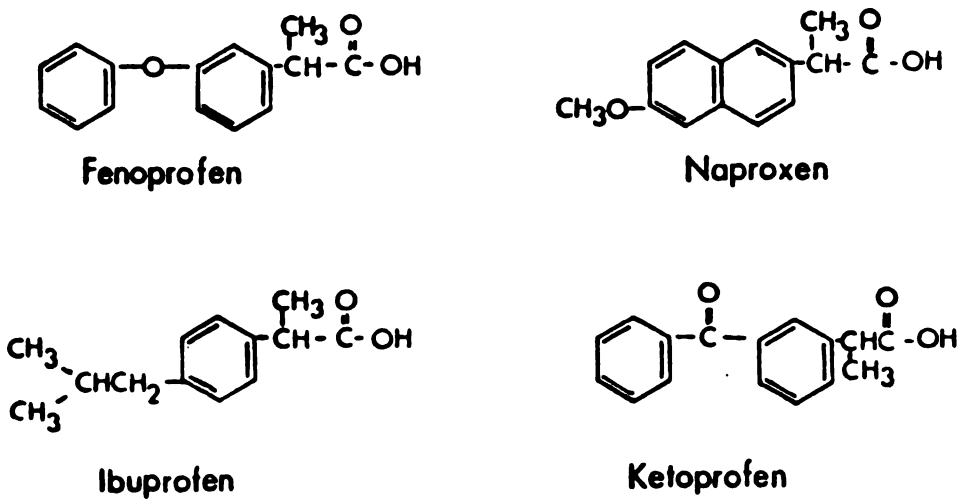


Figure 26

This phenomenon has been reported by several investigators.[31,32,33] Some authors have even drawn analogies between the anti-inflammatory agents and plant growth regulators because of their structural similarity as well as predicting that they both must have similar active sites.[33]

An initial report by Adams et al.,[34] however, showed that the individual S(+) and R(-) isomers of ibuprofen (isobutylhydratropic acid) were essentially biologically equivalent *in vivo*. This anomalous behavior was shown by Wechter et al.,[35] to be due to an unrecognized enzymatic pathway in man and other species which allows for optical inversion (epimerization) at the saturated carbon. Wechter proposed a detailed enzymatic pathway for this optical inversion (Fig. 27)

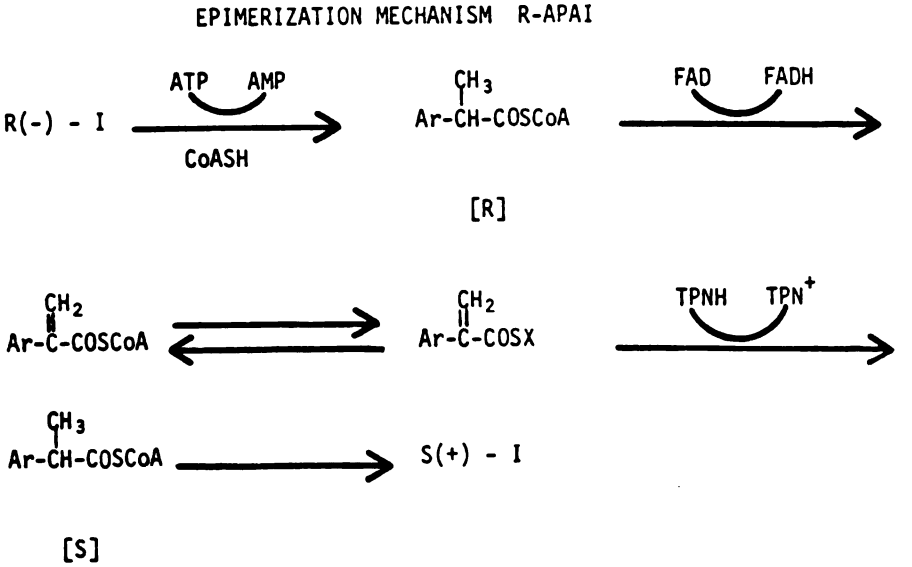


Figure 27

and provided further proof of the mechanism by the in vivo metabolism of the R(-) d_4-analog of ibuprofen to the S(+)-d_2-ibuprofen (Fig. 28).

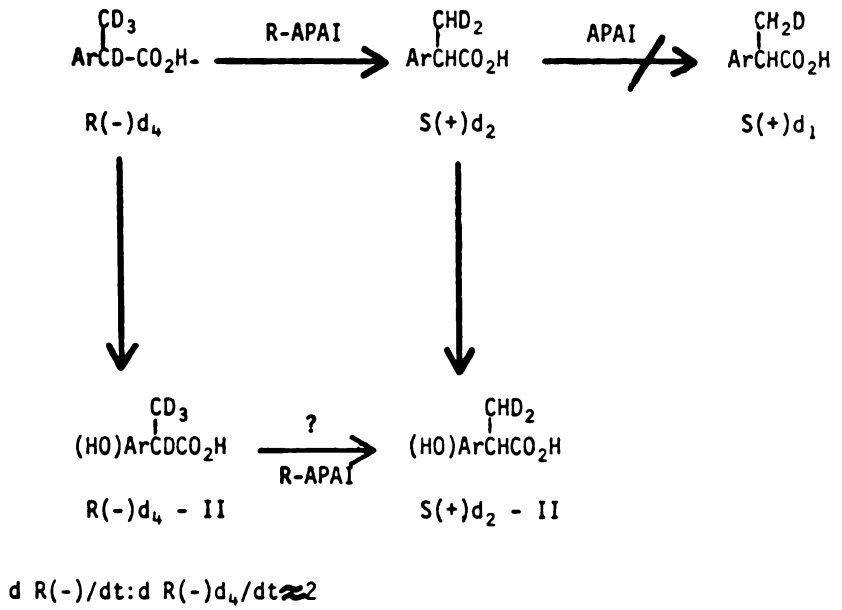


Figure 28

Kripalani[36] provided additional evidence of this type of inversion in his investigation of the in vivo metabolism of the anti-inflammatory α-methylfluorene-2-acetic acid. He observed that 16 days after the administration of the dl racemic drug only the d-enantiomer was present in the dog's blood (Table 2). Administration of only the R(-) isomer gave similar results (Table 3).

Table 2: Percentage of the d-isomer of α-methylfluorene--2-acetic acid Isolated from Plasma of Male Dogs at Various Times After Intravenous Administration of dl-α-[^{14}C]methylfluorene-2-acetic Acid.

Time after administration (h)	d-isomer present (mean %)
3	61 ± 1.3
6	69 ± 2.7
24	80 ± 2.6
384	100

The percentage of d-isomer present was calculated from sp. rotation; mean values ± S. D. are given. Specific rotation of d-α-methylfluorene-2-acetic acid is + 58°.

Table 3: Percentage of the d-isomer of α-methylfluorene-2-acetic Acid Isolated from Whole Blood of Dogs at Various Times After Administration of l-α-[^{14}C]methylfluorene-2-acetic Acid.

Time after administration (days)	d-isomer present (mean %)
0.3	25.8 ± 0.8
2	70.9 ± 10.3
6	73.2
6.5	86.3 ± 8.5
10.5	87.2 ± 4.8
14	97.2

The percentage of d-isomer present was calculated from the specific rotation; mean values ± S. D. are given. The sp. rotation $[\alpha]_D$ of l-α-methylfluorene-2-acetic acid was -58°.

The fact that IPBP is metabolized (Pathway One) to the pharmacologically active acid 2-(4-biphenylyl)propionic acid raises several questions. Is the propionic acid optically active? The ability of enzymes to differentiate between enantiotopic and diastereotopic groups is well-documented.[37] Therefore, if the acid is optically active, is the activity due to the enzymatic inversion of a racemic modification of the acid or is it due to the enzymatic non-equivalence of the two methyl groups of IPBP? The ability of enzymes to distinguish between chemically-like paired groups can be studied by the use of stable isotope labelling.[37]

The purpose of Section B in Chapter Five will be to report the synthesis of a labelled variant of IPBP to be used in the study of the <u>in vivo</u> oxidative metabolism of IPBP to the arylpropionic acid. A portion of Section B will also be devoted to projected further experiments.

RESULTS AND DISCUSSION

Before proceeding with any synthesis, it was necessary to determine if the acid formed was optically active. The IPBP was administered to rats and then their plasma collected. The 2-(4-biphenylyl)propionic acid was extracted from the acidified plasma. From sixteen rats given 1.2 g of IPBP was isolated 2 mg of the acid 11a with an $[\alpha]_D^{20}$ +55 (MeOH).[38] The configuration of the positive enantiomer of 2-(4-biphenylyl)propionic acid 11a was shown to be (S) (Fig. 29) by comparison of its cd with the cd of S-(+)-hydratropic acid.[39]

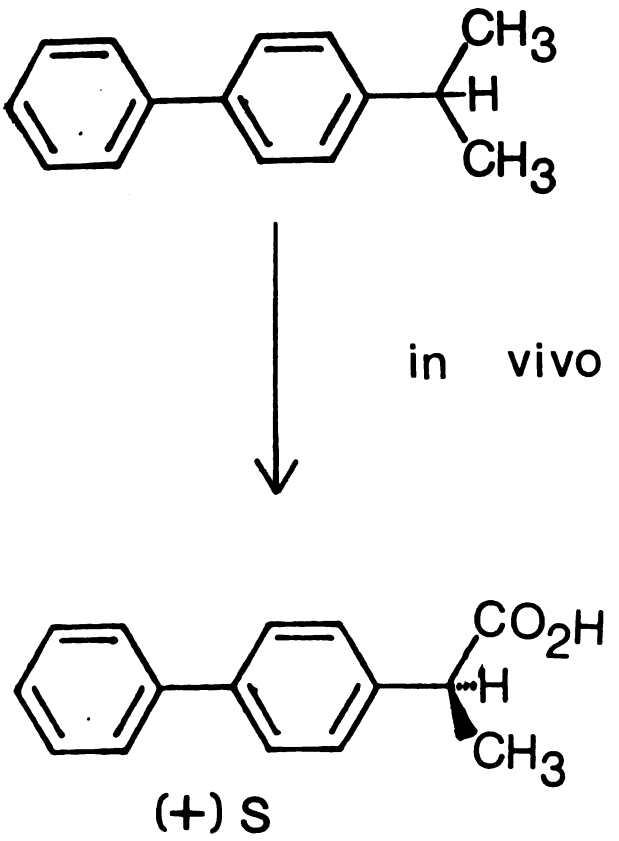


Figure 29

This result was not unexpected from the reports discussed in the introduction. The question was at which step in the metabolic process did the optical activity arise. There are four different steps or combinations thereof where the stereospecificity may have occurred (Fig. 30).

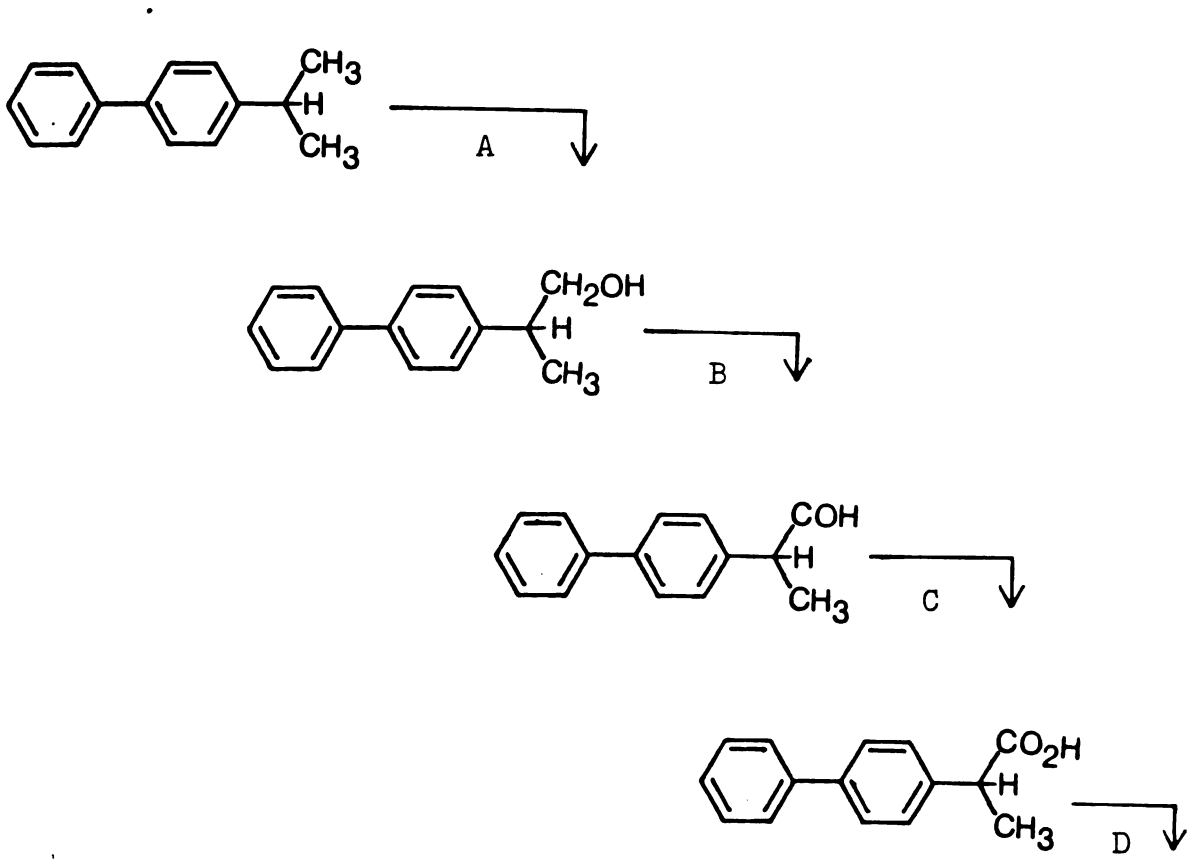


Figure 30

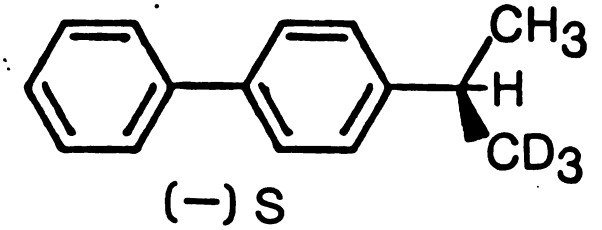
A. The initial hydroxylation at carbon.

B. The stereospecific oxidation of only one enantiomer of the primary carbinols by an alcohol dehydrogenase.

C. The stereospecific oxidation of only one enantiomer of the aldehydes by aldehyde dehydrogenase.

D. The selective epimerization of the R acid to S acid.

In order to determine at which step or steps the stereospecificity arose, it was decided to synthesize an optically active analog of the hydrocarbon IPBP. Since in the final acid, the pro-S methyl group of IPBP was oxidized, the (R)-2-(4-biphenylyl)propane-1,1,1-d_3 was the prime candidate for synthesis (Fig. 31).



5f

Figure 31

If the initial step A is the hydroxylation of the pro-S methyl group and the steps B and C are substrate stereospecific (one isomer only will serve as substrate) and that one isomer is the product of the preceding enyzmatic oxidation, then only the acid S-(+)-2-(4-biphenylyl) propionic acid-1,1,1-d_3 will be formed (Fig. 32).

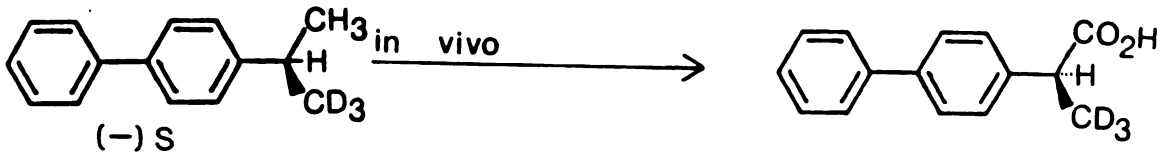
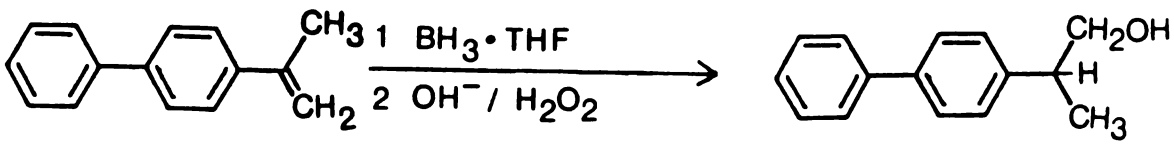


Figure 32

Should any of the steps A, B, or C not be stereospecific or should step D be invoked, due to the formation of the R enantiomer, the acid formed would have less than three deuterium atoms.

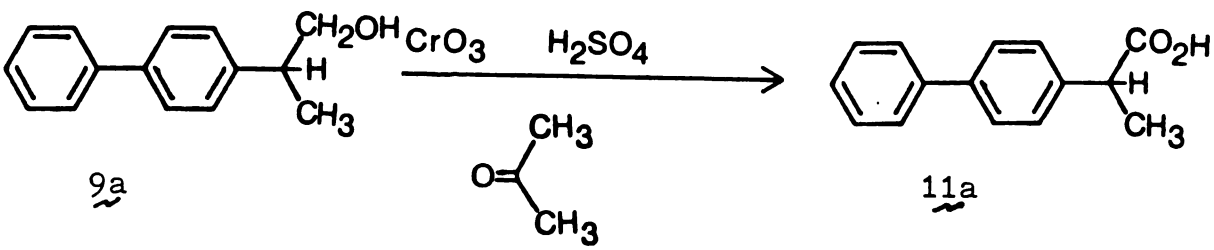
The starting material for the optically active hydrocarbon 5f was the R-(-)-2-(4-biphenylyl)propionic acid.[40] The R(-) acid was synthesized first by the hydroboration of styrene 4a (oxidative workup)[22] to give the primary alcohol 9a (Fig. 33).



4a 9a

Figure 33

The alcohol 9a was oxidized to the racemic acid 11a via a Jones oxidation with chromium trioxide[41] (Fig. 34).



9a 11a

Figure 34

The acid 11a was resolved by repeated recrystallization of its diastereomeric (-)-α-methylbenzylamine salt[39] (Fig. 35).

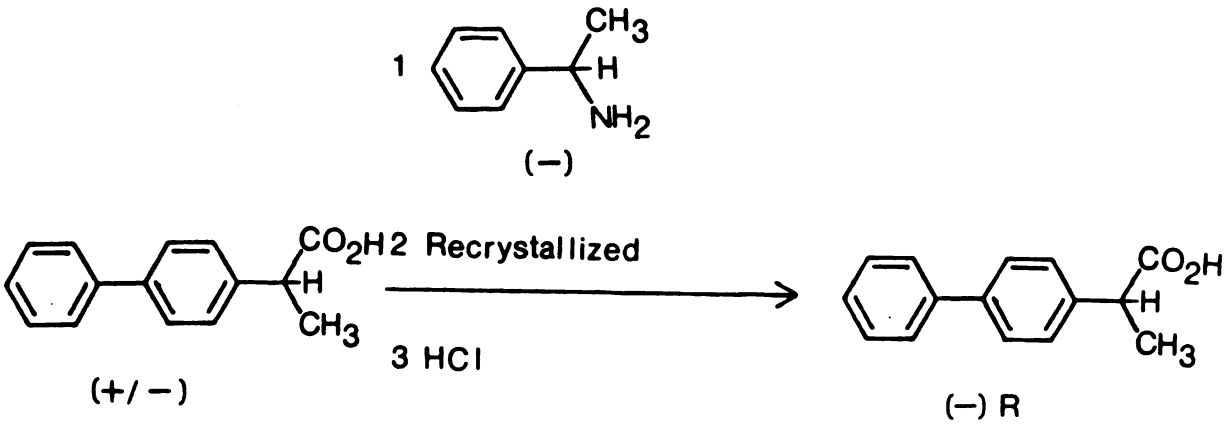


Figure 35

After liberation from the salt, the configuration was confirmed to be R by comparison of its ord and cd with (R)-hydratropic acid. The (R)-11a was reduced with lithium tetradeuteroaluminate to the primary carbinol 9b[42] (Fig. 36).

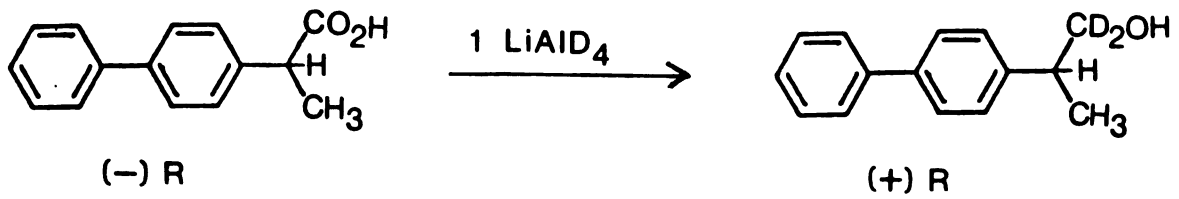


Figure 36

The sign of the rotation ($[\alpha]_D$) of the alcohol (R)-9b changed from the -51° for the acid to a +17° for the alcohol; however, this phenomenon has been previously reported in similar systems.[42]

The alcohol (R)-9b was tosylated and reduced with lithium tetradeuteroaluminate or super deuteride (lithium triethylborodeuteride) to give the R-(-) hydrocarbon 5f whose sign and magnitude of rotation is in agreement with analogous systems[43] (Fig. 37).

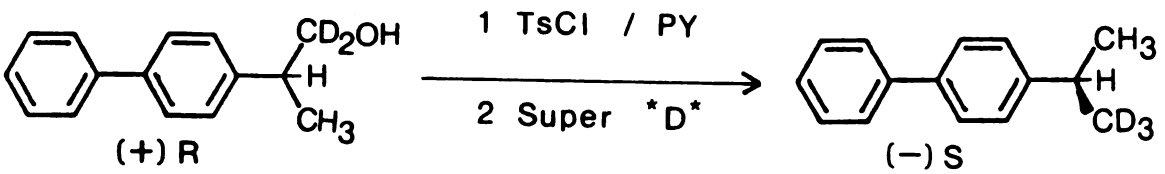


Figure 37

The optical properties of this hydrocarbon will be further studied by use of Raman CID,[44] in conjunction with the U.S.D.A.

Since large amounts of this hydrocarbon would be needed to do the in vivo testing, and since there are no in vitro systems available, this project was terminated with the synthesis of the hydrocarbon 5f.

FUTURE PROJECTS

If the *in vivo* oxidation of 5f gave inconclusive results, the following experiments could be carried out to determine which step or steps were not stereospecific. Each step must be examined individually by synthesizing and resolving (asymmetric synthesis or conversion of an asymmetric precursor) the substrates for that step. Each enantiomer could be tested to determine if it serves as a substrate for the enzymic oxidation under study. The results would have more validity if both enantiomers were simultaneously incubated with the enzyme system. The ideal procedure would therefore be the synthesis of a quasi-racemate. A quasi-racemate is a mixture of one enantiomer plus its isotopically labelled antipode. The mixture of (S)-2-(4-biphenylyl)propan-1-ol-1-^{14}C and (R)-2-(4-biphenylyl)propan-1-ol or *vice versa* would constitute a quasi-racemate (Fig. 38).

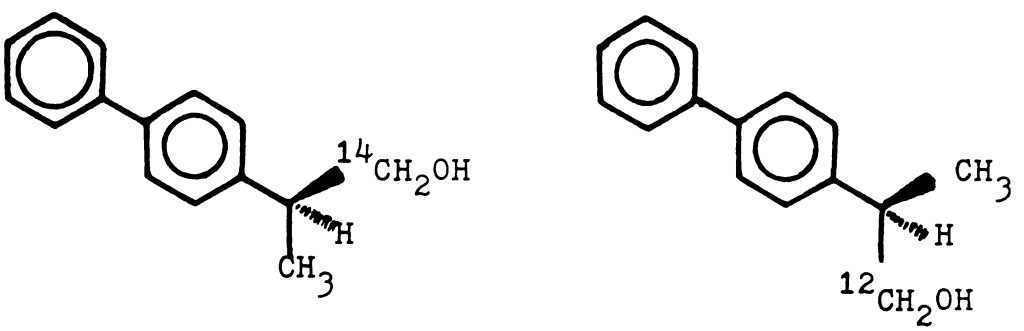


Figure 38

This racemate ((S)-^{14}C- and (R)-^{12}C) could be incubated with the alcohol dehydrogenase. If the product (aldehyde) is radioactive, then the (R)-alcohol (^{14}C) was the preferred substrate or if the product is non-radioactive, the (S)-alcohol was the substrate (Fig. 39).

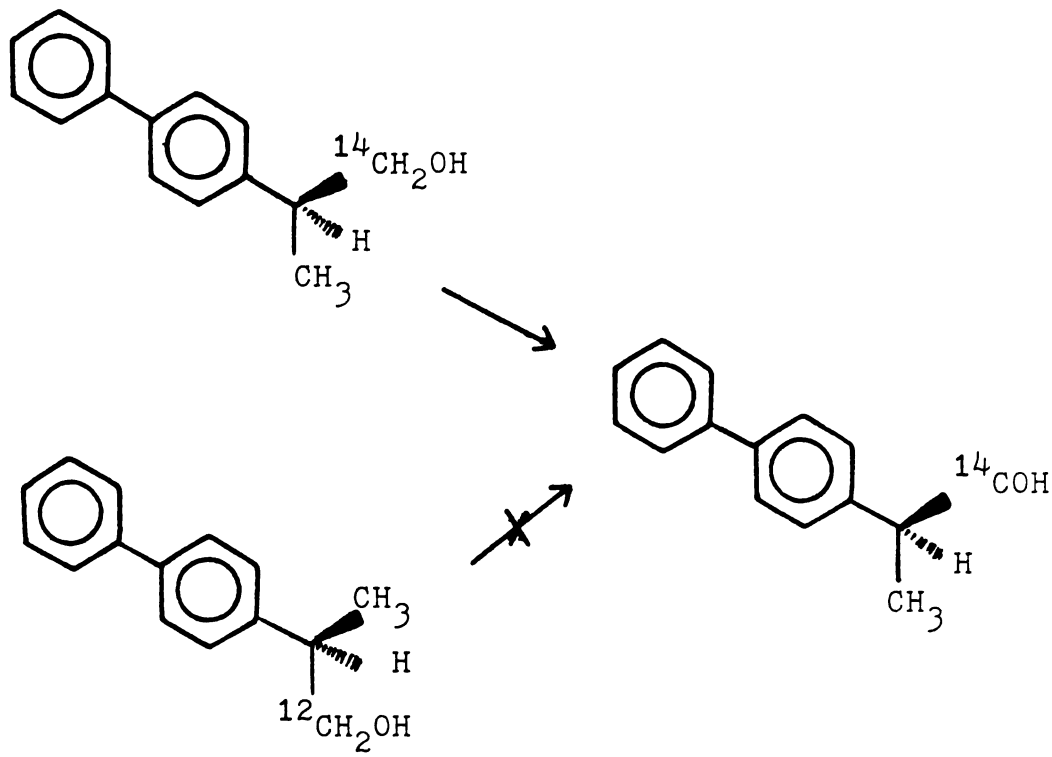


Figure 39

The same technique could be used for steps B and C. The feasibility of the inversion (epimerization) of the (R)-acid to the (S)-acid could be determined by the synthesis of (R)-2-(4-biphenylyl)propionic acid-1,1,1,4-d_4. If, after incubation, the (S)-acid were isolated and contained

only two deuteriums, then the mechanism of Wechter would be further substantiated (Fig. 40).

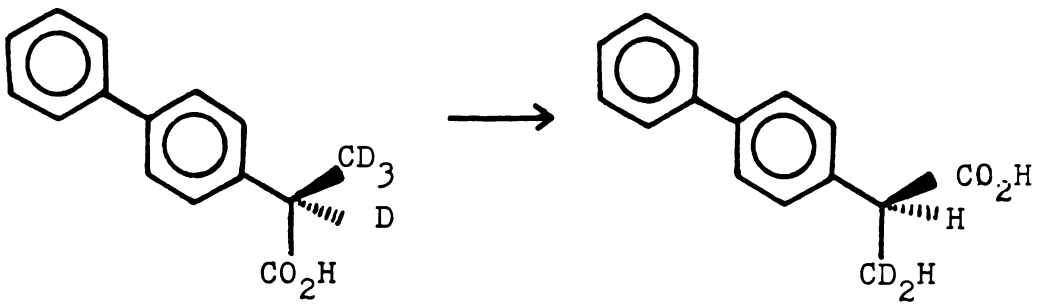
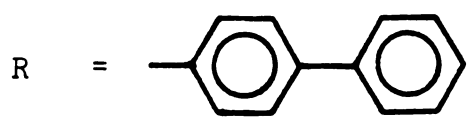
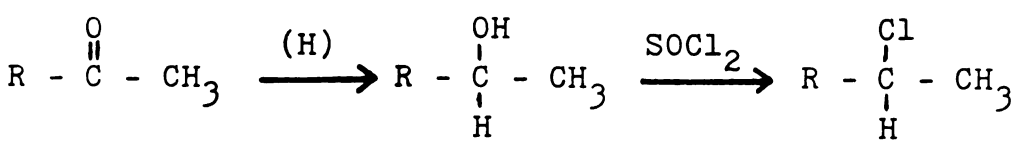


Figure 40

The compounds needed for the above experiments could be synthesized as projected in the following figures.





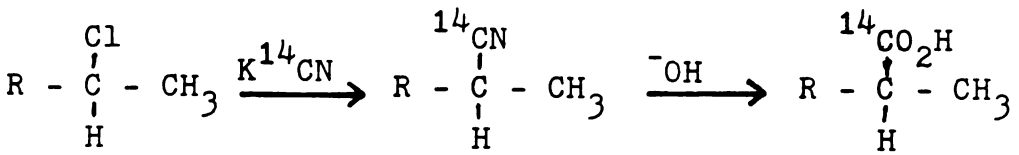
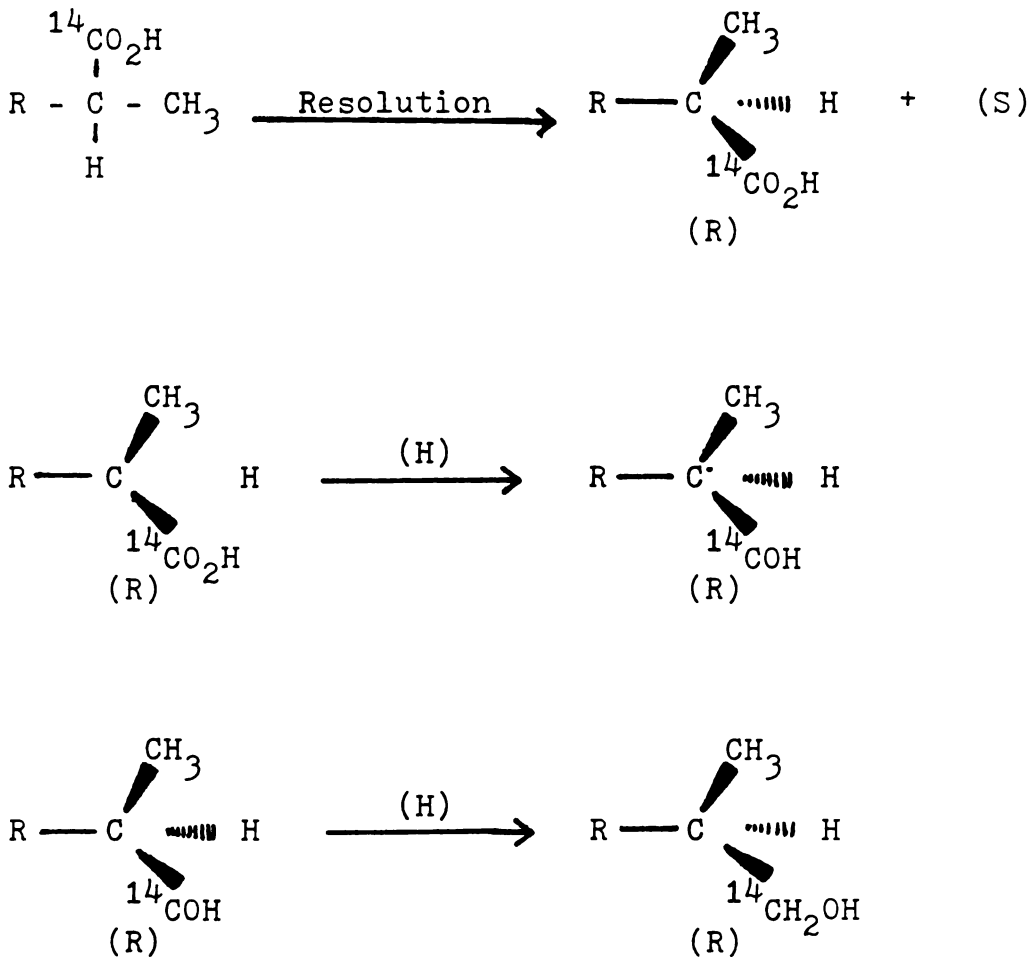


Figure 41



The syntheses of the (S)-^{14}C, the (S)-^{12}C, and the (R)-^{12}C derivatives will be carried out as above.

Figure 41 continued

EXPERIMENTAL

Melting points, determined with a Thomas-Hoover Uni-Melt melting point apparatus, are uncorrected. Nmr spectra at 60 MHz were determined with a Varian A-60A instrument unless otherwise stated, and 100-MHz nmr spectra were recorded on a Varian XL-100-15 instrument operating in the Fourier transform mode using an internal deuterium lock. Nmr chemical shift values are expressed in δ units (parts per million) relative to TMS in organic solvents and sodium 2,2-dimethyl-2-silapentane-5-sulfate in D_2O. For the presentation of nmr spectra, the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, compm = complex multiplet. Infrared (ir) spectra were recorded on a Perkin-Elmer 337 spectrometer. The electron impact mass spectra (eims) were obtained on an AEI MS-12 instrument at 70 eV, and the chemical ionization mass spectra (cims) were obtained on an AEI MS-902 instrument modified for chemical ionization using isobutane as the reagent gas. Glc analyses were performed on a Varian Aerograph Model 2100 gas chromatograph with a 6 ft. U-shaped Pyrex column packed with 3% SE-30 on Chromosorb W. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley. Thin layer chromatography (tlc) was carried out on pre-coated silica gel sheets.

4-Acetylbiphenyl (2a).

The method of Ferriss and Turner[45] was used to prepare the compound 2a in 90% yield, mp 115-117° (lit.[45] 116-118°); tlc (uv) R_f (D:hexane) = 0.04; nmr ($CDCl_3$) δ 2.6 (s, 3H, $COCH_3$), 7.7 (m, 9H, aromatic H).

4-Acetylbiphenyl-1',1',1'-d_3 (2b).

To 1.0 g of the ketone 2a in 30 ml of glyme was added 2 ml of D_2O containing 100 mg of $NaOCH_3$. The mixture was heated at reflux for two days under nitrogen. Upon three successive exchanges, the nmr showed complete deuteration of the methyl group, mp 116-117° (mixed melting point with undeuterated 2a was undepressed); tlc (uv) R_f (D:hexane) = 0.04; nmr ($CDCl_3$) δ 7.7 (m, 9H, aromatic H); eims: 200 (5.0), 199 (35.1), 198 (14.6), 197 (2.5), 181 (100), 153 (29.8), 152 (43.6).

2-(4-Biphenylyl)-2-propanol (3a).

(A). General method from ketone. A solution of 49 g (0.25 mol) of the ketone 2a dissolved in 300 ml of Et_2O and 300 ml of benzene, was added dropwise, with stirring, to 250 ml of a commercial 2.2 molar solution of methyl magnesium bromide which had been diluted with an equal volume of Et_2O, at such a rate that the reaction refluxed gently. After the addition was completed, the reaction was heated at reflux overnight.

The reaction was then allowed to cool to room temp-

erature, then to ≈ 5° in an ice-salt bath, and the excess Grignard reagent was decomposed by the dropwise addition of 100 ml of sat NH_4Cl solution. The organic layer was decanted from the resulting organic residue and poured into ice water. The organic layer was then separated, washed with dilute sodium bicarbonate solution and water, and dried over sodium sulfate. Evaporation of the solvents *in vacuo* left a white solid residue which was subjected to column chromatography on silica gel (100 g), eluting with hexane to remove any styrene and then Et_2O to remove the alcohol. The alcohol **3a** was recrystallized from hexane to yield 40 g (0.19 mol, 76%), mp 89-90° (lit.[20] 88.5-90.5°); tlc (uv) R_f ($D:CHCl_3$) = 0.28; nmr ($CDCl_3$) δ 1.6 (s, 6H, $-(C\underline{H}_3)_2$), 1.95 (s, 1H, -O<u>H</u>), 7.5 (m, 9H, aromatic <u>H</u>).

<u>Anal</u>. Calcd for $C_{15}H_{16}O$: C, 84.87; H, 7.60. Found: C, 84.84; H, 7.53.

(B). General method from bromo compound. A solution of acetone (15 g, 259 mmol) in 25 ml of THF was added during 1 h to the Grignard reagent prepared from 4-bromobiphenyl (40 g, 171 mmol) and Mg (4.1 g, 171 g-atoms) in 100 ml THF. After an additional hour of stirring, the mixture was heated at reflux for 3 h. The mixture was cooled in an ice-salt bath to ≈ 3° and 100 ml of a sat NH_4Cl solution was added slowly. The THF layer was decanted into ice water. The NH_4Cl solution was extracted with

Et_2O and the organic layers combined, washed with sat $NaHCO_3$ solution, washed with a sat NaCl solution, and dried over $MgSO_4$. The solvents were removed under vacuum to leave 22 g (104 mmol, 61%) of the alcohol 3a as a white solid. The solid was purified as above. A mixed melting point of the products from (A) and (B) was not depressed and other physical properties were identical.

2-(4-(Pentadeuterophenyl)phenyl)propan-2-ol (3b).

Using general method (B) described for 3a, 9.5 g (40 mmol) of the bromobiphenyl (7b) in 50 ml of THF was added to 0.9579 g (39.5 mg-atom) of magnesium turnings and then 58 g (1mol) of acetone was added to give 6.9 g of 3b (32.6 mmol, 81%), mp 88-89.5° (lit.[20] 88.5-90.5°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (uv) R_f (D:$CHCl_3$) = 0.28; nmr ($CDCl_3$) δ 1.6 (s, 6H, $-(C\underline{H}_3)_2$), 1.95 (s, 1H, -O<u>H</u>), 7.2-7.7 (A_2B_2, 6H, <u>J</u>= 8.8 Hz, aromatic <u>H</u>); eims: 218 (1.3), 217 (7.5), 216 (0.3), 202 (22.6), 199 (44), 184 (17.7).

2-(4-Biphenylyl)propan-2-ol-1,1,1,3,3,3-d_6 (3c).

A solution of acetone-d_6 (10 ml, 7.91 g, 130 mmol) in 25 ml of THF was added during 1 h to the Grignard reagent prepared from 4-bromobiphenyl (10 g, 42.5 mmol) and Mg (1.043 g, 42.5 mg-atom) in 50 ml THF as described for 3a general method (B) to give 7.1 g of 3c (32.5 mmol, 77%), mp 89-90° (lit.[20] 88.5-90.5°). A mixed melting

point with the undeuterated compound was not depressed; tlc (uv) R_f (D:$CHCl_3$) = 0.28;nmr ($CDCl_3$) δ 2.1 (s, 1H, O<u>H</u>), 7.5 (m, 9H, aromatic <u>H</u>); eims: 219 (2.9), 218 (16), 217 (0.3), 200 (36.8), 90 (76.1), 89 (22.3), 62 (26.5), 46 (100).

2-(4-Biphenylyl)-prop-1-ene (<u>4a</u>).

(A). General method from alcohol. A suspension of 21.2 g (0.1 mol) of the alcohol <u>3a</u> in 50 ml of 4 <u>N</u> H_2SO_4 and 50 ml of EtOH was heated at reflux and stirred vigorously for 1.5 h. Then 50 ml of EtOH was added, and the reaction was heated at reflux with stirring for an additional 2.5 h. After being cooled to room temperature, the reaction mixture was poured into ice water. The white solid was filtered and the aqueous filtrate extracted with ether, benzene, and chloroform. The organic extracts and solid were combined, washed with $NaHCO_3$ and water, and dried over $MgSO_4$. After evaporation of the solvents <u>in vacuo</u>, the residue was subjected to chromatography on silica gel, eluting with hexane. The styrene <u>4a</u> was recrystallized from hexane to yield 9 g (0.046 mol, 46.4%), mp 115-117° (lit.[20] 116-118°); tlc (uv) R_f (D:hexane) = 0.33; nmr ($CDCl_3$) δ 2.25 (m, 3H, -C<u>H</u>$_3$), 5.2 (m, 1H, vinylic <u>H</u>), 5.6 (m, 1H, vinylic <u>H</u>), 7.6 (m, 9H, aromatic <u>H</u>).

<u>Anal</u>. Calcd. for $C_{15}H_{14}$: C, 92.74; H, 7.26. Found: C, 94.45; H, 6.98.

(B). General method from alcohol. A suspension of

21.2 g (0.10 mol) of the alcohol 3a in 200 ml of freshly distilled Ac_2O was stirred for 1 h. The mixture was slowly heated until solution occurred (ca 50°) and then stirred at this temperature until no alcohol remained as analyzed by tlc. After 5 h the mixture was poured into 1000 ml of ice water. The white solid was filtered and washed with copious amounts of cold water. The solid was dissolved in Et_2O and washed with $NaHCO_3$, water, and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the styrene 4a was recrystallized from hexane to yield 17 g (0.0876 mol, 87.6%). All physical properties were identical to the product from procedure (A).

2-(4-Biphenylyl)prop-2-ene-1,1,3,3,3-d_5 (4b).

The alcohol 3c was dehydrated as previously described for the styrene 4a by general method (B) to give an 85% yield of 4b, mp 116-118° (lit.[20] 116-118°); a mixed melting point with non-deuterated compound was not depressed; tlc (uv) R_f (D:hexane) = 0.33; nmr ($CDCl_3$) δ 7.6 (m, 9H, aromatic H); eims: 200 (15), 199 (100), 198 (13.7), 182 (45), 180 (30), 84 (32), 49 (45).

2-(4-Biphenylyl)-propane (5a).

(A). General method from alcohol. A mixture of 5 g (23.5 mmol) of the alcohol 3a, 0.5 g of Adam's catalyst (PtO), and 200 ml of ethyl acetate was hydrogenated until the calculated amount of hydrogen had been absorbed. After

the catalyst was separated by filtration through filter aid, the reaction mixture was evaporated under vacuum to leave an oily residue which was distilled to yield 4.3 g (22 mmol, 94%) of the hydrocarbon 5a, bp 83-85°/0.015 mm (lit.[20] 100-112°/0.09 mm); tlc (uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 1.35 (d, 6H, J= 7 Hz, $-(CH_3)_2$), 2.85 (m, 1H, J= 7 Hz, -C-H), 7.53 (m, 9H, aromatic H).

Anal. Calcd. for $C_{15}H_{16}$: C, 91.78; H, 8.22. Found: C, 91.96; H, 7.97.

(B). General method from styrene. A solution of 5 g (25.5 mmol) of the styrene 4a and 1 g 10% Pd/carbon in 250 ml of EtOH was hydrogenated until the theoretical amount of hydrogen had been absorbed. The solution was filtered and the solvent removed under vacuum to leave 4.9 g (25.0 mmol, 98%) of the hydrocarbon 5a as an oil. The oil was chromatographed on silica gel with hexane as eluting solvent, the hexane removed and the residue distilled by bulb-to-bulb. The analytical data were consistent with those described in (A).

(C). General method from styrene. A solution of 10 g (51 mmol) of the styrene 4a in 50 ml of THF was hydroborated by the slow addition of 20 ml of a 1 M BH_3·THF (20 mmol (60 mmol in H)) over a period of 15 min. The mixture was heated at reflux for 3 h and allowed to stand overnight at ≈ 20° (R.T.). After the careful addition of 5 ml of H_2O, 20 ml of propionic acid (272 mmol) was added,

the solution heated at reflux for 5 days. The pH of the solution was adjusted to ≈ 7 by the addition of 1 N NaOH and the aqueous phase extracted with hexane. After removal of the hexane, the residue oil (5a) was further purified as described above to yield 3 g (15 mmol, 30%) and analytical data were consistent with those described for (A).

(D). General method from alcohol. To a stirred solution of 0.4246 g (2 mmol) of alcohol 3a and 0.2674 g (2.3 mmol) of triethylsilane in 8 ml of dry CH_2Cl_2 cooled in a dry-ice-hexane bath was added very slowly 2 ml of trifluoroacetic acid (TFA) in 2 ml of CH_2Cl_2 (cooled to ≈ -10°). The reaction was monitored via tlc and was complete almost immediately but stirring was continued at room temperature for 16 h. Solid Na_2CO_3 was cautiously added to neutralize excess TFA and to dry the CH_2Cl_2 solution. The solid was filtered and the filtrate was reduced under vacuum to give 0.387 g (1.97 mmol, 98.7%) of 5a as an oil. The oil was purified as described for (A) and the analytical data were in agreement.

2-(4-Biphenylyl)propane-2-d_1 (5b).

(A). From styrene 4a. The general method (C) described for 5a was modified as follows: 2 g (10.3 mmol) styrene 4a in 20 ml THF was deuteroborated with 15 ml of 0.88 M (13.2 mmol, 39.6 mmol in D) of $BD_3 \cdot THF$ (95% d_3, Alfa-Ventron). The reaction was quenched with 5 ml D_2O and heated at reflux for 5 days with propionic acid. The reaction

was worked up as previously described to give 0.5 g (2.5 mmol, 24.7%) of 5b; nmr ($CDCl_3$) δ 1.38 (broad s, 6H, $-(C\underline{H}_3)_2$), 7.4 (m, 9H, aromatic H); eims: 198 (6.4), 197 (38.3), 196 (2.6), 182 (100), 167 (19.9), 166 (27.9), 165 (16.7), 152 (19.4).

(B). From alcohol 3a. A solution of 1 g (4.71 mmol) of 3a and 0.8 g (6.8 mmol) triethylsilane-d_1 (96%) in 10 ml of CH_2Cl_2 was cooled and treated with 5 ml of cold trifluoroacetic acid in 5 ml of CH_2Cl_2 as described in general method (D) for 5a to yield 0.877 g (4.45 mmol, 94.5%); nmr ($CDCl_3$) δ 1.38 (broad s, 6H, $-(C\underline{H}_3)_2$), 7.4 (m, 9H, aromatic H); eims: 198 (6.4), 197 (38.3), 196 (2.6), 182 (100), 167 (19.9), 166 (27.9), 165 (16.7), 152 (19.4).

2-(4-(Pentadeuterophenyl)phenyl)propane (5c).

To a stirred solution of 7 g (51.7 mmol) of p-isopropylaniline (8) in 200 ml of benzene-d_6 (99+ atom %d) was added 7.5 ml (6.5 g, 56 mmol) of freshly distilled isoamyl nitrite. The reaction mixture was warmed until a vigorous reaction with evolution of gas ensued. The reaction was allowed to proceed without heating until the exothermic reaction subsided (30 min) and the mixture was heated at reflux for an additional 2 h. The excess benzene and the low-boiling products were removed under reduced pressure and the black residue was subjected to chromatography on silica gel (300 g), eluting with hexane. The solvent

was removed under vacuum to give 7 g (34.8 mmol, 67%) of the hydrocarbon 5c as an oil, bp 85°/0.015 mm (lit.[20] bp 100-112°/0.09 mm); tlc (uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 1.2 (d, 6H, J= 7 Hz, -C-(CH_3)$_2$), 2.9 (m, 1H, J= 7 Hz, -C-H), 7.4 (AB quartet, 4H, J= 7.5 Hz, aromatic H); eims: 202 (7.9), 201 (47.8), 200 (2.3), 199 (0.9), 186 (100), 170 (13.4), 169 (12.8), 32 (16.1), 28 (96).

2-(4-(Pentadeuterophenyl)phenyl)-propane-2-d_1 (5d).

A cold solution (ca -20°) of 1.9078 (8.79 mmol of the alcohol 3b and 1.2 g of triethylsilane-d (96% d_1) (10.25 mmol) in 15 ml of CH_2Cl_2 was treated with 10 ml trifluoroacetic acid in 15 ml of CH_2Cl_2 as described for general method (D) in 5a to yield 1.5 g (7.4 mmol, 84.5%) of the hydrocarbon 5d, bp 83-85°/0.015 mm (lit.[20] 100-112°/0.09 mm); tlc (uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 1.3 (broad s, 6H, -(CH_3)$_2$), 7.45 (A_2B_2, 4H, J= 8.5 Hz, aromatic H); eims: 203 (8.8), 202 (51), 201 (4.6), 200 (1.3), 188 (16), 187 (100), 170 (15.5).

2-(4-Biphenylyl)propane-1,1,1,3,3,3-d_6 (5e).

The alcohol 3c (2.1913 g, 10.05 mmol) and 1.5 g (12.9 mmol) triethylsilane in 25 ml of CH_2Cl_2 was cooled and stirred while 10 ml (129.8 mmol) of trifluoroacetic acid -O-d in 15 ml of CH_2Cl_2 was added dropwise. The reaction was worked up as previously described in general method (D) for 5a to give the hydrocarbon 5e (1.5 g, 7.4 mmol), bp 83-85°/0.015 mm (lit.[20] 100-112°/0.09 mm); tlc

(uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 2.84 (broad s, 1H, methine H), 7.51 (m, 9H, aromatic H); eims: 203 (8.1), 202 (49.5), 201 (2.7), 185 (15.5), 184 (100), 168 (21.5), 167 (13.3).

4-Bromobiphenyl-d_5 (7b).

Using the procedure described for 5a, 6 ml (5.25 g, 45 mmol) of isoamylnitrite was added to a solution of 5 g (36.9 mmol) of 4-bromoaniline (6) in 200 ml benzene-d_6 to give 6 g of 7b (25 mmol, 56%), mp 89-90° (lit.[46] 89-91°). A mixed melting point with the authentic undeuterated sample was not depressed; tlc (uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 7.5 (AB quartet, 4H, J= 3 Hz, aromatic H); eims: 240 (12.6), 239 (97.9), 238 (16.6), 237 (100), 236 (3.2), 157 (48.3), 156 (46.5).

2-(4-Biphenylyl)propan-1-ol (9a).

A 500 ml three-necked flask equipped with a condenser, a pressure-equalizing dropping funnel, and a mechanical stirrer was assembled. The top of the condenser led to a mercury bubbler. The top of the funnel was fitted with a rubber stopper to permit introduction of materials with the aid of a hypodermic syringe. In a dried,flamed, and cooled flask was placed 3.1 g (0.080 mol) of sodium borohydride. A hypodermic needle was inserted into a rubber septum at the top of the dropping funnel and a stream of dry nitrogen introduced to flush the apparatus. A static nitrogen pressure was maintained through the

oxidation stage. The reagents were then introduced into the dropping funnel and then into the flask with the aid of suitable syringes. The styrene 4a, 58.2 g (0.300 mol) was introduced in 100 ml of dry THF. The flask was immersed in a water bath (20-25°), and hydroboration initiated by the dropwise addition of 14.0 ml (0.11 mol) of borotrifluoride etherate to a well-stirred suspension over a period of 1 h, while the temperature was maintained for an additional hour at 25°. Then 20 ml of H_2O was added to destroy residual hydride. The organoborane was oxidized at 30-40° (water bath) by the addition of 33 ml of a 3 M NaOH solution, followed by the careful dropwise addition of 33 ml 30% H_2O_2. Solid NaCl was added to saturate the aqueous phase. The upper THF layer was separated, washed with sat NaCl, and dried over Na_2SO_4. The THF was removed under vacuum to leave 60 g (96%) of 9a as a white solid, mp 58-60°; tlc (uv) R_f ($D:CHCl_3$) = 0.28; nmr ($CDCl_3$) δ 1.3 (d, 3H, J= 7 Hz, -C-CH_3), 1.75 (broad s, 1H, O<u>H</u>), 3.1 (m, 1H, J= 7Hz, -C-H), 3.74 (d, 2H, J= 7 Hz, -CH_2-O), 7.4 (m, 9H, aromatic H).

(±)-2-(4-Biphenylyl)propionic acid (11a).

A chromic acid solution (Jones' Reagent), (prepared from 25 g (0.70 mol) of chromium trioxide in 85 ml cold H_2O to which 20 ml conc H_2SO_4 is added dropwise with stirring and diluted with H_2O to 150 ml) was added to a stirred solution of the alcohol 9a (30 g, 0.14 mol) in

200 ml of acetone over a period of 30 min, maintaining the temperature at 25-30°. The progress of the reaction was monitored by the color change of the Jones reagent from red to green. The addition of chromic acid was stopped when the red color persisted. The stirring was continued for 2 h. The yellow acetone layer was separated from the aqueous green chromium salts and the salts extracted twice with 100 ml Et_2O. The organic layers were combined and washed with sat $NaHSO_3$, sat NaCl, and dried over $MgSO_4$. The solvents were removed in vacuum to give a tacky solid which was dissolved in 1 N NaOH (sodium salt very insoluble). The basic aqueous phase was extracted with ether. The aqueous layer was acidified and filtered to give 14 g (44%) of the acid 11a, mp 155-157° (lit.[40] 159-160°); tlc (uv) R_f (D:$CHCl_3$) = 0.04; nmr ($CDCl_3$) δ 1.5 (d, 3H, J= 7 Hz, $-C(C\underline{H}_3)$), 3.8 (quartet, 1H, J= 7Hz, methine H), 7.57 (m, 9H, aromatic H), 8.1 (broad s, 1H, $-COO\underline{H}$).

Resolution of 2-(4-Biphenylyl)propionic acid (11a).

To a hot solution of 18.5 g (0.08 mol) of the racemic acid 11a in 100 ml of benzene:ethanol (4:1) was added 1-(-)-α-methylbenzylamine ($[\alpha]_D^{20}$ -39° neat) in 100 ml of the solvent system. The reaction mixture was heated to 70° and allowed to cool slowly and undisturbed for 2 days. The precipitate was filtered off, washed with a small amount of cold benzene, dried, and weighed. A small

amount (ca 0.49 g) of the salt was set apart, the acid liberated with dilute sulfuric acid, extracted with Et_2O and isolated by evaporation of the solvent. The acid was dissolved in 95% EtOH and the optical activity measured in a 1 dm tube. The principle part of the salt was recrystallized from a mixture of benzene and ethanol (4:1) until the optical activity remained constant. The pure (-)-phenethylamine salt of the (-) acid 11a was obtained as colorless needles. The acid 11a was liberated as described above to yield 2 g (25%), mp 155-158° (lit.[40] 159-160°); tlc (uv) R_f (D:$CHCl_3$) = 0.04; nmr ($CDCl_3$) δ 1.5 (d, 3H, J= 7 Hz, -C($C\underline{H}_3$)), 3.8 (quartet, 1H, J= 7 Hz, methine H), 7.57 (m, 9H, aromatic H), 8.1 (broad s, 1H, -COO<u>H</u>); $[\alpha]_D^{20}$ -51° (C 0.63, 95% EtOH). Lit.[40] +55° (C 0.49, EtOH) (no (-) reported). CD (C 0.063, 95% EtOH) $[\theta]_{266}$ 0, $[\theta]_{249}$ 3553, $[\theta]_{247}$ 3080 (tr), $[\theta]_{242}$ 4264, $[\theta]_{232}$ 3080 (tr), $[\theta]_{216}$ 7345.

R-(+)-2-(4-Biphenylyl)propan-1-ol-1,1-d_2 (9b).

To a stirred solution of 0.5 g (12.5 mmol) of lithium tetradeuteroaluminate (98.5% d_4) in 100 ml of dry Et_2O was added 1 g (4.4 mmol) of the acid 11a in 50 ml of Et_2O over a period of 0.5 h, followed by refluxing for 10 h. After cooling, the excess hydride was decomposed with H_2O and 50 ml of 10% H_2SO_4 was added. The ether layer was separated and the aqueous layer extracted with Et_2O. The combined ethereal extracts were washed with 5%

$NaHCO_3$ solution, followed by H_2O, dried over Na_2SO_4, and the ether removed at room temperature, under vacuum, to yield a clear liquid which solidified on standing. The solid was recrystallized from hexane to yield 0.7 g (3.2 mmol, 74%) of the alcohol 9b, mp 58-60° (a mixed melting point with undeuterated 9a was not depressed); tlc (uv) R_f (D:$CHCl_3$) = 0.28; nmr ($CDCl_3$) δ 1.3 (d, 3H, J = 7Hz, -C-CH_3), 3.1 (quartet, 1H, J= 7 Hz, -C-H), 7.4 (m, 9H, aromatic H); eims: 215 (4.6), 214 (27.9), 213 (0.8), 186 (100); $[\alpha]_D^{20}$ +17.2° (C 0.46, 95% EtOH). CD (C 0.09, 95% EtOH) $[\theta]_{285}$ 0, $[\theta]_{268}$ 1759, $[\theta]_{259}$ 765 (tr), $[\theta]_{253}$ 1835.

R-(-)-2-(4-Biphenylyl)propane-1,1,1-d_3 (5f).

The alcohol 9b (1 g, 4.6 mmol) was mixed with 5 ml of anhydrous pyridine and 4-toluenesulfonylchloride (1.1 g, 4.9 mmol) was added. After 2 h needles separated, but stirring was continued for 18 h at room temperature and then at 65° for 15 min. The solution was cooled and poured into 100 ml of ice-cold 5 N HCl. It was extracted with Et_2O. The combined ethereal extracts were washed with H_2O and dried over Na_2SO_4. The Et_2O was removed under vacuum to yield a solid. Tlc indicated no alcohol.

The crude sulfonate was reduced with lithium tetradeuteroaluminate in ether by the usual method. The excess hydride was decomposed with H_2O and dil H_2SO_4, and the ether layer separated. The aqueous solution was extracted with Et_2O. The combined ethereal solution was

washed with 0.1 N NaOH, and then with H_2O. The solution was dried over Na_2SO_4 and the ether distilled off. A light yellow oil was obtained which was purified by chromatography on silica gel with hexane as solvent. The hydrocarbon 5f was obtained in 53% yield, bp 85-87°/0.017 mm (lit.[20] 100-112°/0.09 mm); tlc (uv) R_f (D:hexane) = 0.46; nmr ($CDCl_3$) δ 1.3 (d, 3H, J= 7 Hz, -(CH_3)), 2.85 (quartet, 1H, J= 7 Hz, methine H), 7.50 (m, 9H, aromatic H); eims: 200 (13.6), 199 (80), 198 (6.2), 197 (5.4), 184 (97.6), 181 (100); $[\alpha]_D^{20}$ -0.54° (C 3.726 95% EtOH).

Triethyldeuterosilane-d_1[25]

To a stirred mixture of lithium aluminum deuteride (96% d, 1.0 g, 23 mmol) in 60 ml of Et_2O contained in a three-necked flask, equipped with a water condenser, addition funnel, and drying tube, was added triethylchlorosilane (12.3 g, 82 mmol) dropwise over a 30 min period. After the addition was complete the mixture was heated at reflux for 2 h. Ether and 5 g of ice were slowly added to the reaction mixture followed by 50 ml of a cold 25% aqueous H_2SO_4 solution. The Et_2O layer was separated, and the aqueous solution was washed with two 20 ml portions of Et_2O. The combined Et_2O solution was washed with 20 ml portions of 25% aqueous H_2SO_4, 10% NaOH, and water, and then dried over $MgSO_4$. Fractional distillation through a 10 cm Vigreux column gave 7.2 g (61.5 mmol, 75%) of tri-

ethyldeuterosilane, bp 105-106.5°; ir (film) 1550 cm^{-1} (Si-D), no Si-H adsorption observed at 2100 cm^{-1}; nmr ($CHCl_3$) δ 1.0 (m, 6H, $-C\underline{H}_2-$), 0.62 (m, 9H, $-C\underline{H}_3$).

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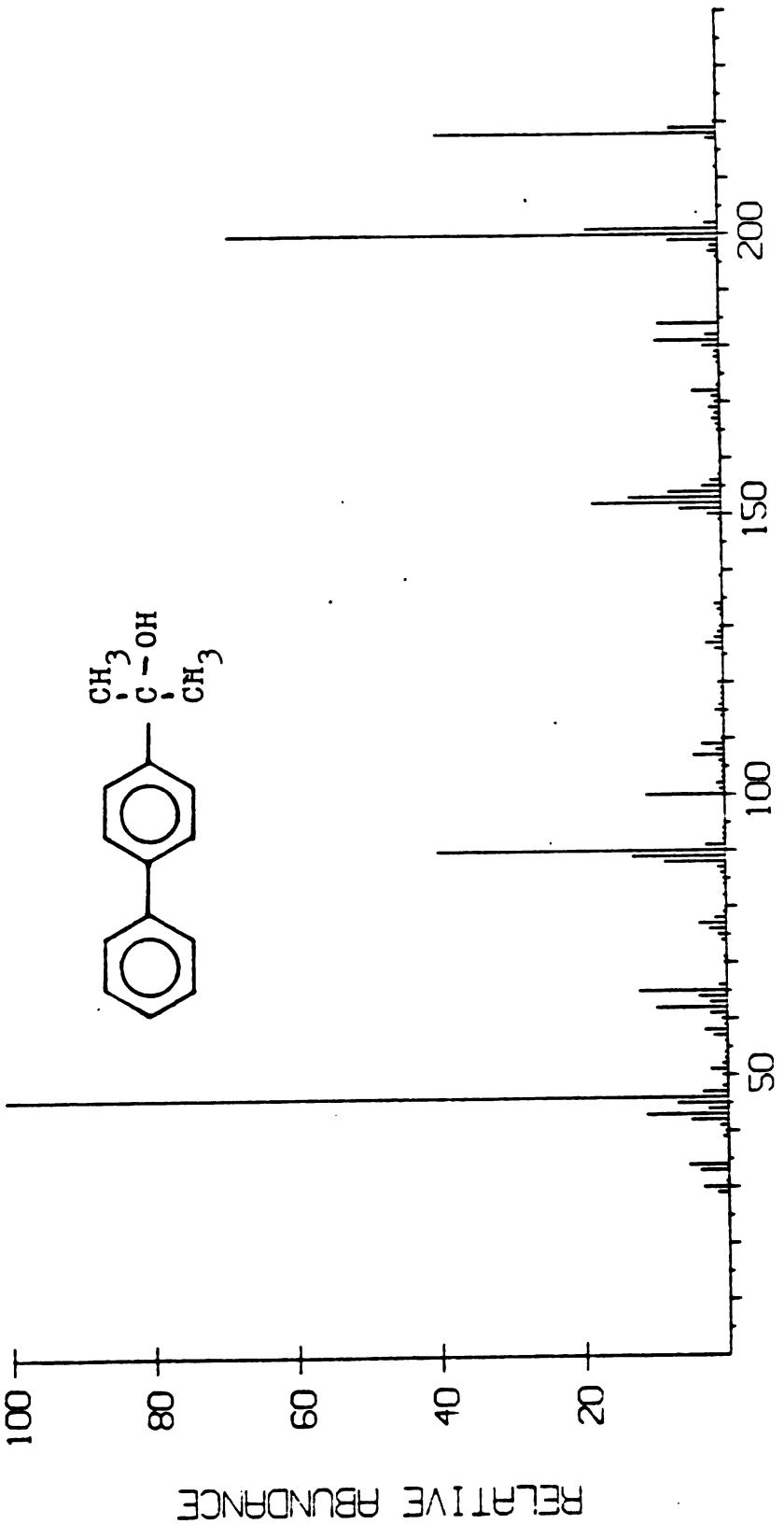
APPENDIX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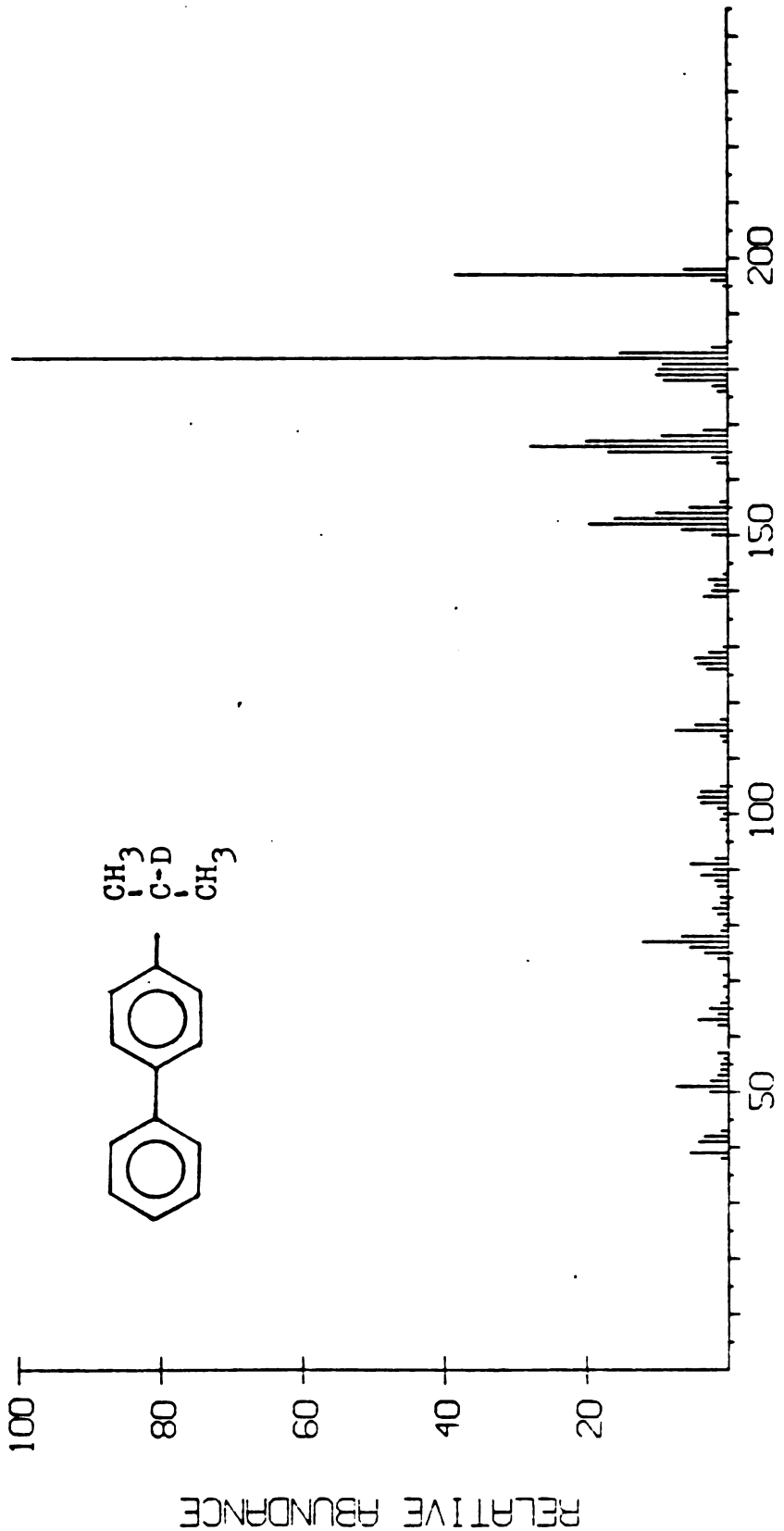
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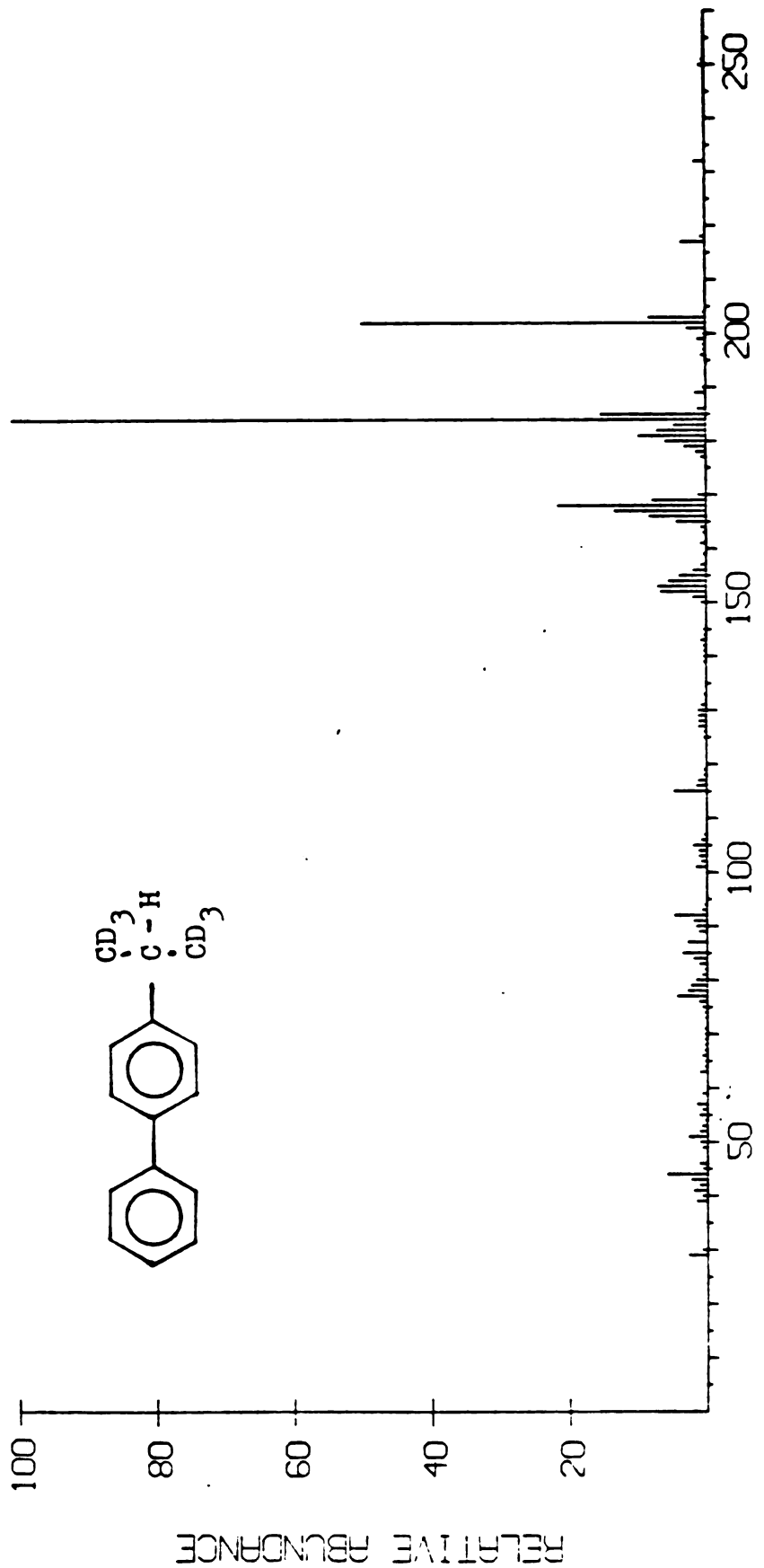
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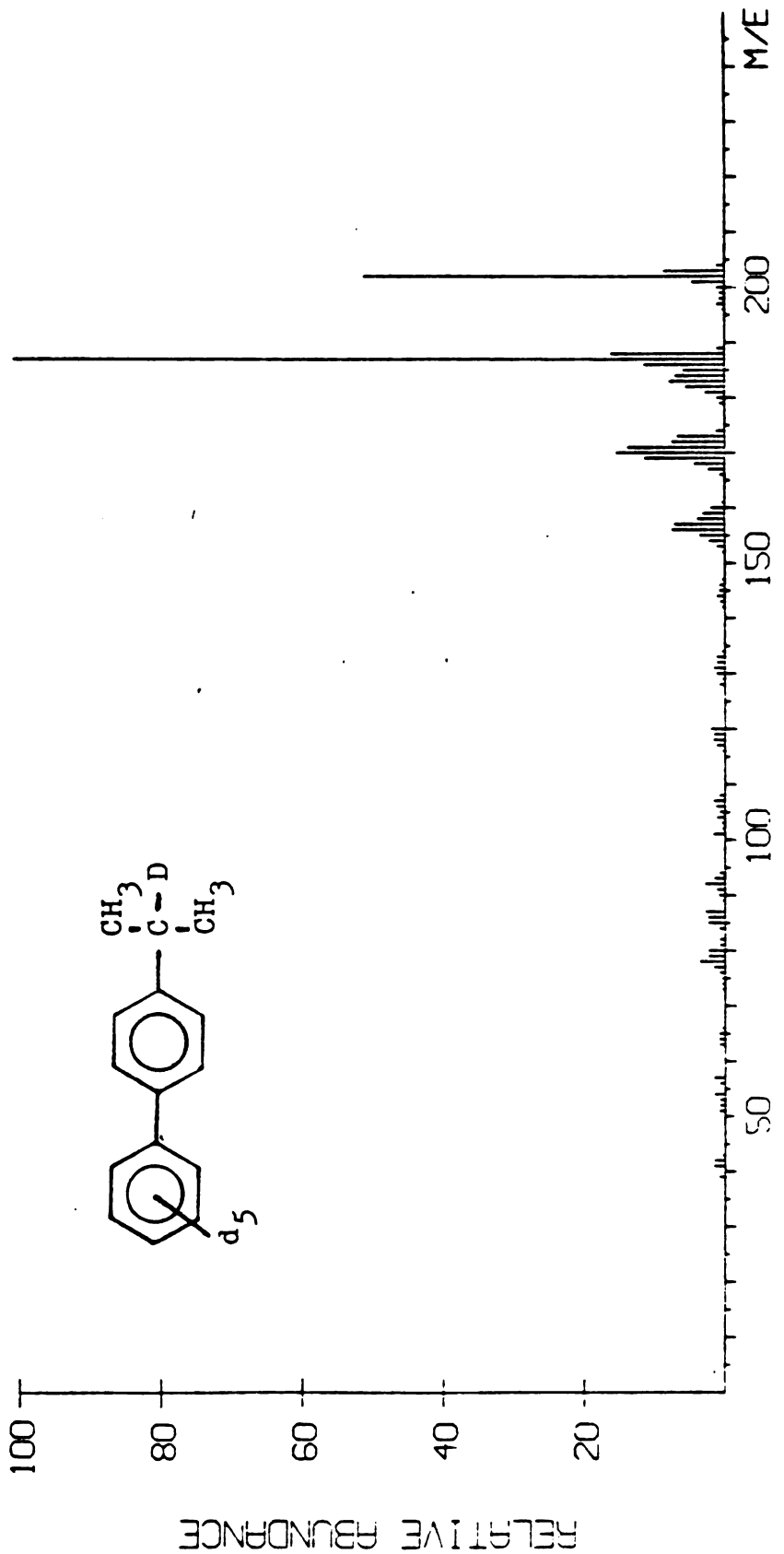
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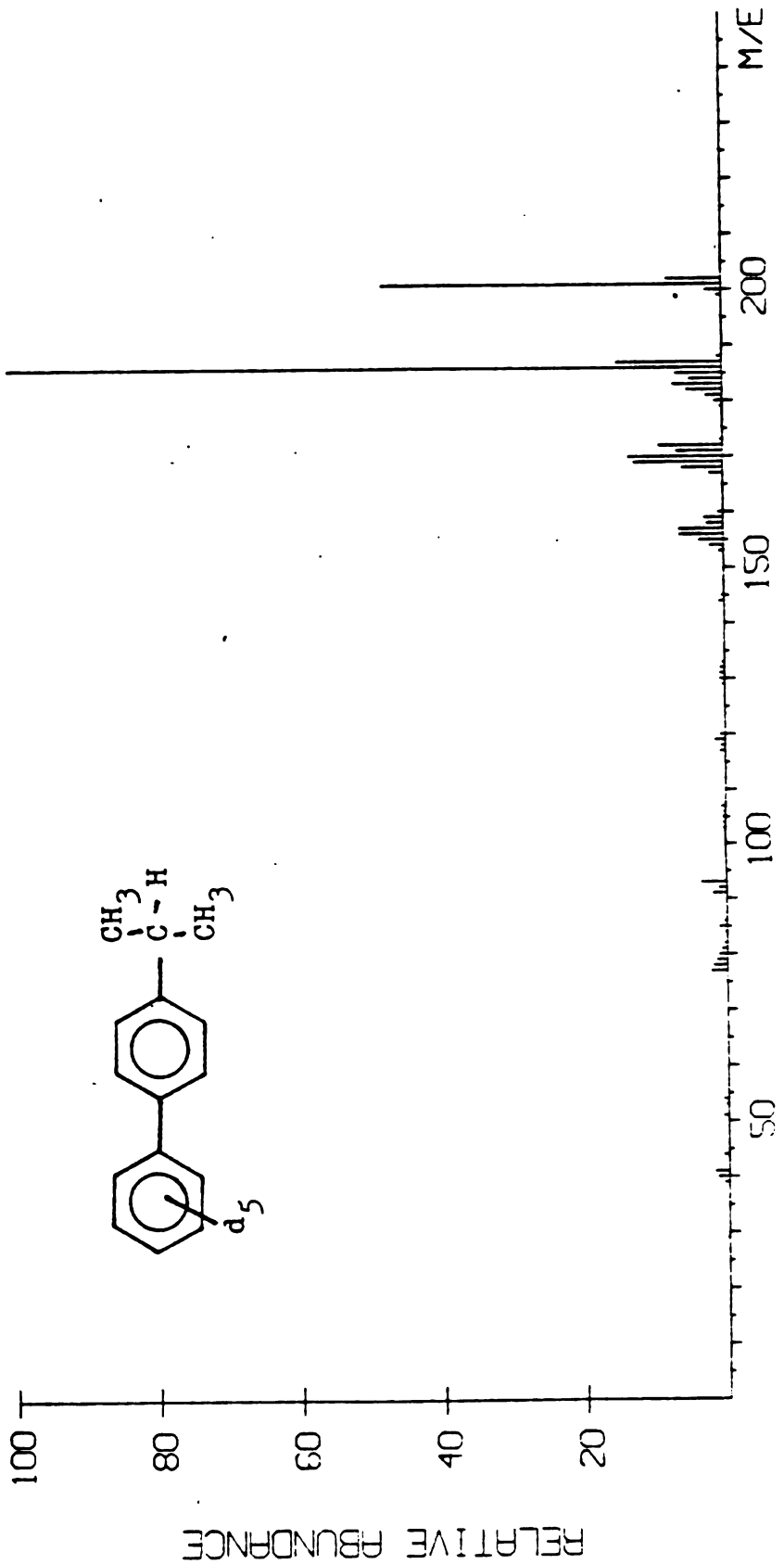
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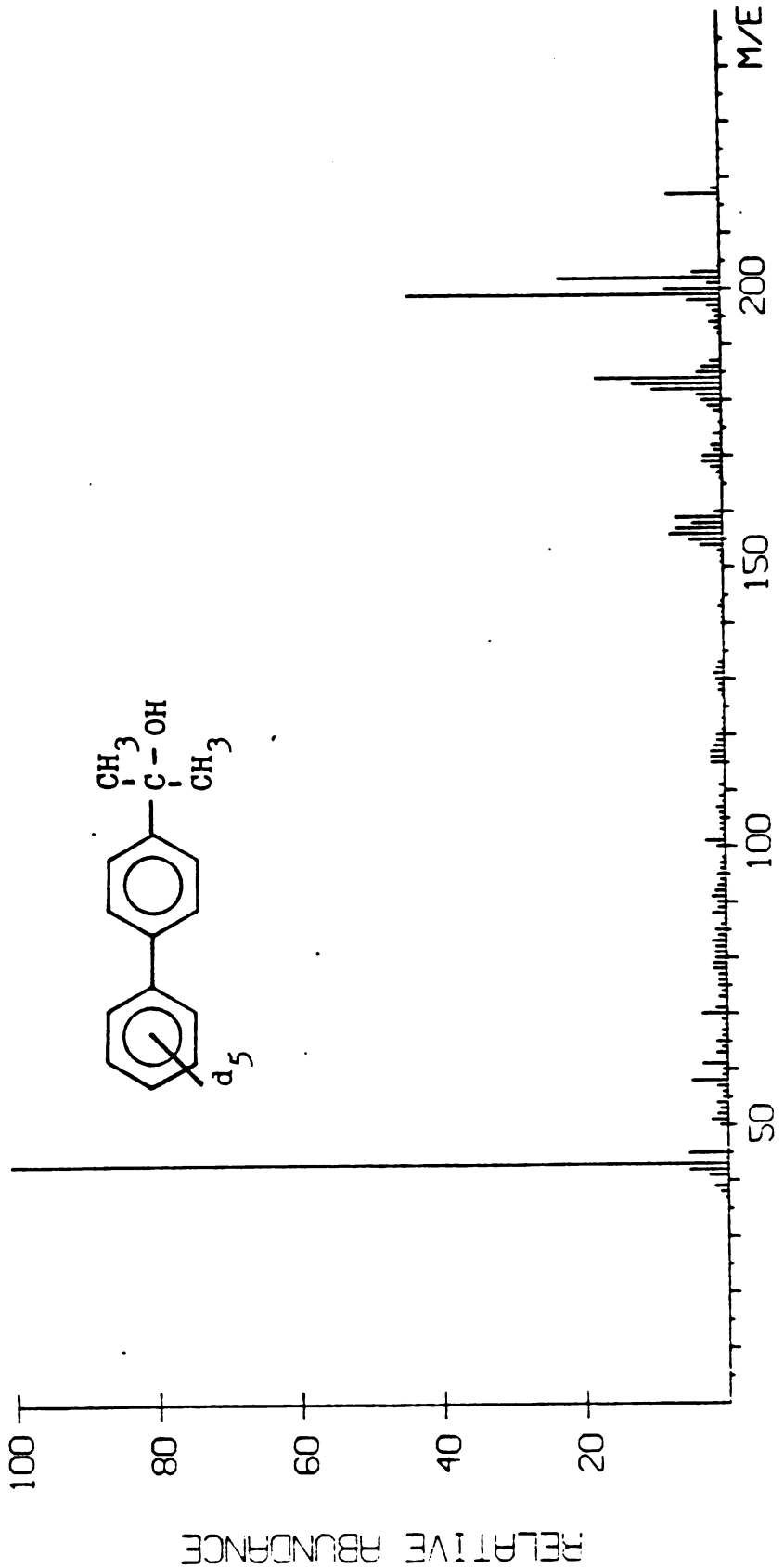
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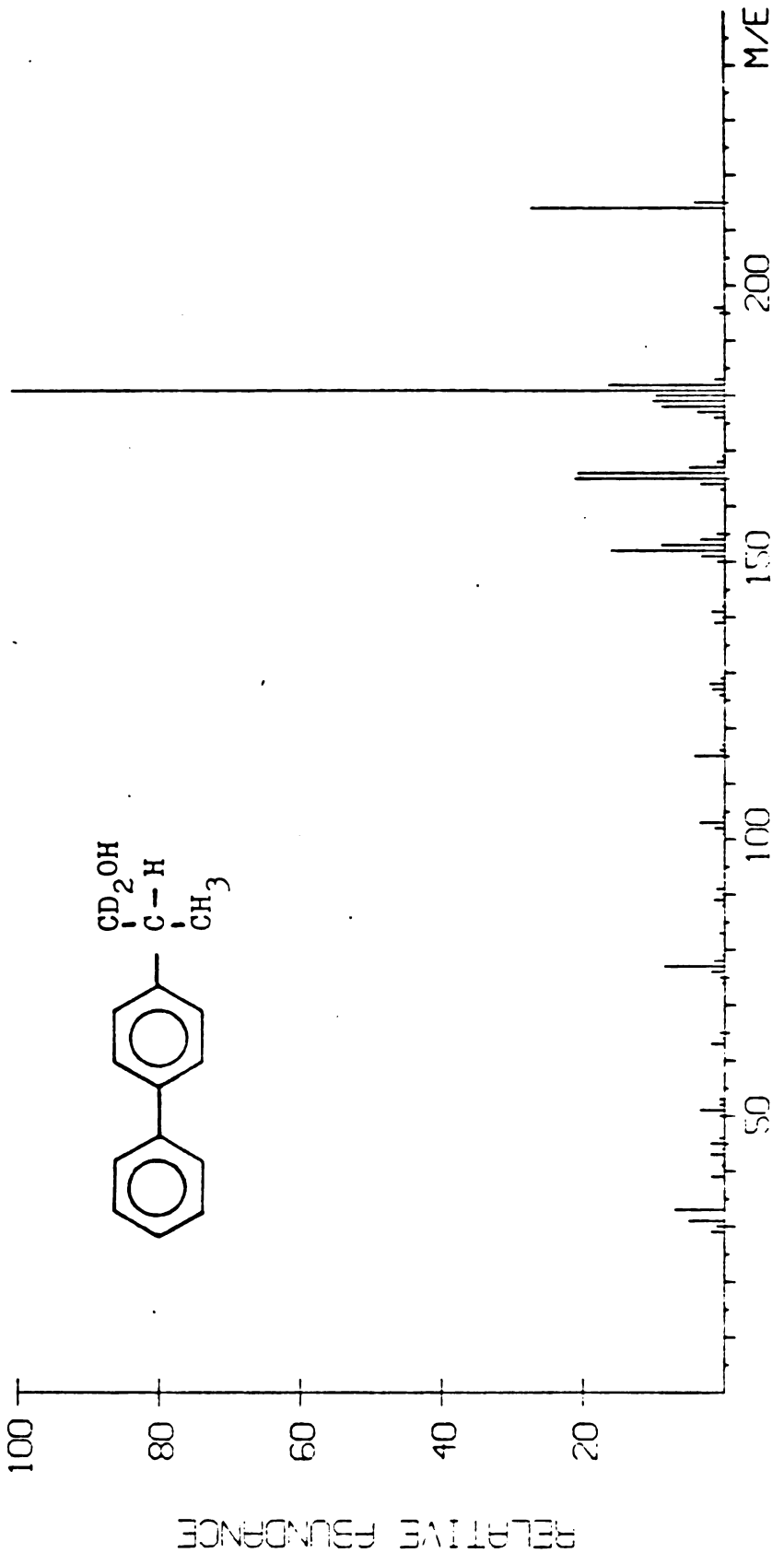
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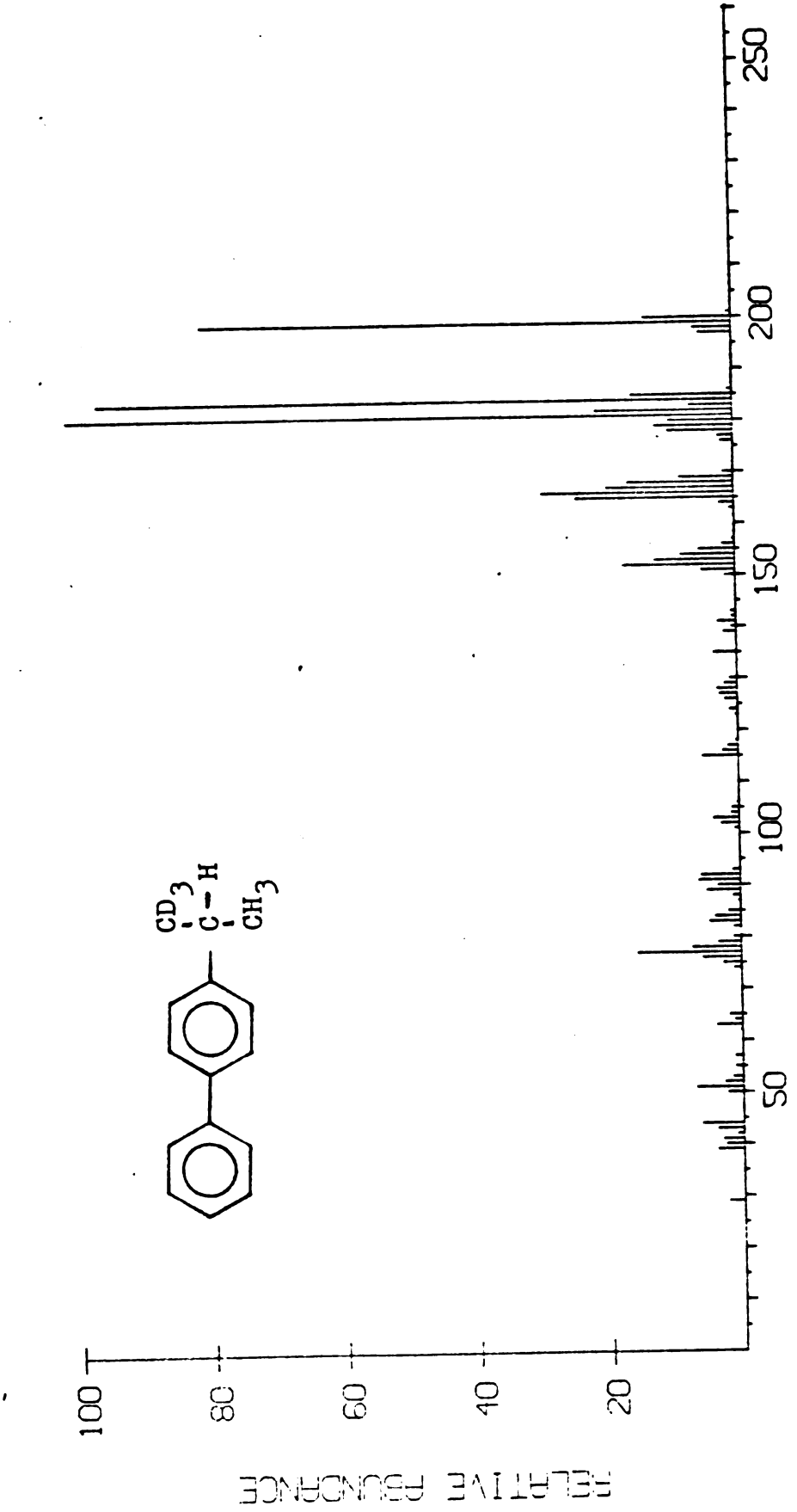
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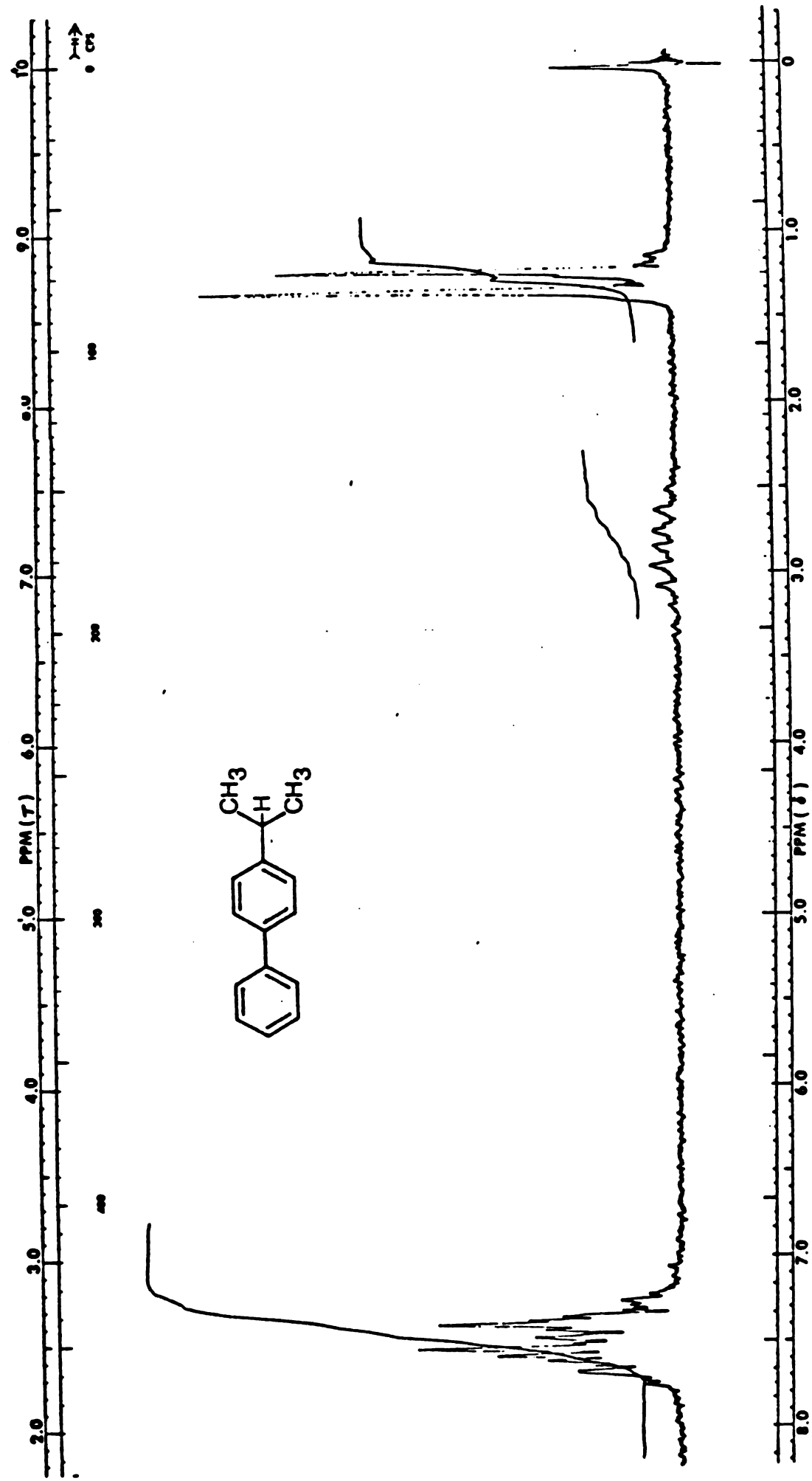
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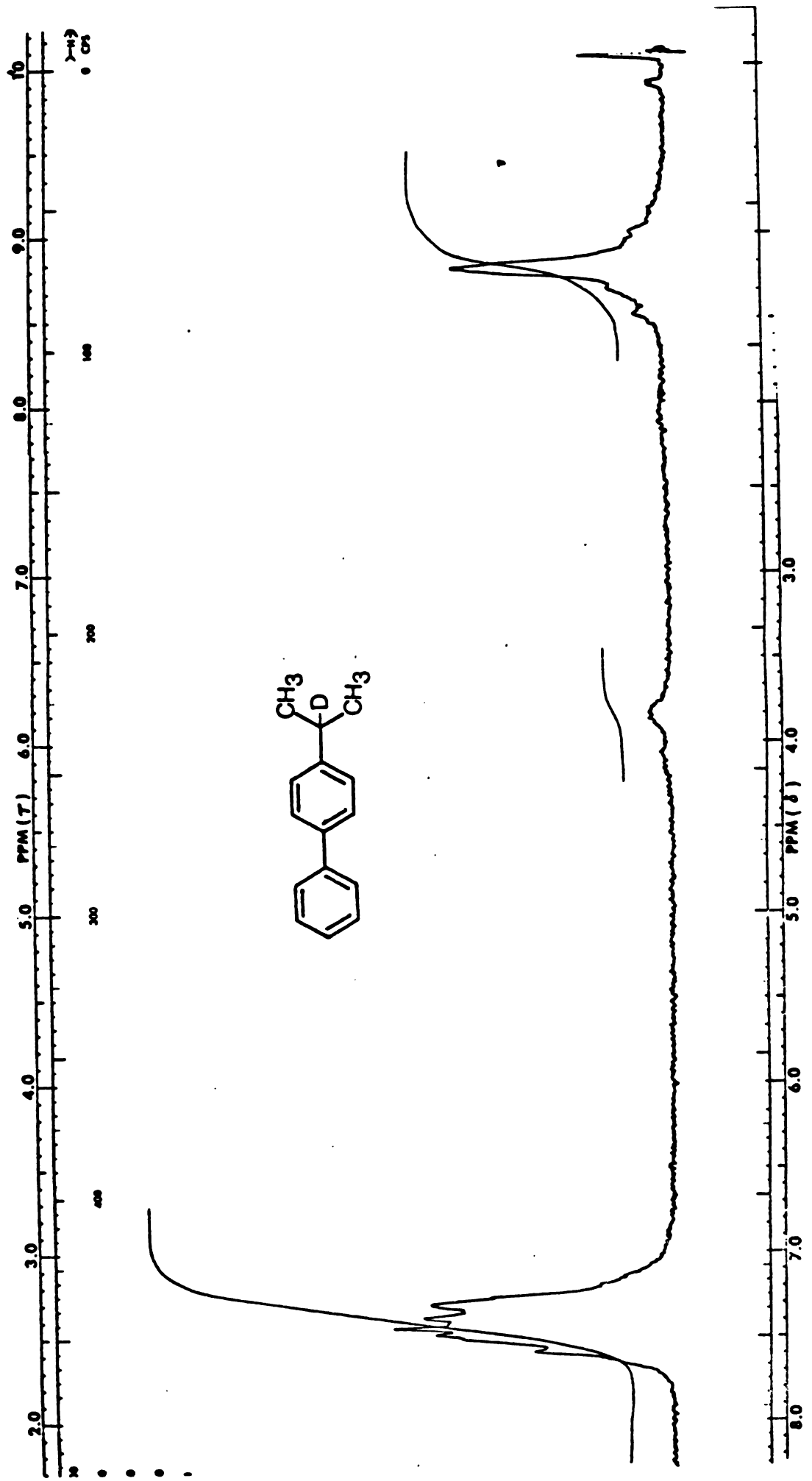
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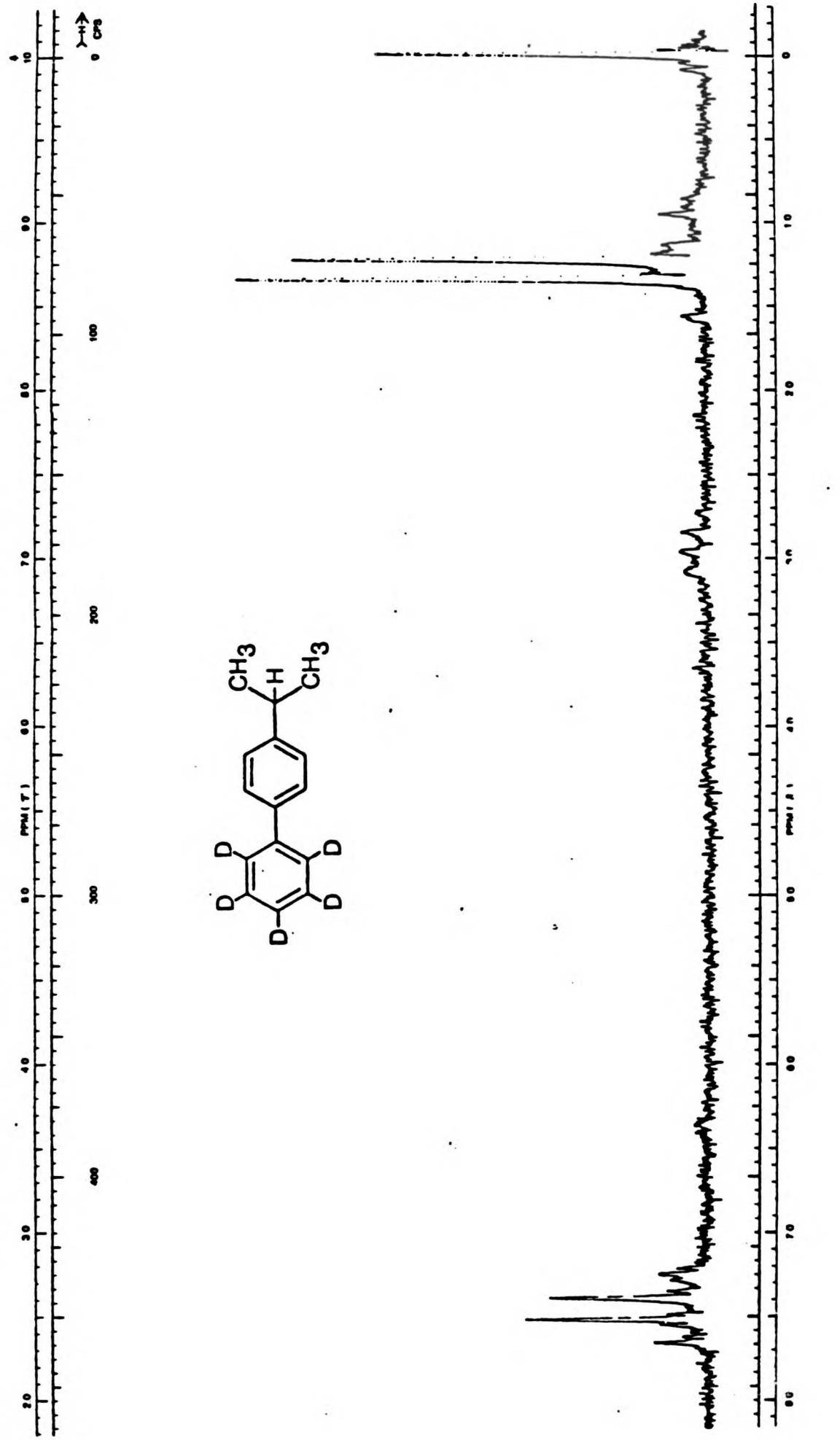
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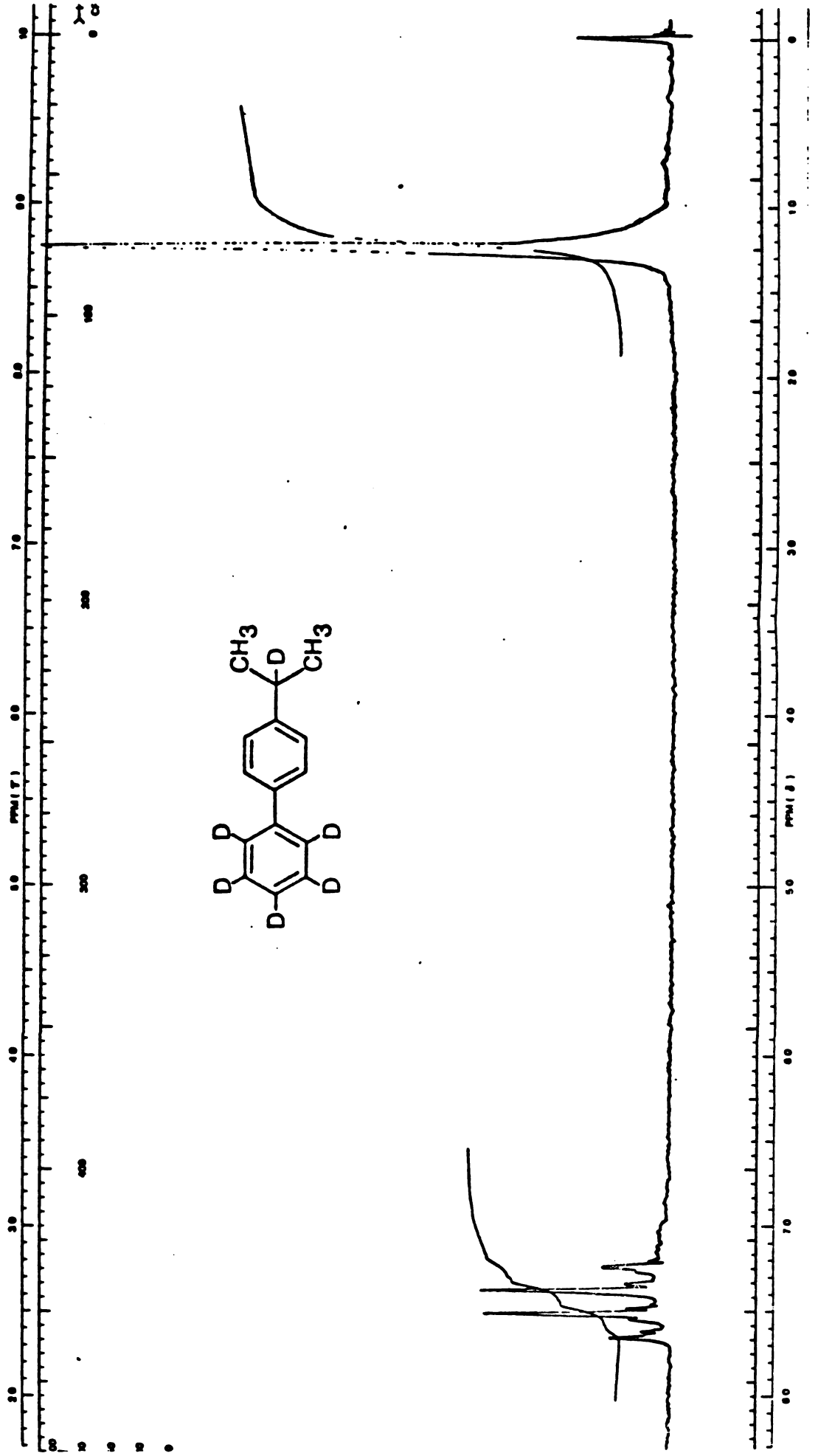
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BIOGRAPHICAL SKETCH

Ronald Wesley Woodard was born in Ozark, Alabama, on May 29, 1947. He graduated from Saks High School in Anniston, Alabama, in May 1965, as an honor graduate. The author received his Bachelor of Science degree in Chemistry from Jacksonville State University in June, 1968 and a Master of Science degree from Georgia State University in Organic Chemistry in June of 1971.

The author entered Georgia Institute of Technology in June of 1970 and studied there until September of 1972, when he was called to take his commission in the United States Army Chemical Corps. After completing his tour of duty, the author moved to San Francisco to engage in studies leading to the Doctor of Philosophy degree at the University of California, Department of Pharmaceutical Chemistry. During his graduate studies, he was supported by the National Institutes of Health, University of California, and the Eli Lilly Company.

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