SYNTHESIS AND DETECTION OF POTENTIAL N- AND α-C- OXIDIZED METABOLITES OF METHADONE AND RECIPAVRIN

By

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ABSTRACT

This thesis describes the attempted characterization of a new metabolite of methadone which was observed by GCMS following β-glucuronidase hydrolysis of conjugated metabolites in the bile of methadone dosed rats. Kang (1) proposed a methylene nitrone structure for this metabolite and suggested that it arose from a glucuronide conjugated secondary hydroxylamine. The nitrone structure was also assigned to a chemical oxidation product of methadone's major metabolite EDDP which had identical GCMS characteristics to the metabolite (1).

This thesis shows that the chemical oxidation product was in fact an oxaziridine which, under GC conditions, isomerizes to a formamide, with mass spectrum and retention time identical to the observed metabolite. Attempts to synthesize the potentially thermolabile methylene nitrone structure proposed by Kang failed due to instability of key intermediates or by facile isomerization to the formamide.

A model compound, recipavrin, which lacks the ethyl ketone side chain of methadone, was selected to characterize potential N- and α-C-oxidized metabolites and to establish whether formation of the formamide-like metabolite was general to the many drugs containing the 4,4-diphenyl -N,N-dimethyl-2-butylamine structure.

The nitrone, oxaziridine and formamide were synthesized as potential N- and α-C oxidized metabolites of recipavrin and were characterized by GCMS, LCMS, NMR, IR and UV spectroscopy. By exact analogy to methadone, rats treated with recipavrin produced a conjugated metabolite, which upon β-glucuronidase hydrolysis had GCMS characteristics identical to that of the synthetic oxaziridine and formamide. LC and LCMS were not sensitive enough to determine whether the formamide or a thermolabile precursor was responsible for the observed metabolite.

While it was not possible to determine which precursor was responsible for the formamide observed by GCMS, there is a good possibility that it is a formamide arising from a tertiary formamide carbinolamine glucuronide.

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LIST OF ABBREVIATIONS

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Ac	Acetyl
Ar	Aryl
BDDP	2-butylidene-N,5-dimethyl-3,3-diphenyl pyrollidine
BMDP	2-butyl-5-methyl-3,3-diphenyl pyrollidine
BSTFA	N,O-bis-(Trimethylsilyl) Trifluoroacetamide
CAS	Chemical abstracts service
CI GCMS	Chemical ionization gas chromatography mass spectrometry
DMCS	Dimethyl chlorosilane
d	doublet
dd	doublet of doublets
DPAN	diphenylacetonitrile
EDDP	2-ethylidene-N,5-dimethyl-3,3-diphenyl pyrollidine
EI	Electron impact
EMDP	2-ethyl-5-methyl-3,3-diphenyl pyrollidine
EtMgBr	Ethyl magnesium bronide
GCMS	Gas chromatography mass spectrometry
GCQ	Gas chrom Q
GLC	Gas liquid chromatography
GLU	Glucuronic Acid
H-Bond	Hydrogen bond
HPLC	High performance liquid chromatography
IR	Infrared
LAH	Lithium aluminum hydride
LC	Liquid chromatography
LCMS	Liquid chromatography mass spectrometry

LIST OF ABBREVIATIONS (CONT'D)

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m	medium (IR), multiplet (NMR)
M^+	molecular ion
MC	Mass chromatogram
MCPBA	metachloroperbenzoic acid
MeOH	methanol
MOX	O-methyl hydroxylamine hydrochloride
MS	mass spectrometry
MIZ	mass to charge ratio
NADP	Nicotinamide adenine dinucleotide phosphate
NOE	Nuclear Overhauser Effect
MDP	5-methyl-3,3-diphenyl pyrollidine
NMR	Nuclear magnetic resonance
DDP	N,5-dimethyl-3,3-diphenyl pyrollidine
pet.ether	petroleum ether (30°-60°)
ppm	parts per million
py	pyridine
q	quadruplet
rf	TLC mobility relative to solvent front
s	singlet (NMR), strong (IR)
SIM	selected ion monitoring
Str	stretch
TMAH	Trimethylanilinium hydroxide
UV	ultra violet

ACKNOWLEDGEMENT

The author wishes to thank Dr. F.S. Abbott for his guidance and support throughout this program. Thanks also go to Roland Burton for able assistance in GCMS and LCMS analysis, Sheila Ferguson for assistance with surgery, Margaret Heldman and the staff of the NMR lab in the Department of Chemistry for fast and excellent NMR service, Geoff Sunahara and Ahmad Fawzi for discussion and encouragement, and Lisa Wong for preparation of the manuscript. Lastly the financial support provided by the Faculty of Pharmaceutical Sciences, and the University Graduate Fellowship Committee are gratefully acknowledged.

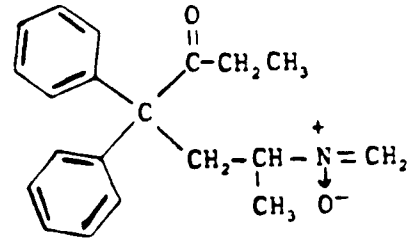
DEDICATION

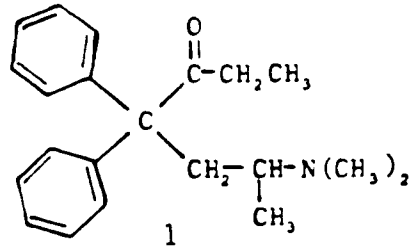
To my family and friends

I. INTRODUCTION

1. OBSERVATION OF A NEW METHADONE METABOLITE

In his PhD thesis, Kang (1) recently described an unknown metabolite of methadone (1). The metabolite was characterized following the β-glucuronidase hydrolysis of the conjugated fraction of bile from methadone dosed rats. He proposed the nitrone structure (2), and presumed that it arose from the secondary hydroxylamine (3), as a result of oxidation during alkaline workup. This was similar to the in vitro formation and extraction of secondary hydroxylamine metabolites of amphetamines described by Beckett et al (2).





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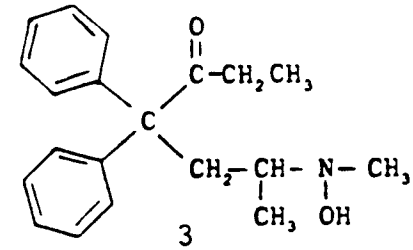

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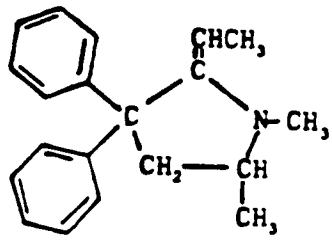
Identification of the metabolite (2) was made on the basis of a m-chloroperbenzoic acid oxidation of EDDP (4) the major metabolite of methadone. The EDDP oxidation product had the same GC retention time and mass spectrum, as the metabolite and was also assigned the nitrone structure. Kang alluded that the oxidation product could also be the oxaziridine (5), if thermal isomerization to the nitrone in the GC inlet occurred.

In a continuation of this work, this thesis examines the possibility of an N- or α-C- oxidative metabolic pathway for methadone (1) and the structurally related model compound, recipavrin, (6) in rats.



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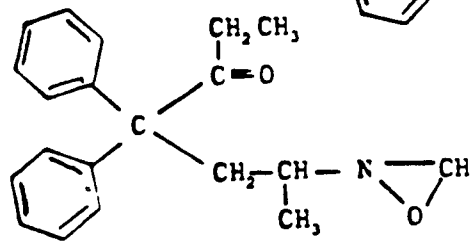

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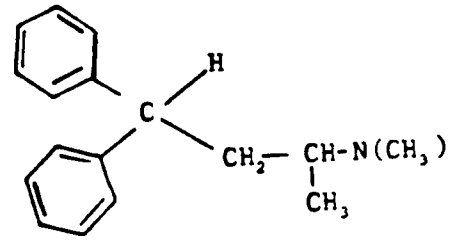

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The major objectives were four fold:

1) To identify and synthesize the methadone metabolite proposed by Kang.

2) To synthesize and screen for potential N- and α-C- oxidized metabolites of recipavrin, and thus establish whether the pathway is general to compounds containing the 4,4-diphenyl-2- (N,N-dimethylamino) butane structure.

3) To determine the stability of potential N and α-C oxidized metabolites of methadone and recipavrin to GLC analysis.

4 To investigate the NMR spectra of methylene nitrones and oxaziridines as an aid to structure elucidation of thermolabile metabolites.

2. Potential Significance of The New Metabolite

The structure elucidation of new metabolites of drugs has theoretical justification in the need for better understanding of the pharmacokinetics and toxicities of the drug.

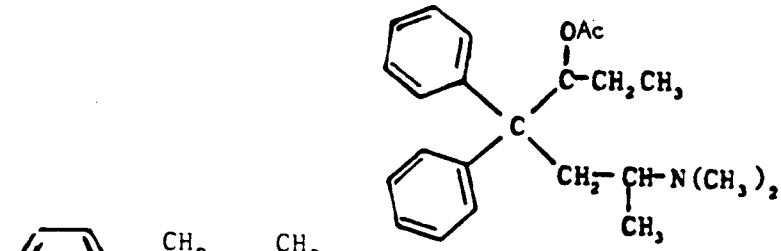
Methadone is a potent analgesic but its use is viewed with some trepidation in certain countries due to its affiliation with the treatment of heroin addiction. Its other use as a narcotic analgesic mostly involves pain relief in terminal cancer patients. Thus although the metabolic pathway is novel, and the metabolites potentially toxic or carcinogenic, public sympathy does not lie with heroin addicts, and long term toxicity is certainly not a concern of terminal cancer patients.

Methadone is however being reviewed in Britain as a post operative analgesic, and its basic side chain is a component of many other drugs. Thus the generality of a novel metabolic pathway, and the possibilities that toxic or active metabolites are formed is worth investigating. The toxicity of aromatic amine N-oxidized metabolites is well documented (3). Several workers have tried to implicate covalent binding of N-oxidized metabolites of various aliphatic amines in their toxicity and carcinogenicity and N-oxidized metabolites have been postulated as potential mediators in the pharmacology of endogenous amines (4) and as the cause of the undesirable side effects of amphetamine abuse (5).

From a theoretical standpoint, the mechanism of N-dealkylation, and the trapping of reactive intermediates in N-dealkylation reactions may be further elucidated.

3. Methadone, Recipavrin and Related Compounds

Methadone (1) was first synthesized by Bockmuhl in the 1940's (6). The (-)-isomer had analgesic potency equal to morphine and this observation prompted the synthesis and testing of several hundred diphenylpropylamine analogues (7), among them, the analgesics methadol (7), 1-acetylmethadol (8), and propoxyphene (9).



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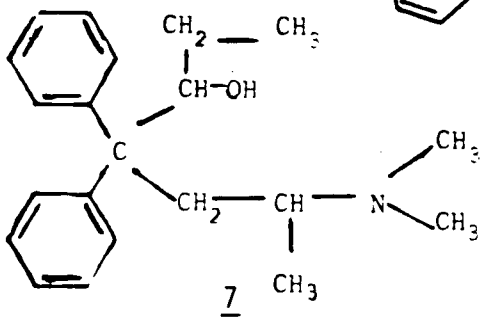

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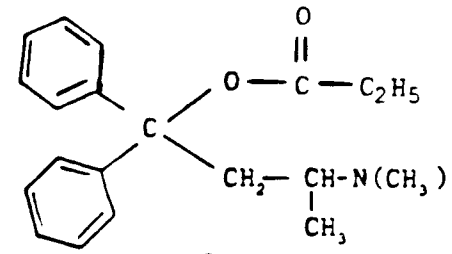

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Because of its long half life (25 hours) relative to morphine and a less severe abstinence syndrome, methadone is currently used in the treatment of heroin addiction. Recipavrin (6) was synthesized by the Wellcome company in 1949 (British Patent 624117) (8). It was found to be inactive as an analgesic, but its antihistaminic and anticholinergic properties resulted in its use as an antispasmodic under the trade names Robetyl and Preparyl (9) during the late 1950's.

In this thesis recipavrin was chosen as a model compound to determine whether other tertiary 4,4-diphenyl-2-butylamines undergo N-oxidation, in a manner similar to that described by Kang (1). The pka value, (9.48) is similar to that of methadone, (8.99) (4), and its metabolism although not previously investigated, was expected to be simpler, due to the lack of an ethyl ketone side chain. This precludes the formation of pyrollidines like EDDP, the major metabolite of methadone (Figure 1) (10).

4. Metabolic Pathways of Methadone and Recipavrin

As outlined in Figure 1, N-demethylation of methadone, results in spontaneous cyclization of normethadone to EDDP (4), which may be in turn

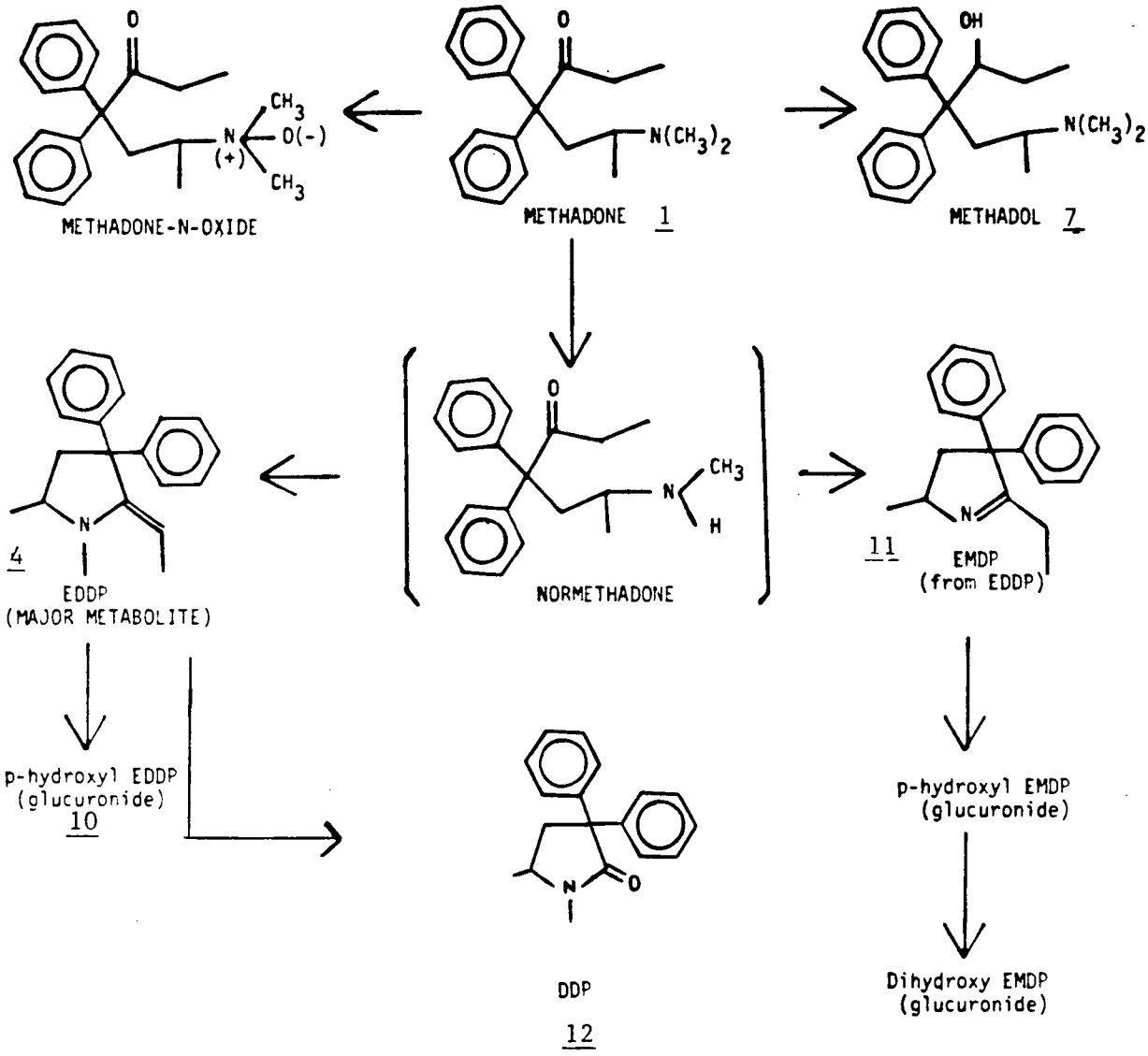


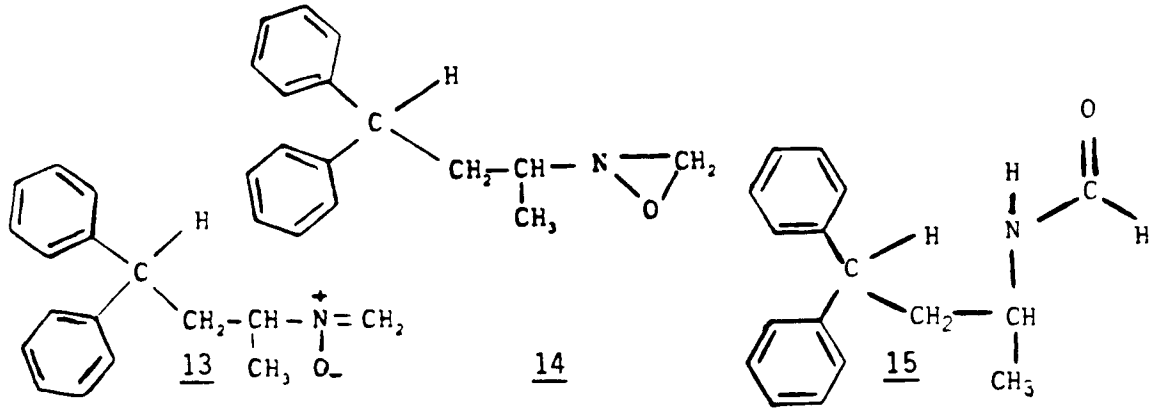
Figure 1. Major metabolic pathways of methadone

be ring hydroxylated (10), further dealkylated to EMDP (11), oxidized to DDP (12), or excreted intact in bile and urine. The structure elucidation of these metabolites was performed by Beckett et al (11) and Sullivan and Due (12). Biliary metabolism has been investigated by Kreek et al (13) and Roerig (14). A tertiary N-oxide, has been described by Beckett (15), and is observed by GCMS at short retention time as the Cope elimination product 4,4-diphenyl-5-hept-2-enone. The remaining metabolites arise from oxidation of the ethyl ketone side chain or by ring hydroxylation.

The new metabolite observed by Kang was not ring hydroxylated, since administration of phenyl ring labelled $^{2}H_{10}$ - methadone resulted in total retention of deuterium. This suggested glucuronidation on or near the nitrogen atom, since the keto function was intact in the chemical oxidation product of EDDP. The long GC retention time of the metabolite suggested a very polar compound, therefore the nitrone structure was a good proposal in light of the nitrone and hydroxylamine metabolites of N-methyl amphetamine observed in vitro by Coutts et al (16).

There are problems with the proposal however. Firstly, attempts to detect the secondary hydroxylamine were unsuccessful, and secondly, there are few examples in the literature of the in vivo N-oxidation of aliphatic amines (17), and no examples of aliphatic secondary hydroxylamine N-O-glucuronides (18). However, glucuronide conjugates of primary aliphatic hydroxylamines (19, 20), aromatic hydroxylamines and aryl hydroxamic acids are well known (18). Lastly, the requisite secondary amine precursor (normethadone) is not a stable compound, and EDDP did not produce the required metabolite when administered to rats (1).

To further elucidate the structure of the metabolite observed by Kang, the well documented thermolability of N-oxidized metabolites prompted attempts to synthesize the recipavrin nitrone (13) and oxaziridine (14). The thermal rearrangement of oxaziridine to amides observed by Emmons (21) indicated that the formamide (15) could also account for the observed metabolite. This introduction will review the literature precedents for potential N and α-C oxidized metabolites and their glucuronide precursors.



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5. Metabolism of tertiary aliphatic amines

A. N-Dealkylation

It is generally held that carbinolamines formed metabolically from tertiary amines decompose to formaldehyde and the corresponding desalkyl amine as outlined in Figure 2 (22).

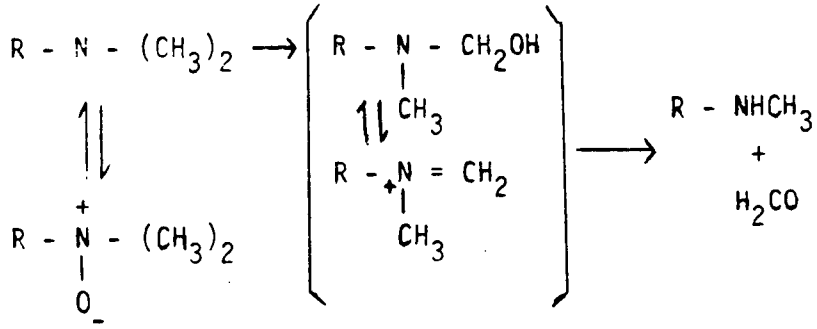


Figure 2. N-dealkylation of tertiary amines showing proposed reactive intermediates.

The intermediates arise by a cytochrome P-450 mediated oxene [:Ö:] insertion mechanism, or by dehydrogenation of the amine to an iminium ion. The stoichiometry and the mechanism of oxidation are outlined in Figure 3 and 4 (23).

Tertiary N-oxides, once thought to be the reactive intermediates in N-dealkylation, are now thought to be excreted intact, or reduced back to the parent tertiary amine prior to N-dealkylation (24).

3. $R_2N\text{-}CH_2R' + NADPH + O_2 + H^+ \rightleftharpoons R_2N\text{-}CHOH\text{-}R' + NADP^+ + H_2O$

4. $R_2\ddot{N}CH_2R' + Fe^{3+}{:}\ddot{O}{:} \dashrightarrow R_2\overset{+\cdot}{N}CH_2R' + Fe^{3+}{:}\ddot{O}{:}^-$

$\downarrow$

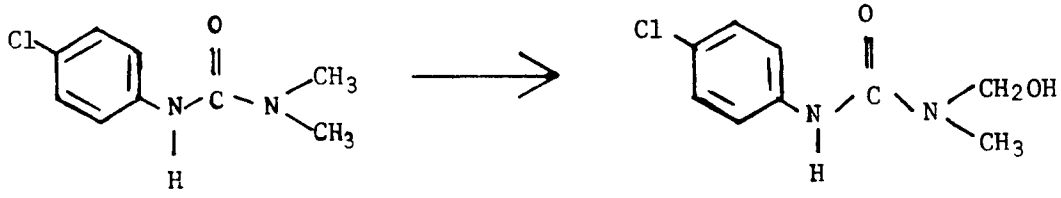
$R_2N\text{-}CHOH\text{-}R + Fe^{3+} \dashleftarrow R_2\overset{+}{N}{=}CH\ R' + Fe^{3+}{:}\ddot{O}{:}H^-$

Figure 3. Stoichiometry of Cytochrome P-450 catalyzed α-C oxidation.

Figure 4. Mechanism of tertiary amine oxidation via an iminium ion.

B. Stable carbinolamines

There are a number of examples of weakly basic drugs, whose carbinolamine in vivo metabolites are stable enough to isolate. These include N-Methyl Carbazole (25), Monuron (16, 17), (26) N-methyl benzamide (26), clebropride (27) and hexamethylmelamine (26).



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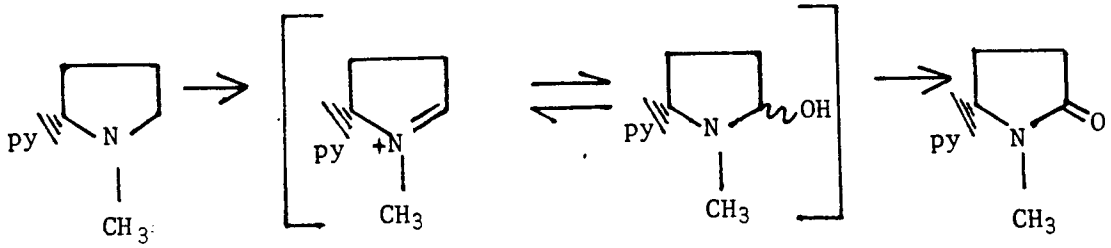
The existence of less stable carbinolamines has been inferred from the discovery of α-oxo metabolites such as the lactams formed metabolically from nicotine, (18, 19, 20), (28) prolintane (29), medazepam (30) and methyprylon (31). The preference for dehydrogenation to the lactam over dealkylation to open chain compounds is difficult to rationalize in light of the basic nature of the nitrogen atom in these compounds.



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In each of these three examples the carbinol group is adjacent to a basic nitrogen atom, therefore even mildly acidic conditions, should dealkylate the intermediate by the mechanism shown in Figure 5 (22).

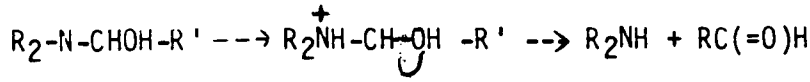

Figure 5. Instability of carbinolamines in acid solution.

C. Carbinolamine Glucuronide Conjugates

In the metabolism of benzyl-N-benzylcarbethoxy hydroxamate (32), N-Methyl-α-phenyl-α-ethyl glutarimide (33), diphenamid (34), N-methyl carbazole (25) and various dimethylaryltriazenes (26) (21, 22, 23), semi stable carbinolamine metabolites are trapped by glucuronidation prior to dealkylation.

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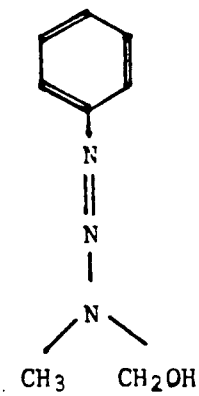

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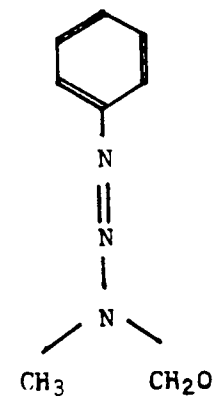

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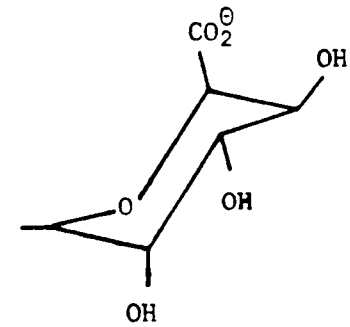
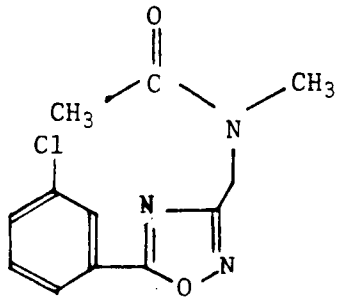


The anticonvulsant N-2-[5-m-chlorophenyl)-1,2,4-oxadiazol-3-yl] ethyl-N-methyl acetamide (oxadiazol) (24), is metabolized to a glucuronide conjugate of the carbinolamine (25), which after enzymatic hydrolysis loses formaldehyde and is analyzed as the corresponding secondary amide (26) (35). This may be the mechanism whereby a formamide metabolite of methadone or recipavrin could arise, concomitant upon initial α-C oxidation of a tertiary N-methyl to a formamido group.



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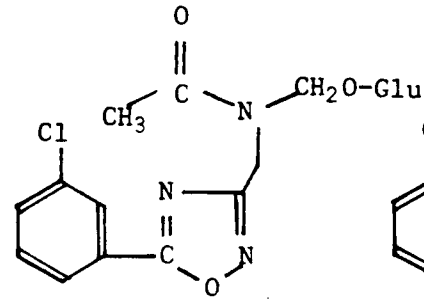

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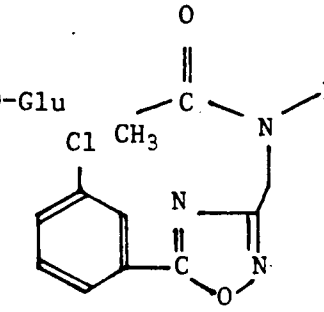

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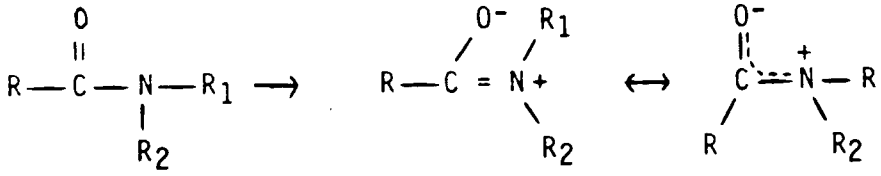
D. Factors influencing carbinolamine stability

All of the above carbinolamines are stabilized by conjugation to α-aryl, or α-carbonyl groups. These stabilizing groups lower the pKa value of an amido nitrogen to one or less (36), as a result of delocalization of and non bonding electrons in the equilibrium:

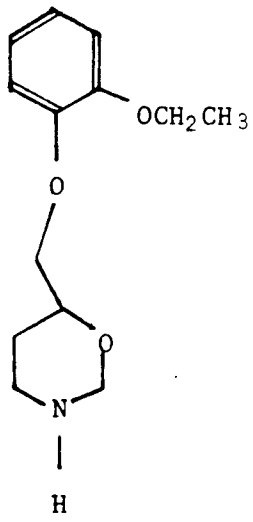


The resultant planar sp^2 geometry at nitrogen contrasts the pyramidal geometry of amines, and precludes oxidation of the nitrogen since the lone pair is not available for bond formation with electrophiles. This evidence supports carbinolamine intermediacy in the N-dealkylation of tertiary amides (37).

E. N-Formyl metabolites

i) Formyl conjugates

Just as N-acetylation of primary amines is known to be a metabolic pathway, there are examples (albeit few) of N-formyl metabolites. In the metabolism of viloxazine (27) (38), 2-aminoanthraquinone (28) (39) 2-aminonaphthalene (29) (40), and caffeine (41), the N-formyl group is a conjugate supplied by the organism. Sante et al (42) have postulated a transformylation mechanism involving the enzyme kynurenine formidase to account for these metabolites.



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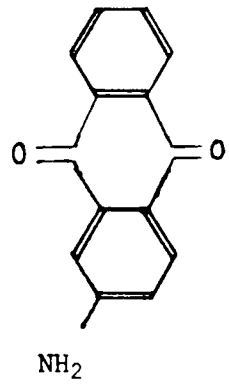

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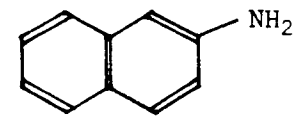

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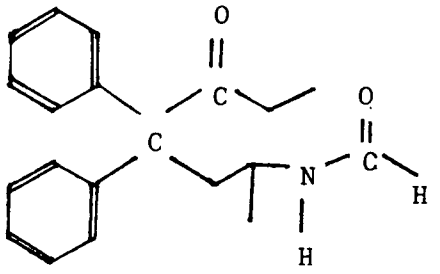
ii) Formyl metabolites of tertiary aliphatic amines

There is one example of a N-formyl major metabolite arising from the tertiary dimethylamine, aminopyrine (30) (43) (figure 6). By analogy, a formamide metabolite of methadone (31) or recipavrin (15) can be proposed.



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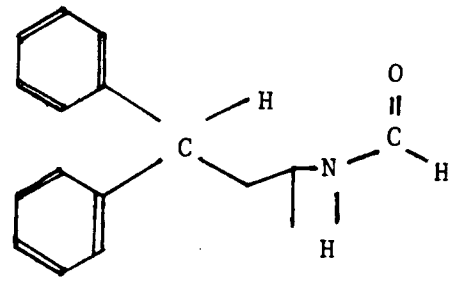

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The pathway is unusual in light of the fact that carbinolamines of aminopyrine are thought to decompose spontaneously (44) (as should those of methadone and recipavrin). In fact, aminopyrine has long been used as a substrate to measure N-demethylase activity in microsomal preparations by quantifying the amount of formaldehyde produced versus time (45, 46).

The authors demonstrated that $(^{13}CH_3)_2$-N-labelled aminopyrine retained the ^{13}C label on the formyl carbon after metabolism by rats. The appropriate M+1 ions in the mass spectrum and a formyl resonance at 161.3 ppm in the ^{13}C NMR of a urine extract were present.

Pathway B (figure 6) is supported by SIM detection of the formamide arising in vitro from the desalkyl compound (32) (47).

In vivo metabolism of the didesmethylaminopyrine (33) failed to produce any formamide; this was further evidence against a formyl conjugative pathway. A free radical mechanism may be responsible, as proposed for the synthesis of formamides from tertiary amines (48), and the cumene hydroperoxide mediated N-dealkylation of aminopyrine (49). Proof that the

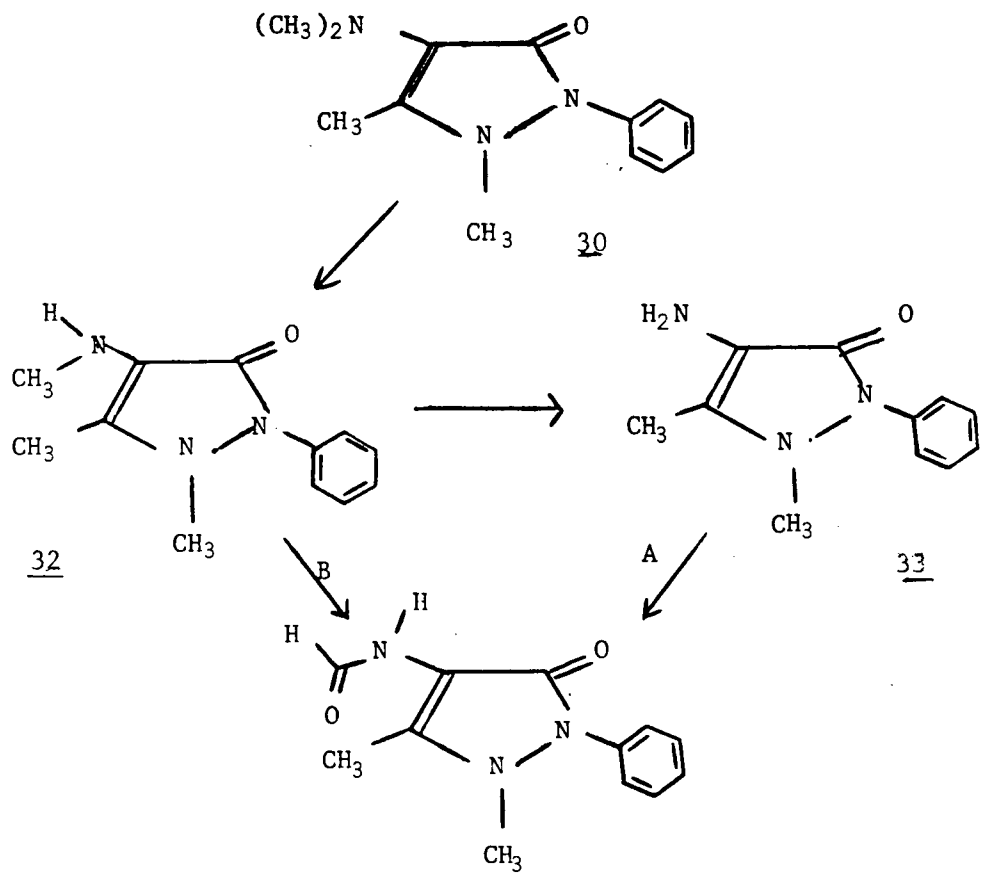
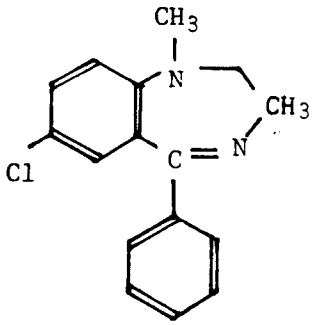


Figure 6. Possible metabolic pathways to a formyl oxidation product of aminopyrine

further oxidation of a carbinolamine is enzymatic is only available for those carbinolamines which form lactams. Schwartz and Kolis (50) have shown that the microsomal oxidation of 2-hydroxymedazepam (34) to diazepam (35)



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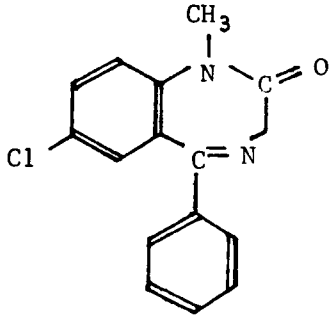

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involves a NAD+ dependent dehydrogenase, and recently, Brandange and Lindblom (51) have suggested that an aldehyde oxidase (EC 1.2.3.1) was responsible for the oxidation of nicotine to cotinine. They claim that an iminium ion is a more likely substrate for the enzyme than the carbinolamine.

On the basis of the observations of Noda et al and in light of thermal isomerizations of N-oxidized compounds observed by Emmons (21). The synthesis of the methadone and recipavrin formamides was necessitated to compare to the unknown metabolite.

iii) Potential significance of formamide metabolites

Gescher et al (26) have recently discovered that carbinolamines of intermediate stability, such as hydroxymethylpentamethylmelamine are positive in the analysis of formaldehyde by the Nash method (routinely used to measure metabolic N-demethylation (52). He infers that stable carbinolamines may have been overlooked in the past and states:

> "Oxidative metabolism of the N-methyl moiety in xenobiotic molecules is more complex than simply a pathway leading to the desmethyl compound. Depending on as yet unknown factors associated with the structure of the molecule, N-hydroxymethyl groups can be generated with widely different stabilities."(26)

He postulates that carbinolamines of intermediate stability may liberate formaldehyde extrahepatically, or react with tissue nucleophiles such as cysteine or histidine. The carbinolamine, hydroxymethyl pentamethylmelamine is a cytotoxic major metabolite of hexamethylmelamine and is active as an antitumor agent (26).

6. Metabolic N-Oxidation

A. Substrates and mechanisms

Biological oxidation of nitrogen in aliphatic amines has been an active area of research for the past 13 years (17, 18). The metabolic products are determined by the substitution on nitrogen, (primary, secondary or tertiary), and by the availability of α-hydrogen atoms. The products formed by representative amphetamines, which have available α-hydrogen atoms are outlined in Figure 7.

While this pathway is well known in vitro, N-oxidation products of secondary or primary aliphatic amines are rarely conclusively demonstrated in vivo, possibly because of facile reduction to the precursor amines or by instability to GC analysis. The mechanism of N-oxidation of secondary aliphatic amines proposed by Beckett (53) involves intermediate N-hydroperoxides which decompose to imines, or nitrones, or a reduced flavoprotein:O_2 complex which loses water to form a secondary hydroxylamine.

B. Metabolic Fate and Detection of N-Oxidized Metabolites

i) Source of Methylene Nitrones

Besides arising from unstable N-hydroperoxides, nitrones may also form by condensation of a primary hydroxylamine with formaldehyde, or by oxidation of a secondary hydroxylamine in alkali as occurs during the extraction of N-hydroxy amphetamine metabolites (54). Condensation with formaldehyde is a fundamental reaction in endogenous metabolism (55) and in drug metabolism (56). Coutts has presented evidence that the methylene nitrone rather than the isomeric methanimine N-oxide is the in vitro metabolite of methamphetamine despite similarities in their analysis by GCMS

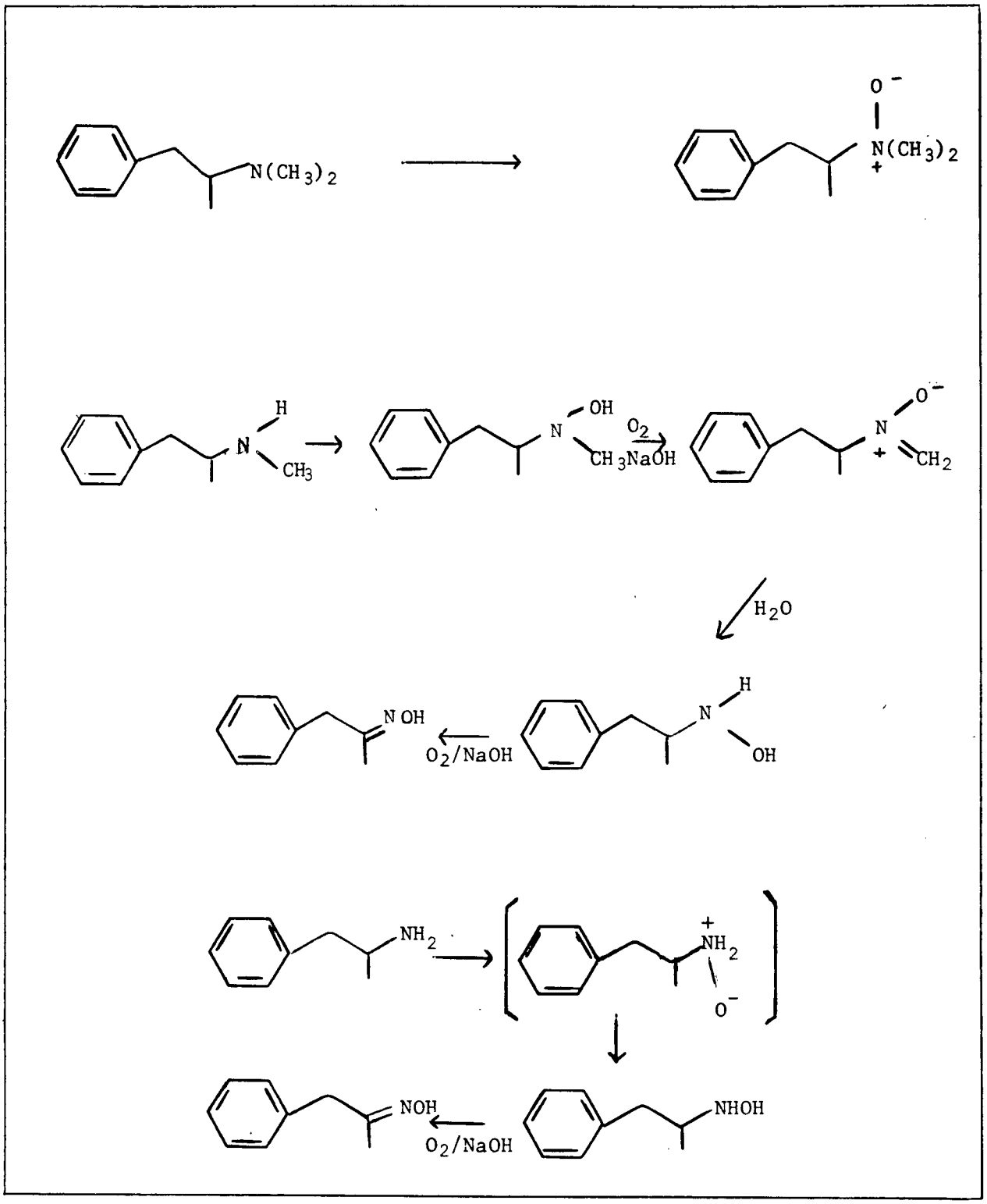
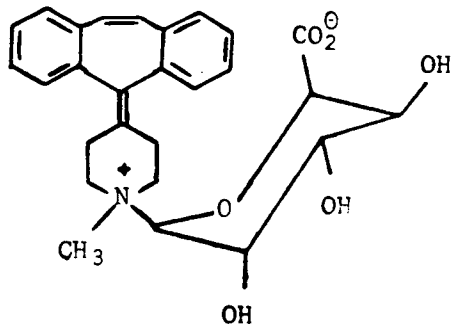


Figure 7. Metabolic N-oxidation products of tertiary, secondary and primary amphetamines.

(57). Non enzymatic oxidation and hydrolysis of secondary hydroxylamines has been implicated in the relatively rapid N-dealkylation of secondary amines, particularly in light of the facile demethylation of secondary hydroxylamines (58), but conclusive evidence that aliphatic nitrones are formed enzymatically rather than chemically is lacking.

The nature of the glucuronide precursor limits the source of the methylene nitrone to a 2° hydroxylamine. There are several examples of tertiary amines such as cyproheptadine which when glucuronidated, exist as quaternary amine internal salts (36) (59, 60), and it is known that glucuronides are formed at electron dense centres, but there are no known examples of glucuronide conjugates of nitrones with the structure (37).



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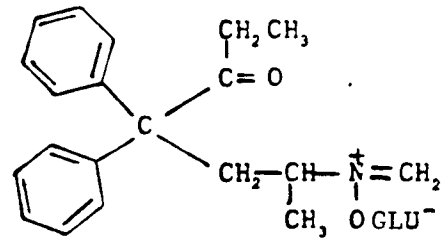

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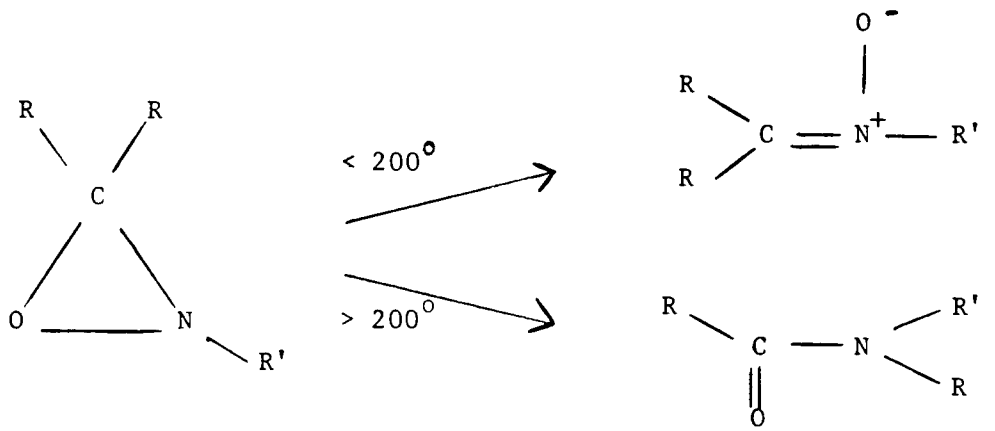
ii) Potential metabolic sources of methylene oxaziridines

There are no references to oxaziridine drug metabolites in the literature. This might be due to the unstable nature of the oxaziridine ring, which decomposes under mild conditions. Condensation of a primary amine with formaldehyde, followed by peroxidation (as in synthetic procedures) or the peroxidation of iminium dealkylation intermediates could result in the required structure. The structures of the glucuronides

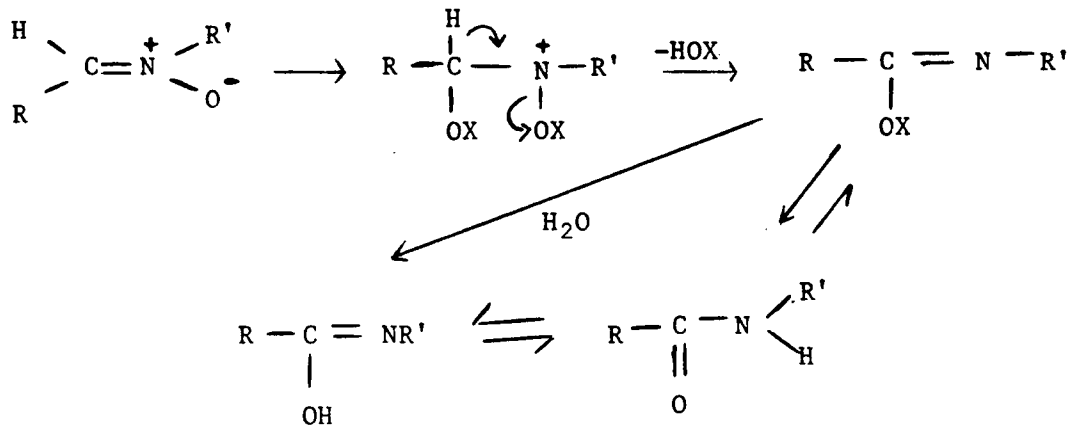
precursor is limited to a N-glucuronidated imine which are only known to occur in heterocycles like sulfisoxazole. By analogy, if an imino glucuronide of methadone were present, hydrolysis with β-glucuronidase would probably result in cyclization to EDDP or its unsaturated analogues (see synthetic section).

7. Thermolability and interconversion of potential metabolites during GCMS analysis

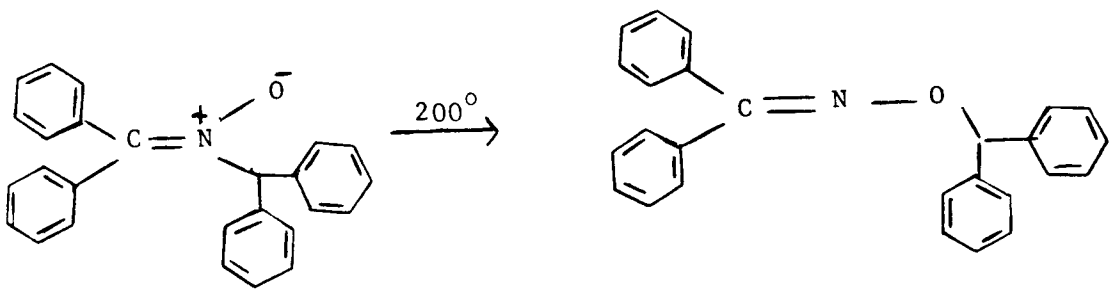
Studies by Emmons (21) have shown that oxaziridines thermally isomerize to both nitrones and amides, depending on the pyrolytic conditions, according to the pathway (61):



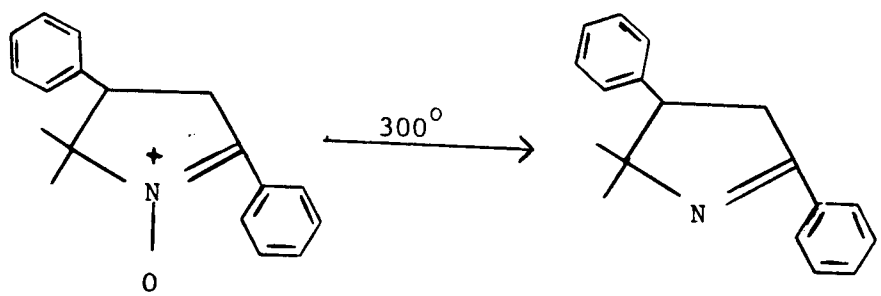
Nitrones, known to produce the higher energy oxaziridines by photolysis, are susceptible to a variety of thermal and chemical rearrangements. These include amide formation (under the influence of alkali or acylating agents), and base catalyzed double bond migration;



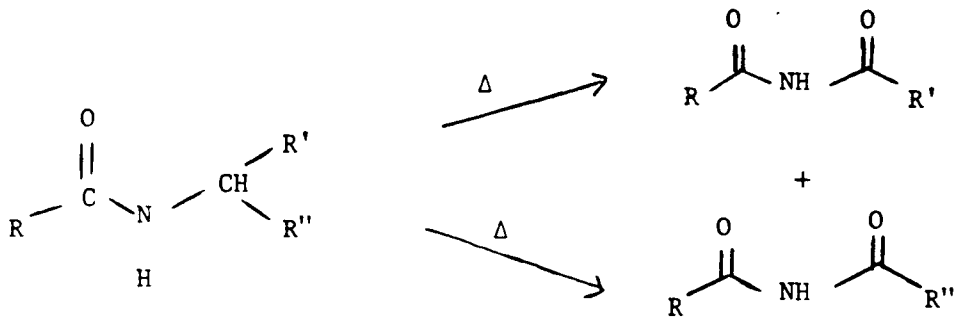
as well as thermal oxime O-ether formation (62):



or alternatively, desoxygenation (61):



Lastly, even amides are known to autoxidize thermally into N-formylamides and N-acylamides by C_1-C_2 bond scission (63):



There are several possible structures available for the metabolite which could arise both by chemical changes during workup and by thermal changes in the GC. Therefore synthesis was centred on a variety of potential metabolites and their most probable glucuronide precursors.

8. Synthesis of N-Oxidized and related drug metabolites

The synthetic methods used in this thesis parallel those developed for N-oxidized amphetamine metabolites by Morgan and Beckett (64), Beckett et al (65) and Coutts et al (57).

Carbon-13 and ^1H NMR investigations of the nitrone and oxaziridines synthesized in this study were carried out as an aid to structure elucidation, because no examples of ^{13}C NMR spectra of methylene oxaziridines containing α-protons exist in the literature, and as a potential means of identifying the metabolites of $(^{13}CH_3)_2$-N-labelled recipavrin by ^{13}C NMR.

II. EXPERIMENTAL

A. REAGENTS AND MATERIALS

Chemicals were reagent grade and purchased from the following sources:

1. Aldrich Chemical Co. (Milwaukee, Wisconsin)

Allyl chloride, Aluminum chloride (anhydrous), Benzalacetone, Calcium chloride (anhydrous), Deuterochloroform (gold label), Diethylene glycol, Diphenylacetonitrile, Ethylbromide, Lithium aluminum hydride, Magnesium turnings, N-methylhydroxylamine hydrochloride, Sodium Cyanoborohydride, Sodium hydride (50% oil dispersion).

2. Allied Chemical, (New York, New York)

Sodium acetate, Ferrous sulfate

3. American Scientific and Chemical Co. (Seattle, Washington)

Formaldehyde (38% aqueous), Hydrochloric acid, Potassium hydroxide, Sodium hydroxide, Sulfuric acid.

4. Applied Science Laboratories (State College, Pennsylvania)

Dexsil 300

5. J.T. Baker Ltd. (Philipsburg, New Jersey)

Silica Gel for Flash Chromatography, Silica Gel precoated plates, Sodium metal.

6. British Drug Houses (Poole, U.K.)

Acetonitrile, Acetone, Benzene, Calcium carbonate, 2,4-dinitrophenylhydrazine, Ethyl formate, Ethyl phthalate, Hydroxylamine hydrochloride, Ligroine, Magnesium sulfate, Petroleum ether (30°-60°), Potassium chloride, Propylene glycol, Pyridine, Sodium chloride, Toluene.

7. Brinkman Instruments (Toronto, Ontario)

Dragendorf's Reagent.

8. Caledon Laboratories (Georgetown, Ontario)

Chloroform (distilled in glass grade), Ether, Ethyl acetate (distilled in glass), Methanol (distilled in glass grade), Methanol (HPLC grade), Water (HPLC grade).

9. Eastman Kodak Co. (Rochester, New York)

Methylamine 40% Aqueous

10. Fisher Chemical, (Fairlawn, New Jersey)

Benzophenone, Chromium trioxide, Magnesium chloride, Sodium bicarbonate.

11. Linde Co. (Union Carbide, Vancouver, B.C.)

Molecular sieves, Nitrogen gas.

12. Mallinckrodt (St. Louis, Missouri)

Sodium bisulfate, Sodium sulfate, Xylene, 60% Perchloric Acid.

13. Matheson Limited (Edmonton, Alberta)

Gaseous hydrochloric Acid.

14. Matheson, Coleman and Bell (Norward, Ohio)

Dimethyl sulfoxide, Disodium hydrogen phosphate, Methyl acrylate, Potassium carbonate (anhydrous), Propylene oxide, Sodium dihydrogen phosphate.

15. Merck Co. (Rahway, New Jersey)

Yellow mercuric oxide

16. Merck Sharp and Dohme (Isotopes) (Montreal, Quebec)

Deuterium oxide.

17. Pierce Chemical Co. (Rockford, Illinois)

BSTFA, TMAH, MOX.

18. Sigma Chemical Co. (St. Louis Missouri)

Glucose-6-phosphate, Glucose-6-phosphate dehydrogenase, Glucurase, Glusulase, NADP.

19. Stanchem Ltd. (Winnipeg, Manitoba)

95% Ethanol.

20. Supelco Ltd. (Bellanfonte, Pennsylvania)

DMCS

3% OV-17 on GCQ

21. Synthesized in our laboratory (Abbott et al.(66))

2,2-Diphenyl-4-dimethylamino valeronitrile

Ethylidene dimethyl diphenyl pyrollidine perchlorate.

Ethyl methyl diphenyl pyrollidine.

Methadone hydrochloride.

Methadone-$^{2}H_{10}$ hydrochloride.

22. Terochem, (Edmonton, Alberta)

85% M-chloroperbenzoic acid.

23. Ventron (Beverly, Massachusetts)

Sodium borohydride.

B. INSTRUMENTAL

1. Nuclear Magnetic Resonance Spectra

Routine proton NMR spectra were recorded on a Bruker WP-80 or a Varian XL-100 spectrometer.

Decoupling and high resolution experiments were performed on a Bruker WP-400 spectrometer.

^{13}C NMR spectra were recorded on a CFT-20 and Bruker WP-400 spectrometers. All high resolution SFORD experiments were performed on the 400 MHz instrument.

Spectra were recorded in $CDCl_3$ with TMS as an internal standard. All spectra are included in the Appendix.

All NMR spectra (save one) were recorded at the NMR facility in the Department of Chemistry, U.B.C.

The micro-cell 400 MHz spectrum of eluted GC peaks was recorded at the NMR facility at Simon Fraser University.

2. Infra Red Spectra were obtained with sodium chloride disks either as liquid films, nujol mulls, or in 0.1 mm path length solution cells using

$CHCl_3$ as a solvent, on an Unicam SP-1000 spectrometer. All spectra are included in the Appendix.

3. Gas Chromatography Mass Spectrometry

GCMS analysis was performed on a Hewlett Packard 5700A gas chromatograph interfaced to a Varian Mat-111 Mass Spectrometer via a variable slit separator. Electron impact spectra were recorded at 80eV, ion source pressure 8.0×10^{-6} torr, emission current 300 uA. Direct probe experiments were performed on the Mat-111. Computerized background subtractions were made to plot mass spectra. The scan range was 4 to 500 with one scan every five seconds. TIC plots were based on m/z 50 to 500. Mass chromatograms were plotted in scan mode. The data was processed by an on line Varian 620 L computer system. Chemical ionization, capillary GCMS was performed on a Hewlett Packard 5987A instrument using methane as reagent gas.

Gas Chromatographic Conditions

(a) 3% OV-17 on 80/100 mesh Chromosorb W-HP
200°-280° at 8°/minute, Helium flow 20 ml/min.
Column length 2 m, column internal diameter 2 mm.

(b) 3% OV-17 on 100/120 mesh Gas Chrom Q
150-280° at 4°/minute, helium flow 20 ml/minute.
Column length 2 m, column internal diameter 2 mm.

(c) 3% OV-17 on 100/120 mesh Gas Chrom Q.
200-280° at 4°/minute, helium flow 20 ml/minute.
Column length 2 m, column internal diameter 2 mm.

(d) 3% Dexil 300 on Chromosorb W-AW.

200-280° at 8°/minute. Helium flow 20 ml/minute.

Column length 2 m, column internal diameter 2 mm.

(e) 2.3% Dexil 300 on 80/100 Gas Chrom Q.

200-280° at 8°/minute. Helium flow 20 ml/minute.

Column length 2 m, column internal diameter 2 mm.

(f) Capillary Column: Cross linked Dimethyl Silicone.

(Hewlett Packard part number 19091-60312)

Length 12.5 metres. Internal diameter 0.2 mm.

Column Temperature: 50-200° at 30°/minute. Then

(i) 10 or (ii) 5°/minute to 280°

(g) 3% OV-17 on 100/120 mesh Gas Chrom Q.

150°-280° at 2° per minute. Helium flow 20 mL/minute.

Column length 2 m, diameter 2 mm.

In all of the above, the GC inlet line, injection port and separator temperature were 250°. Injections were in a splitless mode.

4. Liquid Chromatography Mass Spectrometry

Liquid Chromatography was performed on a Hewlett Packard 1082B Liquid Chromatograph either with UV detection at 254 nm or interfaced to a Hewlett Packard 5987A Mass Spectrometer.

A Hewlett Packard RP.8, 10 cm length, 4 mm diameter reversed phase column (Cat.Δ79918B) equipped with a C_{18} guard column was run at a flow rate of 1 mL/minute and column pressure 7 bar.

Using a mixture of 2 solvents (a) 50% water 50% methanol and (b) 100% methanol, the following solvent program gave satisfactory resolution of nitrones, oxaziridines and formamides.

Time (minutes)	% B	%A
0.0	0.0	100
30.0	0.0	100
35.0	25.0	75
50.0	25.0	75
51.0	100.0	0
60.0	stop	

5. Ultraviolet Spectrometry

UV spectra were recorded in methanol on a Beckman Model 24 UV-visible spectrometer in 1 cm path length cells.

6(a) Melting Points

Melting points were determined on a Thomas-Hoover Capillary melting point apparatus and are uncorrected.

6(b) Elemental Analyses

Elemental analyses were performed by the Canadian Micro-Analytical Service Ltd. 5704 University Blvd. Vancouver, B.C. V6T 1K6.

7. Metabolism Experiments

(a) Centrifugation

An International Equipment Co. Model B-20 centrifuge and a Beckman L5-50 ultracentrifuge were employed in the microsomal studies.

(b) Tissue Homogenization

This was performed on a Fisher Dynamix tissue homogenizer.

(c) Lyophilization

A Vir Tis Lyophilizer (Gardiner, N.J.) was used.

(C) IN VIVO METABOLISM

1. BILE DUCT CANNULATION

Sprague-Dawley rats of either sex weighing 200-250 grams were anaesthetized with ether during surgery. A midline abdominal incision was made and the common bile duct was isolated and cannulated with polyethylene-10 tubing. The abdomen was closed with interrupted sutures and the rat was placed in a restraint cage.

After recovery from anaesthesia, each rat was administered subcutaneously one of the following drugs in 0.1 ml of vehicle.

DRUG	DOSAGE	VEHICLE
Methadone HCl	20 mg/kg	sterile water
Methadone $^{2}H_{10}$.HCl	20 mg/kg	sterile water
Recipavrin (free base)	20 mg/kg	propylene glycol
Norrecipavrin HCl	20 mg/kg	propylene glycol
Methadone analogue HCl	20 mg/kg	water

Bile was collected for 18 hours, with a second dose of drug administered after 12 hours.

2. EXTRACTION OF CONJUGATED AND NONCONJUGATED METABOLITES FROM BILE

Following the method of Kang (1) with modifications, each bile sample (10-15 ml) was diluted with an equal volume of water, and adjusted to pH 9 with 0.01 M sodium hydroxide. Non conjugated metabolites were extracted by gentle mixing with three, fifty mL aliquots of chloroform. The organic phase was dried over potassium carbonate and evaporated. The residue was dissolved in 20-50 µL of methanol and 5 µL analyzed by gas chromatography mass spectrometry.

The aqueous phase, containing the conjugated metabolites was lyophilized, the pH adjusted to 5 with dilute acetic acid and the residue taken up in 0.1 M sodium acetate buffer (pH 5). Five thousand units of β-glucuronidase (as Glusulase or Glucurase) was added to the buffered solution. After 12 hours incubation at 37°C, the solution was adjusted to pH 8-9 with 0.01 M sodium hydroxide, then extracted gently with four, fifty ml aliquots of chloroform, dried over potassium carbonate, flash evaporated, and the residue redissolved in 20 uL of methanol. Five to ten uL were injected into the GCMS.

(D) IN VITRO METABOLISM After Beckett (67), Gorrod (68), and Coutts (69).

1. PREPARATION OF MICROSOMES

Sprague-Dawley rats of either sex, weighing 200-250 grams, were stunned and then killed by decapitation. The peritoneum was opened and the liver perfused with cold 1.15% potassium chloride via the portal vein. The liver was removed and placed in cold 1.15% KCl. Five g portions, including the main lobe were homogenized in 20 mL of cold 1.15% KCl, then centrifuged at 4°C for ten minutes at 10,000 r.p.m.

Surface lipid was removed with a cotton swab and 8 ml of supernatant (equal to 2 g liver) was pipetted into an ultra centrifuge tube, and spun at 100,000 times gravity for 65 minutes at 4°C.

The tubes were placed on ice, the supernatant decanted, and the remaining microsomal pellets washed with 3 five mL aliquots of pH 7.2, 0.1 M phosphate buffer. The pellets were resuspended in 4 mL of phosphate buffer.

2. METABOLIC PROCEDURE

After ten minutes equilibration at 37°C, 4 mL cofactor solution and 40 µmol of drug were added to the microsomal suspension and vortexed briefly. After incubation with gentle agitation for 2 hours at 37°, the tubes were placed on ice and adjusted to pH 8 with 0.1 M NaOH. Each tube was extracted gently with three, fifty mL aliquots of chloroform. The combined extracts were dried over K_2CO_3 evaporated, then reconstituted in 20 uL of methanol and 5 ul injected into the GCMS.

Cofactor Solution

$MgCl_2$	8.34 mM	4 mL
NADP	1.32 mM	30.6 mg
Glucose-6-phosphate	13.32 mM	112.8 mg
Glucose-6-phosphate Dehydrogenase	200 units	800 uL
Tris buffer pH 7.5	1.29 mM	11.2 mL

E. GENERAL

1. FLASH CHROMATOGRAPHY (After Still et al (70))

A six inch bed of silica gel (flash chromatography grade) was packed over a 0.5 cm bed of silica sand. The column was topped with an additional 0.5 cm of silica sand. Eluent, proven by TLC to have polarity sufficient to move the desired component to an R_f of 0.3 to 0.4, was forced through the column with nitrogen gas until no more air was evident in the column. The sample was placed on column in 0.5 mL of eluent and 5 mL fractions collected. Nitrones and formamides were eluted with ethyl acetate in 10 mL fractions.

F. SYNTHESIS OF POTENTIAL N-OXIDIZED METABOLITES OF METHADONE

1. SYNTHESIS OF 2,2-DIPHENYLPENT-4-ENE NITRILE (ALLYL DIPHENYL ACETONITRILE) (38)

Following a modified method of Wilson (71), diphenyl acetonitrile (21.6 g, 0.11 mol) was treated with a suspension of 5.25 g (0.11 mol) of sodium hydride (previously washed free of oil with dry benzene) in 90 mL of dry benzene containing 2 drops of dimethylsulfoxide.

Allyl chloride (8.42 g, 0.11 mol) in 10 mL dry benzene was added dropwise. The solution was refluxed for 2 hours and left stirring overnight protected from moisture with a $CaCl_2$ guard tube. The solution was filtered throught sintered glass, and washed twice with 10 mL 1 N HCl, once with 20 mL $NaHCO_3$ and twice with 10 mL H_2O, then dried over $CaCl_2$ and flash evaporated. The product ($C_{17}H_{15}N$, MW 233.32) distilled as a clear liquid at 170°/1.0 mm (literature bp 162/1.5 mm) (71).

Mass Spectrum: m/z 233 (M^+ 12%), 192(100), 165(68).

Reaction of the product with ethylmagnesium bromide afforded 4,4-diphenyl-hepten-5-one (39) (GCMS evidence).

Mass Spectrum: m/z 129(100), 91(85), 207(40) 57(10), 264(3).

2. SYNTHESIS OF 3,3-DIPHENYL-5-METHYL TETRAHYDRO-2-FURANONEIMINE (40) (CAS 17279-17-3)

Following the method of Easton et al (72) but substituting sodium hydride for sodium amide. Diphenyl acetonitrile (1.0 g, 4.7×10^{-3} mol) in dry benzene was added slowly to a stirred solution of 0.3 g (6.3×10^{-3} mol) of sodium hydride (washed free of 50% oil dispersion with benzene) in 20 mL dry benzene which contained a drop of dimethyl sulfoxide. After warming the solution gently, 300 mg (5.2×10^{-3} mol) of propylene

oxide in 5 mL dry benzene was added to the green solution. After a gentle reflux overnight, followed by suction filtration through sintered glass, the solution was poured slowly into 100 mL cold H_2O. The benzene was separated, dried over $MgSO_4$, and evaporated, yielding 0.7 g (54%) of a thick liquid, distilling at 180°/0.5 mm. The product solidified on trituration with petroleum ether.

Analysis For: $C_{17}H_{18}NO$, (Molecular Weight 251.33).

Mass Spectrum: m/z 251 (M^+ 30%), 129(100), 91(85), 207(30), 210(30), 165(20).

IR (mull): ν max 3400 cm^{-1} (Sym. N-H str); 1670s, 1590(m, N-H Bend), 1360(m) 910(m) 685(s).

The hydrochloride salt was precipitated from dry benzene with gaseous HCl. Melting Point 222°-decomposes (literature 220-222° (72)).

3. SYNTHESIS OF 2,2-DIPHENYL-4-VALEROLACTONE (41)

The lactone was synthesized by a modified method of Attenburrow et al (73), with sodium hydride substituted for sodamide.

Diphenylacetonitrile (75 g, 0.4 mol) was added to a stirred suspension of 18.66 g (0.4 mol) sodium hydride (washed free of oil with dry benzene) in dry benzene containing 5 drops of DMSO. The solution was warmed gently on a steam bath until green. Then 23.2 g (0.4 mol) propylene oxide was added dropwise, and the solution was refluxed overnight, protected with a $CaCl_2$ guard tube. After cooling and filtration through sintered glass, the solution was poured slowly into 100 mL of ice cold 2N HCl. The layers were separated and the organic phase extracted with two further 25 mL aliquots of 2N HCl. The imine was hydrolyzed by refluxing the HCl solution for 2 hours on a steam bath. The cooled solution was extracted with ether, washed with water, dried over $CaSO_4$, filtered, and evaporated. Following

recrystallization from benzene and ligroine, 77.8 g (79%) of white crystals melting at 111°C (literature 115-116°(73)) were recovered.

Analysis For: $C_{17}H_{16}O_2$, (Molecular Weight 252.315)

Mass Spectrum: m/z 252 (M^+ 8%), 115(100), 208(90), 193(78), 130(75).

NMR (100 MHz): δ ppm, 1.48 doublet (3H, $-CH_3$); 4.5 multiplet (1H, CH); 2.45-2.75, dd(1H, CH_2); 2.95-3.2, dd(1H, CH_2).

IR (mull): ν max, 1745 cm^{-1}, (s, C=O str), 1480(m), 1440(s), 1370(m), 1330(m), 1170 (s, C-O str), 960(m), 690(s).

4. SYNTHESIS OF 1,1-DIPHENYL-2-BUTANONE (42) (CAS 6336-52-3)

Diphenylacetonitrile (10 g, 5.2×10^{-2} mol) dissolved in 30 mL dry toluene and added to a Grignard reagent of 1.9 g (8×10^{-2} mol) Mg turnings and 8.7 g (8×10^{-2} mol) ethyl bromide in dry ether.

Ether was distilled off until the flask temperature was 96°C. After refluxing overnight, protected with a $CaCl_2$ guard tube, the flask was cooled and the reaction mixture decomposed by the slow addition of 200 mL 2N HCl, followed by 2 hours stirring on a steam bath. The toluene layer was removed, dried over $CaCl_2$, evaporated and the residue distilled. The ketone distilled at 152-155° (0.7 mm), yielding 7.23 g (61.8%) of a clear liquid.

Analysis For: $C_{16}H_{16}O$, (Molecular Weight 224.305).

Mass Spectrum: m/z 224 (M^+ 2%), 167(100), 165(23), 57(22).

5. SYNTHESIS OF 4,4-DIPHENYL-2,5 HEPTANE DIOL (43)

Following the method of Wilson (71), 50 g (0.2 mol) 2,2-diphenyl-4-valerolactone in 25 mL toluene was added dropwise to a freshly prepared Grignard reagent containing 7.23 g (0.3 mol) of magnesium turnings and

32.7 g (0.3 mol) ethyl bromide in dry ether. The solution was left stirring overnight. Water was added dropwise with cooling. The solution was acidified with 2N HCl and extracted with three fifty mL aliquots of benzene. The organic phase was dried over $CaCl_2$ and evaporated. Trituration of the residue with pet. ether, and recrystallization from benzene/ligroine (60-80°) gave 36.6 g (65%) of white crystals melting at 121° (literature M.P. 124-125° (71)).

Analysis For: $C_{19}H_{24}O_2$, (Molecular Weight 284.4).

Mass Spectrum: m/z 264(M^+-20, 5%), 208(100), 193(82), 103(80), 130(55), 115(50), 91(42), 181(40), 45(30).

NMR (400 MHz): δppm, 0.82 m (1H, CH_3-CH_aH_b); 0.97 t(2H, CH_3-CH_2); 1.12 d(3H, CH_3-CH); 1.67 m (1H, CH_3 CH_aH_b); 2.27 dd(1H, CH_3-CH_aH_b-CH); 2.44 dd(1H, CH_3-CH_bH_a-CH); 3.72 m (1H, CHOH-CH_3);
4.38 dd(1H, CHOH-CH_2); 7.2 (10H, Arom).

IR (mull): νmax 3150 cm^{-1}, (broad, s, OH Str), 1580(m) 1440(s) 1360(s) 1330(m, O-H Bend), 1160(s) (s, C-O Str), 750 (s, Ar), 700 (s, Ar).

A petroleum ether wash of the crude product afforded 10 g (17.8%) of 3,3-diphenyl-5-methyl-2-ethylidene tetrahydrofuran (44) (CAS 17494-37-0) as a pale yellow oil.

Analysis For: $C_{19}H_{20}O$, (Molecular Weight 264.37).

Mass Spectrum: m/z 264 (M^+ 100%), 91(45), 179(40), 105(32), 115(25), 131(22), 43(22), 207(20), 222(14), 249(12).

NMR (100 MHz): δppm, 1.38 d(3H, CH_3-CH=); 1.68 d(3H, CH_3-CH); 2.6 dd(2H, CH_2); 3.92 q (1H,=CH-CH_3); 4.12 m(1H, CH_3CH-CH_2); 7.3 (10H, Aromatic).

IR (film): νmax 1680 cm^{-1}, (s, C=C Str, vinylether), 1590(m), 1490(s), 1440(s), 1360(m), 1290(m), 1200-1000 (s, C-O Str), 740 (s, Ar), 700 (s, Ar).

6. SYNTHESIS OF 4,4-DIPHENYL-2,5 HEPTANEDIONE (45)

Jones reagent was prepared by dissolving 500 mg of CrO_3 in a solution of 0.46 mL H_2SO_4 and 0.8 mL water then diluting to 2 mL with water. The Jones reagent was added dropwise to 150 mg of 4,4-diphenyl-2,5-heptanediol in acetone, on an ice bath. The addition was discontinued when the red brown color persisted for 15 minutes. The solution was diluted with water and isopropanol and extracted with ether. The ether phase was washed with dilute $NaHCO_3$ solution, then with water, dried over K_2CO_3 and the solvent evaporated. The yellow oil crystalized upon trituration with petroleum ether (30-60°). Fractional crystalization from ether/petroleum ether (30°-60°) afforded 5% diketone (tedious procedure), with the major product being 2,2-diphenyl-4-valerolactone (41).

Analysis For: $C_{19}H_{20}O_2$' (Molecular Weight 280.37)

Elemental Analysis: Calculated C, 81.397%; H, 7.19%; O, 11.43%

Found C, 81.14%; H, 7.1%; O, 11.76%

Mass Spectrum: m/z 262 (M^+-18, 2%), 43(100), 57(15), 223(12), 181(10), 206(8), 29(10).

^{1}H NMR (80 MHz): δ ppm, 0.94 t (3H, CH_3-CH_2); 2.0 s (3H CH_3 -C=O); 2.39 q (2H, CH_3-CH_2); 3.58 s (2H, CH_2-C -Ar_2) 7.3 (10H, Aromatic).

IR (mull): νmax 1710 cm^{-1}, (s, C-O Str), 1490(m) 1460(s, CH_3 bend) 1370, 1360 (m s,CH-C(=O)-CH) 1180 (m, C-C(=O)-C bend) 1120(m) 700(s,Ar).

^{13}CNMR (20 MHZ): δ ppm, 8.8 (CH_3-CH_2); 31.24 (-CH_2-CH_3); 33.04 (CH_3-C(=O)); 52.6 (CAr-CH_2-C(=O)); 63.92 (Ar_2CR_2); C=O not observed.

7. SYNTHESIS OF 2-(4',4'-DIPHENYLHEPTAN-5'-ONE-2'YL)-OXAZIRIDINE (5)

A modification of Kang's (1) method: To a solution of 100 mg (2.6×10^{-4} mol) ethylidene dimethyl diphenyl pyrollidine perchlorate in 5 mL of $CHCl_3$ at 0°C was added 100 mg (5.2×10^{-4} mol) of metachloroperbenzoic acid in 5 mL $CHCl_3$. After stirring overnight at 0°C, the solution was filtered and washed with two ten mL aliquots of 1.5 N NaOH, then twice with water, dried over $MgSO_4$ and the solvent flash evaporated.

The residue was flash chromatographed using 75% petroleum ether (30°-60°) 25% ethyl ether as eluent (alternatively 10% ethyl acetate in petroleum ether 30°-60°) under standard conditions. The product oxaziridine, as a mixture of diastereomers was eluted in the 15-25 mL fractions. The product was detected on thin layer chromatograms as a pair of partially resolved black spots when sprayed with Dragendorf's reagent. Yield after chromatography, 30 mg (36%). On long standing in $CDCl_3$ in the freezer, large cubic crystals were deposited, melting at 80° and decomposing at 150-160°. The crystals were stable at 0°.

Analysis For: $C_{20}H_{23}NO_2$, (Molecular Weight 309.411).

Elemental Analysis: %C calculated 77.64; found 77.61
%H calculated 7.49; found 7.45
%N calculated 4.52; found 4.52
%O calculated 10.34; found 10.42

NMR (400 MHz): δ ppm (major diastereomer) 0.85, t(3H, $\underline{CH_3}$-CH_2); 0.84 d (3H, $\underline{CH_3}$-CH); 1.8, m (1H,-$\underline{CH}$-CH_3); 2.27, q buried (2H,-$\underline{CH_2}$-CH_3); 2.32, dd(1H, $\underline{CH_a}H_b$-CH); 2.80, dd(1H $\underline{CH_b}H_a$); 3.17, d(1H; oxaziridine ring); 3.62, d(1H, oxaziridine ring); 7.3 (10H, Aromatic).

(100 MHz) δ ppm (minor diastereomer) 0.56, d(3H, $\underline{CH_3}$-CH-N), 0.85, t(3H, $\underline{CH_3}$- CH_2); 1.8, m(1H, CH_3-$\underline{CH}$-); 2.45, q buried (2H, -CH_2-CH_3);

2.35, dd(1H,-CHaHb-CH); 3.2 dd(1H, CH_bH_a-CH), 3.42, d(1H, oxaziridine ring), 3.92, d(1H, oxaziridine ring J=10Hz), 7.3, (10H, Aromatic).

IR (film): ν max 3000 cm^{-1}, (m), 1710 (s, C=O str), 1600(m-w), 1500(s), 1450(m), 1380 (w-m) oxaziridine, 1350 (w-m, -C=O Str), 1250(m), 1150(w-m, C-C=O-C, Str. and Bend), 1110(s-m), 1050(m), 950(w); 770(s, Ar), 710(s, Ar).

UV (Methanol): λ max 296 mμ (ε=502); 265(ε=548); 25.45 (ε=480, π-π *Ar); 207.5 (ε=21, 168 π-π *Ar)

^{13}CNMR: (Broad Band and SFORD 400 MZ) δ ppm 9.18, q(CH_3-CH_2); 21.29, q(CH_3-CH); 33.04, t(CH_2-CH_3); 211.26, s(weak C=O); 65.46, s(weak) (R_2-C-Ar_2); 42.6, t(CH_2-CH); 64.00, d(CH-CH_2); 72.54, t (CH_2-oxaziridine)

Mass Spectrum: By GCMS; m/z 309 (M^+3%), 72(100), 207(72), 73(60), 208(50), 44(46), 253(42), 129(30), 57(22), 291(8).

By Direct Inlet: (Source Temp. 100°) m/z 56(100), 72(78), 57(60), 207(40), 42(37).

8. SYNTHESIS OF 2-(N-FORMYL)-4,4-DIPHENYL-5-HEPTANONE (31)

2-(4',4'-diphenylhept-5'-one-2'-yl) oxaziridine (100 mg, 3.2 x 10^{-4} mol) was added to 10 mL dry oxygen free, m-xylene, and refluxed under nitrogen overnight in the dark.

The pale yellow solution was flash evaporated and flash chromatographed. After a 50 mL prerun of 50% petroleum ether (30-60°) in ethyl acetate, the amide was eluted with pure ethyl acetate in the 15-25 mL fraction. Twenty mg (20%) of product was obtained. The column was stripped with 25 mL methanol to isolate the isomeric nitrone, but none was detected.

Mass Spectrum: By GCMS: Identical to starting material.

NMR (400 MHz): Major Component δ ppm 0.85, t(3H, CH_3-CH_2); 1.11, d(3H, CH_3-CH); 2.1-2.3, q buried (2H, CH_2-CH_3); 2.4-2.5, dd buried (1H, CH_aH_b-CH); 2.8-2.9, dd(1H, CH_bH_aCH); 3.25, m(1H, CH_2-CH-CH_3); 5.98, bs(1H, NH); 7.1-7.4, m(10H, Ar) 7.75, s(1H, C(=O)H).

Minor Component: δ ppm 0.85, t(3H, CH_3-CH_2); 1.12, d(3H, CH_3-CH); 2.14-2.3, q buried (CH_3-CH_2-C(=O)); 2.3-2.4, dd(1H, CH_aH_b-CH_2-); 2.7-2.8, dd(1H, CH_bH_a-CAr_2); 2.9-3.0, m(CH_3-CH-CH_2); 5.52, bs(1H, NH); 7.1-7.4, S(10H, Ar); 7.45, d(1H, C(=O)H).

IR (film): ν max: 3360 cm^{-1}, (m, shoulder), 3260 (m, broad), 1735(w-m shoulder), 1710(s, C=O Str), 1670(s), 1535(m), 1495(m), 1445(m), 1380(mw), 1140(mw), 1100(wm-doublet), 1035(2m), 915(w-m broad), 755(m), 730(m), 700(s).

G. DIKETONE REACTIONS

1. SYNTHESIS OF 4,4-DIPHENYL-2,5-HEPTANEDIONE-2-OXIME (46)

4,4-Diphenyl-2,5-heptanedione, 50 mg (1.78×10^{-4} mol) in 5 mL of methanol was added to a solution of 13 mg (1.9×10^{-4} mol) hydroxylamine hydrochloride in 2 mL H_2O, preadjusted to pH 9.5 with 0.1 M NaOH.

After stirring at room temperature overnight, the solution was diluted with water, saturated with NaCl and extracted with ether. The ether layer was separated, dried over $CaCl_2$ and evaporated to give a yellow oil which solidified on trituration with petroleum ether (30-60°). Recrystallization from ether afforded 25 mg (50%) of product.

Analysis For: $C_{19}H_{21}NO_2$, (Molecular Weight 295).

NMR (80 MHz): δ ppm 0.78, t(3H, CH_3-CH_2); 1.8, broad singlet (3H, CH_3-N=OH); 2.1, broad quadruplet (2H, -C=O-CH_2-CH_3); 3.3, broad singlet (2H, C(=NOH)CH_2), 7.3 (10H, Ar)

IR (film): ν max 1620 cm^{-1}, (s, C=N Str), 1710(C=O Str), 1440(s), 1370(m), 1320(m), 1180(s), 1130(m).

Mass Spectrum: m/z 279 (M^+-16,4%), 42(100), 91(70), 57(65), 29(40), 206(50), 220(55), 238(25), 246(23), 261(20).

2. SODIUM CYANOBOROHYDRIDE REDUCTION OF 4,4-DIPHENYL-2,5-HEPTANEDIONE 2-OXIME

Attempted synthesis of 4,4-diphenyl-2-(N-hydroxylamino)-5-heptanone (47). The previous product (46) (1.4×10^{-4} mol) in 10 mL methanol was adjusted to pH 4-5. $NaBH_3CN$ (10 mg, 2.1×10^{-4} mol) was added with stirring. 2N HCl was added dropwise to maintain pH 4-5. When the pH remained constant, the solution was stirred for one hour, then adjusted to pH 1. Gas evolution was allowed to subside. After adjusting to pH 8, the solution was saturated with sodium chloride, extracted with ether, dried over $CaCl_2$, and evaporated, leaving an amber liquid, possibly 4,4-diphenyl -2-(N-hydroxylamino)-5-heptanol.

Analysis For: $C_{19}H_{25}NO_2$, (Molecular Weight 299 (expected for the heptanol (48)).

Mass Spectrum: m/z 279(M^+-20,35%), 91(100), 105(50), 115(40), 250(40), 129(30), 193(30), 208(30), 280(10), 281(2 (M-18)).

NMR (80MHz) (impure): δ ppm 0.7, t(3H CH_3-CH_2); 1.8, broad(2-3H, CH_3-CH_2-); 1.5, d(3H, CH_3-CH); 1.2, s(2H, NH), 2.4 m(-CH_2-CH(-N)); 3.0, m(1H-CH_2-CH(NHOH)-CH_3); 4.0, t(1H, -CH(OH)- CH_2); 7.1-7.3-(10H Aromatic).

3. REACTION OF DIKETONE WITH N-METHYLHYDROXYLAMINE HYDROCHLORIDE

Attempted synthesis of 4,4-diphenyl-2-(N-hydroxy-N-methylamino) -5-heptanone (3)

To a solution of 20 mg (5×10^{-4} mol) N-methylhydroxylamine hydrochloride in 1 mL H_2O was added 70 mg (2.5×10^{-4} mol) diketone (45) in 10 mL methanol. After adjusting to pH 6 with 5% KOH, $NaBH_3CN$ 30 mg (5×10^{-4} mol) was added, and the pH maintained below 6 by dropwise addition of 5% HCl. When pH was stable, the solution was acidified to pH 1, with 5N HCl. Gas bubbles were allowed to subside. After adjusting to pH 8 with 1 N NaOH, the solution was saturated with sodium chloride, extracted with ether, and the ether phase dried over $CaCl_2$. After evaporation, GCMS revealed a mixture of products. Treatment of this mixture with yellow mercuric oxide failed to produce the methadone nitrone, 1-methyl-(N-methylene)-3,3-diphenyl-4-oxohexanamine-N-oxide (2) as determined by GCMS. The products were not further characterized.

Mass Spectrum: Major Product: m/z 276(8%), 199(100), 200(20), 183(10), 42(8), 247(5), 262(4), 220(4).

H. REACTIONS OF METHADONE OXAZIRIDINE (5)

1. REDUCTION WITH LITHIUM ALUMINUM HYDRIDE

Methadone oxaziridine (10 mg) was stirred over excess LAH in dry ether for 12 hours. After filtration and a water wash, the ether solution was dried over $CaCl_2$ and evaporated.

Analysis For: DINORMETHADOL (49): $C_{20}H_{27}NO$. (Molecular Weight 297.44).

Mass Spectrum: m/z 222(2%) 44(100), 58(82), 91(20), 208(18), 193(15), 115(15), 179(10).

NMR: (impure sample) δ ppm 0.88, t(3H, $C\underline{H}_3$-CH_2); 1.4 m, 1.25 m(2H, CH_2-CH_3), 4.04, dd(1H, $C\underline{H}$-OH), 1.07, d(3H, $C\underline{H}_3$-CH); 2.68, m(1H, $C\underline{H}$-CH_3); 2.2, dd, 2.4, dd(2H, CAr_2-$C\underline{H}_2$-CH); 1-2, bs(2H, NH_2); 7.2-7.5, (10H, Aromatic).

IR (film): ν max 3400 cm^{-1}, (w, broad, NH or OH Str), 2970 (m, OH intramolecular H-bond), 1600(w-m), 1490(m, s CH_2), 1440(m), 1260(2), 1120(w, C-N Str), 750(m), 730(m), 700(s).

4. REDUCTION WITH SODIUM CYANOBOROHYDRIDE

To a solution of 5 mg (1.6×10^{-5} mol) methadone oxaziridine (5) in 5 mL methanol was added 2 mg (3.2×10^{-5} mol) $NaBH_3CN$. The pH was maintained at 6-7 by the dropwise addition of 0.1 M methanolic HCl. When pH remained constant the solution was acidified to pH 1 and stirred until gas evolution ceased. After adjusting to pH 8 with 0.1 N NaOH, the solution was diluted with water, saturated with sodium chloride and extracted with chloroform. After drying and evaporation the product was analysed in methanol by GCMS. The diketone and a product which by GCMS appeared to be an EDDP analog (50) were present. The product did not react with BSTFA in CH_3CN and was not further characterized.

Analysis: MASS SPECTRUM OF MAJOR PRODUCT: m/z 280(1%), 99(100), 84(40), 250(22), 208(20), 193(18), 42(18), 115(15), 130(13), 251(5), 279(3).

I. SYNTHESIS OF RECIPAVRIN AND RELATED COMPOUNDS

1. SYNTHESIS OF γ-PHENYL-N,N,α-TRIMETHYL BENZENEPROPANAMINE PERCHLORATE (6) (CAS 13957-55-6)

Following the procedure outlined by May and Mossetig (74), 2-dimethylamino-4,4-diphenylvaleronitrile (1 g, 3.73×10^{-3} mol)

was dissolved, in a solution of 1 g KOH in 10 mL diethylene glycol (bp 250°) and refluxed overnight. When cool, the reaction mixture was diluted with 20 mL H_2O, extracted with ether, dried over $CaSO_4$ and concentrated to 10 mL. The perchlorate salt was precipitated by adding 60% perchloric acid to the ether solution. After two recrystallizations from ethanol 0.73 g (81%) white/beige crystals were obtained.

Analysis For: $C_{18}H_{24}NO_4Cl$, (Molecular Weight 353.84).

Melting Point: 160°C (decomposes).

Mass Spectrum: m/z 253(4%), 72(100), 73(12), 167(12), 44(8), 238(1).

IR (nujol mull, perchlorate salt): ν max 1600 cm^{-1} (s), 1500(m), 1470(s), 1380(s), 1150-1000(broad, strong), 950(m), 810(m), 770(m), 750(m), 670(s).

Analysis For: $C_{18}H_{23}N$, (Molecular Weight 253.39) (free base).

NMR: (80MHz) δ ppm 0.90, d(3H, CH_3CH); 2.0-2.5, m (3H, CH_2-CH); 2.20, s (6H, N-$(CH_3)_2$); 4.2, t(1H, Ar_2-CH-); 7.25 (10H, Ar).

2. SYNTHESIS OF 1,1-DIPHENYL-3-BUTANONE (51) (CAS 5409-60-9)

The synthesis followed was that of Burckhalter et al (75). In a one litre, three neck round bottom flask fitted with a sealed stirrer, thermometer, and 500 mL dropping funnel, were placed 300 mL dry benzene and 29 g (0.223 mol) $AlCl_3$. After cooling to 10°C on an ice bath, the suspension was maintained below 20° during the dropwise addition of a solution of 25 g (0.112 mol) benzalacetone in 60 mL dry benzene (approximately 30 minutes required).

The ice bath was removed and stirring continued overnight. The dark brown solution was decanted into 160 mL of 0.8 M HCl, and filtered with suction. The benzene layer was separated, washed twice with water, dried over CaSO4 and evaporated.

Distillation afforded 28.5 g (74%) diphenylbutanone boiling at 125° (0.3 mm) (Literature 164.5-168° at 4.5 mm (75)). The clear distillate solidified in the receiver.

Analysis For: $C_{16}H_{16}O$, (Molecular Weight 224.305).

Melting Point: 46° (literature 46°) (75).

Mass Spectrum: m/z 224 (M^+ 32%), 43(90) 167(100), 103(65), 165(38), 181(35), 152(22), 77(20).

NMR (100 MHz) δ ppm 2.08, s(3H, CH_3); 3.22, d(2H, CH_2); 4.63 t(1H, CH), 7.2 (10H, Aromatic).

IR (mull): ν max 3425 cm^{-1}, (w), 1712 (s, C=O Str), 1440(m), 1236(m) 1162(s).

3. SYNTHESIS OF 1,1-DIPHENYL-3-BUTANONE OXIME (52) (CAS 36317-57-4)

To a methanolic solution of 0.403 g (5.8×10^{-3} moL) hydroxylamine hydrochloride (adjusted to pH 8.5 with 2N NaOH) was added, 1 g (4.46×10^{-3} moL) 1,1-diphenyl-3-butanone in methanol. After stirring overnight at room temperature, the sample was diluted with water, extracted with $CHCl_3$, dried over K_2CO_3 and evaporated. The residue distilled at 193° (0.06 mm).

Analysis For: $C_{16}H_{17}NO$, (Molecular Weight 239.319).

Mass Spectrum: m/z 239 (M^+ 8%), 167(100), 165(25), 152(15), 103(18), 42(12), 118(11), 181(11), 220(6).

NMR (100 MHz): 2:1 mixture of syn-and anti-oximes SYN: δ ppm 1.8, s(3H, CH_3); 2.97, d(2H, CH_2); 4.35, t(1H, CH), 7.3, (10H, Ar). ANTI: δ ppm 1.57, s(3H, CH_3); 3.15, d,(2H, CH_2); 4.4, t(1H, CH); 7.3 (10H, Ar).

IR (film): ν max 3280 cm^{-1}, (s, broad OH-internal H bond), 3050(s), 2910 (s), 1665(w-m), 1610(m, C=N Str), 1595(w shoulder), 1500(s), 1460(s),

138(m), 1270(m), 1100(w), 1050 (w-shoulder), 1040(m), 970(m), 940(w-m shoulder), 760(s), 710(s).

4. SYNTHESIS OF α-METHYL-γ-PHENYL BENZENEPROPANAMINE-(DINORRECIPAVRIN) (53) (CAS 29869-77-0)

A solution of 2 g (8.9×10^{-3} mol) 1,1-diphenyl-3-butanone, 6.8 g (8.9×10^{-2} mol) ammonium acetate, and 0.78 g (1.25×10^{-2} mol) $NaBH_3CN$ in 50 mL methanol were stirred overnight at room temperature.

The solution was adjusted to pH 2 with 5N HCl. After bubbles had subsided, the methanol was evaporated and the residue taken up in 50 mL 2N NaOH. The amine was extracted with chloroform. The amine salt was extracted into 2N HCl. The HCl extract was made alkaline with 5N NaOH and the free base extracted with ether, dried over $CaSO_4$ and evaporated to give 0.6 g (29%) product.

Alternatively, after extraction from 2N NaOH with chloroform, drying and evaporation yields 1.4 g (70%) crude amine, which solidifies upon trituration with petroleum ether. This product may be recrystallized from benzene: ligroine. The HCl salt was precipitated from dry ether with gaseous HCl.

Analysis For: $C_{16}H_{19}N$, (Molecular Weight 225.34).

Melting Point: 172°C (HCl salt).

Mass Spectrum: m/z 225 (M+ 2%), 44(100), 208(12), 167(10), 165(5), 58(5), 115(4), 193(3).

NMR (100 MHZ): (free base) δ ppm 1.1, d(3H, CH_3); 1.24, s(broad) (2H, NH_2); 2.06, m(2H, CH_2); 2.76, m(1H, $\underline{CH}$-CH_3), 4.11, t($\underline{CH}$-CH_2), 7.3 (10H, Aromatic).

5. SYNTHESIS OF N, α-DIMETHYL-γ-PHENYL BENZENEPROPANAMINE (NORRECIPAVRIN) (54) (CAS 29869-78-1)

To a solution of 4.15 g (0.133 mol) of methylamine (11.25 g of 40% aqueous solution) in 50 mL methanol, was added 1.63 g (4.5×10^{-2} mol) of hydrochloric acid in 10 mL methanol followed by 5 g (2.2×10^{-2} mol), 1,1-diphenyl-3-butanone and 2.1 g (3.35×10^{-2} mol) $NaBH_3CN$.

The mixture was stirred over molecular sieve for 48 hours. The solution was filtered and acidified to pH one. After bubbles subsided, the solution was filtered and the methanol removed by flash evaporation. The residue was dissolved in 25 mL water, adjusted to pH 8 with 6N NaOH, saturated with sodium chloride, extracted with ether, dried over $CaSO_4$, and the hydrochloride salt precipitated with gaseous HCl. Product, 4.25 g (67%) of bluish granules, was recrystallized from ethyl acetate and acetonitrile.

Analysis For: $C_{17}H_{22}NCl$, (Molecular Weight 275.82).

Melting Point: 125°.

Analysis For: $C_{17}H_{21}N$, (Molecular Weight 239.363).

Mass Spectrum: m/z 239 (M+ 3%), 58(100), 167(8), 59(5), 115, 134, 152, 193, 208(2).

NMR (80 MHZ): δ ppm 1.06, d(3H, CH_3-CH); 1.36, bs(NH, H bonded), 1.87-2.5, m(3H CH_3-CH-CH_2), 2.34, s(3H, N-CH_3); 4.04, t(1H,-CH-Ar_2); 7.1(10H, Ar).

IR (film): νmax 3300 cm^{-1}, (b, weak, NH Str), 3000(s), 1615(m, NH bond), 1505(s), 1460(s, CH_2), 1380(m), 1160(m), 1050(m, C-N Str), 760(m-s doublet, NH wag), 705(s).

6. SYNTHESIS OF N-HYDROXY-α-METHYL-ϒ-PHENYL BENZENEPROPANAMINE (55)

To 2 g (8.9×10^{-3} mol) 1,1-diphenyl-3-butanone in methanol, was added 0.8 g (1.1×10^{-2} mol) hydroxylamine hydrochloride. The solution was adjusted to pH 6 with 6N KOH and 0.72 g (1.1×10^{-2} mol) $NaBH_3CN$ was added. The pH was maintained at 5-6 by dropwise addition of 4N HCl. When pH remained constant, the solution was stirred for an additional hour then adjusted to pH 1 with 4N HCl. After gas evolution ceased, the solution was diluted with water, and adjusted to pH 8 with 6N KOH, saturated with NaCl, and extracted 6 times with ether. The ether extract was dried over $MgSO_4$ and evaporated. Trituration with petroleum ether (30°-60°) and recrystallization from benzene/ligroine gave 0.59 g (30%) of a white solid. A TMS derivative was prepared for GCMS by treating the hydroxylamine with BSTFA in CH_3CN at room temperature.

Analysis For: $C_{16}H_{19}NO$, (Molecular Weight 241.335).

Combustion Analysis:

Calculated: C, 79.63; H, 7.94; N, 5.8; O, 6.63

Found: C, 78.93; H, 8.42; N, 5.45; O, 7.20

Mass Spectrum: Decomposes on column to Dinorrecipavrin (80).

TMS Derivative (56): ($C_{19}H_{27}NOSi$): m/z 313(M+ 8%), 132(100), 44(52), 116(50), 75(30), 167(30), 118(20), 91(10), 208(10), 298(8), 223(4).

NMR (100 MHz): δ ppm 1.1, d(3H, CH_3); 2.4 and 1.96, m(2H, CH_2); 4.1, t distorted (-CH-Ar_2); 2.9, m(1H, CH_2-CH-CH_3); 7.1-7.4 (10H, Aromatic), NH, OH not observed.

IR (nujol mull): ν max: 3250 cm^{-1}, (s(N-H/OH Str), 2858(b,s), 1696(w-m), 1610(m, NH bend), 1505(s), 1460(s, -CH_2), 1373(N-O Str), 1230(s), 1108, 1031, 750-780(s), 710(s)

7. SYNTHESIS OF N, α-DIMETHYL-N-HYDROXY-γ-PHENYL BENZENEPROPANAMINE (57)

To a solution of 0.42 (5×10^{-3} mol) N-methylhydroxylamine hydrochloride in 0.5 mL H_2O was added 1.0 g (4.4×10^{-3} mol) 1,1-diphenyl-3-butanone (51) in 26 mL methanol. The solution was adjusted to pH 6 and 0.35 g (5.5×10^{-3} mol) $NaBH_3CN$ was added. HCl (5%) was added dropwise to maintain pH 5-6 until pH remained constant. After stirring for an additional hour, the solution was adjusted to pH 1 with 6N HCl. After gas bubbles subsided the solution was diluted with 10 mL water, washed with ether, and adjusted to pH 8 with 5% KOH. After saturating with NaCl and extraction with ether, the ether was dried over $CaSO_4$ and evaporated. Trituration with petroleum ether afforded 0.24 g (50%) of a white solid, which melts at 115°C. A TMS derivative was prepared for GCMS with BSTFA in CH_3CN at room temperature.

Analysis For: $C_{17}H_{21}NO$, (Molecular Weight 255.357).

Combustion Analysis Calculated: C:79.96%, H:8.29, N:5.48, O:6.27.

Found: C:79.67%, H:8.44, N:5.47, O:6.42.

Mass Spectrum: Decomposes to N-methyl-1,1-diphenyl-3-butylamine in the GC.

TMS Derivative (58): ($C_{20}H_{29}NOSi$), (Molecular Weight 327.36).

m/z 327(M+ 4%), 146(100), 58(28), 167(15), 132(5), 73(5), 165(5), 208(3), 312(3).

NMR (100 MHZ): δ ppm 1.06, d(3H, CH_3-CH); 2.56, s(3H, CH_3-N); 4.1, t(1H, CH-Ar_2) 2.0, m(1H,N-CH-CH_3); 2.4-2.8, m buried (2H, -CH-CH_2-CH); 7.1-7.4 (10H, Aromatic); 9.7, (OH).

Infrared (nujol mull): ν max: 3200 cm^{-1}, (m broad N-H:OH Str), 1950(w), 1590(m, N-H Bend), 1580(m), 1490(s, sharp), 1450(s broad -CH_2), 1370(s, N-O Str), 1360(m shoulder), 1220, 1190, 1160, 1130(m), 1060, 1045, 1030(m), 930(w), 910(w), 800(m), 780, 750, 730(m-s), 700(s).

8. SYNTHESIS OF N-METHYLIDENE-1,1-DIPHENYL-3-AMINOBUTANE (59)

A solution of 1.0 g (4.18×10^{-3} mol) 1,1-diphenyl-3-aminobutane (53) and 0.51 mL (6.25×10^{-3} mol) of 38% aqueous formaldehyde solution in 20 mL methanol was stirred over molecular sieve at room temperature for 2 days. After filtration, GCMS revealed the correct molecular weight for the imine, which probably exists as the triazine (60).

Analysis For: $C_{17}H_{19}N$, (Molecular Weight 237.35).

Mass Spectrum: m/z 237(5%), 57(100), 222(20), 56(15), 91(10), 58(9), 167(9), 165(9), 44(5), 152(5).

INFRARED (film): νmax 2950 cm^{-1}, (m(broad)), 1600(w-m, C=N Str), 1490(m-s, C=N Str), 1450(m-s), 1380(w-m broad), 1150(m-s), 1070-1100(m-broad), 910(w-m), 740(s), 700(s).

9. SYNTHESIS OF 1,1 DIPHENYL-3-NITROSOBUTANE (61)

1,1-Diphenyl-3-aminobutane (1.0 g (4.4×10^{-3} mol)) (53) in a minimum of CH_2Cl_2 was treated with 1.06 g (5.33×10^{-3} mol) 85% metachloroperbenzoic acid at 0° for 24 hours.

The solution was filtered and the residue purified by flash chromatography in 98% petroleum ether 12% ethyl acetate. Product, 100 mg (9.3%) was recovered as a white waxy solid. The product visualized as black spots on TLC plates sprayed with Dragendorf's reagent. The product probably exists as the dimer (62).

Analysis For: $C_{16}H_{17}NO$, (Molecular Weight 239.319).

GCMS: Decomposes on column.

NMR - δ ppm:1.43, d(3H, CH_3); 2.0-2.4, m(1H, H, $C\underline{H}_aH_b$); 2.45-2.8, m(1H, $C\underline{H}_bH_a$); 3.2-3.4, q-m(1H, CH_3-$C\underline{H}$-CH_2); 4.13, t(1H, $Ar_2C\underline{H}$-CH_2).

IR (nujol mull): ν max: 1602 cm^{-1}, (w-m) (N-O monomer Stretch (or Aryl)), 1378(m, cis nitroso dimer), 787(m, N-O Stretch), 760(m), 738(m), 702(s).

10. SYNTHESIS OF N-FORMYL-α-METHYL-γ-PHENYL BENZENEPROPANAMINE (15)

1,1 Diphenyl-3-aminobutane (53) was refluxed in ethyl formate for 4 days. After flash evaporation, the residue was taken up in 1 mL 20% ethyl acetate in petroleum ether (30°-60°) and flash chromatographed with this solvent on a 10 cm by 1 cm column. Forty mg (18%) of product was recovered.

Analysis For: $C_{17}H_{19}NO$, (Molecular Weight 253.346).

Mass Spectrum: (GCMS) m/z 253(25%), 73(100), 208(42), 167(40), 165(28), 193(27), 130(37), 181(23), 72(23), 115(18), 58(12), 44(10). (IDENTICAL TO OXAZIRIDINE (14)).

NMR: (400 MHz) Mixture of 2 Compounds. Chemical shift varies with relation to nitrogen lone pair.

A. (major) δppm 1.19, d(3H, CH_3); 2.25-2.35, m(1H, $C\underline{H}_aH_b$); 2.0-2.1, m(1H, $CH_b\underline{H}_a$) 4.02, t(Ar_2-$C\underline{H}$); 4.02, m buried (1H CH_2-$C\underline{H}$-CH_3) 5.16, s, broad (NH); 7.1-7.3 (10H, Ar); 8.05, s(C(=O)$\underline{H}$).

B. (minor) 1.24, d(3H, CH_3); 2.1-2.2, m(1H, $C\underline{H}_aH_b$); 2.25-2.35, m buried (1H, $CH_b\underline{H}_a$); 3.3-3.4, m(1H, CH_2-$C\underline{H}$-CH_3); 4.04, t buried (1H,-$C\underline{H}$- Ar_2); 5.35 broad s(1H, NH); 7.1-7.3 (10H, Aromatic) 7.82; broad doublet (1H, C(=O)H).

Infrared (film) (poor spectrum): ν max: 3350 cm^{-1}, (broad m, N-H str), 1730(m, (C=O contaminant formate ester), 1675 (m shoulder C=O Stretch-Amide I band), 1460(m), 1390(m), 1265(w shoulder), 1190(broad m-s formate ester contaminant), 1050(w), 750, 770(w), 710(m).

The sample (pure by GCMS) was derivatized on column with TMAH as described in the Pierce catalogue (76). The formamide (1 mg in 10 µL CH_3CN), was treated with 20 µL TMAH and 5 µL was injected in the GCMS. The product, eluted at longer retention time, had a mass spectrum consistent with the tertiary amide, N, α-dimethyl-N-formyl-γ-phenylbenzenepropanamine (63).
Mass Spectrum: m/z 267 (M+, 10%), 87(100), 85(70), 58(28), 72(25), 167(22), 165(20), 208(18), 193(17).

11. SYNTHESIS OF 2-(4',4'-DIPHENYL-BUT-2'-YL) OXAZIRIDINE (14)

Following a modification of the method of Krimm (87), 500 mg (1.9×10^{-3} mol), of Dinorrecipavrin (80) in 10 mL water was cooled to 0°. Aqueous formaldehyde (38%) 0.4 mL (4×10^{-3} mol) and 50 mL of $CHCl_3$ were added. To the stirred mixture was added 0.79 g (3.9×10^{-3} mol) MCPBA (85% pure). After 2 hours, 10 mL of 1.1 m $CaCO_3$ solution was added dropwise with stirring.

The $CHCl_3$ phase was separated, dried over potassium carbonate, and evaporated.

Flash chromatography of the residue in 9:1 petroleum ether (30°-60°): ethylacetate afforded 165 mg (34%) mixture of 2 diastereomeric oxaziridines which gave black spots when visualized by Dragendorf's reagent on TLC plates. The chromatography was repeated and the major isomer isolated in pure form for NMR analysis. Samples were stored in the freezer but were unstable.

Analysis For: $C_{17}H_{19}NO$, (Molecular Weight 253.346).

Mass Spectrum: 1. GCMS: identical to N-formyl-1,1-diphenyl-3-aminobutane.
2. Direct Inlet: m/z 253(M+, 4%), 139(100), 156(90), 111(50), 167(48), 141(32), 158(30), 75(20), 50(18), 57(18), 208(12), 224(6), 237(2).

NMR (400 MHz): Major Isomer δppm 1.2, d(3H, CH_3); 1.86, (sextuplet) (1H, CH_3-C<u>H</u>); 2.34, dd(1H,-C<u>H</u>$_a$H$_b$-CH, J_{AB}=18 Hz); 2.21, dd(1H CH$_b$<u>H</u>$_a$-CH); 3.82, d(1H, oxaziridine <u>H</u>$_a$, J_{AB}=10 Hz); 3.28, d(1H, oxazirdine H_b) 4.01, t(1H, Ar_2-CH-) 7.1-7.4 (10H, Aromatic).

Minor Isomer (100 MHz): δ ppm 1.12, d(3H, CH_3); 1.85, m(1H, CH_3-C<u>H</u>-CH_2); 2.24, dd(1H, CH-C<u>H</u>$_A$H$_B$-CH, J_{AB}=18 Hz); 2.64, dd(1H CH-C<u>H</u>$_B$H$_A$-CH); 3.46, d(1H, oxaziridine (H_A J_{AB}=10 Hz); 3.95, d(1H, oxaziridine H_B); 4.25, t(Ar_2-C<u>H</u>-CH_2); 7.1-7.4 (10H, Aromatic).

^{13}C NMR (BB and SFORD): Major Isomer: δ ppm: 19.73(q, CH_3); 40.12 (t, -C<u>H</u>$_2$-CH); 65.09 (d, CH_3-C<u>H</u>-CH_2); 48.23 (d, CH-Ar_2); 71.97 (t, oxaziridine CH_2).

IR (film): ν max 3100-2900cm^{-1}, (m), 1750(w), 1610(m), 1590(w shoulder), 1520(s), 1480(s), 1390(m, oxaziridine), 1270(m-s), 1170(w-m), 1120(w-m), 1080(w-m), 1050(w-m), 970(w-m), 770(m-s), 700(s).

SYNTHESIS OF α-METHYL-(N-METHYLENE)-γ-PHENYLBENZENEPROPANAMINE N-OXIDE (Recipavrin Nitrone) (13)

Aqueous formaldehyde 38% w/v (0.4 mL, (5 x 10^{-3} mol)) in 20 mL dry benzene was refluxed for 2 hours in a Dean and Stark apparatus. Then 0.5 g (2.1 x 10^{-3} mol) N-hydroxy-1,1-diphenyl-3-aminobutane in benzene was added slowly with stirring. The apparatus was flushed with N_2 and refluxed for 20 minutes. After cooling, the benzene was evaporated and the product flash chromatographed in ethyl acetate. The polar product (R_f. 0.2) was eluted slowly (100-150 mL) yielding 250 mg (47%) of a clear thick liquid.

Analysis For: $C_{17}H_{19}NO$, (Molecular Weight 253.346).

Mass Spectrum: GCMS: Decomposes on column to N-methylidene-1,1-diphenyl-3-aminobutane (major) (59), 1,1-diphenyl-3-butanone oxime (52), and N-formyl-1,1-diphenyl-3-aminobutane (minor) (15).

Direct Inlet: m/z 253 (M+, 2%) 56(100), 236(15), 208(25), 91(40), 222(4).

^{1}H NMR: δ ppm, 1.42, d(3H, CH_3-); 2.23, dd(1H, CH-C$\underline{H_A}$HB-CH); 2.74, dd(1H CH-C$\underline{H_B}H_A$-CH-); 3.79, m(1H, CH_AH_B-C$\underline{H}$ J_{AB}=18 Hz); 5.98, d(1H, N=C$\underline{H_A}H_B$); 6.41, d(1H, N=C$\underline{H_B}H_A$); 7.1-7.4 (10H, Aromatic).

^{13}C NMR (Broad Band andSFORD): δ ppm 19.92(q, CH_3); 39.41 (t, $CHAr_2$-C$\underline{H_2}$-CH); 47.74(d,-C$\underline{H}Ar_2$); 69.03(d, CH_3-C$\underline{H}$-); 122.29(t, N=C$\underline{H_2}$).

Infrared (film): ν max 3395 cm^{-1}, (weak, broad) 1566(C=N), 3100-2900(m), 1580(w-m), 1490(s), 1450(s), 1296 (m), 1200(w), 1060(s), 920(w), 800(w), 760(doublet(m)), 710(s).

Ultraviolet: (methanol) λmax 235 mμ(ε=6210), 220(ε=10,120).

13. SYNTHESIS OF 1,1-DIPHENYL-3-BUTANONE-O-METHYL OXIME (64)

Following the procedure in the Pierce Catalogue (76).

To 50 mg (2.23×10^{-4} mol) 1,1-diphenyl-3-butanone in 0.1 mL pyridine was added 1.0 mL MOX. After heating for 3 hours at 60° in a reactivial with a teflon lined cap, the solution was diluted with 4 mL water, and extracted with three 10 mL aliquots of benzene. The organic phase was washed with 1N HCl and 1% $NaHCO_3$, then dried over $MgSO_4$, and concentrated to a volume of 0.5 mL. Two µL was injected into the GCMS.

Mass Spectrum: m/z 253 (M+, 8%) 167(100), 165(22), 152(12), 103(12), 118(12), 42(10), 77(9), 181(8), 222(3).

NMR (80 MHZ): Mixture of syn and anti oximes (2:1)- impure sample

A: δppm 1.72, s(3H, CH_3-C=N); 2.9, d(2H, CH_2-C=N); 3.76, s(3H,-O-CH_3); 4.35, t(1H, Ar_2-CH-); 7.25, s(10H, Ar).

14. ALKALINE OXIDATION OF N, α-DIMETHYL-N-HYDROXY- γ-PHENYL-BENZENEPROPANAMINE (57)

A. Bile from a rat was worked up by the procedure outlined earlier. After the extraction of non-conjugated metabolites, 10 mg of the secondary hydroxylamine was added to the conjugated fraction, and the sample work up continued. GCMS analysis of the conjugated fraction indicated the presence of a compound with mass spectrum identical to N-formyl-α-methyl-γ-phenyl benzenepropanamine (15).

B. Secondary hydroxylamine (42 mg) was treated with ethanolic NaOH for one week. The sample was diluted with water, extracted with $CHCl_3$, dried over K_2CO_3, evaporated and examined by HPLC.

J. ATTEMPTED CHARACTERIZATION OF THE RECIPAVRIN METABOLITE BY LCMS

Liquid chromatographic conditions were determined, to optimally separate the nitrone (13), amide (15) and oxaziridine (14). A sample of the conjugated fraction of rat bile from a recipavrin dosed rat was examined, but concentrations were too low for detection by selected ion monitoring of the m/z 254 (M+1) ion.

LCMS

Mass Spectrum: N-formyl-α-methyl-γ-phenyl benzenepropanamine (15)
(t_R=24.41 min) m/z 254 (M^++1, 100%); 255(M^++2, 20%)

Mass Spectrum: 2-(4',4'-Diphenyl-but-2'-yl) oxaziridine (14)
(t_R=40.87 min) m/z 254 (M^++1, 100%); 238(20); 255(20); 226(15); 73(4)

Mass Spectrum: α-methyl-(N-methylene)-γ-phenyl benzenepropanamine N-oxide (13).

(t_R=17.20 min) m/z 254 (M^++1, 100%), 238 (M+1-16, 20%), 74(20).

K. ATTEMPTED GAS CHROMATOGRAPHY/ CHEMICAL IONIZATION MASS SPECTROMETRY CHARACTERIZATION OF THE RECIPAVRIN METABOLITE

The recipavrin nitrone and oxaziridine CI mass spectra and capillary GC chromatograms were obtained. The conjugated fraction of bile from a recipavrin dosed rat was examined.

Chemical Ionization

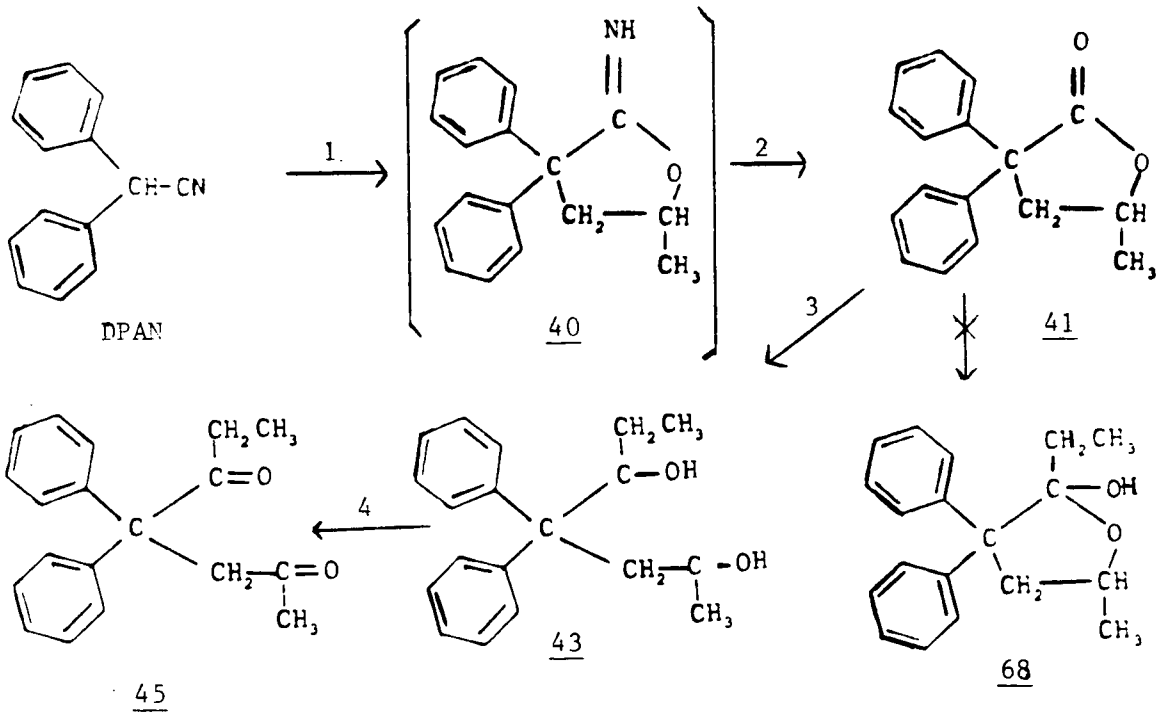
Oxaziridine (14) same as formamide (15): 254(M^+ +1, 100%); 255(M^+ +2, 20%); 282(M^+ +29, 20%), 57(10), 176(8).

III. RESULTS AND DISCUSSION

1. CHEMISTRY: SYNTHETIC METHODS AND ANALYSIS

A. Synthetic Pathways

In the synthetic portion of this thesis, the diketone (45) was a key intermediate in the attempted synthesis of the methadone nitrone (2). The diketone was obtained in low yield via the synthetic pathway:



1. a) NaH, C_6H_6, DMSO
   b) Propyleneoxide

2. H_3O^+/Δ

3. a) EtMgBr/Ether/toluene/Δ
   b) H_3O^+

4. a) $CrO_3/H_2SO_4/H_2O$/Acetone
   b) Isopropanol

In Figure 8 the attempted synthesis of the methadone nitrone (2) is outlined. The oxime (46) was synthesized but was unstable even when stored at 0°. Attempts to selectively reduce the oxime portion of 46 with $NaBH_3CN$ resulted in a mixture of pyrollidine like products or concomittant reduction of the keto group. Since the desired primary hydroxylamine (47) was unavailable, attempts to synthesize the methadone nitrone (2) were discontinued.

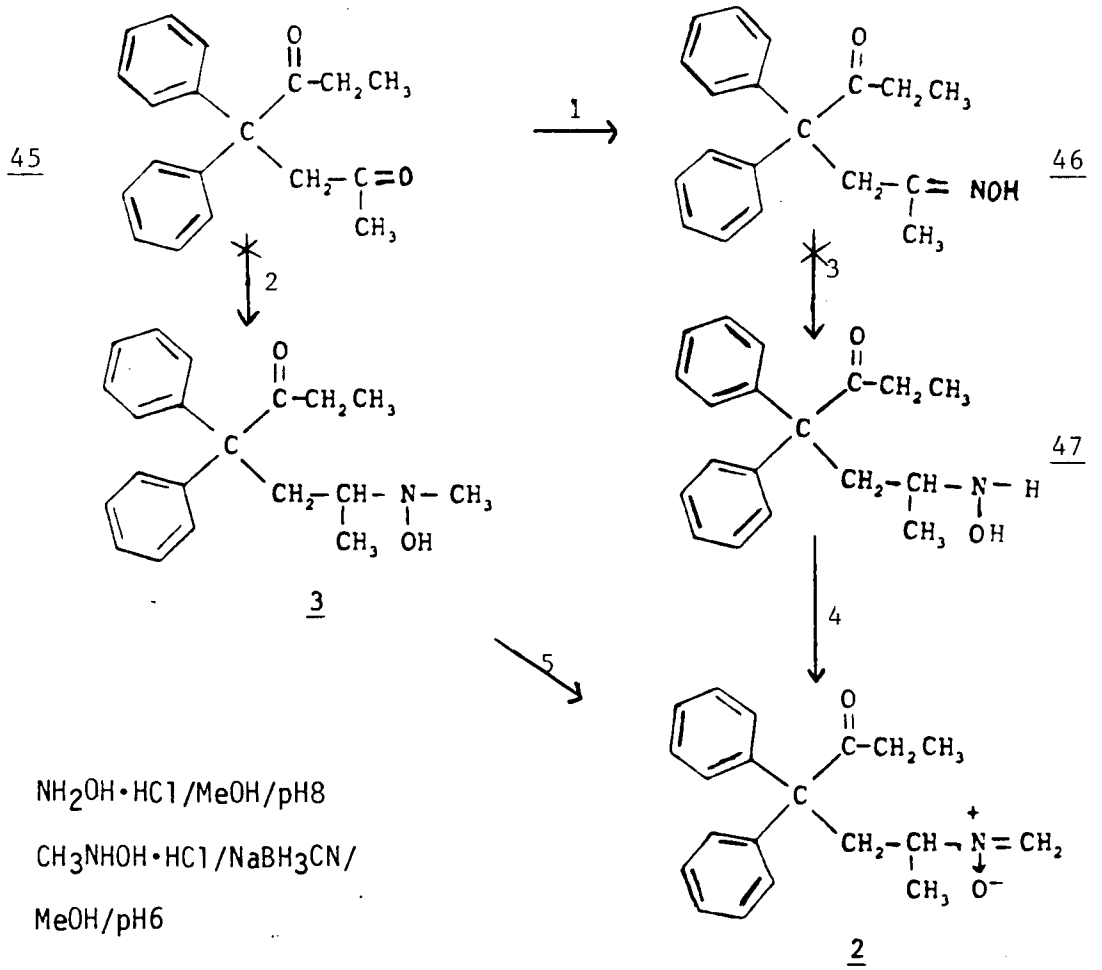


Figure 8: Attempted syntheses of the methadone nitrone (2).

The methadone oxaziridine (5) was made by a modification of Kang's method for the oxidation of EDDP. The formamide (31) was obtained by thermal isomerization of the oxaziridine (Figure 9).

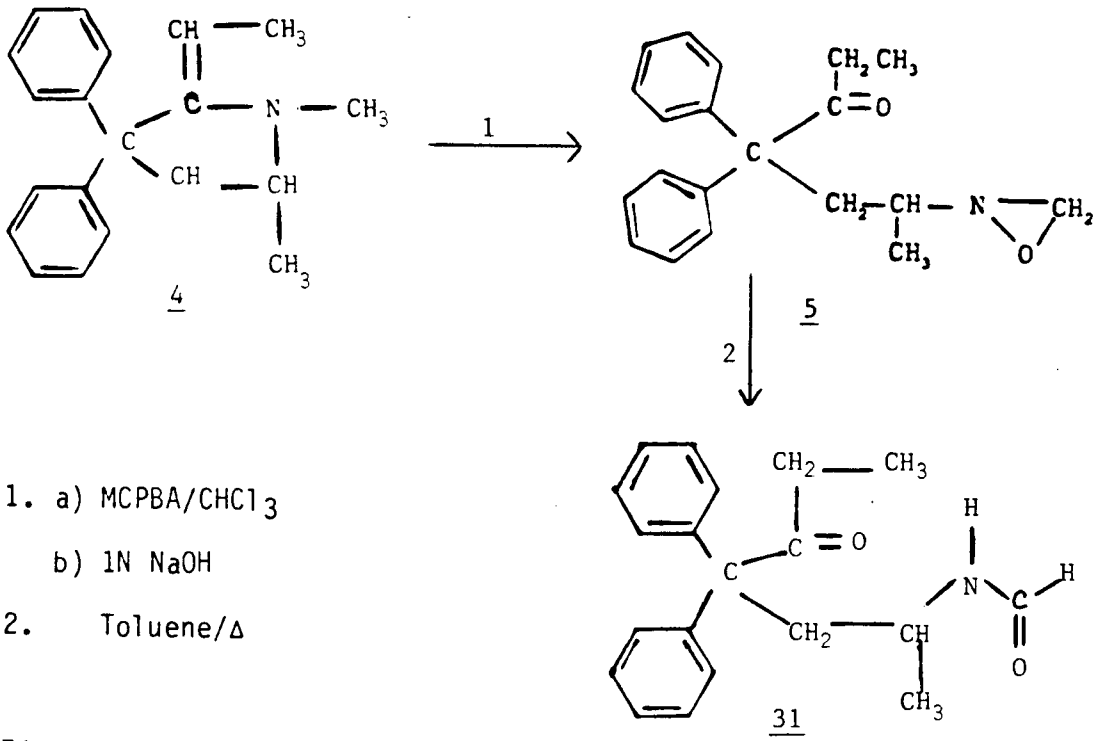


Figure 9. Synthesis of methadone oxaziridine (5) and formamide (31).

Since the methadone nitrone (2) proposed by Kang was not successfully synthesized, recipavrin was chosen as a model for synthetic and metabolic studies.

In the recipavrin series, the N and α-C oxidised potential metabolites were obtained by the reactions outlined in Figure 10. The stem compound diphenyl butan-3-one (51) was synthesized by the method of Burckhalter (75). Recipavrin was synthesized by the alkaline hydrolysis and decarboxylation of methadone nitrile as described by May and Mossetig (74). A low yield of the recipavrin formamide was obtained by refluxing the primary amine (53) in ethylformate.

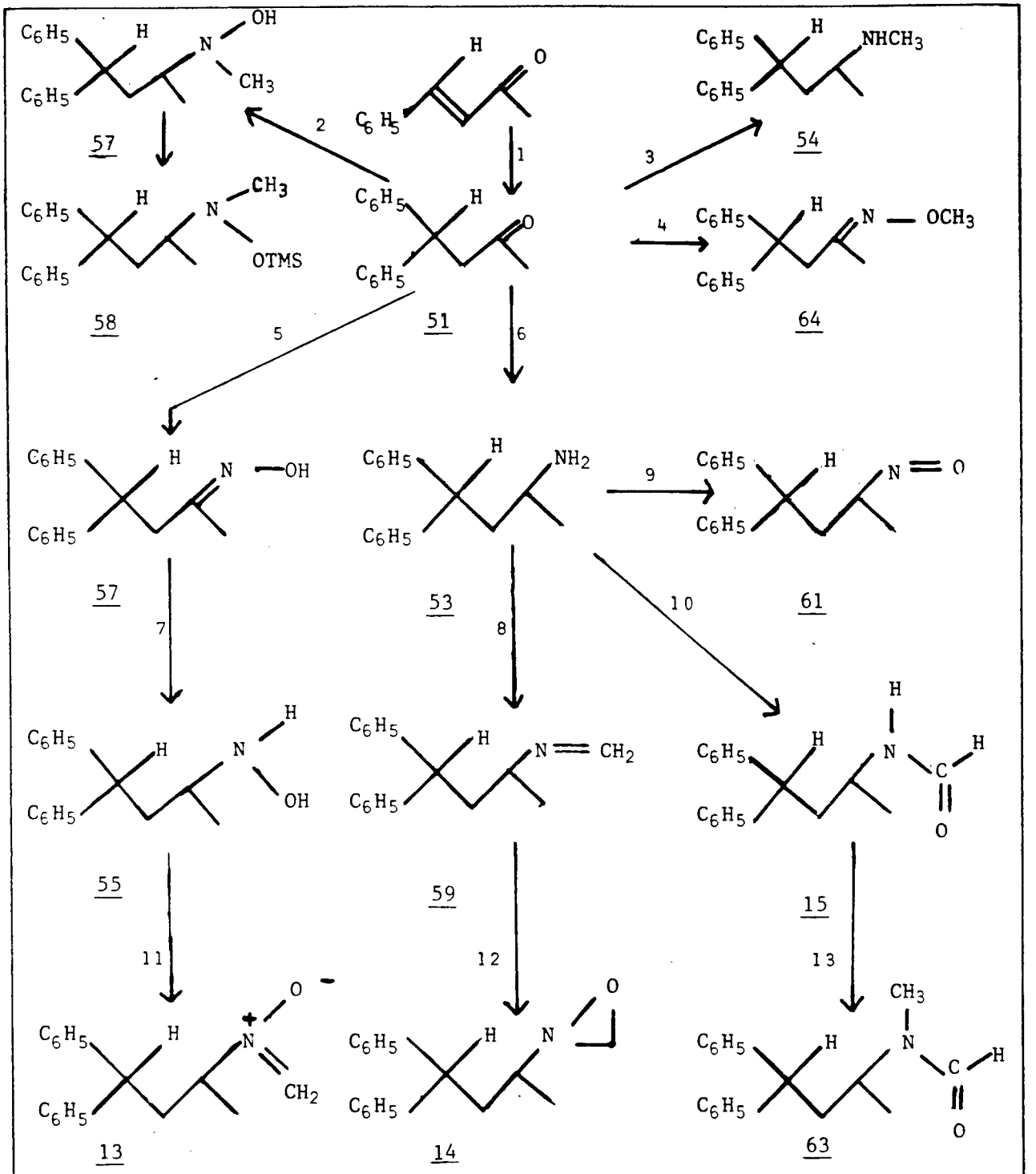


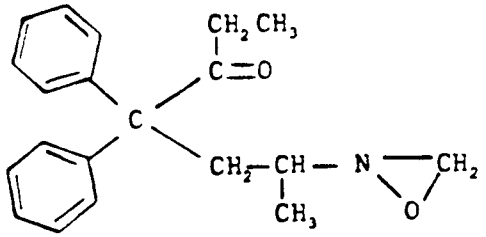
Figure 10. Synthesis of N and α-C Oxidized Potential Metabolites of Recipavrin.

Since we were concerned with compounds which appeared by GCMS to be identical to the unknown metabolites of methadone and recipavrin, the analysis of these oxaziridines, formamides and nitrone are discussed first. The related N-oxidized compounds and their synthetic precursors are discussed in subsequent sections.

B. OXAZIRIDINES

i) Synthesis and properties

Oxaziridines are three membered heterocycles containing oxygen (position 1), nitrogen (position 2) and carbon (position 3). They were first described by Krimm (77) and Emmons (21) in the late 1950's. The recipavrin and methadone oxaziridines (14, 5) were synthesized by peroxidation of the imine 59 (which probably exists as the triazene) and the pyrollidine EDDP (4) respectively.



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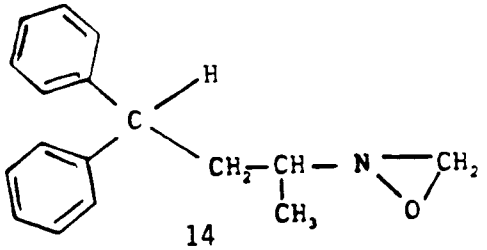

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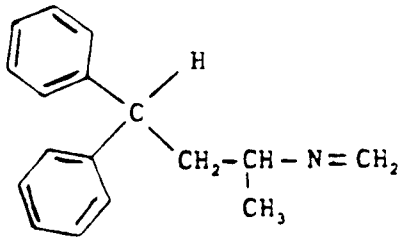


59

A mechanism for the peroxidation of EDDP to oxaziridine (5) which also accounts for the presence of EMDP (11), and the diketone (45), can be proposed based on the investigations of Milliet et al (78) into peracid oxidation of pyrollidine alkaloid salts (Figure 11). Initial peroxidation of the immonium salt is followed by two possible proton abstractions A and

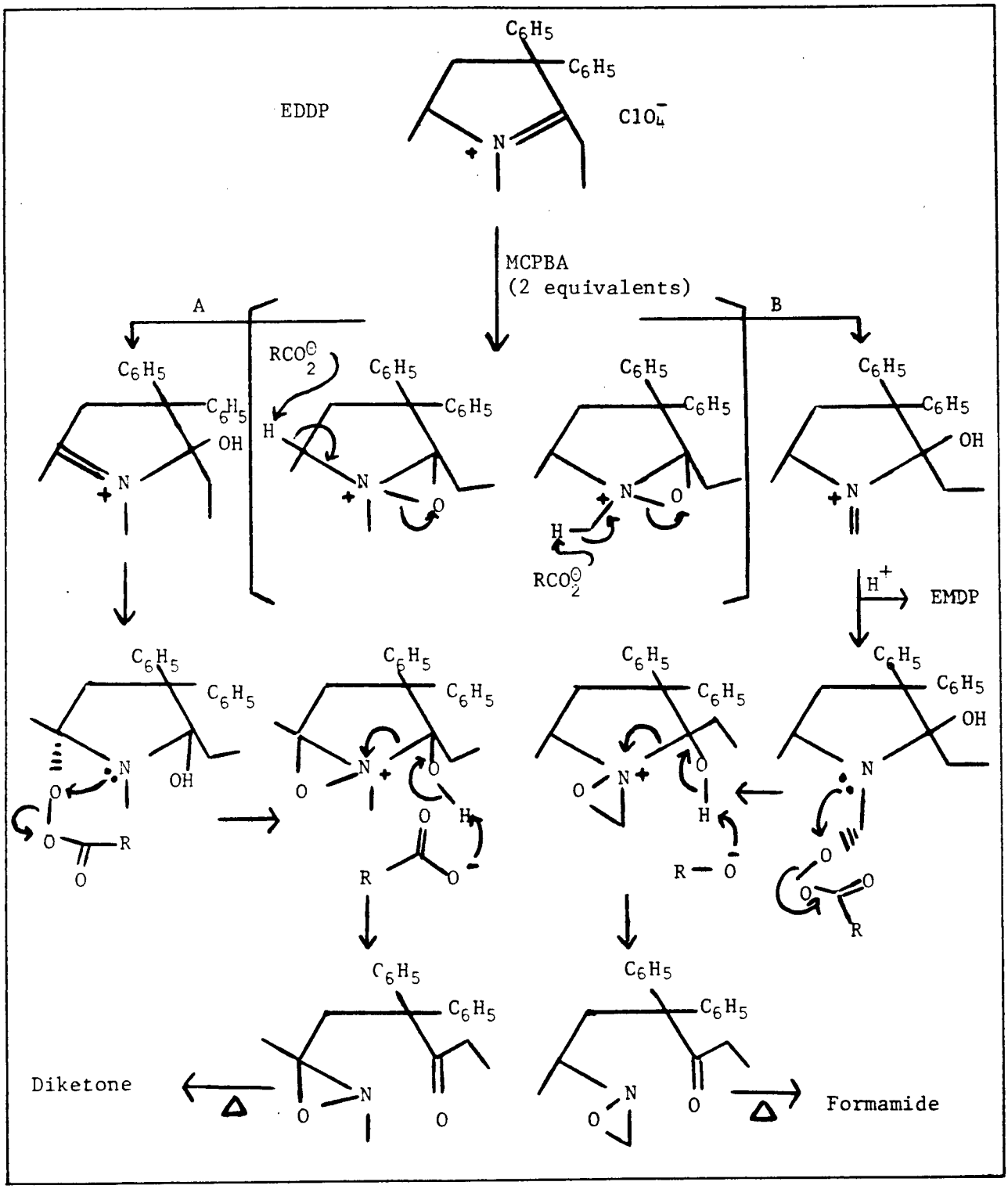


Figure 11. Mechanism for the formation of a methylene oxaziridine from EDDP

B. Pathway A leads to consumption of a second mole of peroxide and ring opening to a keto oxaziridine. Milliet said pathway B leads to the desalkyl product (in our case EMDP). However, by following a pathway analogous to A, the methylene oxaziridine could be obtained. Two equivalents of MCPBA are required in the oxidation (1).

The yield of oxaziridine was substantially improved, and the amount of byproducts reduced by hydrolyzing the perester reaction products with 1N NaOH rather than by Kang's procedure using aqueous $NaHCO_3$ or $NaHSO_3$. Kang had proposed a mechanism supporting a nitrone structure for this compound. NMR, LCMS and GC data presented here prove that the corresponding oxaziridine is the correct structure.

The synthesis of the recipavrin oxaziridines followed the method of Krimm (77), who found that although methylene imines exist in the trimeric (triazene) form (60), they undergo peroxidation to oxaziridines in a manner similar to secondary imine monomers. Emmons states that the acidic reagent apparently depolymerizes the triazene to the imine which is then oxidized (21).

ii) Detection and isolation

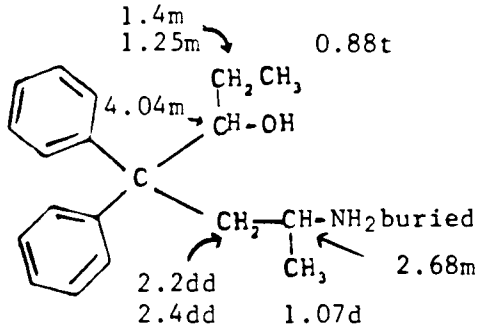
Almost all the reactions of oxaziridines involve fission of the ring. The fact that they were assayed iodometrically by Emmons showed that they oxidize iodide to iodine. Thus oxaziridines are easily detected as black spots when TLC plates were sprayed with Dragendorf's Reagent. The non polar nature of oxaziridines relative to nitrones and amides was reflected by their TLC mobility (R_f=0.3-0.4) in 15% ethyl acetate in petroleum ether. Purification by flash chromatography (70) allowed the partial purification of two diastereomeric forms of each oxaziridine similar to results obtained

by Morgan and Beckett (64). Diastereomers of oxaziridines can be isolated as a result of two contiguous asymmetric centres. These are the methine carbon α to the nitrogen, and the pseudoasymmetric centre of nitrogen, which arises from the high inversion barrier (138 Kj/mol at 120°) (79) of the oxaziridine ring.

Emmons states that methylene oxaziridines containing α-protons, for example 2-n-butyloxaziridine, are the least stable (21). This was true of the recipavrin oxaziridines which decomposed at 0° in a matter of weeks to the primary amine (53) and methylene imine (59) as determined by GCMS and the formamide (5) as observed by LCMS. The methadone oxaziridine deposited cubic crystals from deuterochloroform that were stable indefinitely at 0°. Solutions tended to decompose to EMDP.

iii. Reactions of methadone oxaziridine

Reduction with excess lithium aluminum hydride produced dinormethadol (49) quantitatively.



IR: Primary Amine 3400(m,broad), 1590(m) 740(s)
: Secondary Alcohol:1110 cm^{-1}(s)
No C=O stretch

MS: m/z 44 (100%) Primary Amine

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It was hoped that reduction with $NaBH_3CN$ at pH 6-7 would selectively reduce the oxaziridine ring. Two equivalents of reducing agent were required for complete reaction. The product did not react with BSTFA, therefore it was not the expected secondary hydroxylamine. By GCMS the

product fragmented similar to ethyl dimethyl diphenyl pyrollidine (molecular weight 279).

iv) ^{1}H and ^{13}C NMR of oxaziridines

The ^{1}H and ^{13}C NMR of the major diastereomers and structures showing ^{1}H NMR chemical shift values for major and minor diastereomers of methadone and recipavrin oxaziridines are outlined in Figure 12. Spectra from decoupling experiments and the ^{1}H NMR of minor isomers are included in the Appendix. Chemical shift values of three to four ppm (for the oxaziridine ring proton doublets) agree with those observed by Crist et al (80) for 2-t-butyl oxaziridine. Jordan and Crist (81) have observed that the proton trans to the lone pair resonates at higher field. The coupling constant (J_{AB} = 10 Hz) and the chemical shift value of the α-methine proton (1.8 ppm) were consistent between methadone and recipavrin oxaziridines. However, the oxaziridine ring has marked effects on β substituents, and differences in chemical shift between isomers demonstrated long range effects on the protons α- to the carbonyl group in the case of the methadone oxaziridine. The results shown for the major diastereomer correlates with the 100 MHz results of Kang, chemical shifts, are more accurately determined here as a result of high resolution, better sample purification and decoupling experiments.

SFORD and broad band decoupled ^{13}C NMR were obtained for the major diastereomers of each oxaziridine (figure 12). The oxaziridine carbon was observed as a broad triplet at 72 ppm in the SFORD spectra, as compared to 65.5 ppm in 2-t-butyloxaziridine (80). The large coupling constant indicates adjacent electronegative atoms. These are the first examples of ^{13}C NMR of methylene oxaziridines with protons α to the nitrogen atom.

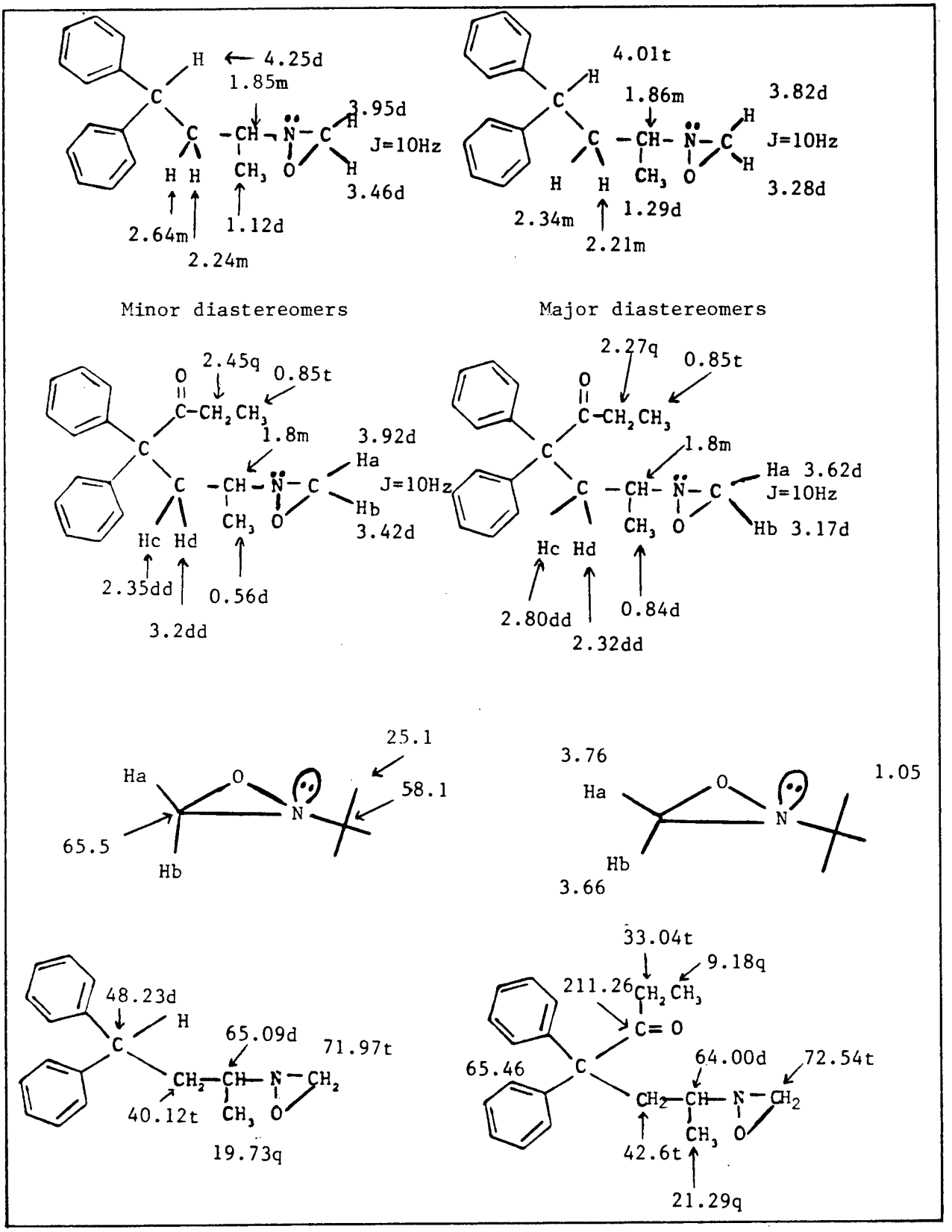


Figure 12(a). ^{1}H and ^{13}C NMR results for methadone and recipavrin oxaziridines compared to those of 2-t-butyl oxazirdine.

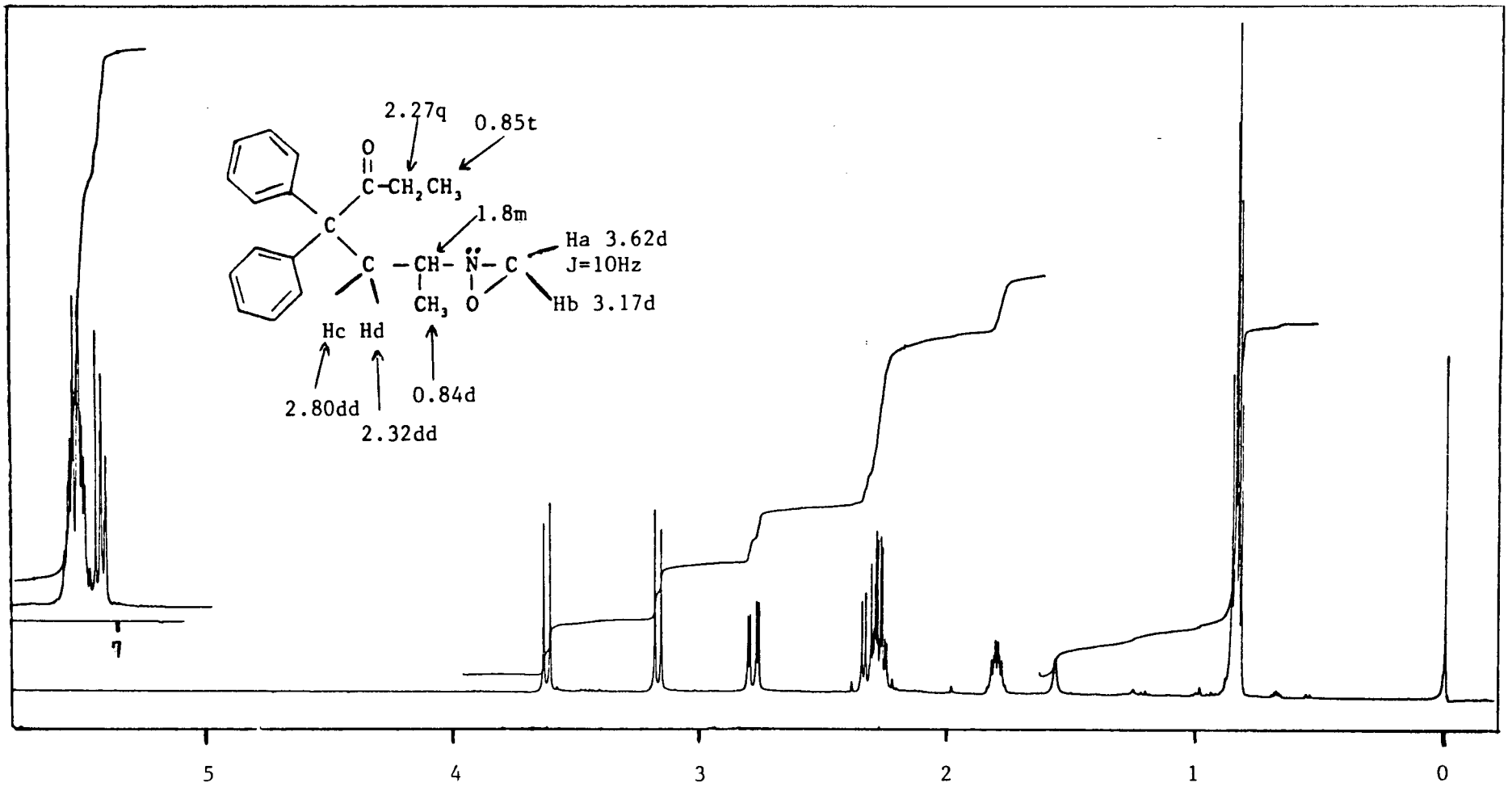


Figure 12 (b) 400 MHz ^{1}H NMR of 2-(4',4'-diphenylheptan-5'-one'2'-yl) oxaziridine major diastereomer (5)

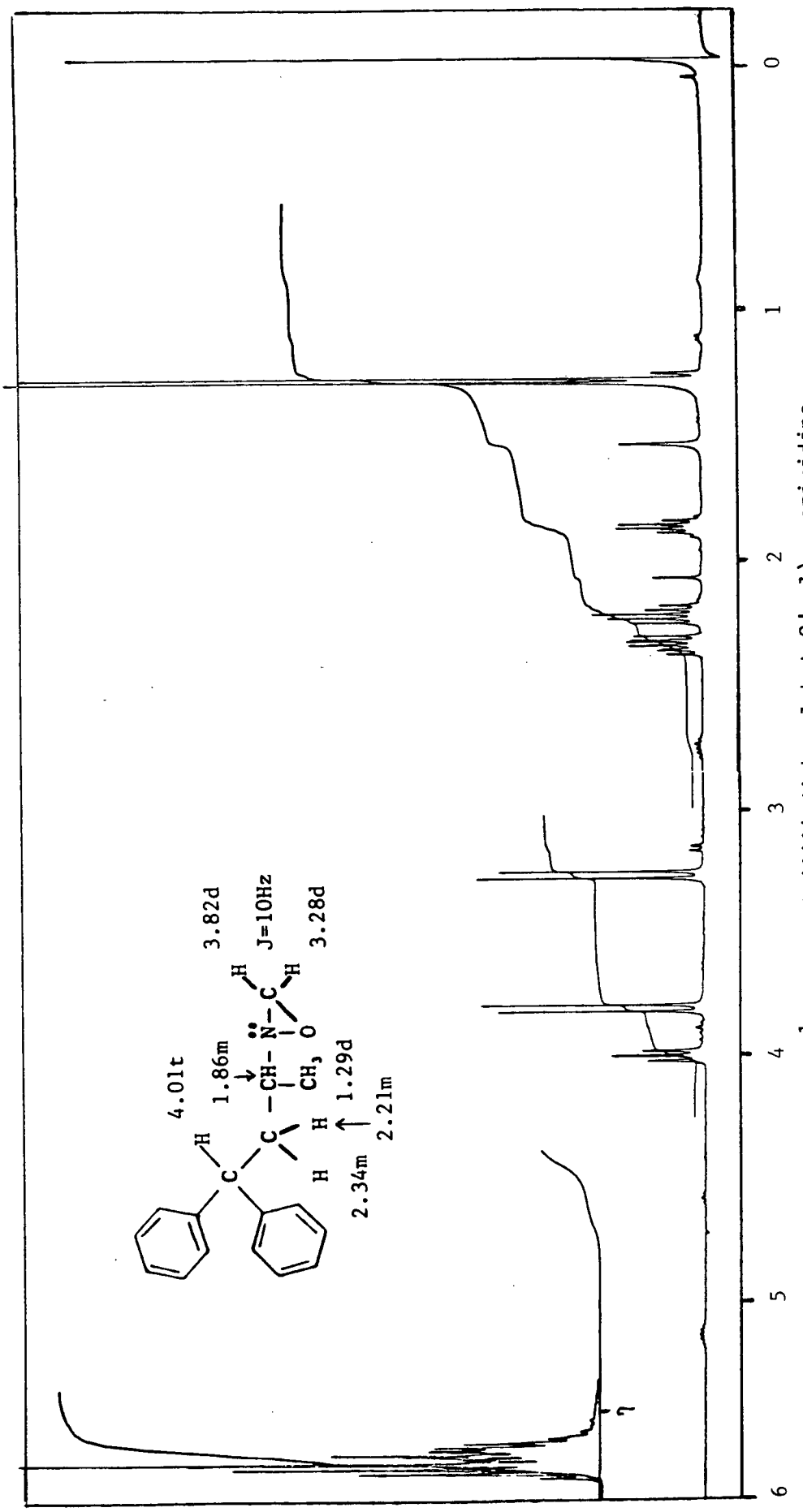


Figure 12 (c) 400 MHz ^1H NMR of 2-(4'4'-diphenyl-but-2'-yl) oxaziridine major diastereomer (14)

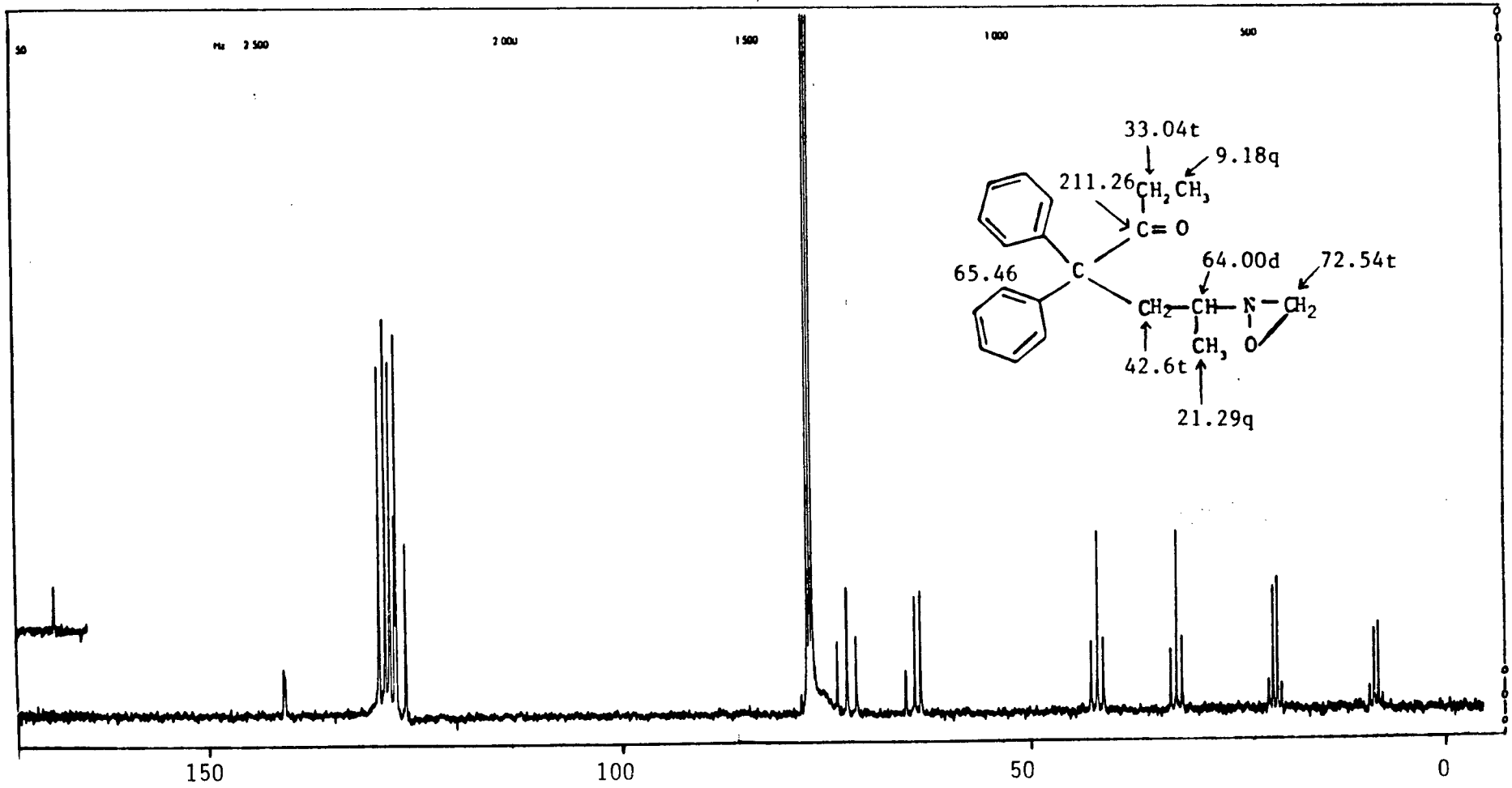


Figure 12 (d) SFORD 400 MHz ^{13}C NMR of 2-(4',4'-diphenylheptan-5'-one -2'-yl) oxaziridine major diastereomer (5)

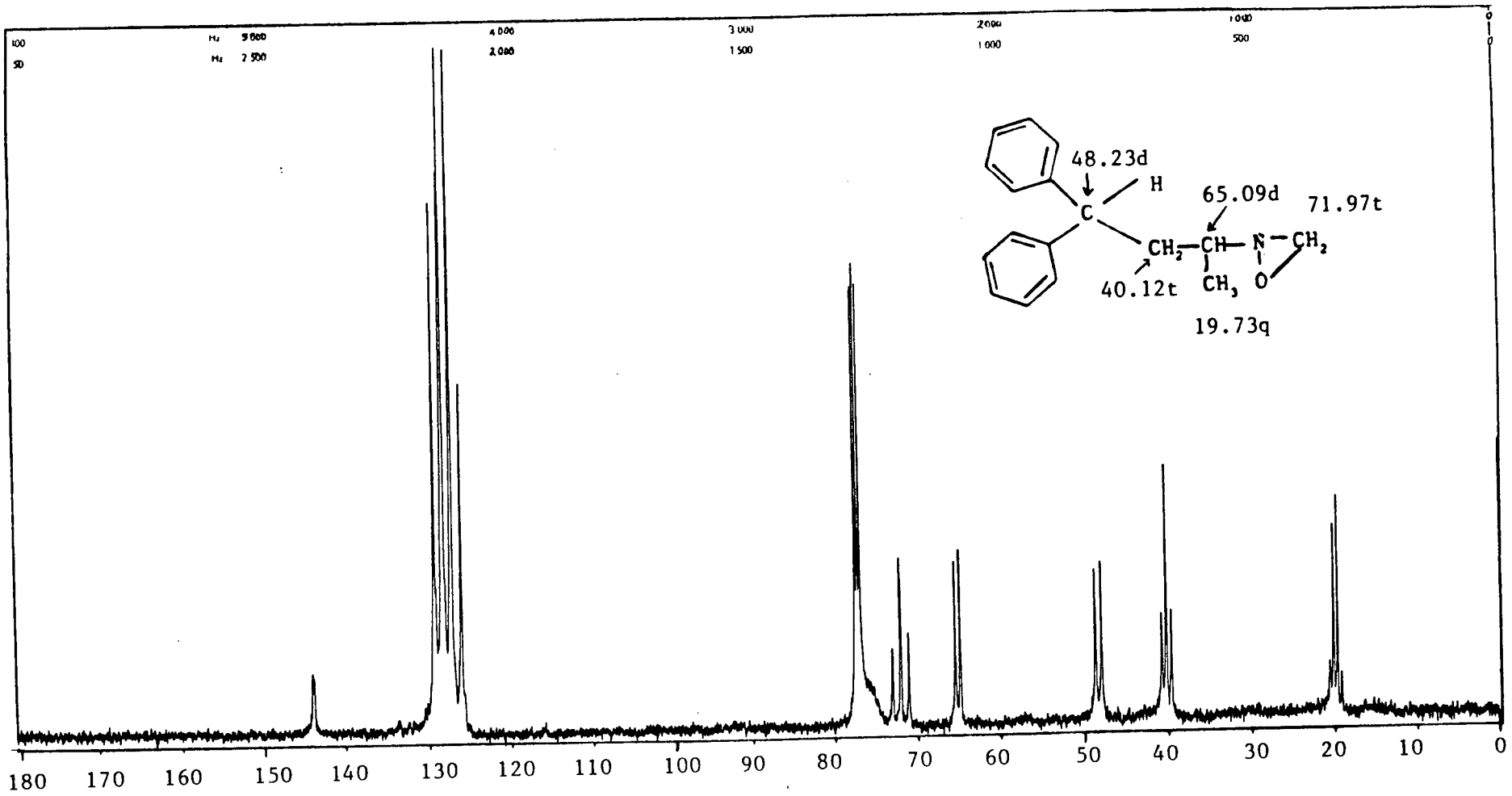


Figure 12 (e) SFORD 400 MHz ^{13}C NMR of 2-(1',1'-diphenyl-but-2'-yl) oxaziridine major diastereomer (14)

v) Infrared spectra

By comparison of infrared spectra of the oxaziridines with the diketone and diphenylbutanone spectra, a number of bands appear to be associated with the oxaziridine ring. An unusual medium intensity absorption at 1730 cm^{-1} in the recipavrin oxaziridine, usually associated with a strong carbonyl absorption of esters is present. The band is very strong in poorly purified samples and probably is a contaminant, perhaps an unhydrolyzed N-3-chlorobenzyl ester of MCPBA as described by Beckett (64) in the MCPBA oxidation of secondary amines or possibly a decomposition product of the formamide (15). A sharp medium band at 1600 cm^{-1} is similar to an amide band. The band at 1250 cm^{-1} is likely an N-O (or C-N) stretch similar to that of an aliphatic nitroso dimer. Bands at 1035, 935 cm^{-1} are likely associated with the C-O bond. The infrared spectra of methadone and recipavrin oxaziridines are shown in Figure 13.

vi) Ultraviolet spectra

In the ultraviolet spectrum of methadone oxaziridine two bands at 296.5 mμ (ε=502) and 265.5 mμ (ε=548) were observed in addition to aromatic bands at 254 and 207 mμ. These are possibly N - O transitions of each heteroatom in the oxaziridine ring.

vii) Thermolability and mass spectral analysis

The thermal isomerization of oxaziridines to amides has been well documented by Emmons (21). This study shows that both methadone and recipavrin oxaziridines isomerize in the GC to the respective formamides (5) and (15). St. Claire Black et al (82) have indicated that oxaziridines thermally isomerize to nitrones at temperatures below 200° and to amides above 200°. Thermal isomerization of methadone oxaziridine under reflux in

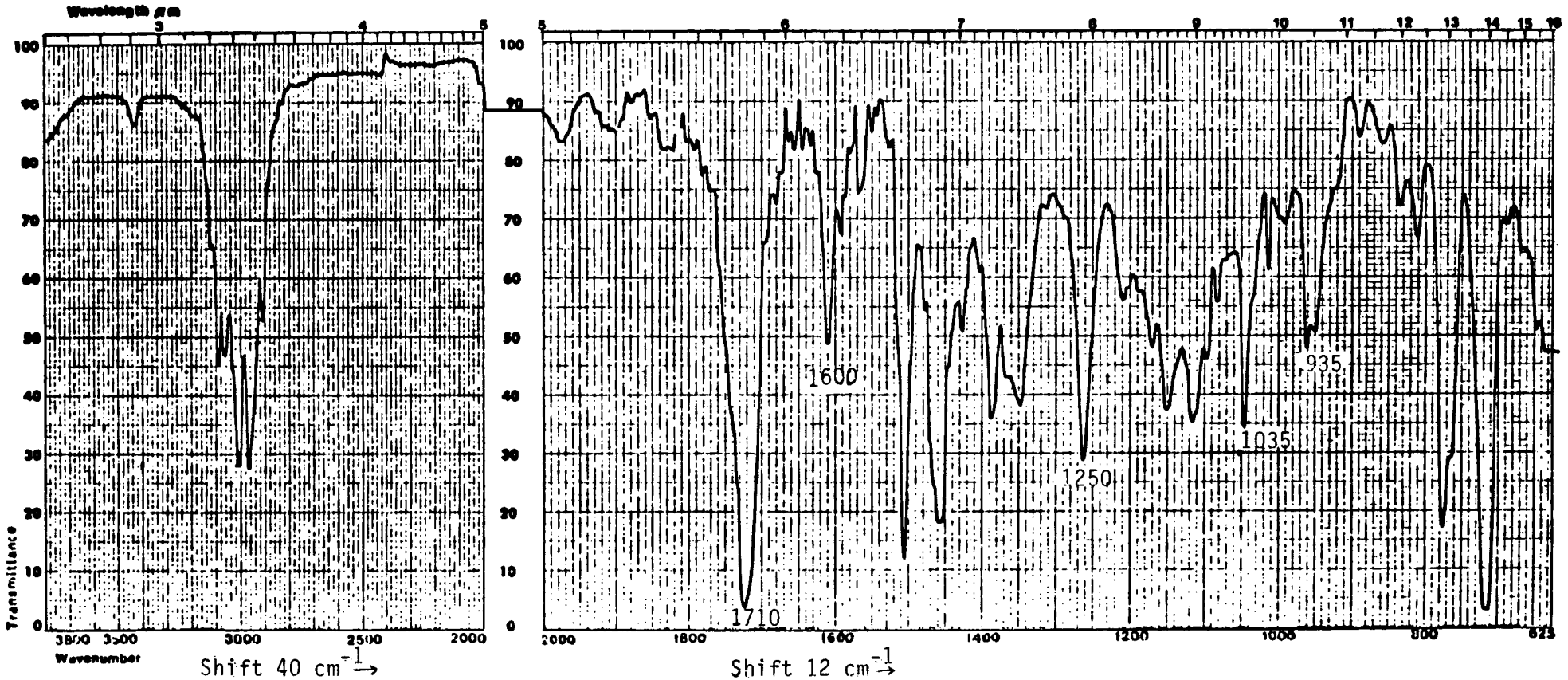


Figure 13(a) Infrared spectrum (film) of 2-(4',4'-diphenylheptan-5'-one-2'-yl) oxaziridine (5)

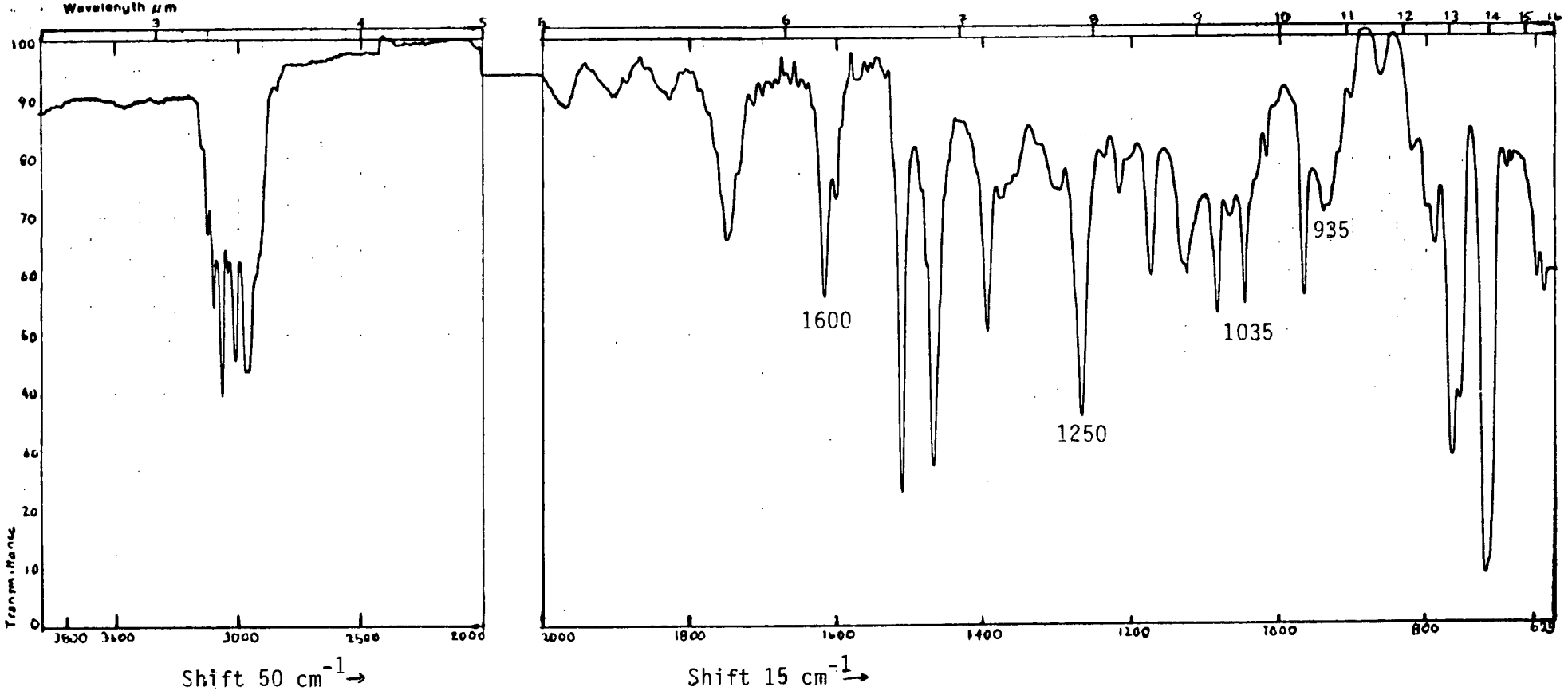


Figure 13 (b) Infrared spectrum (film) of 2-(4',4'-diphenyl-but-2'-yl) oxaziridine (14)

m-xylene under nitrogen afforded the formamide (68) but no nitrone. The methadone oxaziridine is non volatile and thermolabile. Thus even moderate heating of the source in direct probe mass spectral analysis produces ions characteristic of the formamides. The only different ions in the direct probe mass spectrum occur at m/z 42 (C_3H_6) and 56 (C_4H_8), these suggest isomerization to the nitrone upon gentle heating of the source (see discussion of the recipavrin nitrone mass spectrum).

The marked similarity of the benzylidine nitrones and oxaziridines studied by Morgan and Beckett (64) were attributed to thermal isomerization of oxaziridine to nitrone. While this is possible, especially at the lower GC temperatures required to elute these compounds, GC retention times and GCMS were not detailed, and a similar isomerization to formamides or benzamides is possible.

C. FORMAMIDES

i) Synthesis and NMR spectra

The recipavrin formamide (15) was synthesized in low yield by refluxing the primary amine (53) in ethyl formate for several days. After purification by flash chromatography, peak areas in the NMR spectrum of the product showed that two species were present in a ratio of 8:6. Comparison with the NMR of isopropyl formamide (83) showed that the relation of substituent groups to the nitrogen lone pair had marked effects on chemical shifts of substituents resulting in two distinct spectra. The NMR of major and minor components of the methadone and recipavrin formamides and isopropyl formamide are shown and summarized in Figure (14a). Decoupled spectra are included in the appendix. Decoupling by the multiplet at 4.05 ppm of the recipavrin formamide collapsed the methylene protons

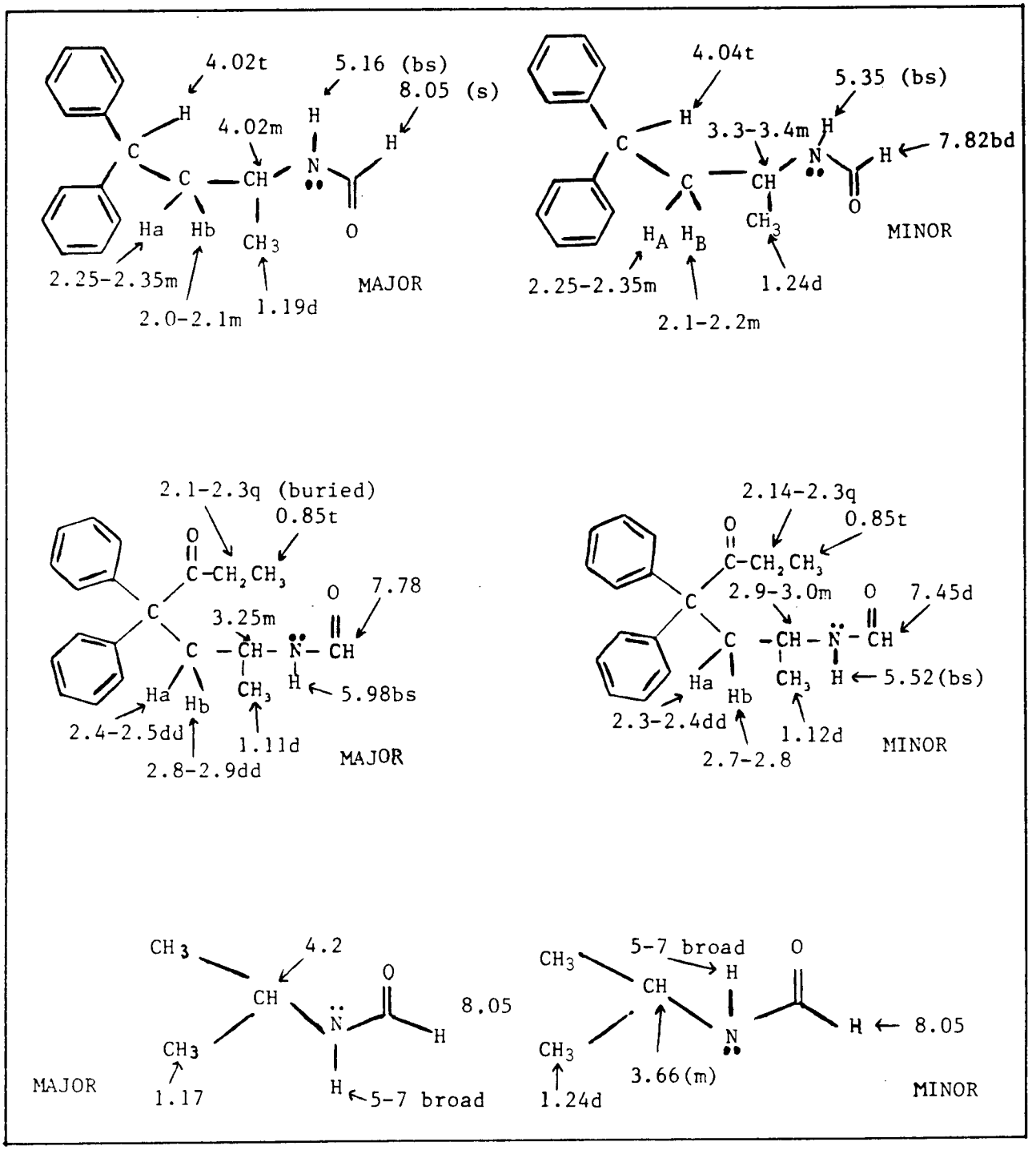


Figure 14(a) Comparison of ^{1}H NMR results for recipavrin and methadone formamides with those of isopropyl formamide

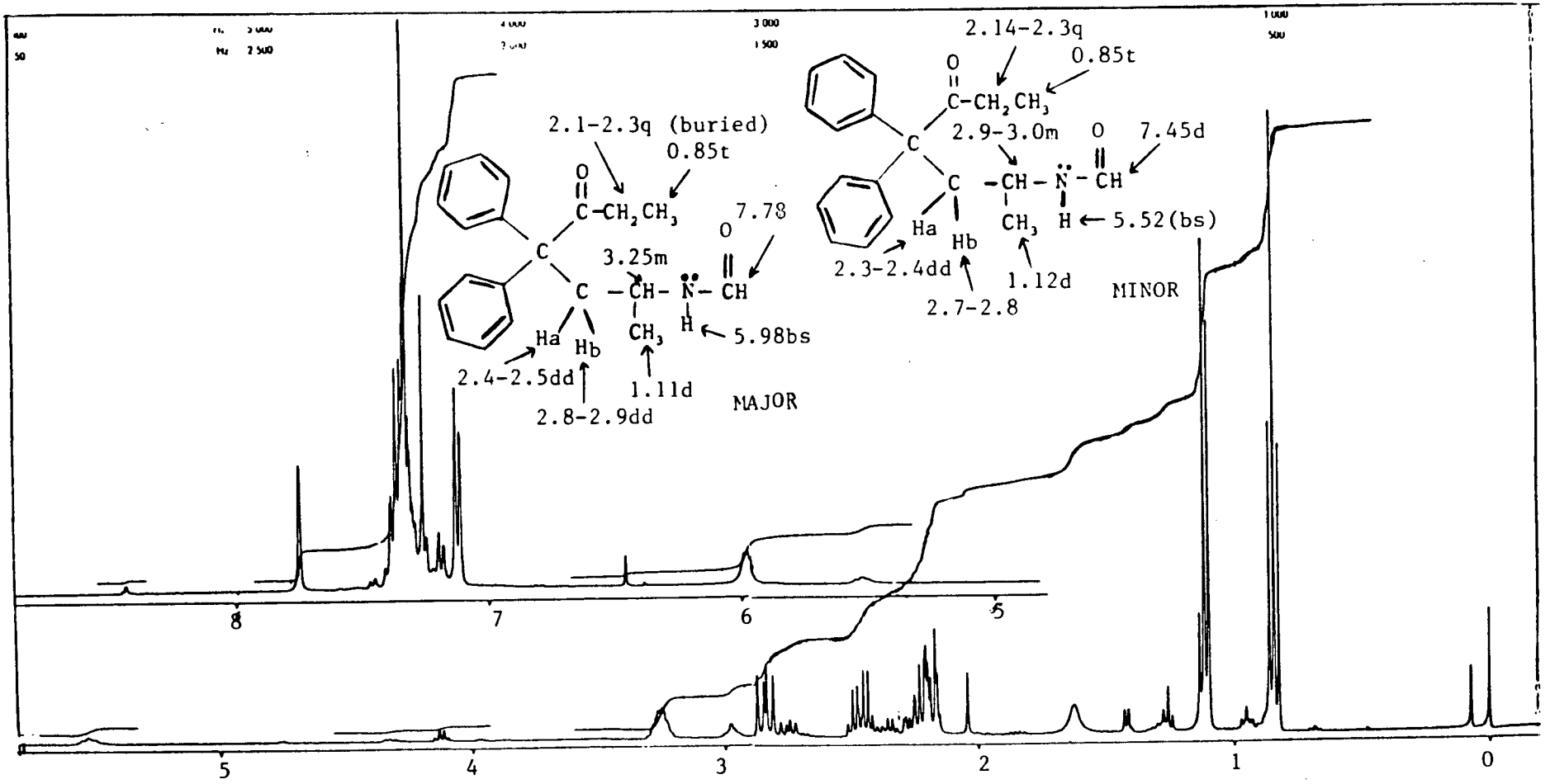


Figure 14 (b) 400 MHz ^{1}H NMR of 2-(N-formyl)-4,4-diphenyl-5-heptanone

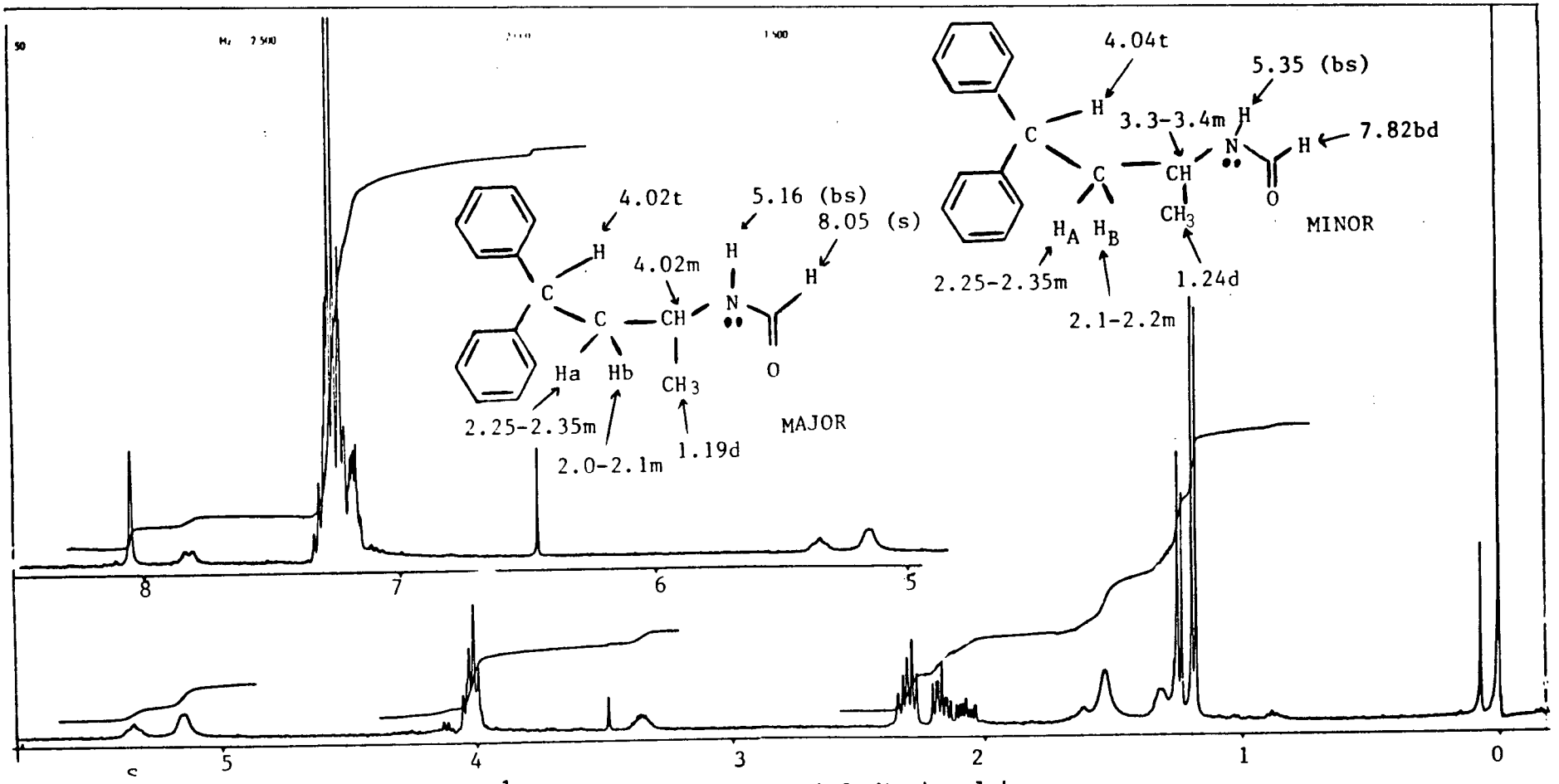


Figure 14 (c) 400 MHz ^{1}H NMR of N-formyl-α-methyl-γ-phenyl benzene-propanamine (15)

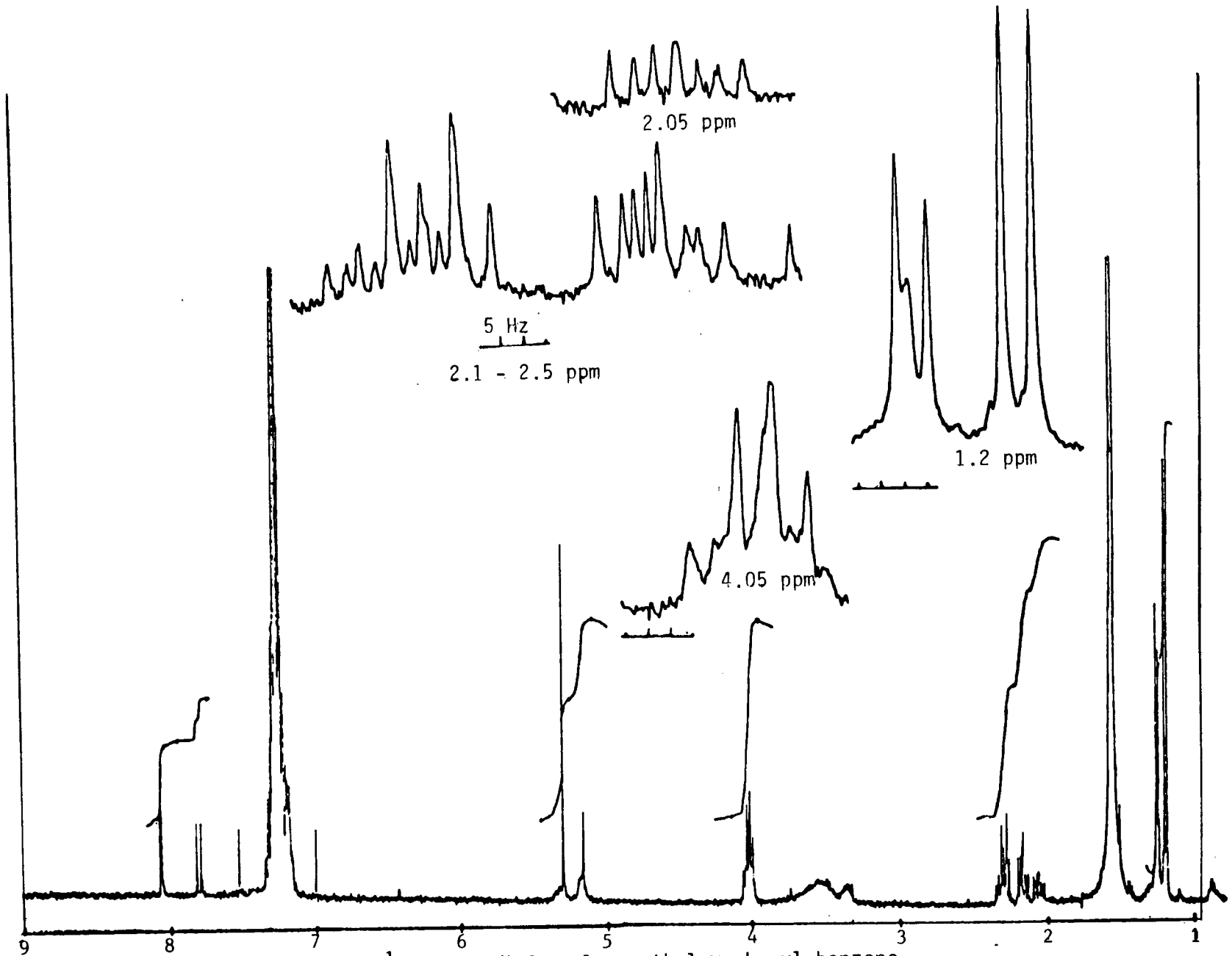


Figure 14 (d) 400 MHz ^{1}H NMR of N-formyl-α-methyl-γ-phenyl benzene-propanamine collected off a GC column

in both diastereomers as well as reducing the N-H peak intensity and collapsing the methyl doublet in the major diastereomer. Thus, the proton α to the nitrogen of the major diastereomer was hidden by the diphenylmethine protons of major and minor diastereomers.

The singlet formyl resonance at 8.05 ppm appears upfield at 7.82 as a broad doublet in the minor diastereomer of the recipavrin formamide. The corresponding peak in the methadone formamide occurred upfield at 7.78, possibly due to interaction of the formyl and keto side chains. Since the observed metabolite of methadone and recipavrin had an identical GC retention time and mass spectrum to the oxaziridine and the formamide of each drug, it was important to know that the formamide survived GC analysis. The recipavrin formamide was injected into the GC and the peaks were collected in a cold capillary tube. The NMR spectrum (obtained in a 1 mm NMR tube on 500 μg of material), of the eluted peaks were identical to that of the formamide (figure 14d), therefore although the structure of the metabolite remains unclear, this is evidence that it is observed by GCMS as the formamide.

ii) Infrared Spectra

The infrared spectrum of the formamides (15) and (31) (Figure 15) have a broad hydrogen bonded NH stretch at 3200-3300, a strong secondary amide I band at 1670-1660 cm^{-1}, a weak amide II band at 1605 cm^{-1}, amide C-N stretching at 1385 cm^{-1} and a partially resolved amide peak below 800 cm^{-1} which obscured by phenyl ring bending (84). The recipavrin formamide sample saved for IR was weak and contaminated with an ester (1730 cm^{-1}, 1175 cm^{-1}), possibly methyl formate, however the low intensity peaks of the weak sample correlated with those observed for the methadone formamide.

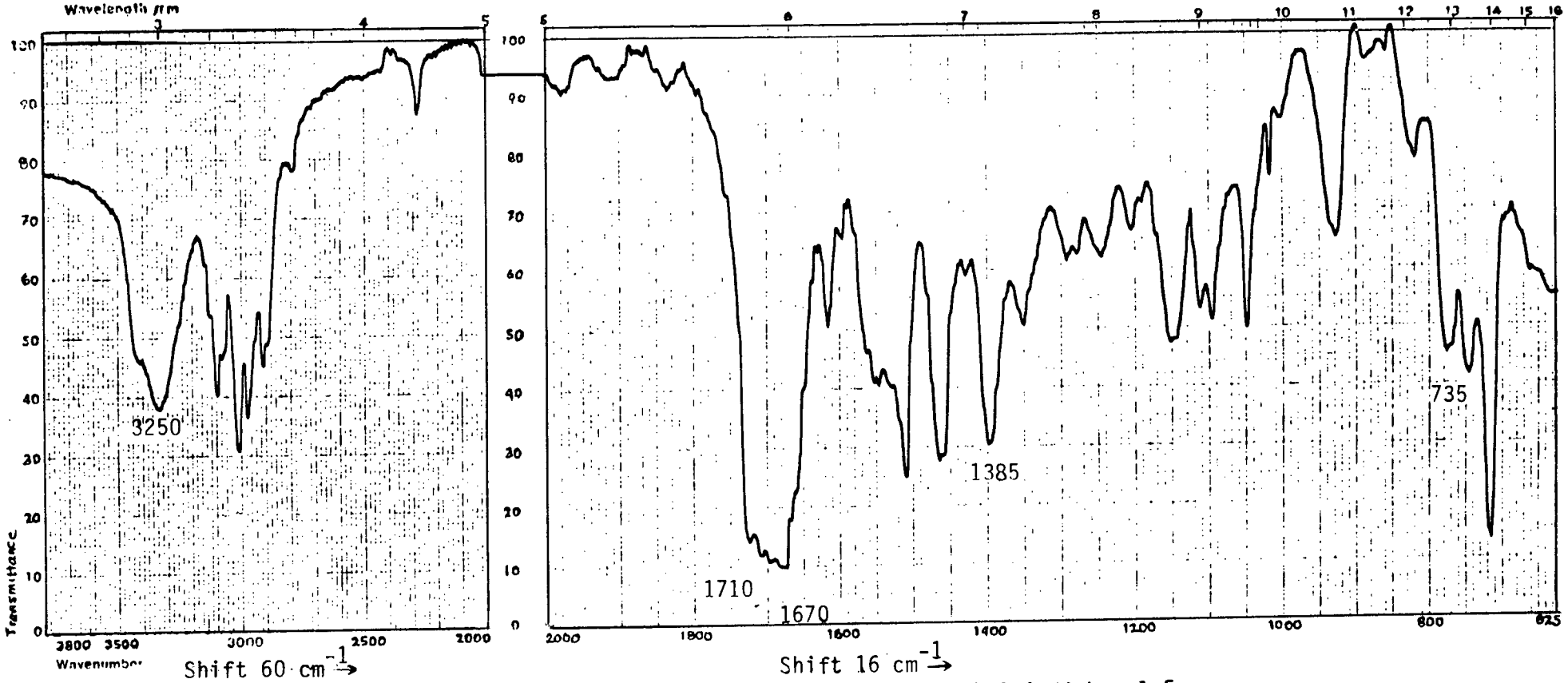


Figure 15(a) Infrared spectrum (film) of 2-(N-formyl)-4,4-diphenyl-5-heptanone (31)

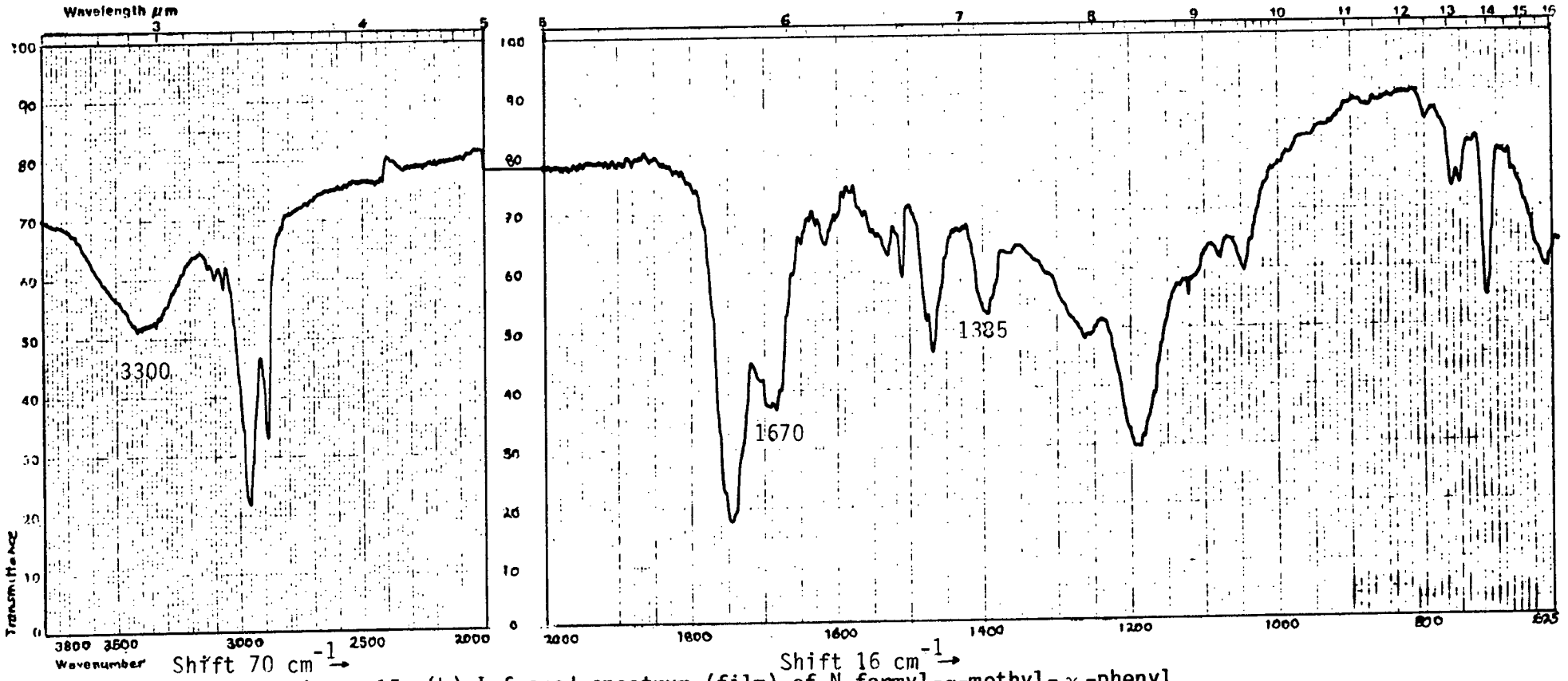
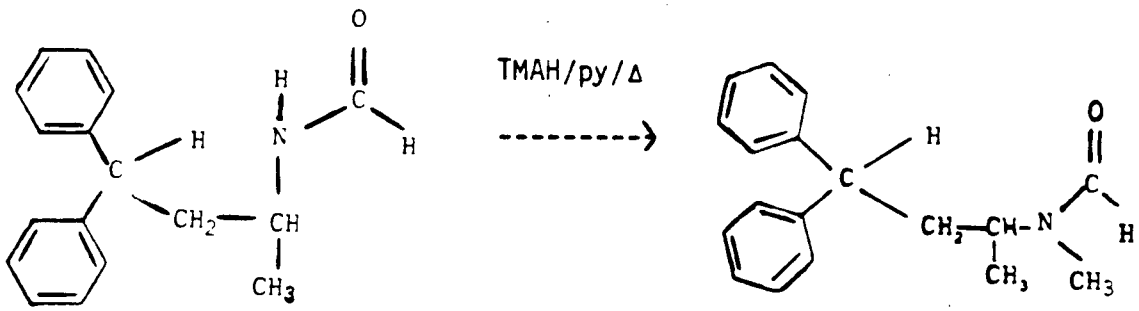


Figure 15 (b) Infrared spectrum (film) of N-formyl-α-methyl-γ-phenyl benzenepropanamine (15) impure sample

iii) Mass spectra

The mass spectra of the formamides (identical to the metabolites of methadone and recipavrin) each show a molecular ion m/z 309 and m/z 253, with base peaks at either m/z 72 or 73. LCMS and CI GCMS revealed a M^++1 base peak at m/z 254 for recipavrin. The mass spectrum and possible fragmentation pathways of the recipavrin formamide are shown in Figure (16). Beta cleavage accounts for series derived from m/z 208 as described by Abbott et al (66). All other major fragments can be rationalized as arising from γ-cleavage of the formamide.

Methylation on the GC column using trimethylanilinium hydroxide (TMAH) produced the corresponding tertiary amide (63) (Figure 16,17) with a molecular ion at m/z 267 and base peak at m/z 87. This compound was synthesized as a potential metabolic precursor of the recipavrin formamide metabolite.



15 63

The methadone formamide (Figure 18) can undergo two possible McLafferty rearrangements. The first (pathway A) results in a neutral mass 264 fragment (not observed), which, if cleaved α to the carbonyl group, could give rise to m/z 57, and the m/z 207, 129 series characteristic of both this compound and the synthetic diphenylheptenone (39). The strong ion at m/z 253 consistant with recipavrin appears to arise by a proton transfer process which we have tentatively shown as a 7 centre rearrangement.

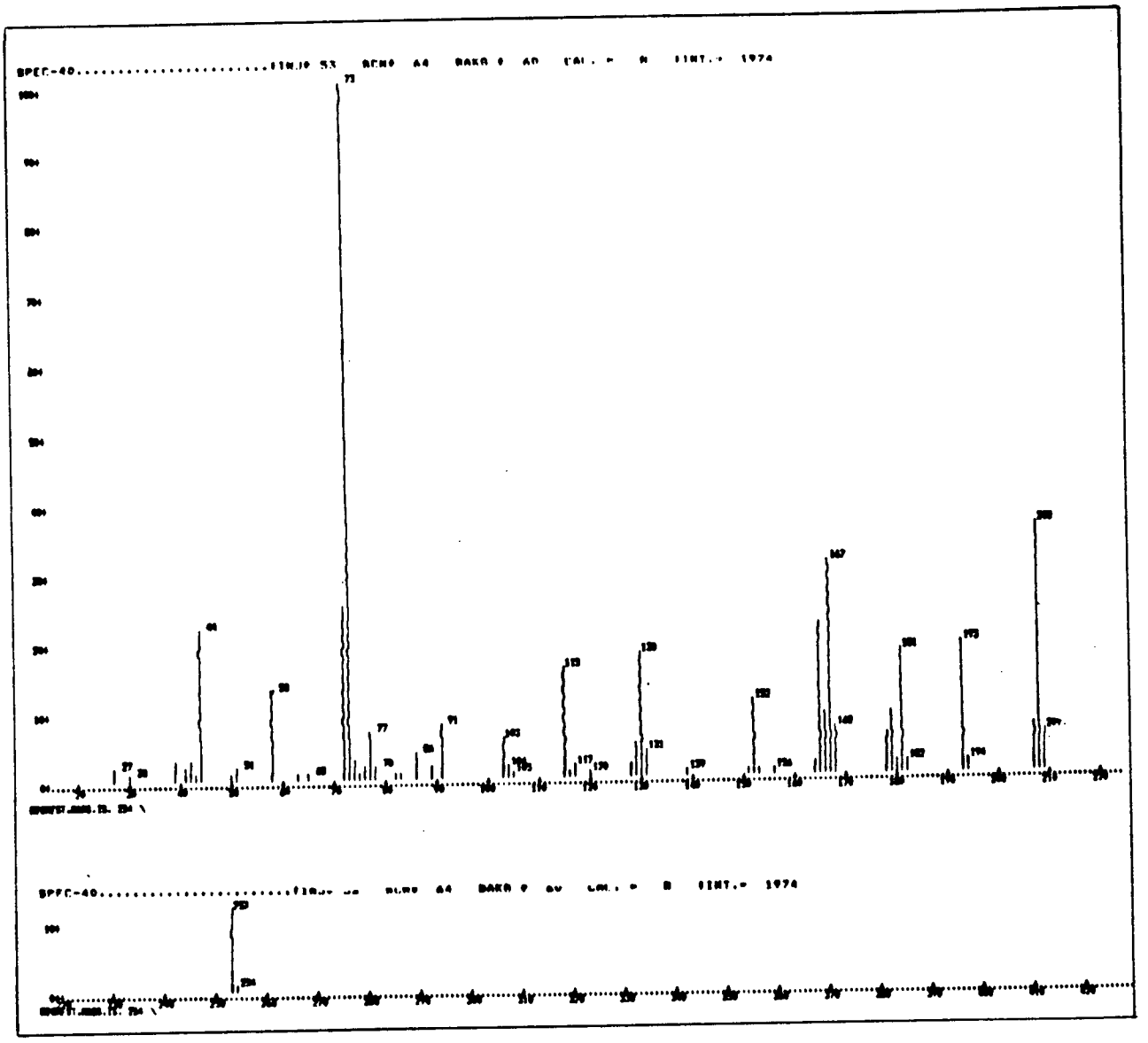


Figure 16(a) Mass spectrum (GCMS) of N-formyl-α-methyl- γ -phenyl benzenepropanamine (15) (identical to oxaziridine (14) and the recipavrin metabolite

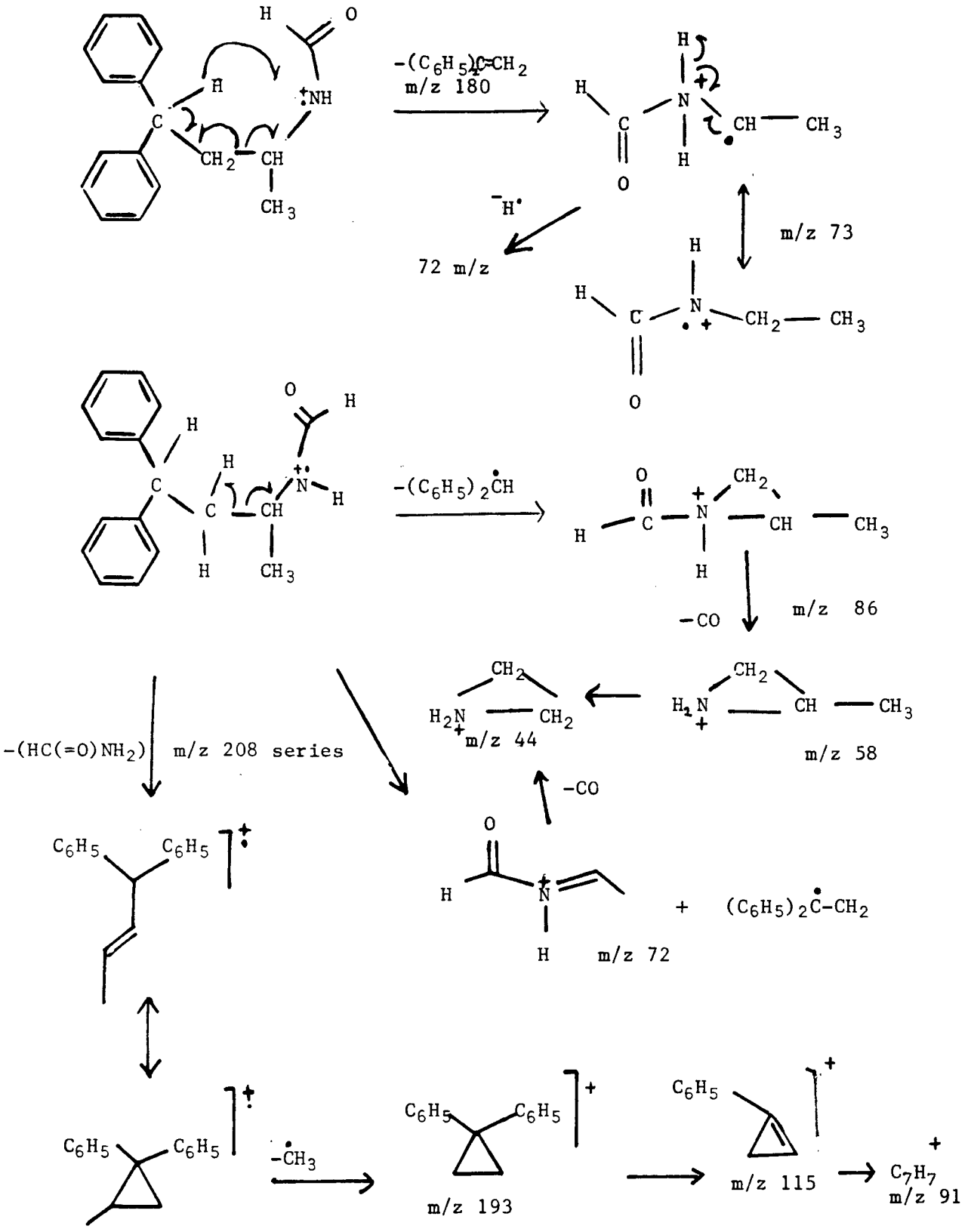


Figure 16 (b) Mass spectral fragmentation pathway of N-formyl-α-methyl -γ -phenyl benzenepropanamine (15)

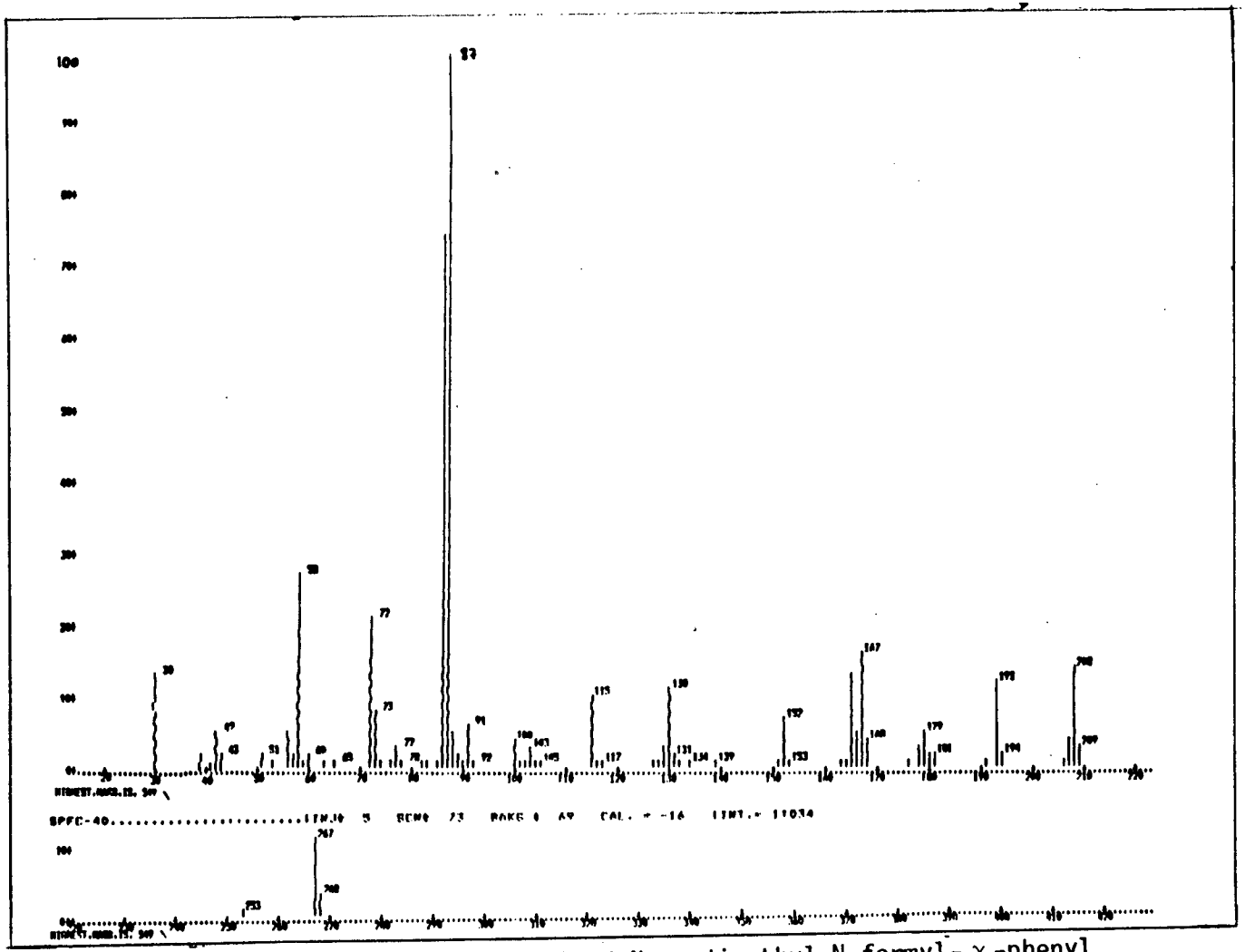


Figure 17. Mass spectrum (GCMS) of N, α-dimethyl-N-formyl- γ -phenyl benzenepropanamine (63)

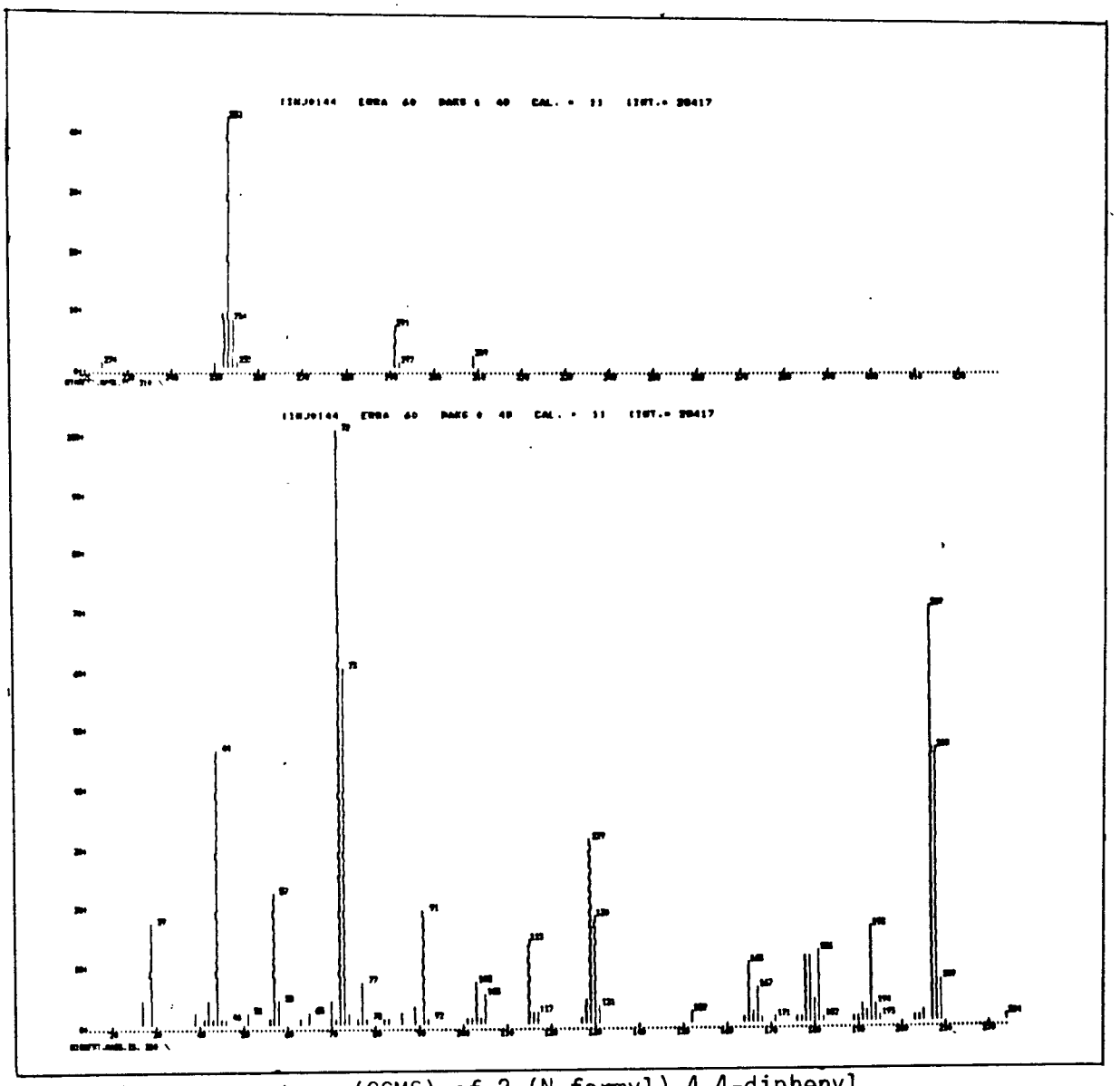


Figure 18(a) Mass spectrum (GCMS) of 2-(N-formyl)-4,4-diphenyl -5-heptanone (31) (identical to oxaziridine (5) and the methadone metabolite

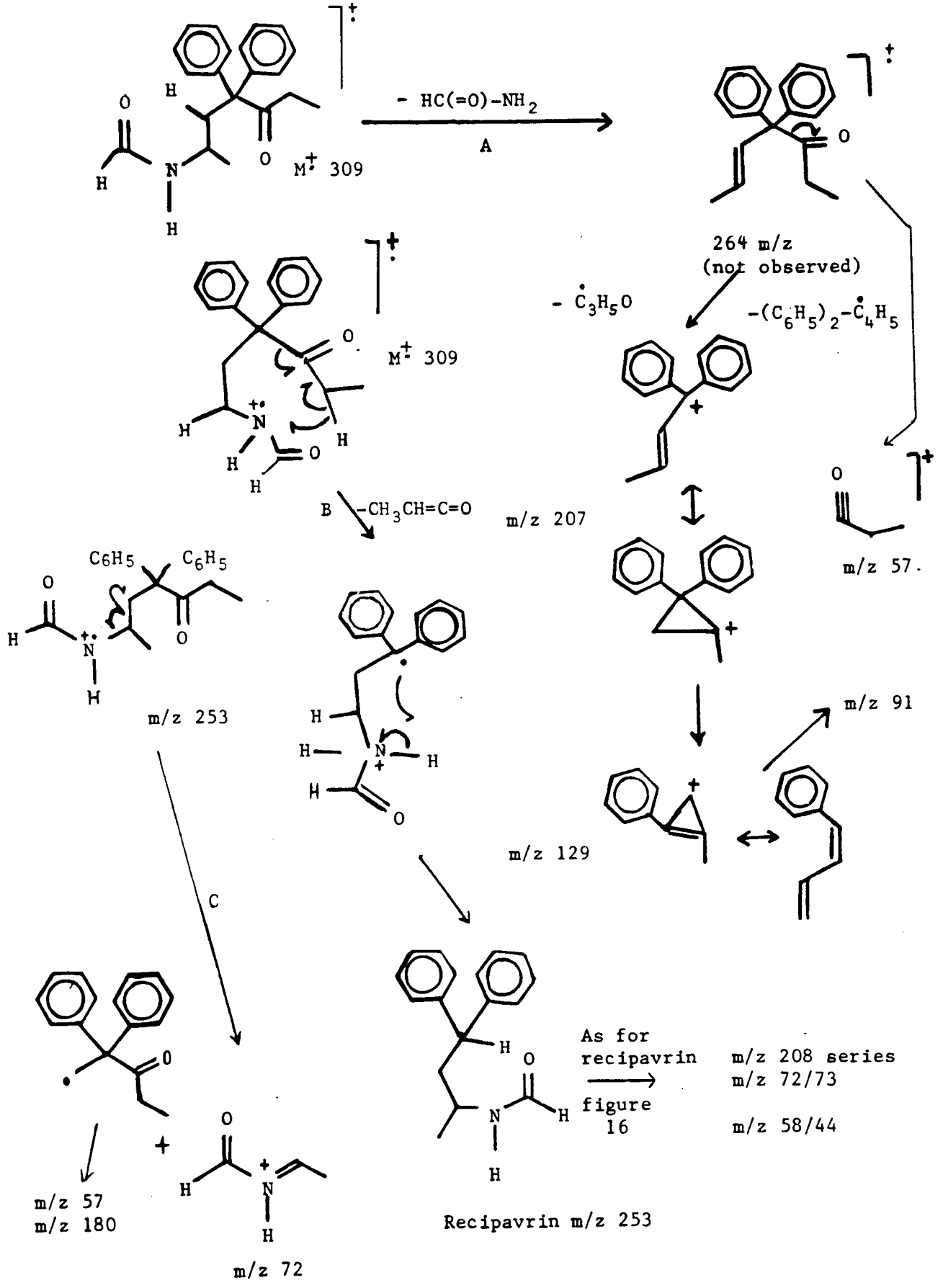


Figure 18(b) Mass spectral fragmentation of methadone formamide

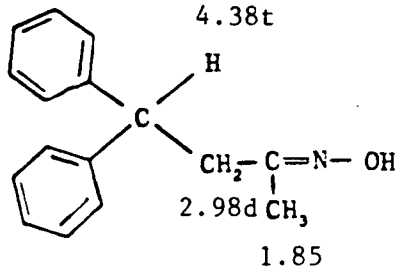
The m/z 253 fragment then leads to all ions present in the recipavrin spectrum.

The base peak at m/z 72 or 73 can be rationalized on the basis of scission of the carbonyl group of the formamide. While γ scission of carbonyl groups with or without proton rearrangement does not normally produce intense peaks (85), the fact that m/z 178, 180 fragments are of high intensity in all compounds studied, indicates that, for steric or electronic reasons, the phenyl rings direct fragmentation at this bond. Gamma cleavage of the ethyl group of sec-butylacetamide (86) by this mechanism gives rise to a 30% relative intensity peak at m/z 86. In the methadone formamide, this bond is γ to both carbonyl groups and may be doubly activated. The methadone ethyl ketone chain does not normally give rise to McLafferty β-cleavage (66), further substantiating this scission pathway.

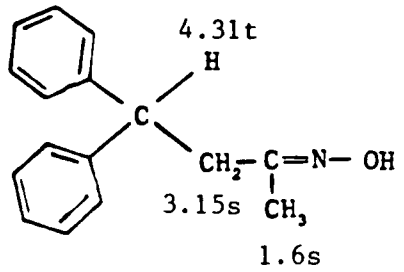
D. NITRONES

i) Attempted synthesis of the methadone nitrone via the primary hydroxylamine (47)

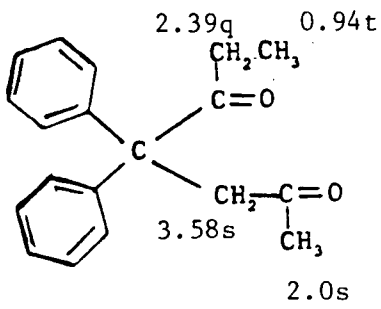
Since the methylene nitrone (2) was proposed by Kang as a plausible structure for the unknown metabolite of methadone, several attempts to synthesize it, using the diketone (45) as a starting material were tried. The pathway outlined earlier via the oxime (46), and hydroxylamine (47) to the nitrone (2) appeared most promising.



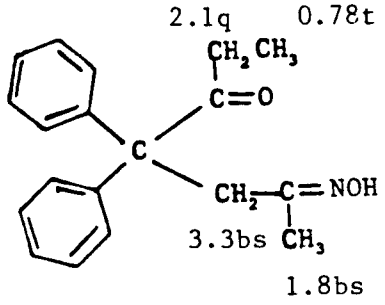
52 syn



52 anti



45



46

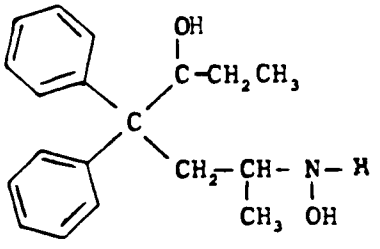
NMR (80MHz) results showed broad bands which when compared with the NMR of syn and anti recipavrin oximes (52) and diketone (45), support the keto oxime structure (46). The infrared spectrum of the keto oxime (46) had a strong C=N stretch at 1620 cm^{-1}, a broad intermolecular hydrogen bonded OH band at 3200 cm^{-1} and medium bands at 1025 and 950 cm^{-1}, in common with the recipavrin oxime (52). The C=O doublet as seen in the infrared of the diketone remained as a single peak at 1710 cm^{-1} in the keto oxime.

In the mass spectrum of the oxime (65), the alkyl fragment m/z 42 (100%) is also found in the diphenyl butanone oxime mass spectrum (neither compound

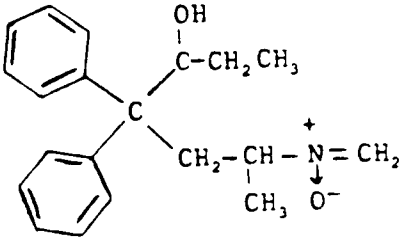
has the γ protons required for a McLafferty rearrangement) and m/z 29, 57 and 239, α-scission products of an ethyl ketone are evident. The highest mass evident is 279 (M^+-16), and the series m/z 206, 191, 128, 115, 91 analogous to the 208 series of EMDP-like compounds are prominent. The high mass fragments (279, 261, 246, 206) may arise from dealkylation of pyrollidine type fragments.

The reduction of the keto oxime was attempted using sodium cyanoborohydride at controlled pH. One equivalent of reducing agent should have selectively reduced the oxime at pH 5, however complete conversion of the starting material required two moles of reducing agent suggesting concomittant reduction of the carbonyl group. A NMR triplet at 4.04 ppm (-CH(OH)-) of the crude product indicated that both groups were reduced.

The product is thought to be the dinormethadol primary hydroxylamine (47). Subsequent reactions with formaldehyde to produce a nitrone gave rise to mixtures of products by GCMS and were not pursued. It is possible that the methadol nitrone (65) or its acetal could be isolated using this procedure.



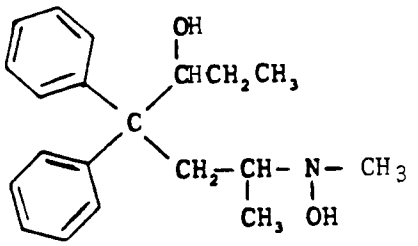
47



65

ii. Attempted synthesis of the methadone nitrone via the secondary hydroxylamine (3)

The attempted synthesis of the methadone secondary hydroxylamine (3) as a precursor of the methadone nitrone, by reductive N-methyl hydroxylamination of the diketone gave products, which were shown by infared spectrometry to have lost the ketone functional group. The mass spectrum resembled that of EDDP in that loss of a phenyl ring is a major fragmentation pathway (66). However, the peaks were one mass unit less and had different relative intensities (i.e.) m/z 276 (5%) and 199 (100%) versus 277 (M^+ 100%) and 200 (15%) for EDDP. The even numbered high mass fragment implies that m/z 276 is not the molecular ion. It is possible that the compound was the methadol secondary hydroxylamine (66). Because of the low yield of the diketone and because yellow mercuric oxide oxidation failed to give the desired keto product, this synthetic pathway was not pursued. Further work should give access to the methadol nitrone. It will be important not to rely heavily on GC analysis since the intermediates are unstable and GCMS results are misleading.



66

iii. Other attempts to obtain the methadone nitrone

Thermal isomerization of the methadone oxaziridine (5) under nitrogen in xylene (the boiling point of xylene approximates the temperature at which the methadone oxaziridine visibly decomposes) gave the formamide (68) as a major product, plus a trace of material at short retention time which, by

GCMS had a molecular weight of 309 a.m.u. with a base peak at m/z 222 (M^+-87). This product was minor, and was not isolated. Flushing the flash chromatography column with methanol followed by NMR analysis of the concentrated eluate failed to detect any nitrone type compounds. Treatment of crude oxaziridine thermal isomerization products with methyl acrylate, in an attempt to trap any nitrone produced as an isoxazolidine 1,3 addition product (57) did give rise to a minor long retention time product. No molecular ion was present in the mass spectrum and the product was not further characterized.

iv) Synthesis of the recipavrin nitrone (13)

The recipavrin nitrone was synthesized in good yield from the corresponding primary hydroxylamine (55). The polar product (R_f 0.05 by TLC in ethylacetate) was purified by flash chromatography and examined by NMR IR, UV, GC and LCMS.

a) NMR Spectra

The ^1H NMR spectrum was comparable to results obtained for N-(1-(3',4'-dimethoxyphenyl) prop-2-yl nitrone (67) (64) as shown in Figure 19. The methylene protons of the nitrones are well downfield in accord with their olefinic nature. Any difference in the chemical shift of the methine proton can be attributed to the insertion of an extra carbon between it and the aromatic portion of the molecule .

No comparable literature values for ^{13}C NMR were available, however the large downfield shift of the azomethine carbon (122.29 ppm) relative to 72 ppm of the oxaziridine ring carbon is in accord with the olefinic nature of this atom. SFORD revealed that this was a broad triplet, as in the recipavrin oxaziridine (14).

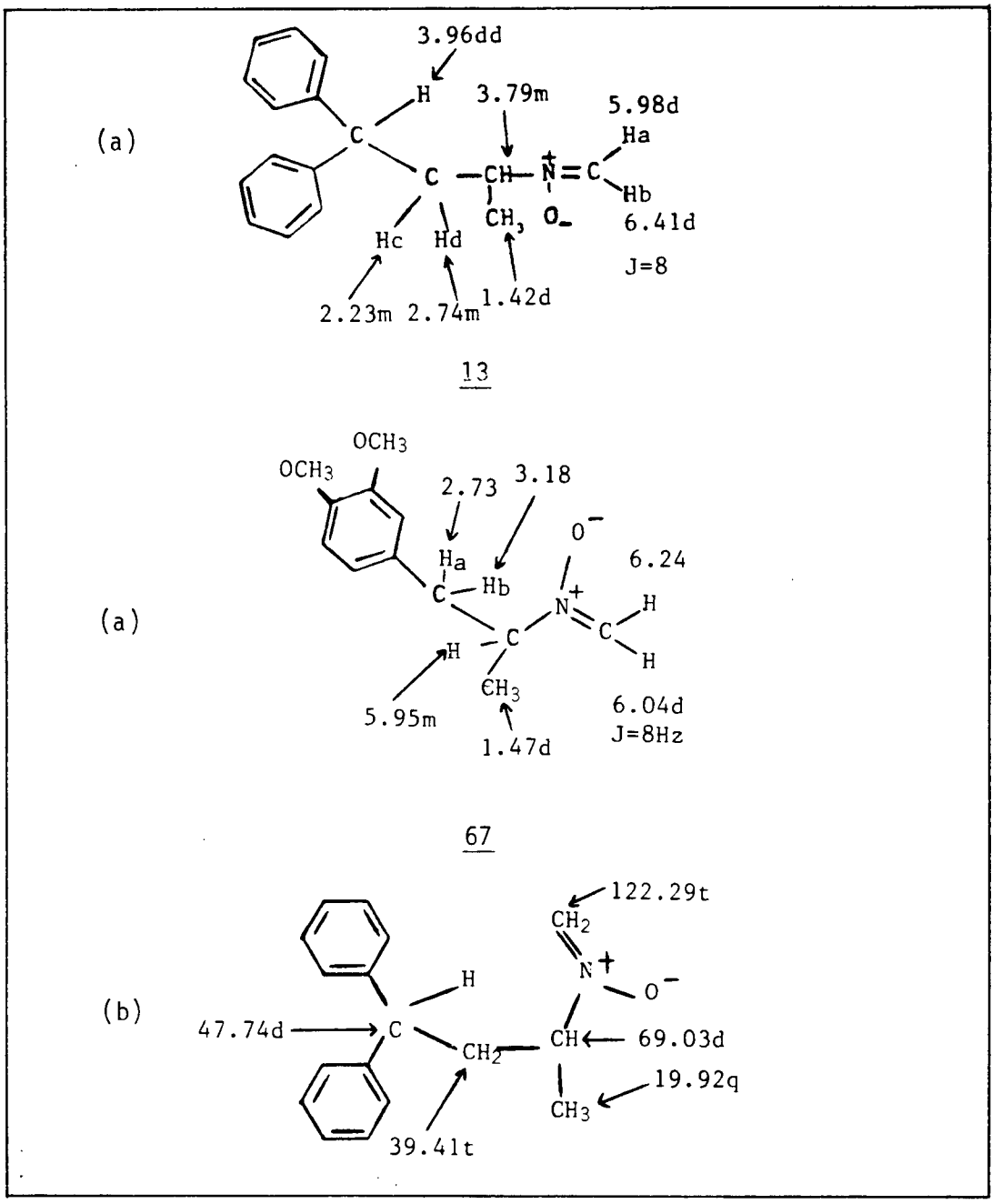


Figure 19(a) Comparison of ^{1}H NMR results for recipavrin nitrone (13) with a literature compound (67)

(b) ^{13}C NMR of the recipavrin nitrone

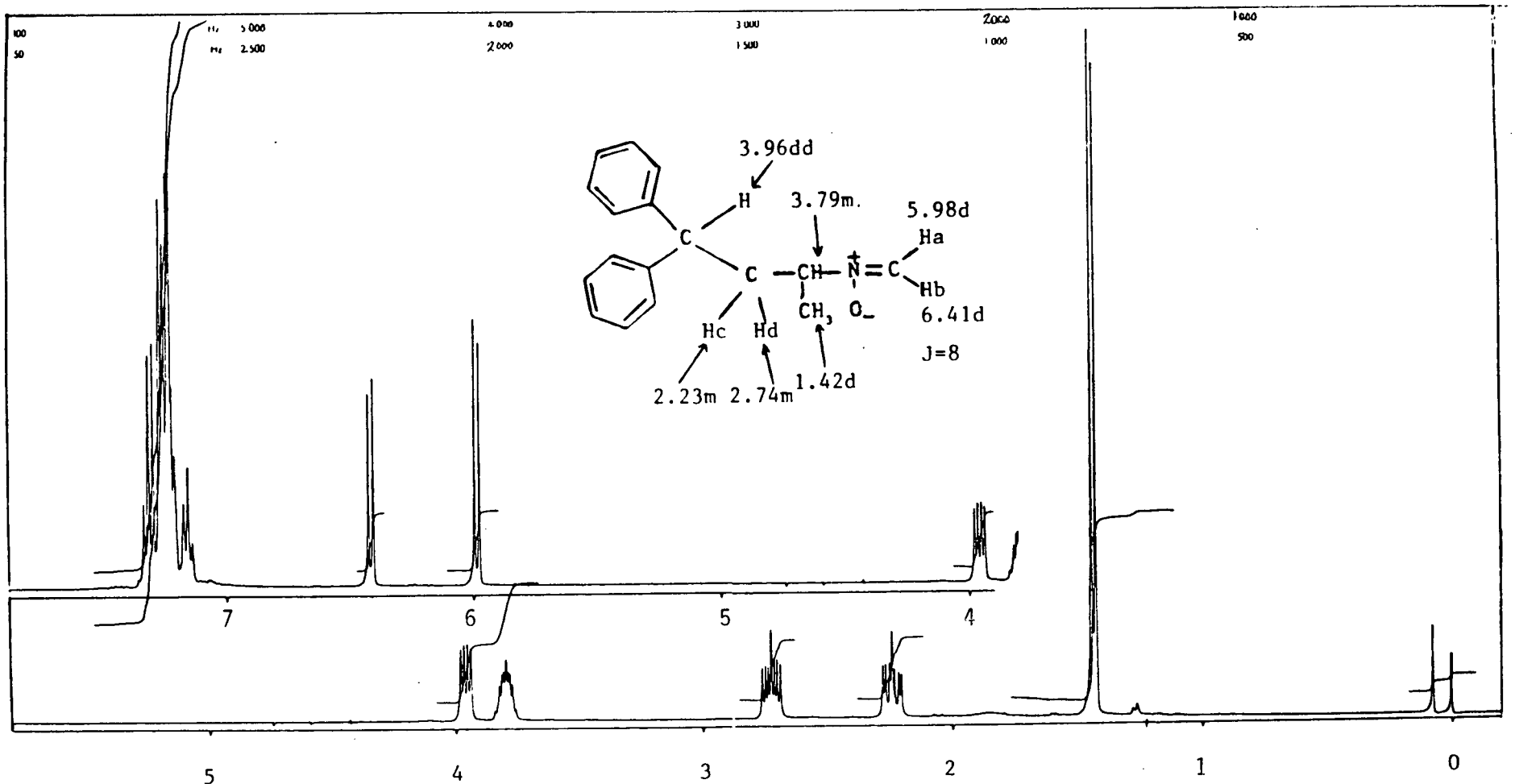


Figure 19 (c) 400 MHz ^{1}H NMR of α-methyl-(N-methylene)-ϒ-phenyl benzenepropanamine N-oxide (13)

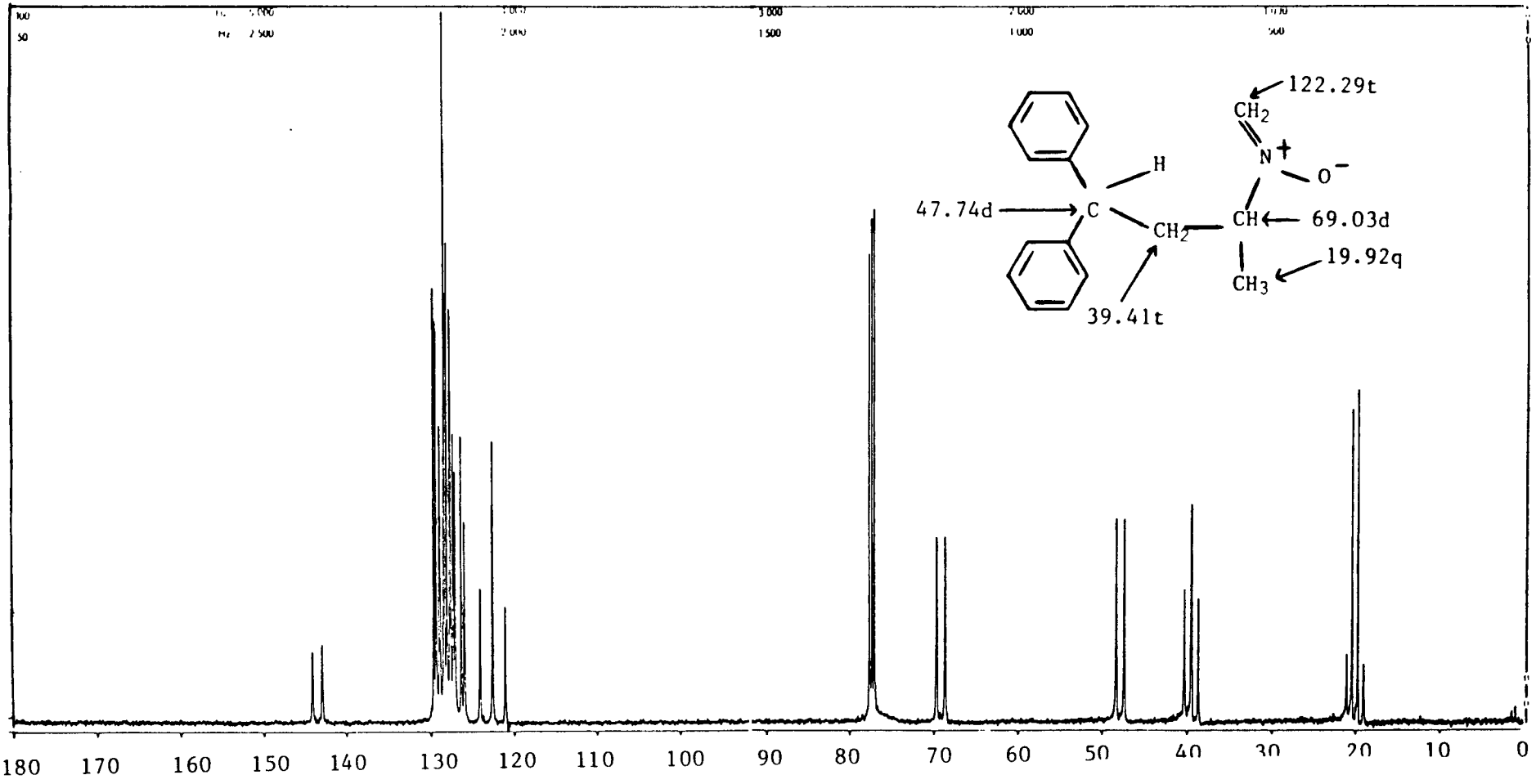


Figure 19 (d) SFORD 400 MHz ^{13}C NMR of α-methyl-(N-methylene)

- γ-phenyl benzenepropanamine N-oxide (13)

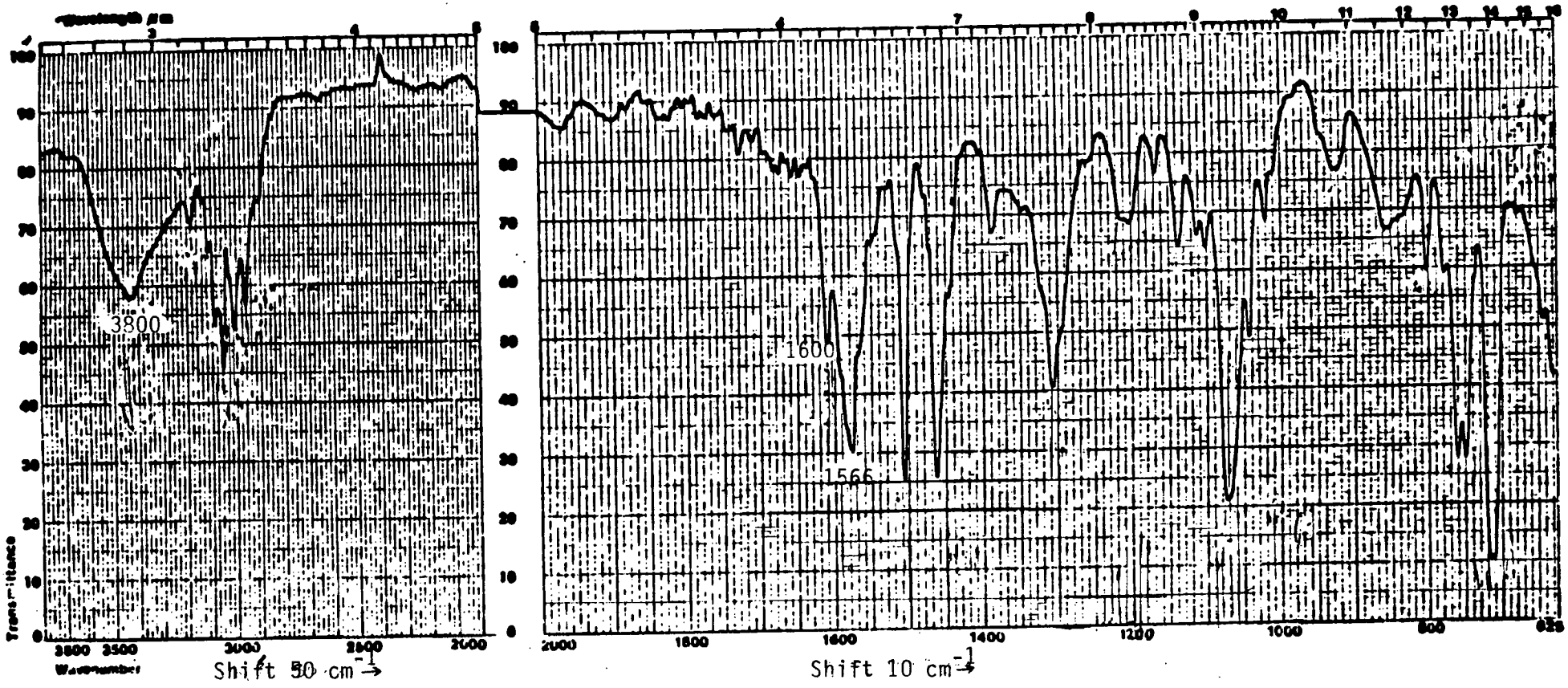


Figure 20. Infrared spectrum (film) of α-methyl-(N-methylene) -γ-phenyl benzenepropanamine N-oxide (13)

b) Infrared and UV spectra

The infrared spectra (Figure 20) also compared well with Beckett's nitrone (67) in that they both had a C=N stretch at 1566 cm^{-1}.
UV spectra revealed an absorption at 233 mμ (ε= 8160) as compared to 235 mμ (ε= 6210) for compound (67) probably a π--π* transition of the azomethine bond. Additonal bands were observed at 282 mμ(ε = 2170) and 340 mμ (ε=870).

c) GCMS and mass spectra

The nitrone decomposed during GCMS to produce three breakdown products. The major compound was the imine (84), with lesser amounts of the oxime (79) and lastly, a small amount of the formamide (15). This correlates well with work by Beckett on the methylene nitrone of N-methyl-3',4'- dimethoxy-amphetamine (67) which, on GLC analysis "behaved anomalously and produced an oxime and an unidentified product". The formamide in our case is not always evident as a sharp peak, unless a freshly packed column is used, sometimes it is necessary to monitor the m/z 73 and 253 peaks to detect it. The isomerization to the amide may not take place in Beckett's case since the more volatile amphetamine derivatives require GLC column temperatures between 100 and 200°. This is much less than the temperatures required to elute or produce the recipavrin formamide. The imine is the deoxygenated product of the nitrone (in accord with literature reports on thermal deoxygenation (61)). Literature precedent for thermal production of the oxime exists only in Becketts work (67). The absence of the former two components in the GCMS of conjugated recipavrin metabolites of rat bile will be demonstrated in the metabolism section, as evidence against the nitrone as the structure of the metabolite. The synthetic nitrone chromatographed at short retention time by reversed phase HPLC, and LCMS revealed the

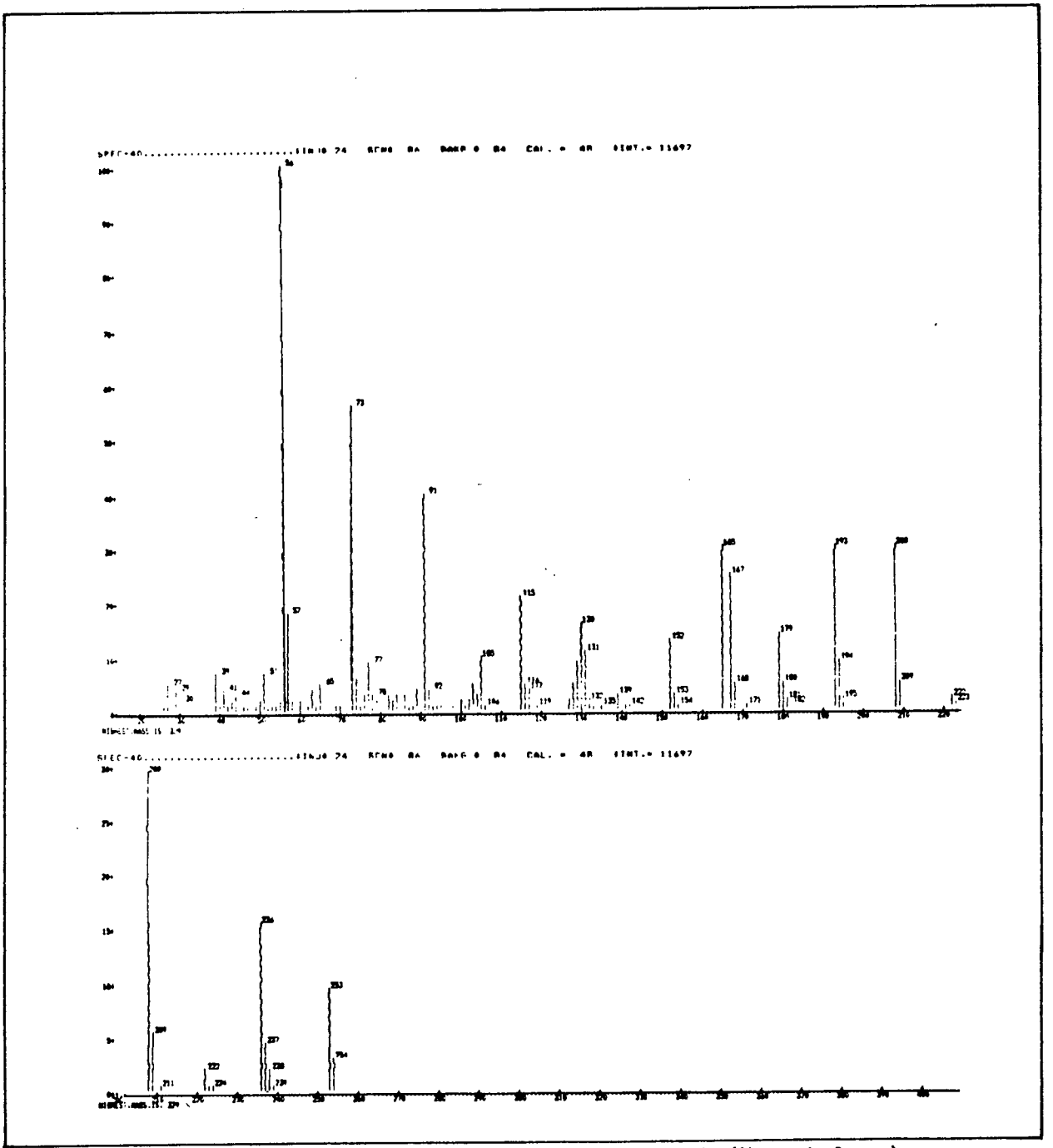


Figure 21(a) Mass spectrum (direct inlet) of α-methyl-(N-methylene) - γ -phenyl-benzenepropanamine N-oxide (13)

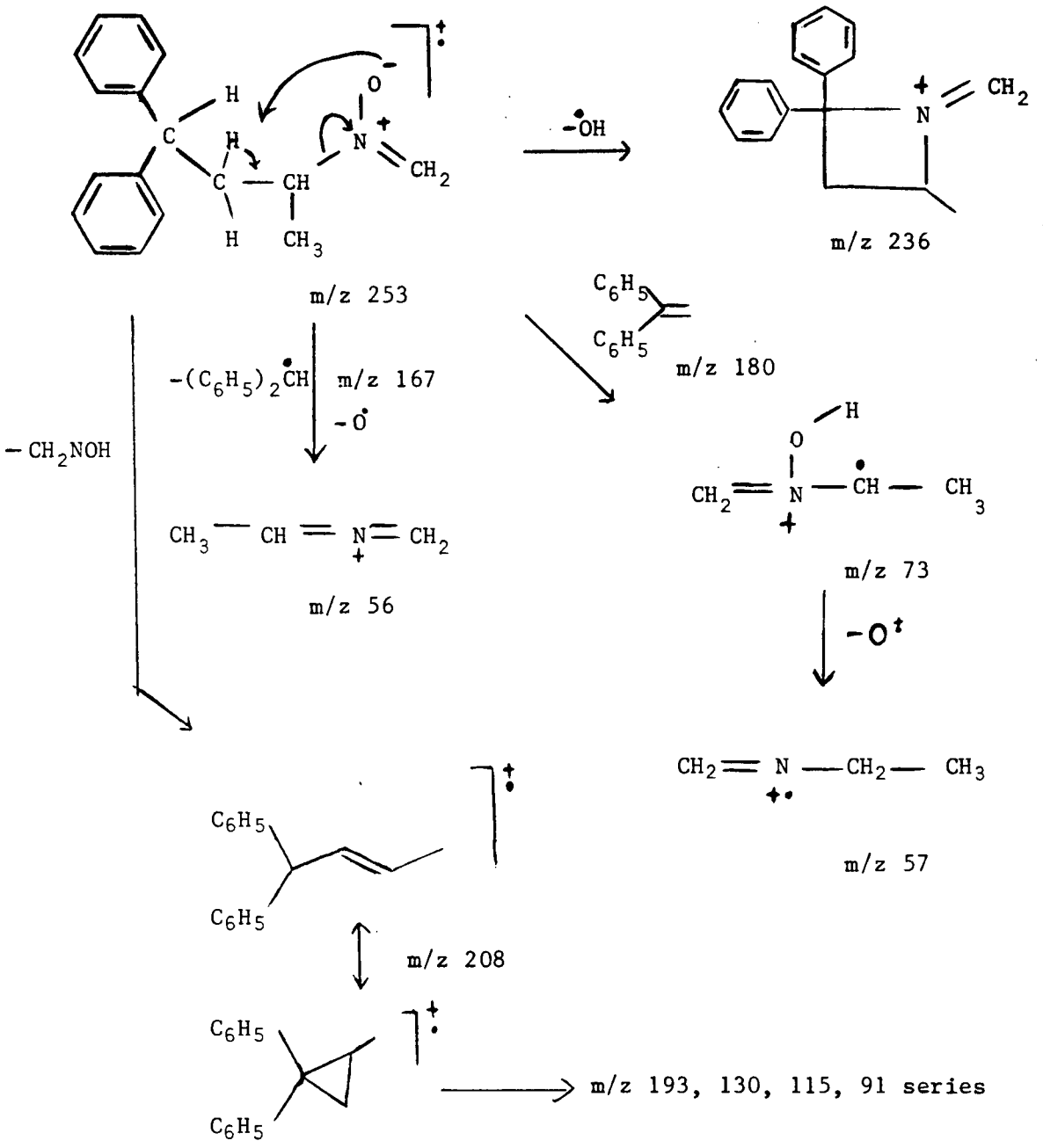


Figure 21 (b) Fragmentation pathways of the recipavrin nitrone (13) by analogy to Coutts et al (57)

M^++1 ion at m/z 254. The direct inlet mass spectrum had strong peaks at m/z 56 and m/z 91 $(C_6H_5\text{-}CH_2)^+$ consistent with the direct inlet mass spectrum of α-methyl- (N-methylidene) benzene ethanamine N-oxide reported by Coutts et al. The M^+-15 (m/z 238) fragment was less important in the mass spectrum of the recipavrin nitrone but a prominent M^+-17 (m/z 236) was present. As in the oxaziridine, the m/z 208 series of ions was prominent. Based on the mass spectra of deuterated methamphetamine nitrones, the fragmentation pathway proposed by Coutts applies to the recipavrin nitrone (Figure 21a).

As the direct probe of the mass spectrometer is heated, the ions characteristic of the formamide become prominent in the direct inlet mass spectrum of both the recipavrin nitrone and oxaziridine.

In light of the occurrence of m/z 56 and 42 fragments of the direct probe mass spectrum of the methadone oxaziridine which appear prior to heating the probe, the evidence here supports isomerization of oxaziridines to nitrones at low temperatures and of the oxaziridines to formamides as the source is heated, similar to results reported by St. Claire Black (82).

E. KETONES

The ketones 1,1-diphenyl-2-butanone (42) (CAS 6336-52-3), 1,1-diphenyl-3-butanone (51) (CAS 5409-60-9) and 4,4-diphenyl-2,5-heptanedione (45) (the latter described by Kang as a byproduct in the peroxidation of EDDP) were made for use as synthetic intermediates. Compounds (45) and (51) were characterized in detail as they are potential metabolites of methadone and recipavrin respectively.

i) NMR Spectra

^1H NMR results, presented in Figure 22 are in accord with the ketone functional groups present. Of all the compounds studied, only the ketones

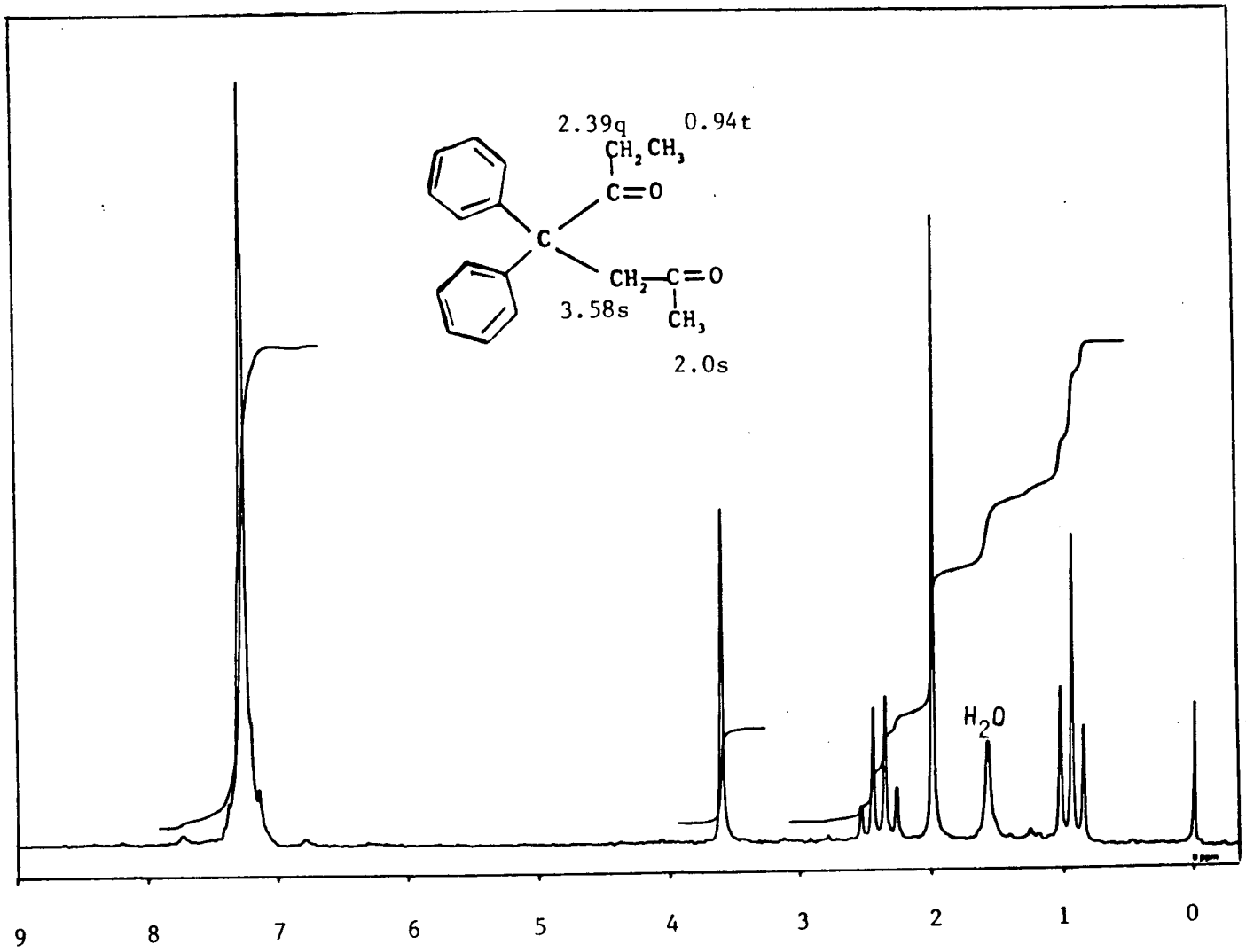


Figure 22(a) 80 MHz ^{1}H NMR of 4,4-diphenyl-2,5-heptanedione (45)

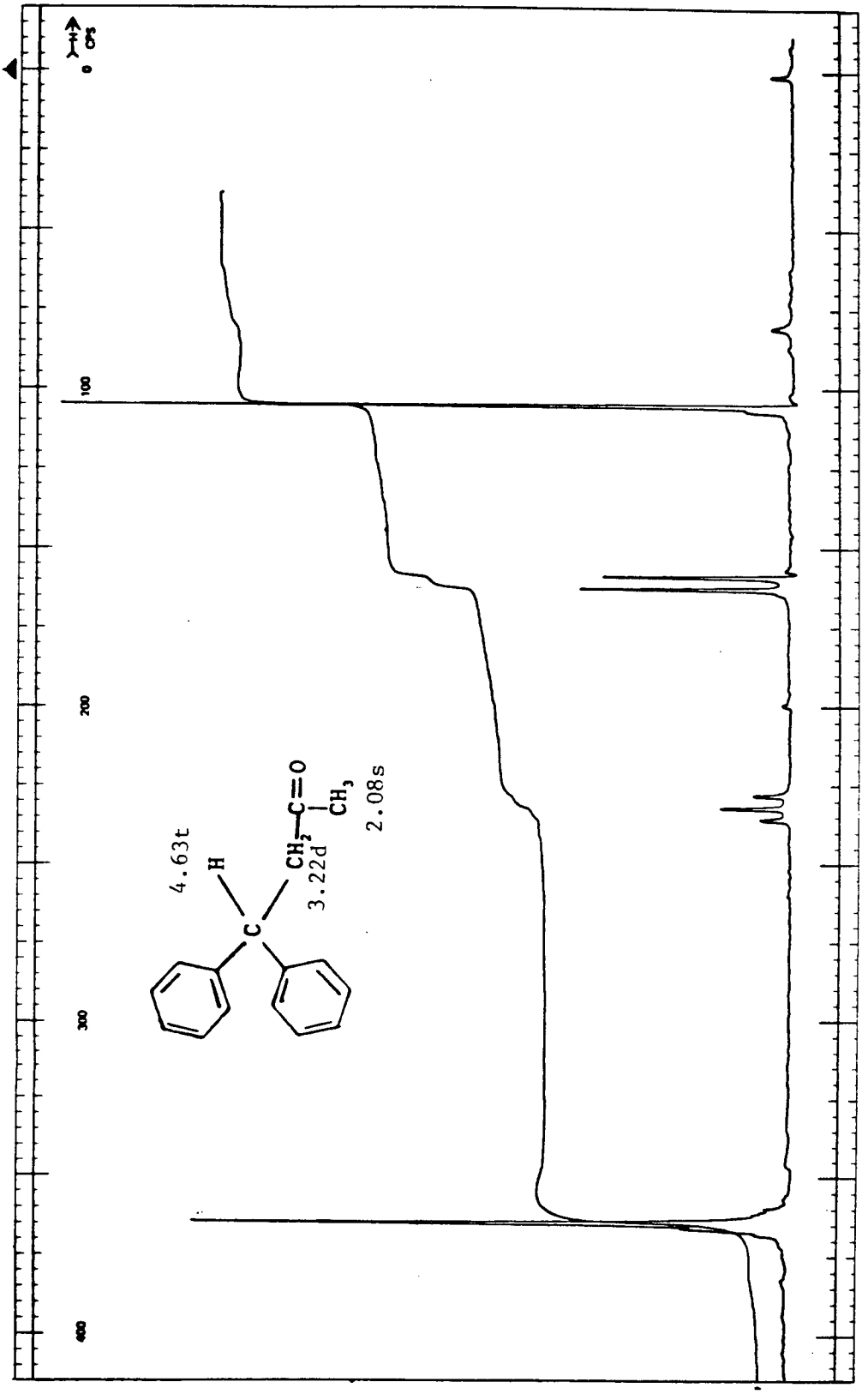


Figure 22 (b) 100 MHz ^1H NMR of 1,1-diphenyl-3-butanone (51)

and oximes (with double bonds) had equivalent chemical shifts for the methylene group α- to the methylketone. The 20 MHz ^{13}C NMR spectrum of compound (45) (results shown in Figure 22c) supports the diketone structure when compared to results for the methadone oxaziridine and the chemical shifts of aliphatic ketones. The carbonyl carbons were not observed, since they lack nuclear overhauser enhancement and were lost in the baseline.

ii) Infrared spectra

In the infrared spectra both ketones exhibited strong C=O stretches, the dione as a doublet at 1710 cm^{-1} and 1695 cm^{-1} (Figure 23) and the 3-butanone as a single peak at 1710 cm^{-1}.

iii) Mass spectra

Mass spectra showed weak molecular ions (m/z 224, M^+) plus α-scission ions of the carbonyl group at m/z 43 and 57 in diphenylbutanones (51) and (42) respectively. Both these ions plus weak M^+ -18 series peaks occurring at m/z 262, 223, 206, were present in the diketone. The mass spectral fragmentation pathways are outlined in Figure (24).

iv) Problems in the diketone synthesis

Much time was spent on the synthetic pathway starting from diphenyl acetonitrile to the lactone (41), diol (43) and diketone (45). All these compounds except the diketone were investigated in the 1950's and little data besides combustion analysis was available for them.

The lactone was obtained in good yield via the in situ acid hydrolysis of the THF imine (40) as described by Attenburrow et al (73). NMR results are summarized in Figure 25. The infrared had a strong δ -lactone C=O stretch at 1750 cm-1 and the mass spectrum showed a weak

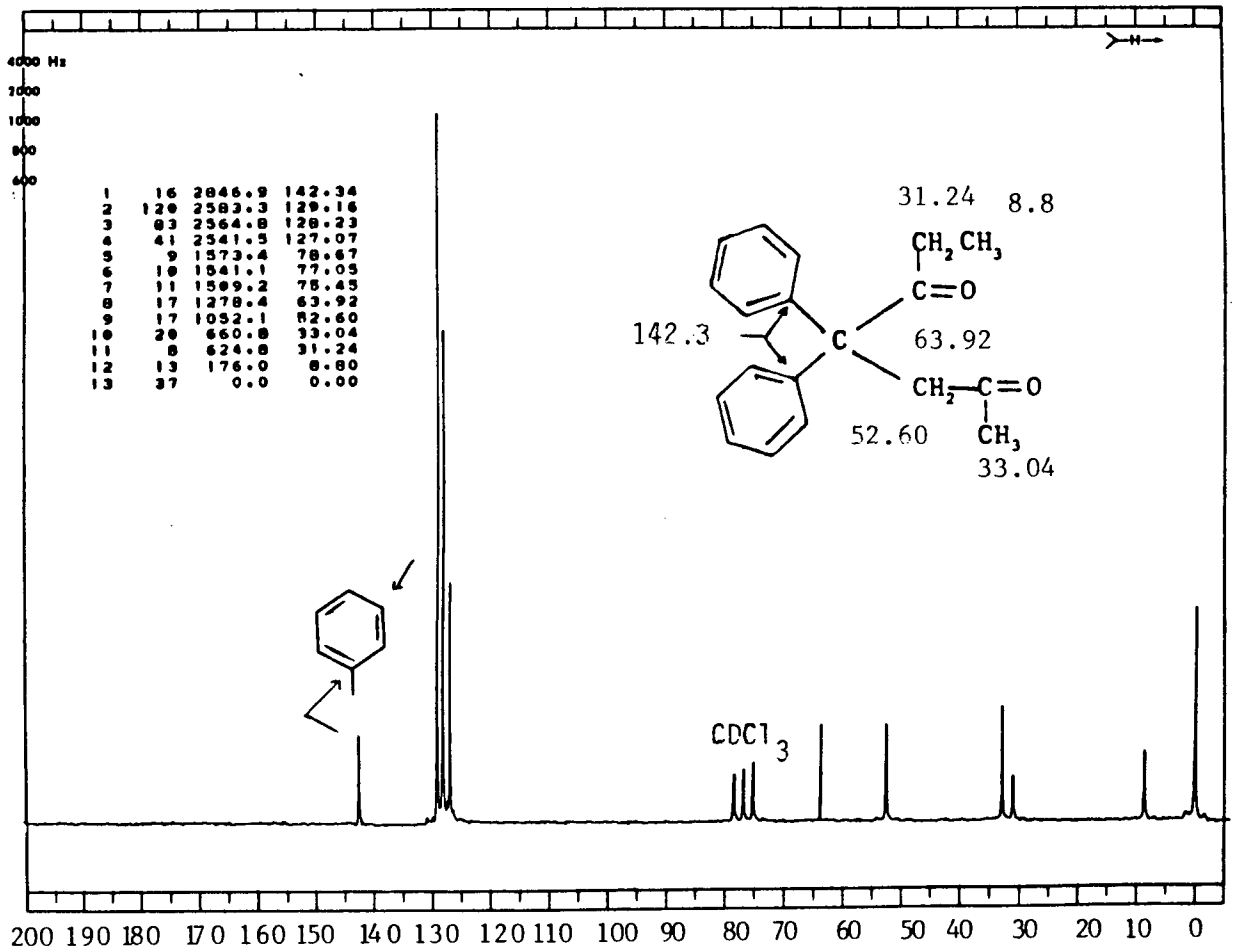


Figure 22 (c) Broadband decoupled 20 MHz ^{13}C NMR of 4,4-diphenyl-2,5-heptanedione (45)

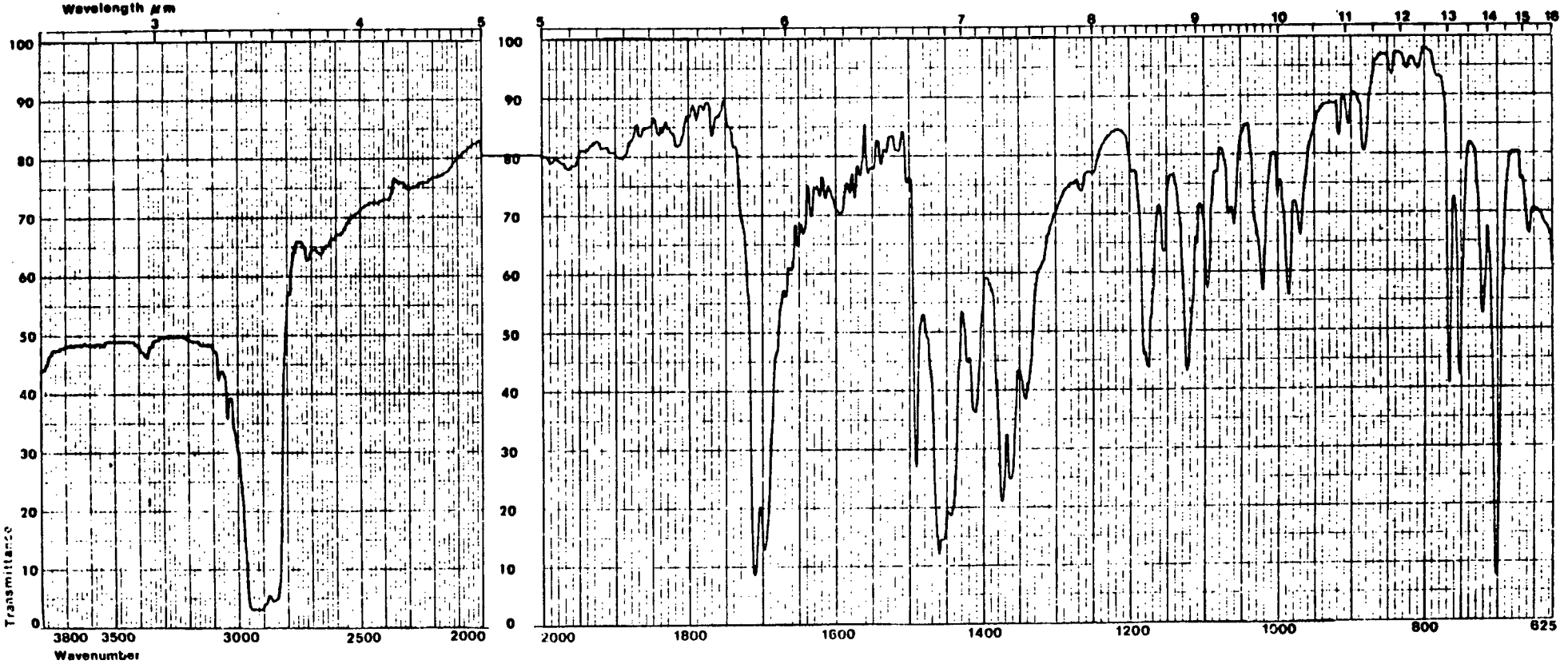


Figure 23. Infrared spectrum (nujol mull) of 4,4-diphenyl-2,5-heptanedione (45)

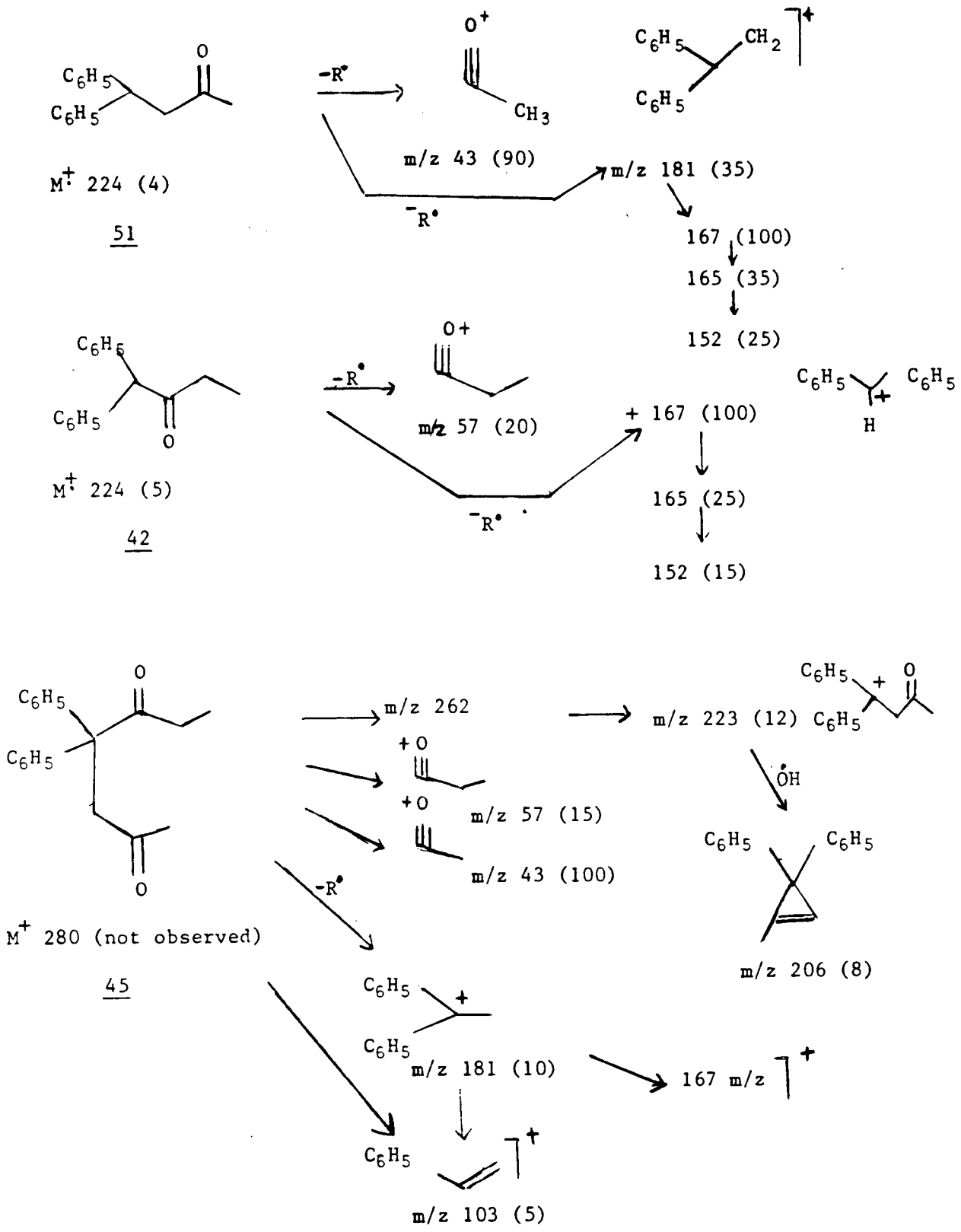


Figure 24. Electron impact mass fragmentography of the ketones (42) (45) and (51)

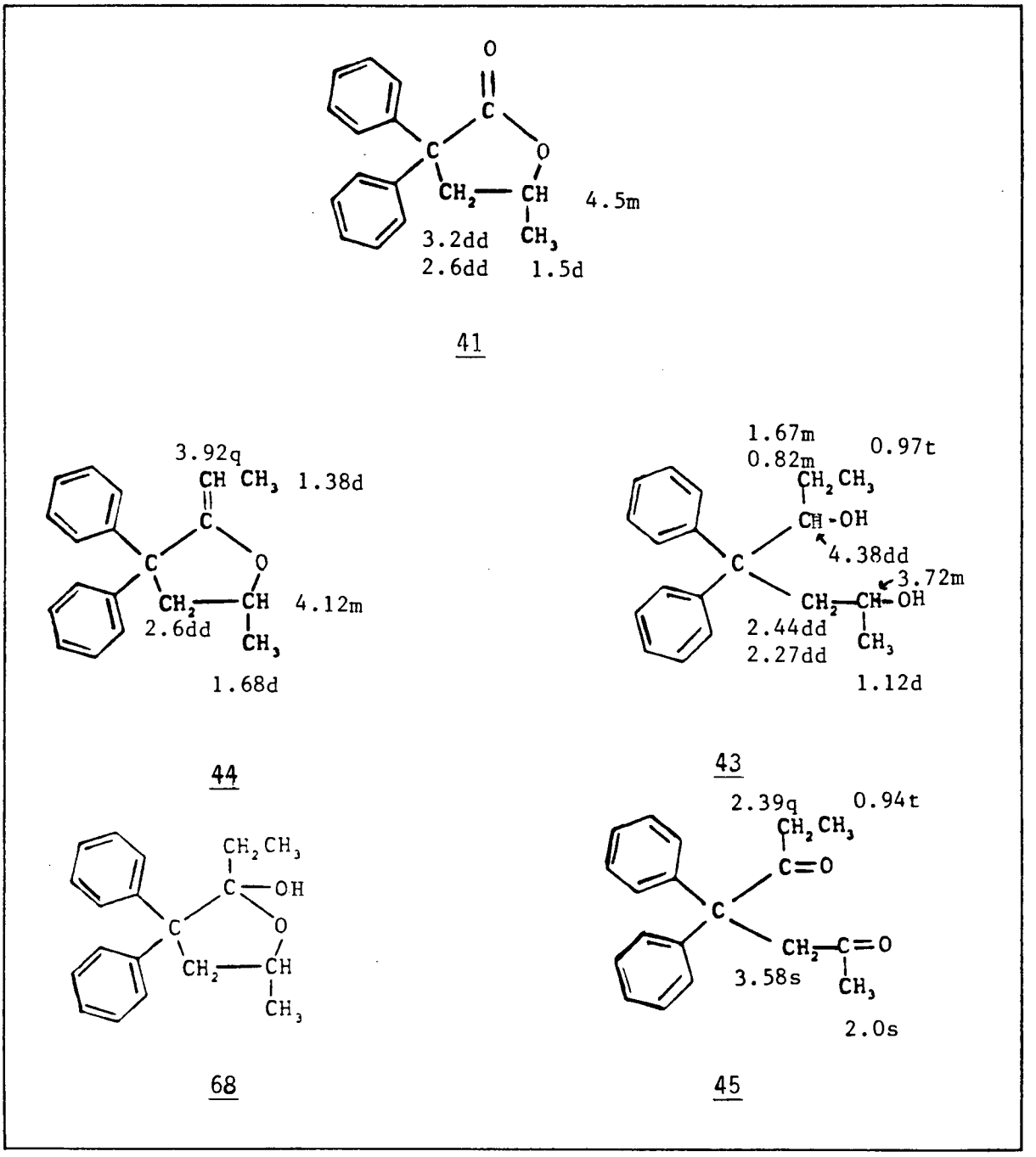


Figure 25. NMR results for the diketone synthetic pathway

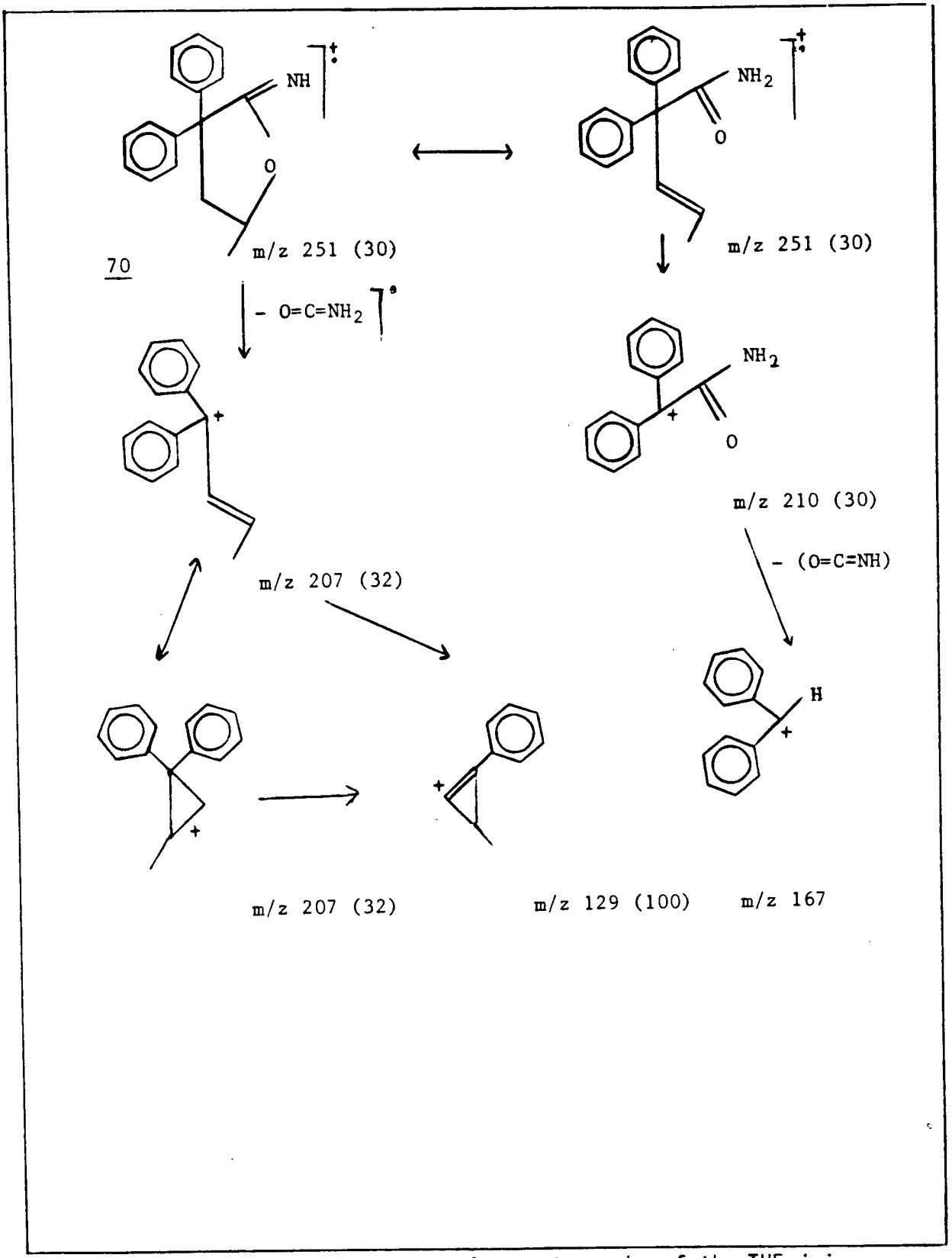


Figure 26(a) Electron impact mass fragmentography of the THF imine

molecular ion at m/z 252 (8%) which loses CO_2 and fragments via the m/z 208 series described earlier. In the intermediate THF imine (40) hydrogen bonding (NH) in the 3400 cm^{-1} region and a C=N stretch at 1670 cm^{-1} were present in the infrared spectrum. The intermediate THF imine (70) had the mass spectral fragmentation pathway outlined in Figure (26a). The ions at m/z 207 and 129 only occur in compounds which appear to eliminate nitrogen to form a double bond in the amino side chain. This pathway is also important in the fragmentation of 4,4-diphenyl-5-hept-2-enone (39) and the methadone formamide (31). The m/z 207, 129 series is also diagnostic of the Cope elimination products of tertiary N-oxide metabolites, which arise in the GC during the analysis of these labile compounds (Figure 26b).

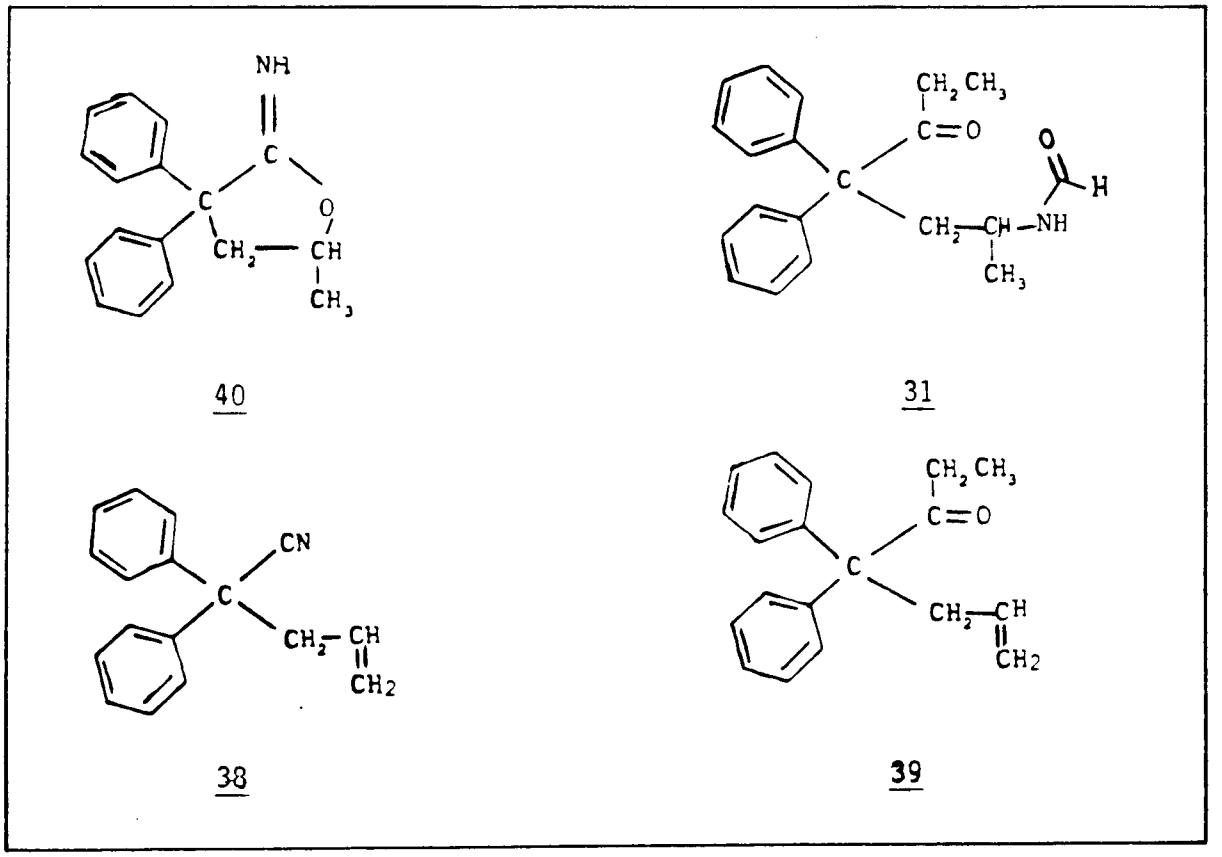


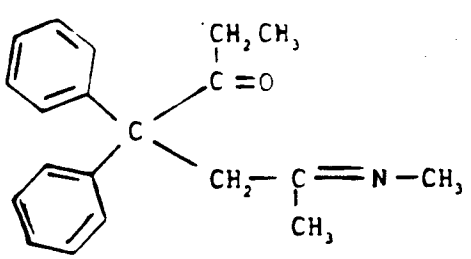
Figure 26 (b). Compounds which fragment via the m/z 207, 129 series.

Reaction of the lactone (41) with one equivalent of EtMgBr was expected to give the lactol (68) as described by Wilson (71). Two equivalents of EtMgBr were required for the complete conversion of the lactone to a product correctly ascribed the diol structure (43) by Craig et al (87). The ethylidene tetrahydrofuran (44) was a major byproduct (10%). The diol exhibited a strong broad O-H intramolecular hydrogen bonding band at 3200 cm^{-1} and a C-O stretch characteristic of saturated secondary alcohols at 1120 cm^{-1}. No lactol (68) was obtained. In the mass spectrometer the diol fragmented via the 208 (base peak) series, with a minor pathway via the ethylidene tetrahydrofuran (M^+-20) as outlined in Figure 25.

The major fragmentation pathway of the ethylidene THF (44) (base peak M^+ 264) is likely via the open chain compounds A and B (Figure 26c) since the fragments m/z 43, 57 and the 207 series are relatively important.

Attempts to oxidize the diol with Jones reagent gave very low yields (5%) and required a tedious fractional crystallization procedure to purify the minor product. The major product was the lactone (41). Syntheses using pyridinium chlorochromate, sodium dichromate, and a number of other oxidants were all unsuccessful.

An attempt to synthesize the oxaziridine intermediate (69) by reacting the diketone (45) with methylamine in methanol produced a major product which by GCMS was assigned the dihydropyrolle structure (70), based on its similarity to the fragmentation of EDDP (66). (Figure 26d)



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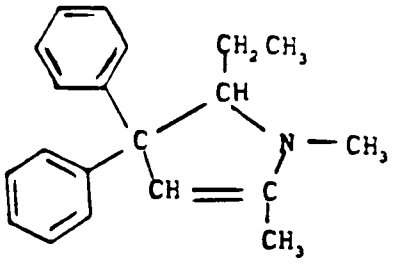


70

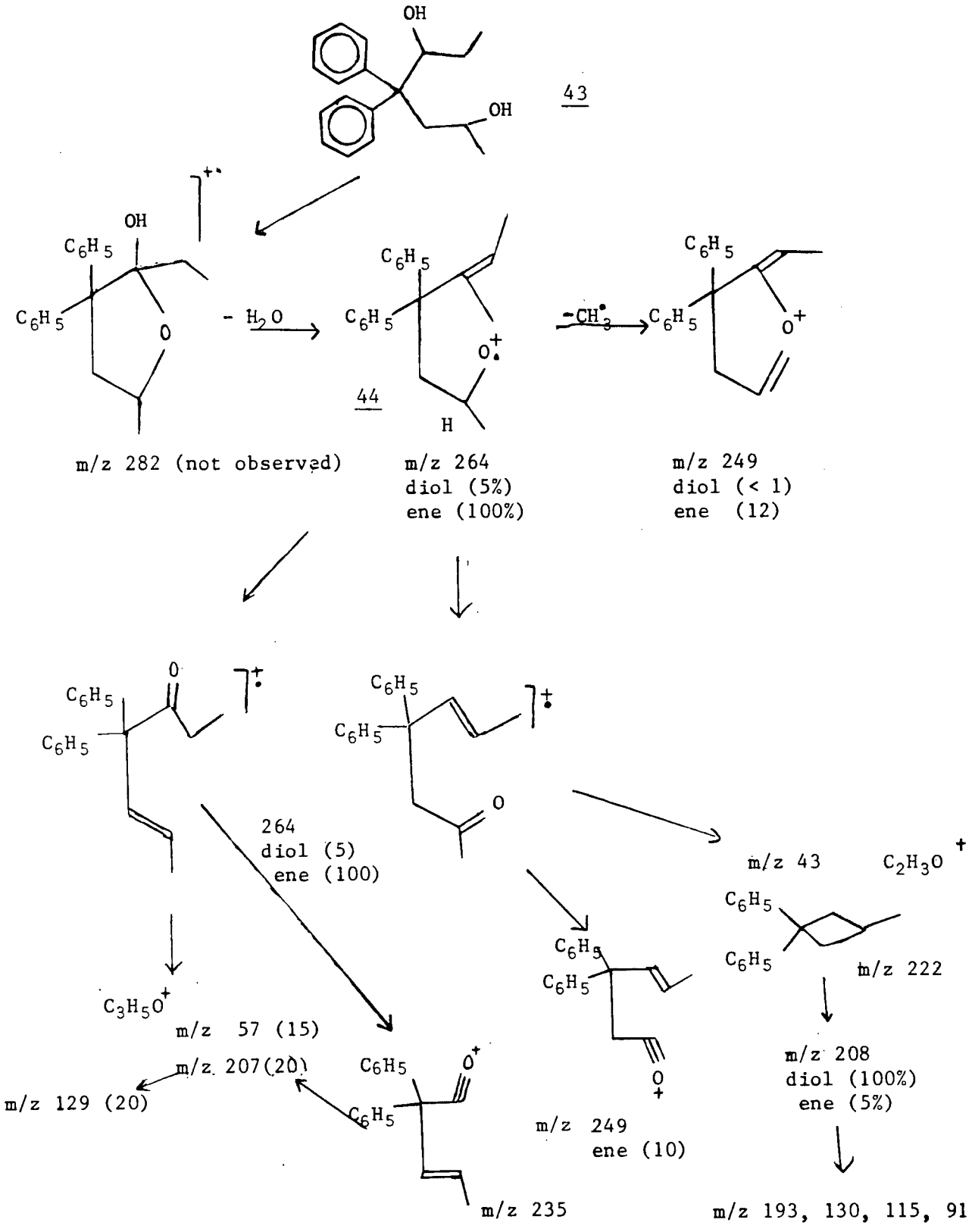


Figure 26 (c) Fragmentation of diol (43) and ethylidene tetrahydrofuran (44)

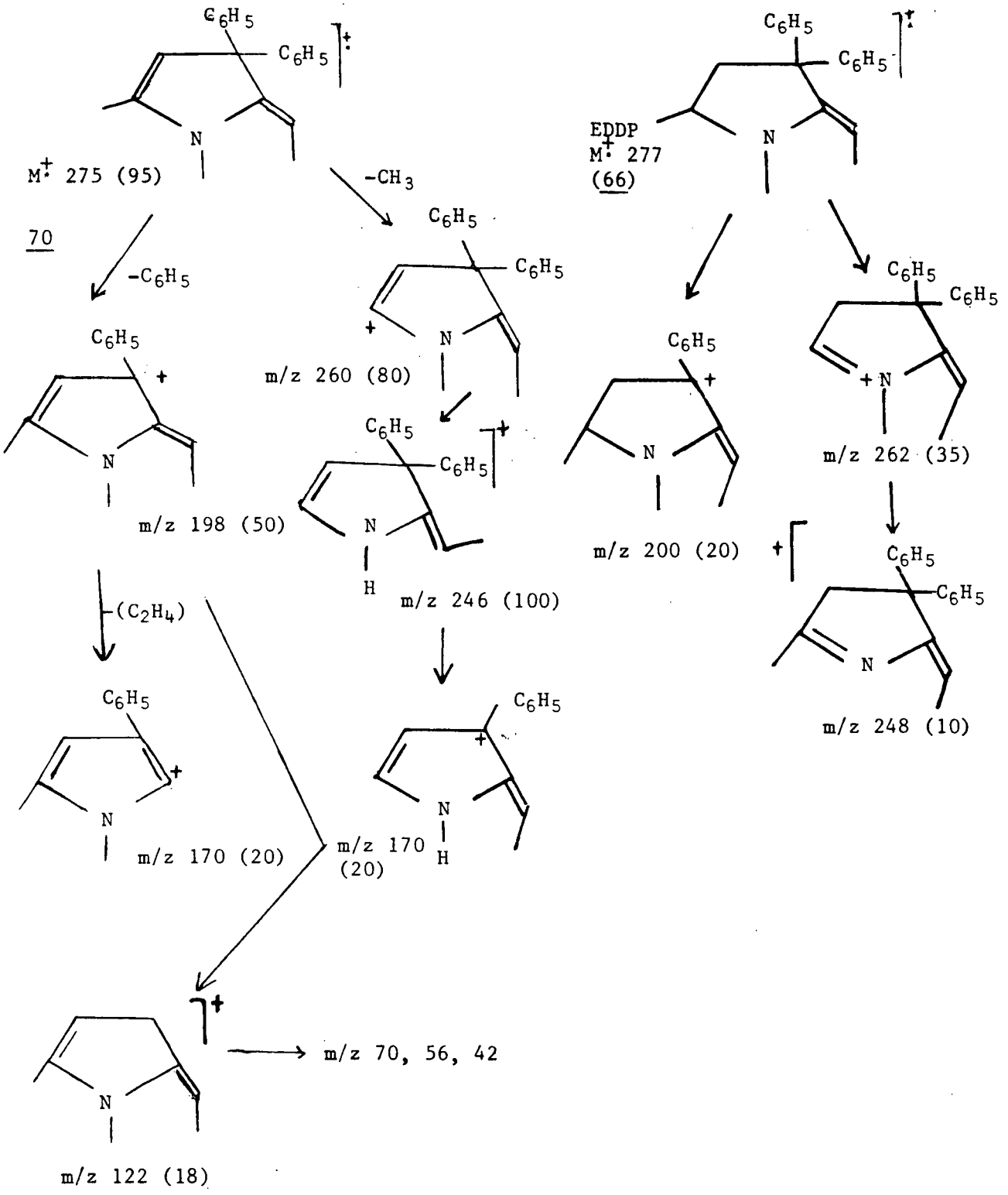
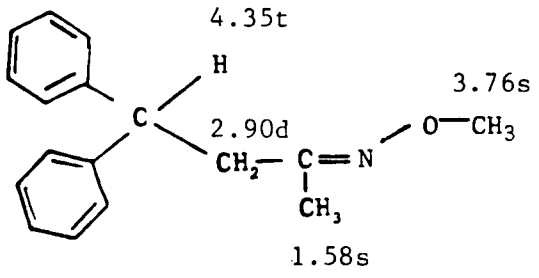


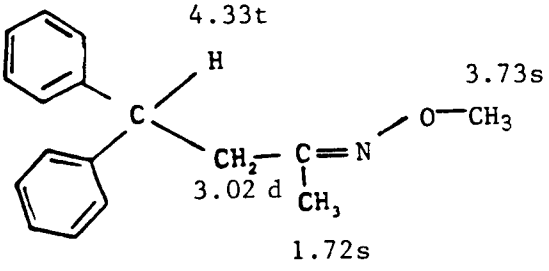
Figure 26 (d) Fragmentation of 2-ethylidene-1,4-dimethyl-3,3-diphenyl-2,3-dihydropyrolle (70) compared to EDDP (66)

F. OXIMES

The oximes (52) and (64) were synthesized in good yield from 1,1-diphenyl-3-butanone. NMR and IR results for (52) have already been discussed by comparison with the keto oxime (46). Like the oxime (52) the NMR of the O-methyloxime (64), (isomeric with the formamides, nitrones and oxaziridines) shows a 2:1 mixture of syn and anti isomers (shown below). The mass spectra are fairly simple, since no γ hydrogens are present to allow McLafferty rearrangement. Both oximes have molecular ions and exhibit β-cleavage to give the diphenyl methane base peak m/z 167 (100%) with a m/z 42 (10%) alkyl fragment present. Alpha-cleavage is a minor pathway in both compounds giving ions at m/z 181 and 103 (10%). The m/z 207 and 220, 206 series (a major pathway in the keto oxime (46) fragmentation pathway) are present to the extent of 5% in (64) and (52) respectively.



64 syn

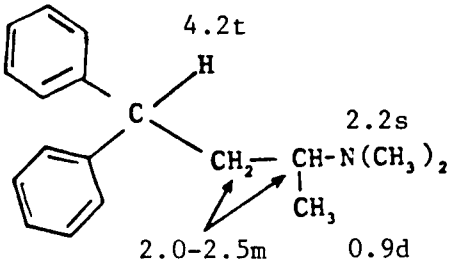


64 anti

G. AMINES

The amines, recipavrin (6) (CAS 13957-55-6), norrecipavrin (54) (CAS 29869-78-1) and dinorrecipavrin (53) (CAS 29869-77-0) were synthesized in good yield, from methadone nitrile in the case of recipavrin (74), or by reductive amination of 1,1-diphenyl-3-butanone (51) in the latter two cases. NMR results for the primary and secondary amines are outlined in the

following section. The NMR results for recipavrin compare favorably with those obtained by Beckett and Casy (88). Mass spectra exhibit base peaks at m/z 72, 58, and 44 resulting from α-cleavage of the tertiary, secondary and primary amines respectively. Weak molecular ions, diphenylalkane residues and evidence of the m/z 208 series were also present to a minor extent.



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H. HYDROXYLAMINES

The hydroxylamines (55) and (57) were obtained by reductive hydroxylamination or reductive N-methylhydroxylamination of diphenyl butanone (51). They are both potential metabolites and synthetic precursors of the methylene nitrone. The NMR results shown below (Figure 27) show the deshielding effects of the hydroxyl group on one of the α-methylene protons, and on the amino methine proton. A similar downfield shift of 0.2 ppm was observed for the α-methylene and N-methyl protons of the secondary hydroxylamine.

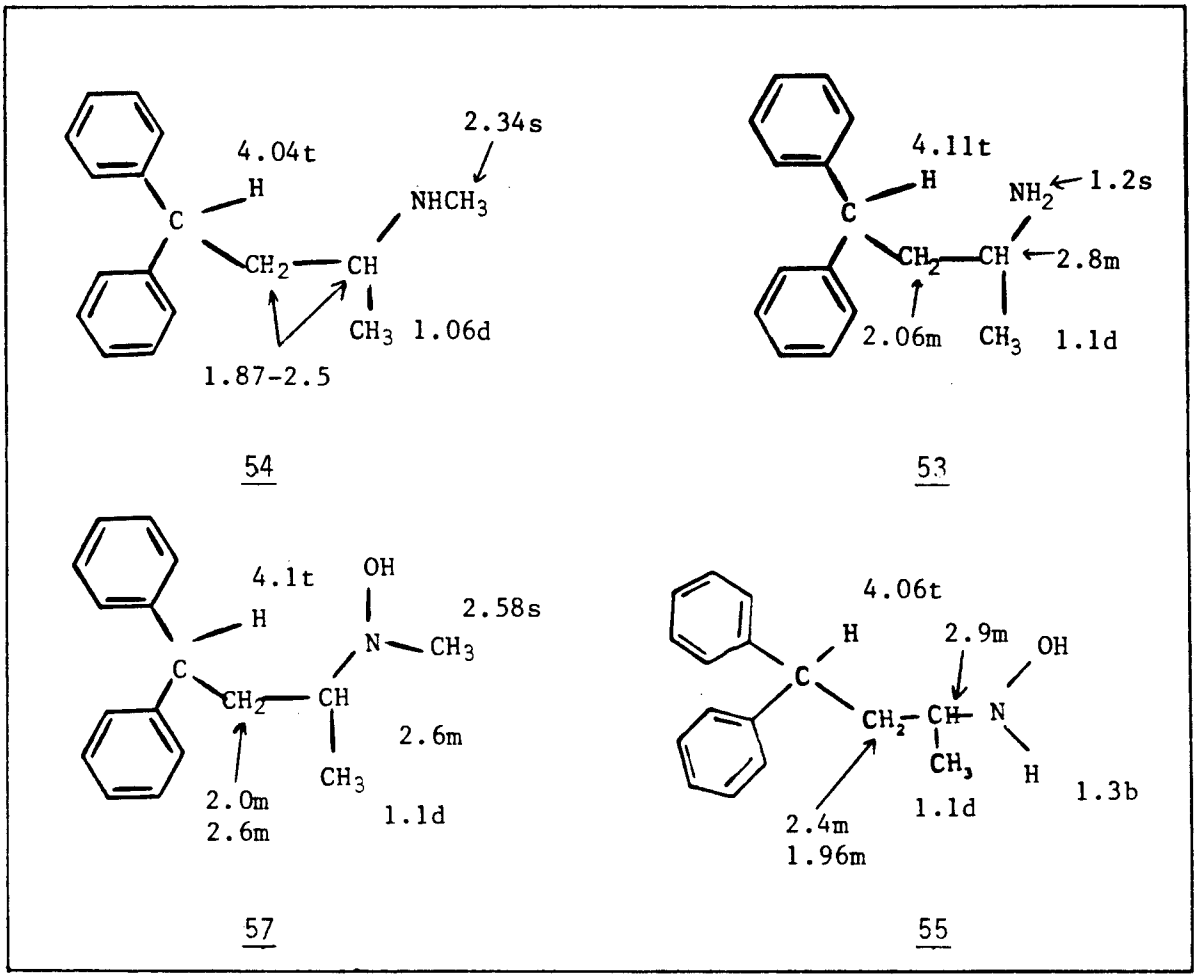


Figure 27. Comparison of NMR results for the primary and secondary hydroxylamines and the corresponding amines.

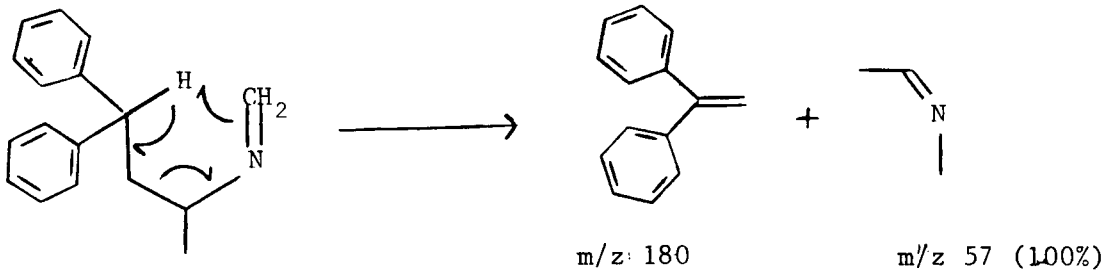
In the infrared spectrum of the hydroxylamines the weak N-H stretch band at 3300 cm^{-1} of the secondary amine is replaced by a strong intermolecularly hydrogen bonded O-H stretch at 3150 cm^{-1}. Two C-N stretches occurring between 1200 and 1000 cm^{-1} (characteristic of secondary aliphatic amines) are also present in the hydroxylamines. A medium intensity doublet at 1220 and 1190 cm^{-1} could be a N-O stretch similar to that of an aliphatic nitroso dimer. Morgan and Beckett (64), who have synthesized the corresponding amphetamine secondary hydroxylamines,

characterized oxalate salts of these compounds, making comparison to their data difficult.

The secondary hydroxylamines decomposed in the GC inlet, to the corresponding amines. When derivatized with BSTFA, each showed a weak molecular ion (m/z 327 and 313 respectively) and base peaks at m/z 146 and 132 respectively arising from α-cleavage of the -N(R)-OTMS group. This is characteristic of aliphatic hydroxylamines (53). These ions were used to search for any hydroxylamine metabolites in derivatized bile extracts.

I. SYNTHESIS OF THE IMINE (59)

The imine (59) as noted previously, probably exists as the triazene (60). A weak molecular ion at m/z 237 (monomer) appeared to fragment by a McLafferty rearrangement to the base peak at m/z 57 as outlined below. Minor amounts of α-cleavage to m/z 222 leading to the m/z 208 series was observed.

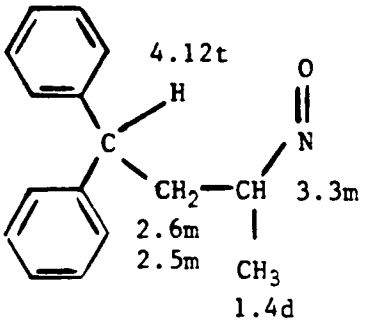


m/z 180 m/z 57 (100%)

J. SYNTHESIS OF THE NITROSO COMPOUND

Treatment of the primary amine (53) with MCPBA produced a thermolabile nitroso compound (61) which by infrared spectrometry was consistent with a dimeric structure.

As in the recipavrin nitrone, N-methine proton was deshielded (3.3 ppm), signifying an electron withdrawing substituent. A sharp infrared band at 1380 cm^{-1} with a shoulder at 1365, is characteristic of aliphatic cis-nitroso dimers.



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K. PEROXIDATION OF EMDP

Treatment of EMDP base with 2 moles of MCPBA gave rise to 5 major products by GCMS. The last three peaks eluted from the GC column may be identified based on the mechanistic schemes of Milliet et al (78). Peak three (Appendix, page 163) is likely 4-methyl-2,2-diphenyl pyrollidone (71). The structure proposed is based on its mass spectral similarity to DDP (12) since it has a strong molecular ion (M^+251) which fragments by the m/z 208 series.

The last two peaks, appear as a potential methyl ketone, and an ethyl ketone as shown by base peaks at m/z 43 and 57 respectively. Knowing that thermal isomerization of oxaziridines to amides in the GC inlet is possible, allows us to predict the EMDP oxaziridine structures (72), (73) for peak 4 and 5 based on their long retention time and the observed molecular ions (Figure 28). These compounds could form the ketones (74) and (75) in the GC and fragment by α-scission in the mass spectrometer since no τ protons are present.

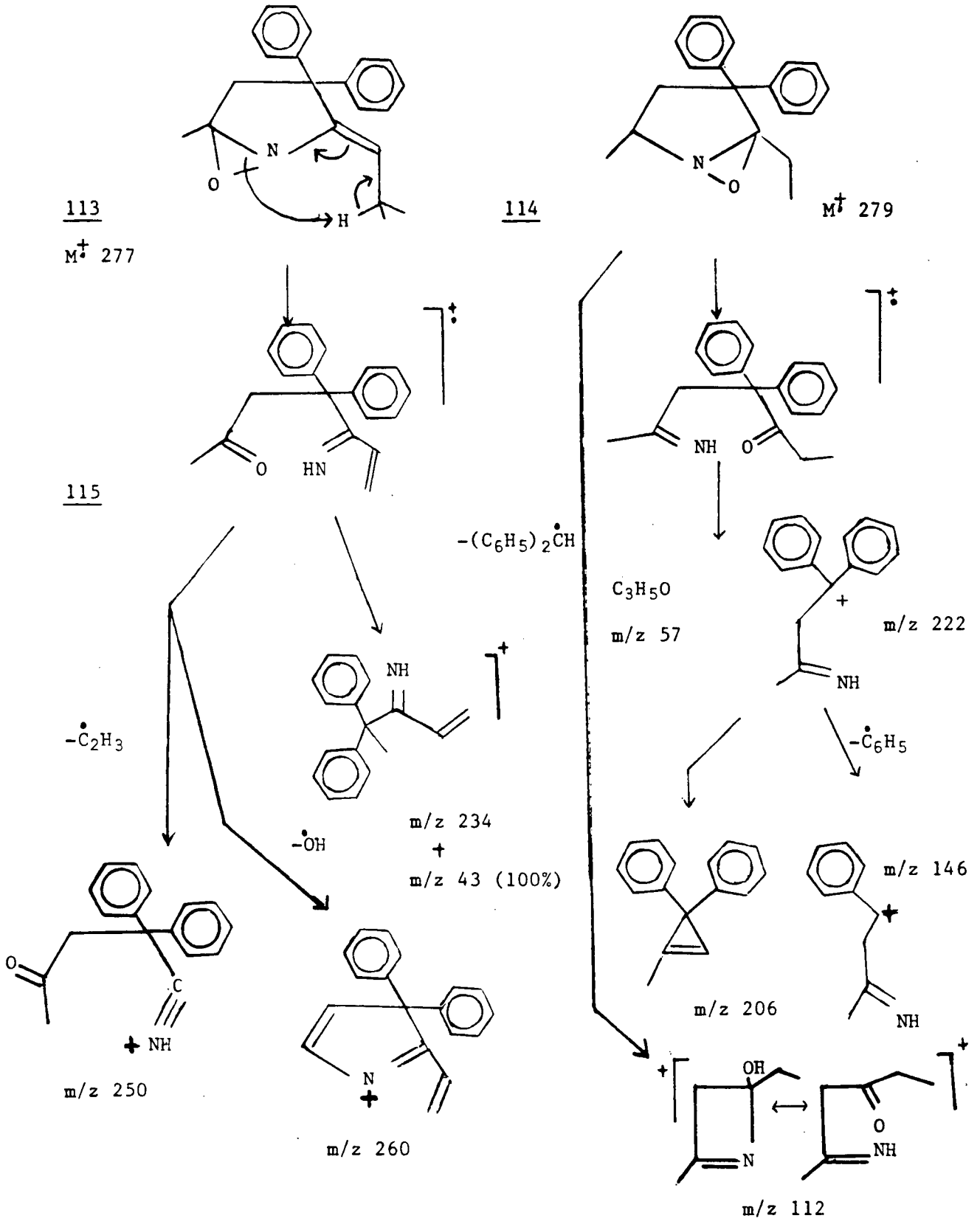


Figure 28. Thermal rearrangement and fragmentation of the EMDP oxaziridines (72) and (73) via the ketones (74) and (75)

2. METABOLISM

A. METHADONE METABOLISM

The GCMS experiments performed by Kang demonstrating the formamide in the conjugated fraction of bile from $^{2}H_{10}$- and $^{1}H_{10}$- methadone dosed rats were repeated. The spectra of the formamide metabolites observed at the correct retention time are shown in Figure 29a and b. The formamide was also formed to a minor extent in vitro as shown by the mass spectrum (Figure 29c) and the total ion current trace in Figure 30. Two other unidentified minor metabolites (including a compound resembling a methadone nonanone analogue non conjugated metabolite) as well as EDDP, EMDP and DDP were also observed in the in vitro experiments.

The in vitro metabolism of EDDP produced EMDP and DDP but no detectable formamide.

B. RECIPAVRIN METABOLISM

i) In Vitro Metabolism

Both nor- and dinor- recipavrin were present as in vitro metabolites but were not completely resolved by packed column GLC (Figure 31). Mass spectra of the partially resolved secondary amine metabolite (M^{+} m/z 239) and of the overlapping tertiary secondary and primary amine metabolites are included in the Appendix. The peaks at longer retention time than the parent drug are likely the phenol (76) and catechol (77) plus an unidentified compound (78). Derivatization and CIMS were not performed so these proposals are made solely on the basis of the m/z 72 base peak which indicates that the tertiary amino group is intact. Compounds (77) and (78) both had detectable m/z 269 ions, corresponding to the molecular ions of

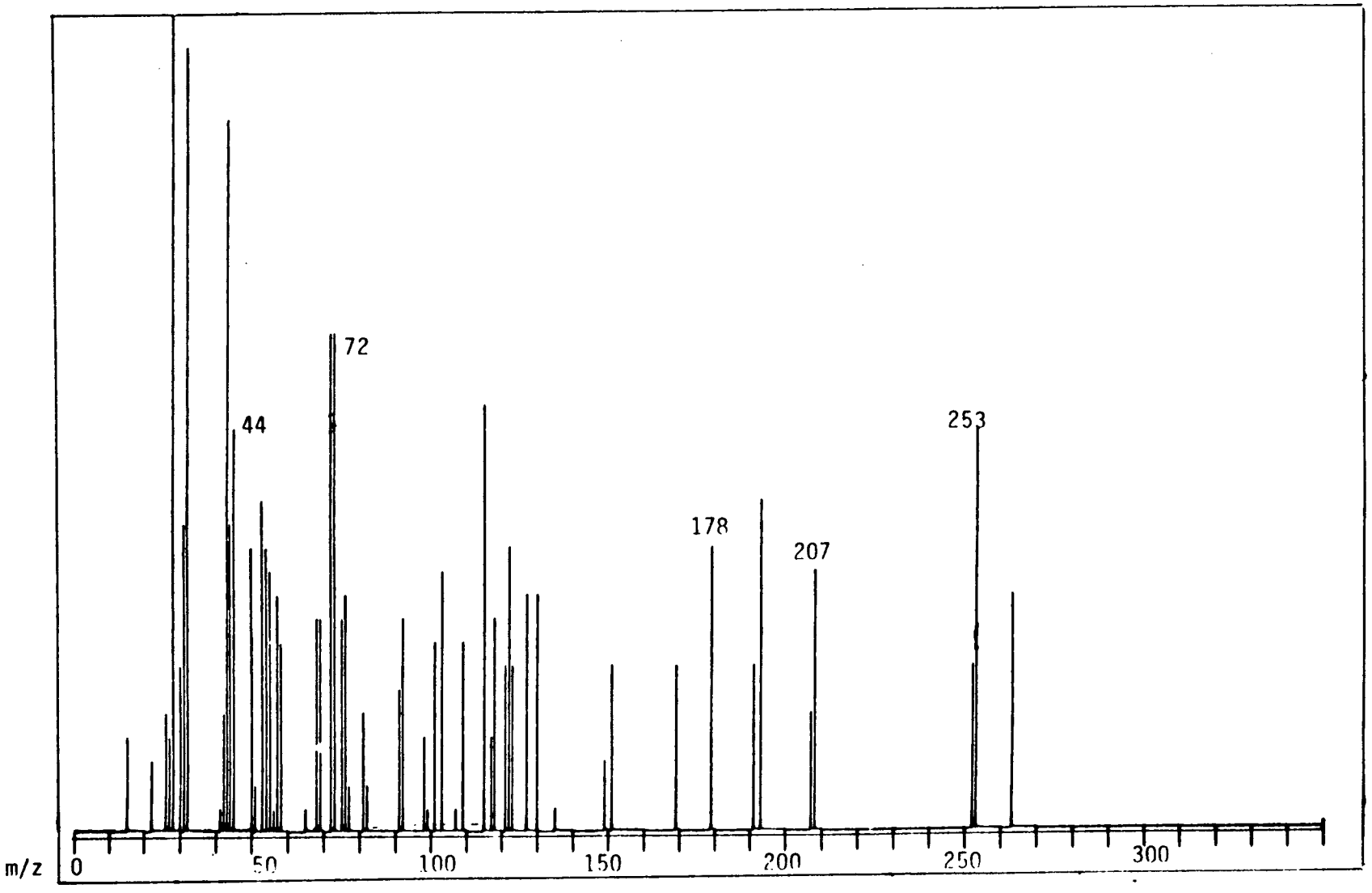


Figure 29(a) Mass spectrum (GCMS) of the methadone formamide metabolite in the conjugated fraction of bile from a methadone dosed rat

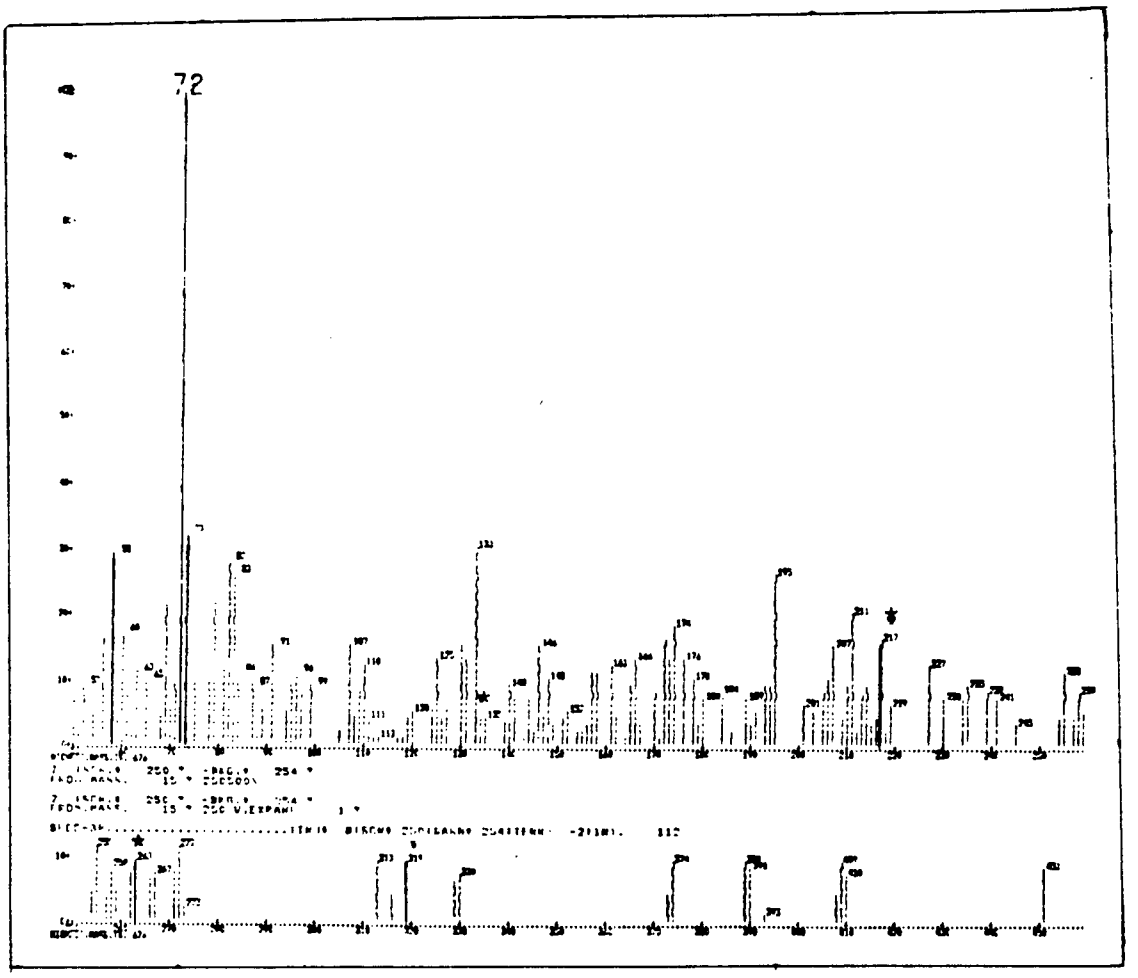


Figure 29 (b) Mass spectrum (GCMS) of the $^{2}H_{10}$ methadone formamide metabolite from the conjugated fraction of bile from a methadone dosed rat

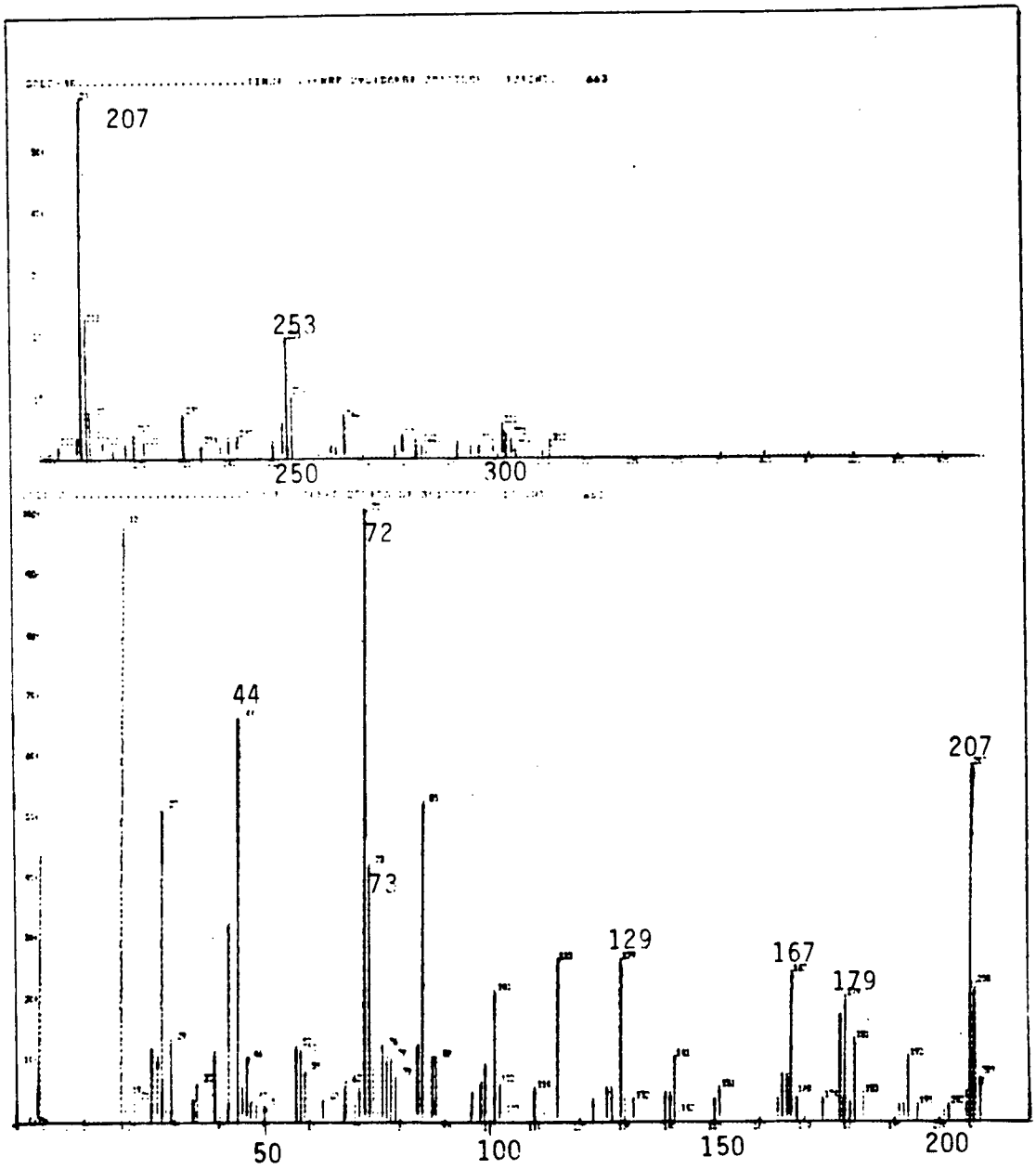


Figure 29 (c) Mass spectrum (GCMS) of the methadone formamide metabolite generated in vitro

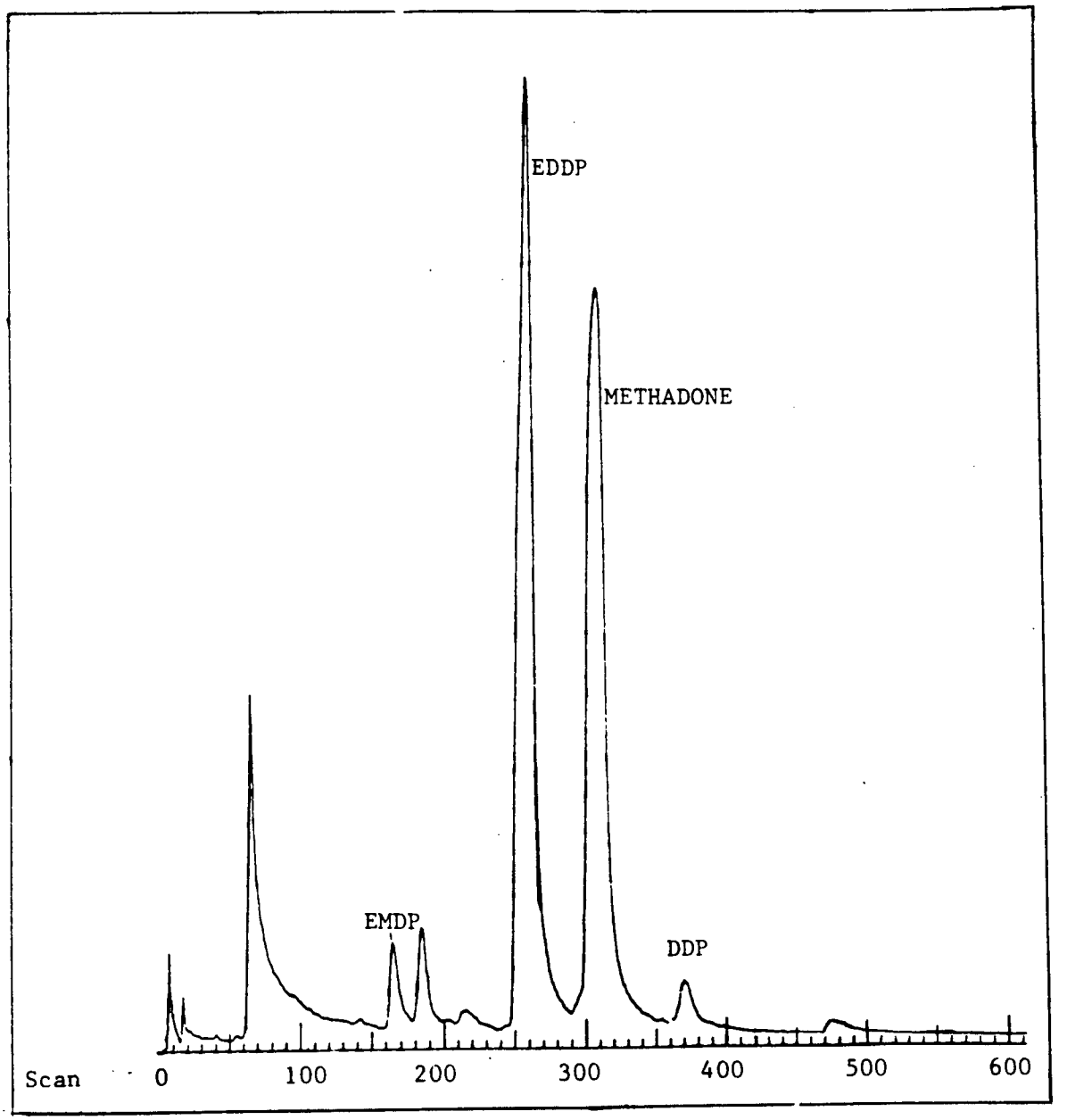


Figure 30. TIC of in vitro methadone metabolic extract
GC conditions(g)with 150° isothermal for 10 minutes
before temperature programing at 2°/minute to 280°

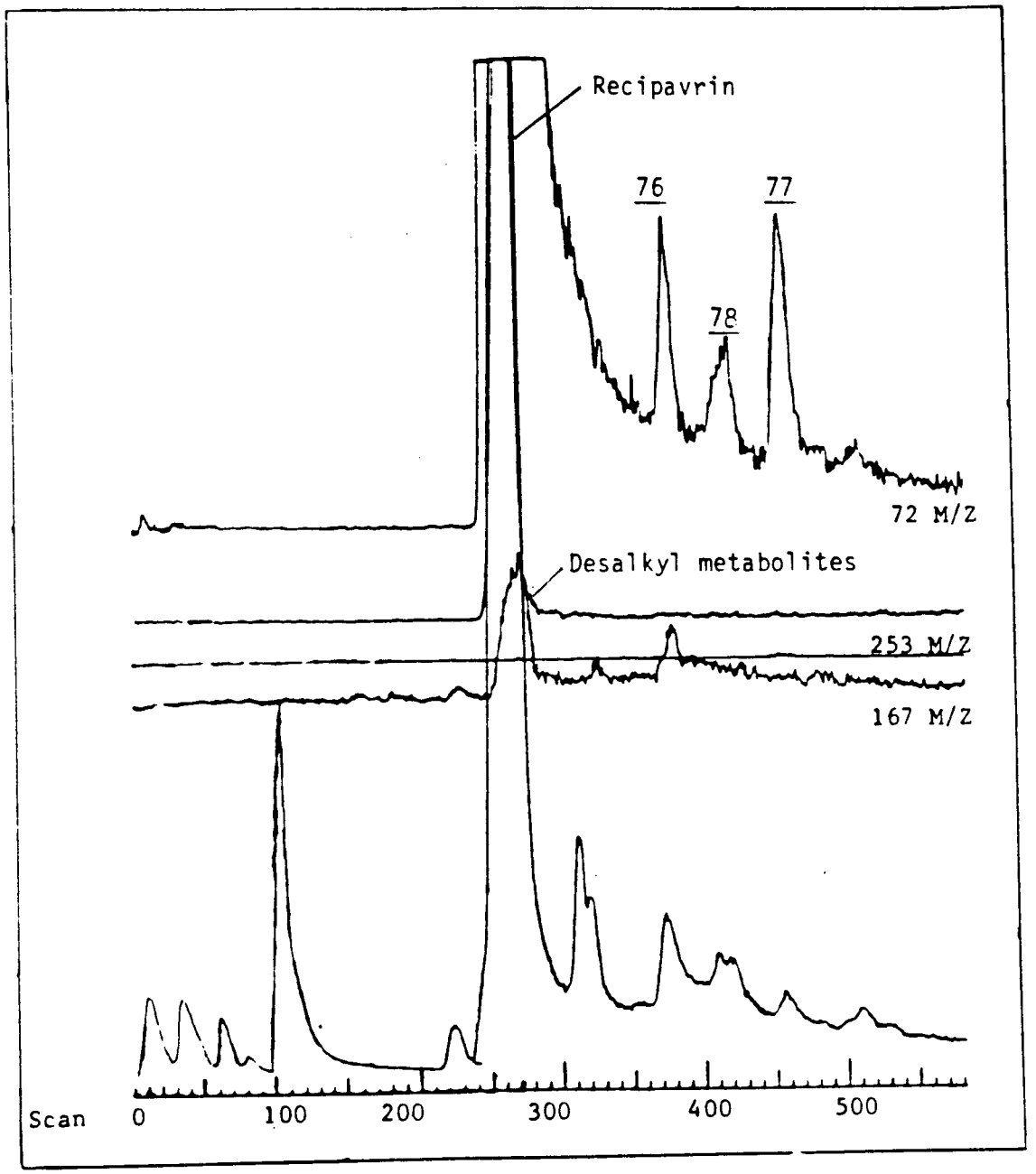
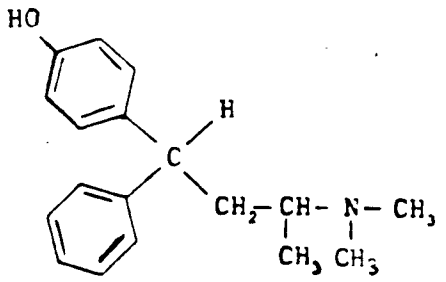


Figure 31. GCMS of an in vitro recipavrin metabolic extract showing metabolites (76) (77) (78)
GC conditions (g) with 150° isothermal for 10 minutes before temperature programing

phenolic metabolites (all metabolite mass spectra are included in the Appendix).



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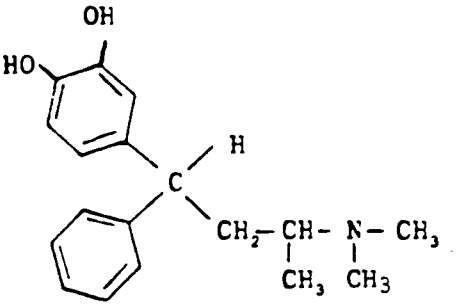

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ii) Recipavrin in vivo metabolism

a) Non conjugated metabolites

The mass chromatograms in Figure 32 demonstrate the presence of the desalkyl metabolites nor- and dinorrecipavrin as for the in vitro experiments. The desalkyl metabolites are not resolved by packed column GLC. No other metabolites were detected.

b) Recipavrin conjugated metabolites

1. Detection of the formamide metabolite

One of the riskier hypotheses of this thesis was that recipavrin would undergo metabolism to a metabolite analogous to that observed for methadone. Fortunately this turned out to be the case. The experiment was repeated with three separate rats and the formamide was present at the correct retention time by GCMS analysis of the conjugated fraction in all three cases (Figure 33a and b).

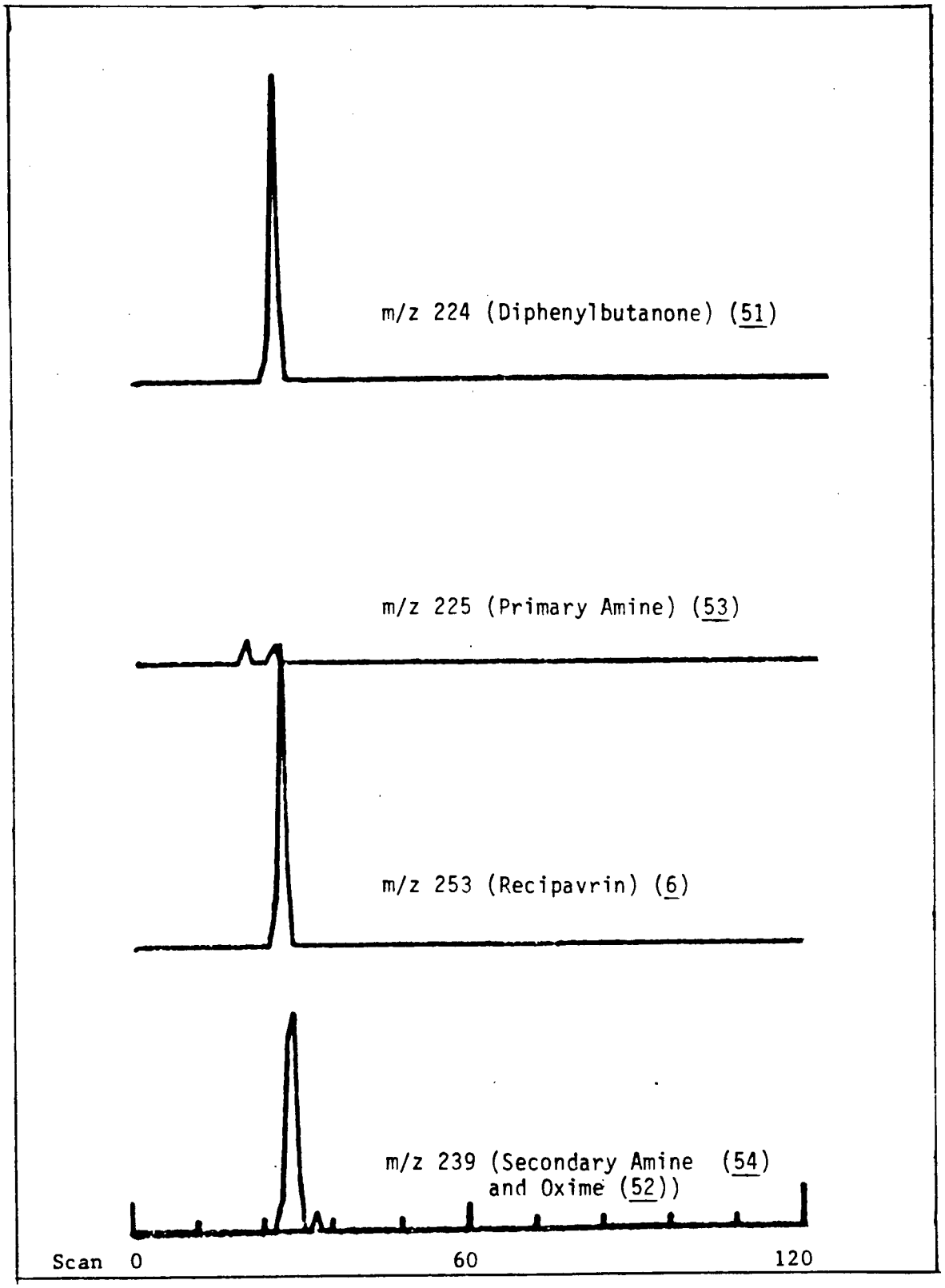


Figure 32. Mass chromatograms of ions present in the non conjugated fraction of bile from a recipavrin dosed rat, showing dealkyl and deaminated metabolites (GC conditions(d))

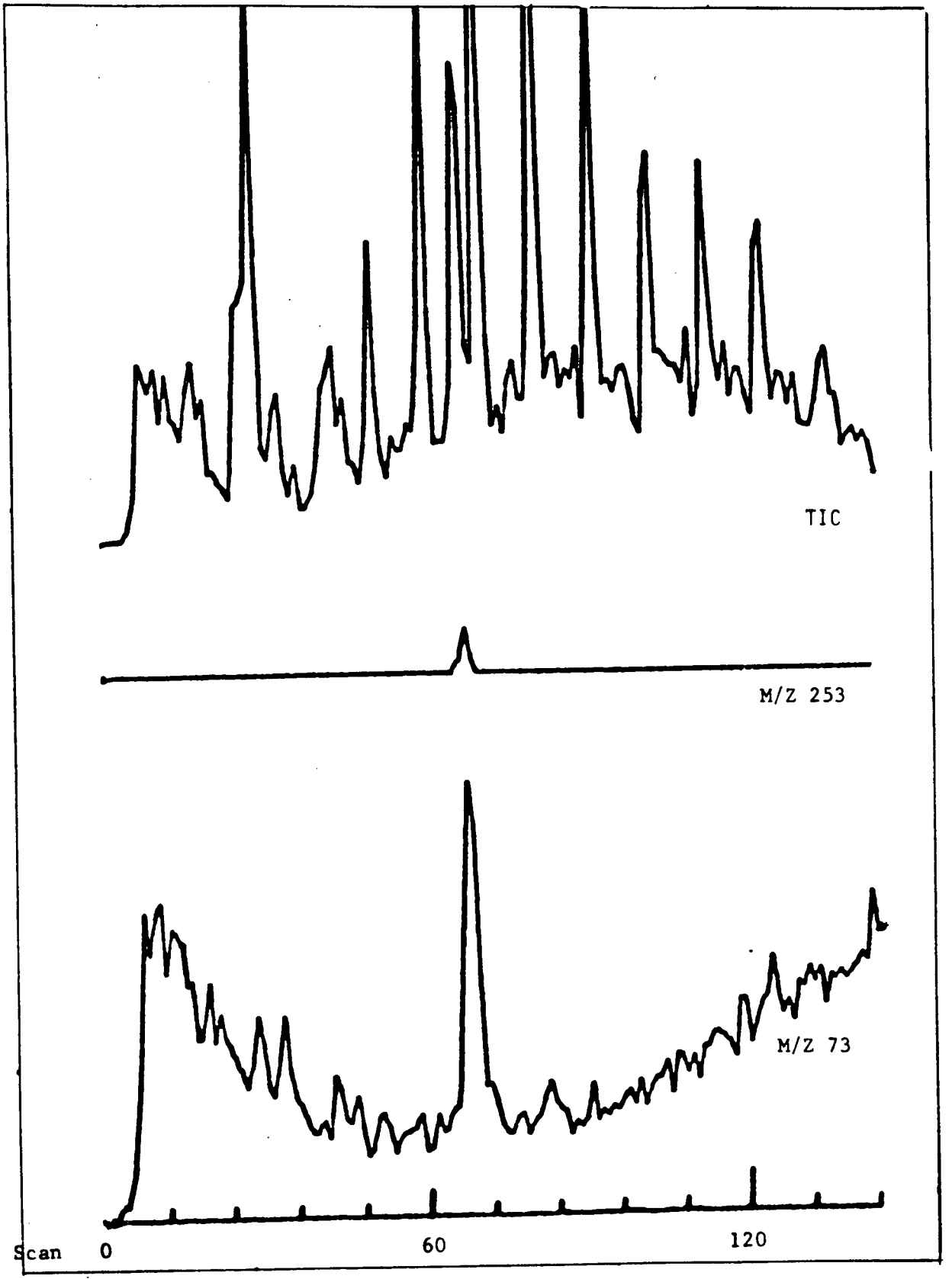


Figure 33(a) GCMS of the conjugated fraction of bile from a recipavrin dosed rat (GC conditions(d), injection delayed 15 seconds)

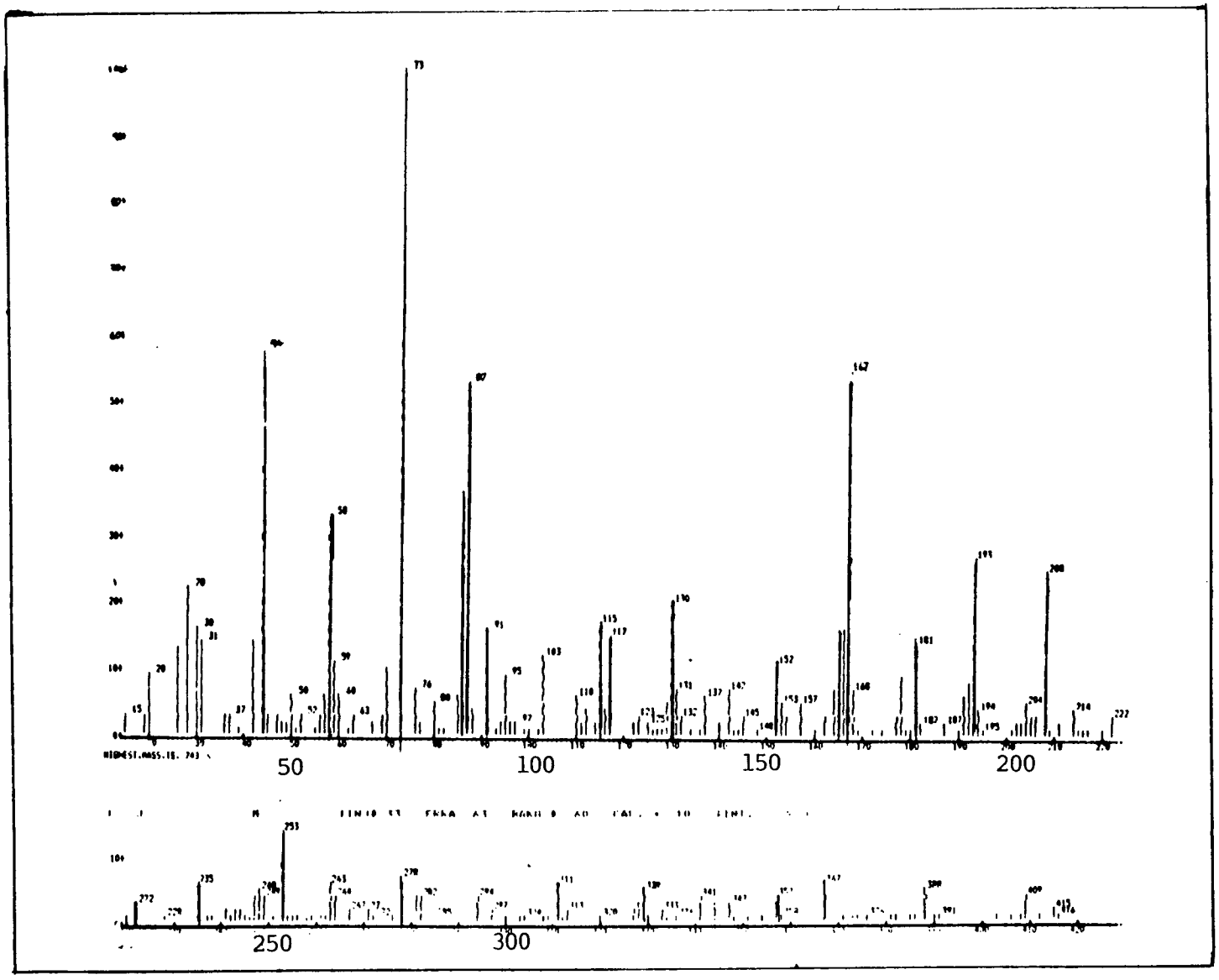


Figure 33(b) Mass spectrum (GCMS) of the recipavrin formamide metabolite in the conjugated fraction of bile from a recipavrin dosed rat

The synthetic samples have demonstrated the minor differences in the mass spectra of the methadone and recipavrin formamides. The long retention time and thermal conversion of the precursor oxaziridines and nitrones to the formamides make them relatively easy to detect even through present in very small quantities. The fact that recipavrin is metabolized to a compound similar to the metabolite observed by Kang supports the hypothesis that other diphenyl-2-aminobutane and diphenyl-2-aminoheptane related compounds, will undergo the same metabolic pathway.

LCMS experiments to deduce which thermolabile precursor was responsible for the formamide observed by GCMS were performed. LCMS of the synthetic nitrone, formamide and oxaziridine (Figure 34 a-c) revealed that a $M^{+}+1$ base peak at m/z 254 occurred in all three compounds with significant m/z 238 (20%) fragments only occurring in the mass spectra of the nitrone and oxaziridine. The nitrone was unique in that it also had a strong m/z 74 ion (75%). The LCMS mass chromatograms show that both the nitrone and oxaziridine decompose to the formamide on standing.

Unfortunately, the small amount of this metabolite in rat bile and the 2% split of sample entering the LCMS combined to put the metabolite below the level of detection. A selected ion monitoring capillary GC chemical ionization GCMS experiment on the conjugated metabolite fraction of recipavrin did indicate that it was not the nitrone. As shown in Figure (35a), monitoring the total ion current of six representative ions resulted in three peaks corresponding to the imine (59) oxime (52) and formamide (15), all decomposition products of GC analysis of the synthetic nitrone (13). When the same ions were monitored during the analysis of recipavrin

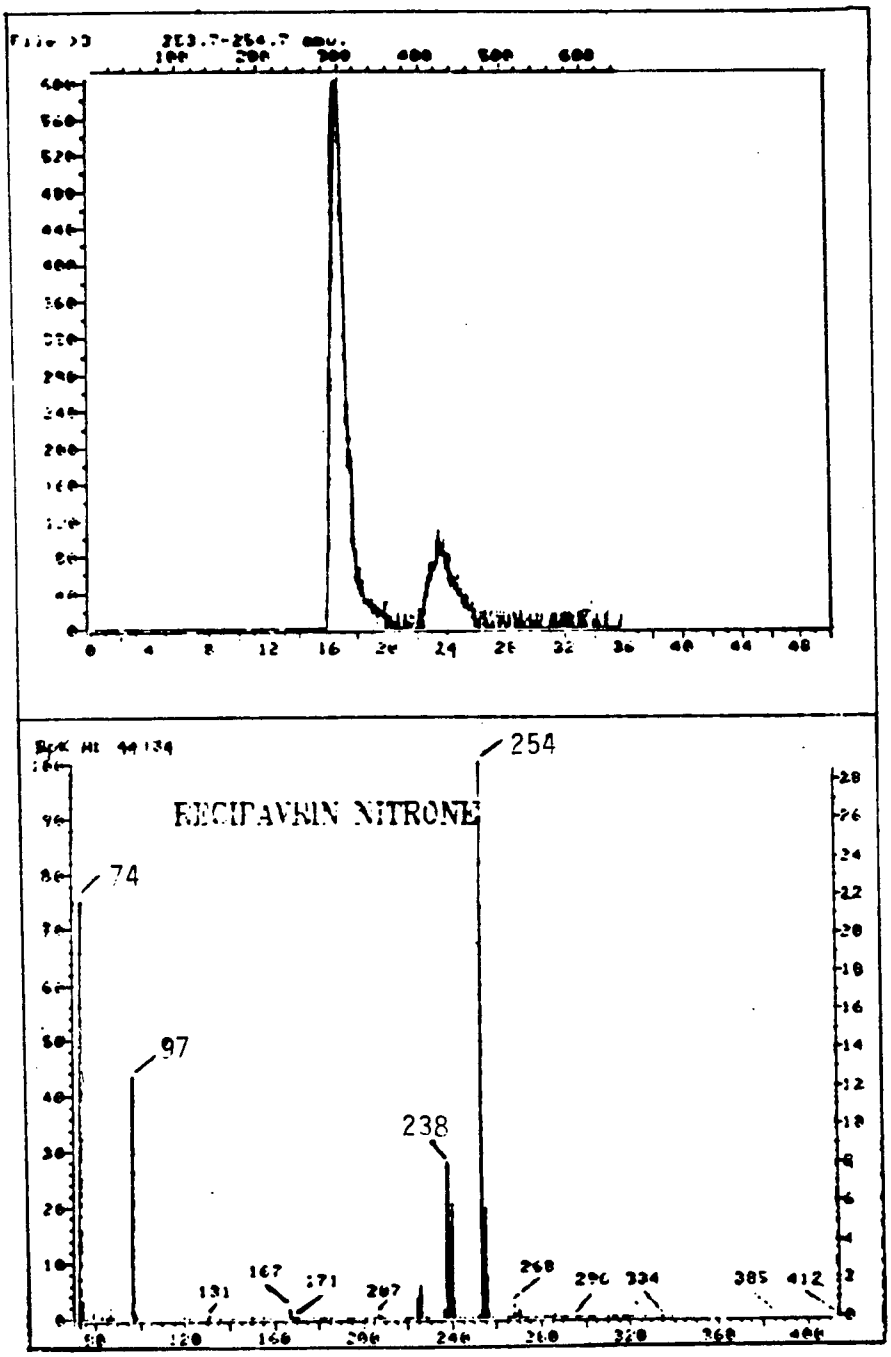


Figure 34(a) LCMS of nitrone (13) with some formamide present as a decomposition product

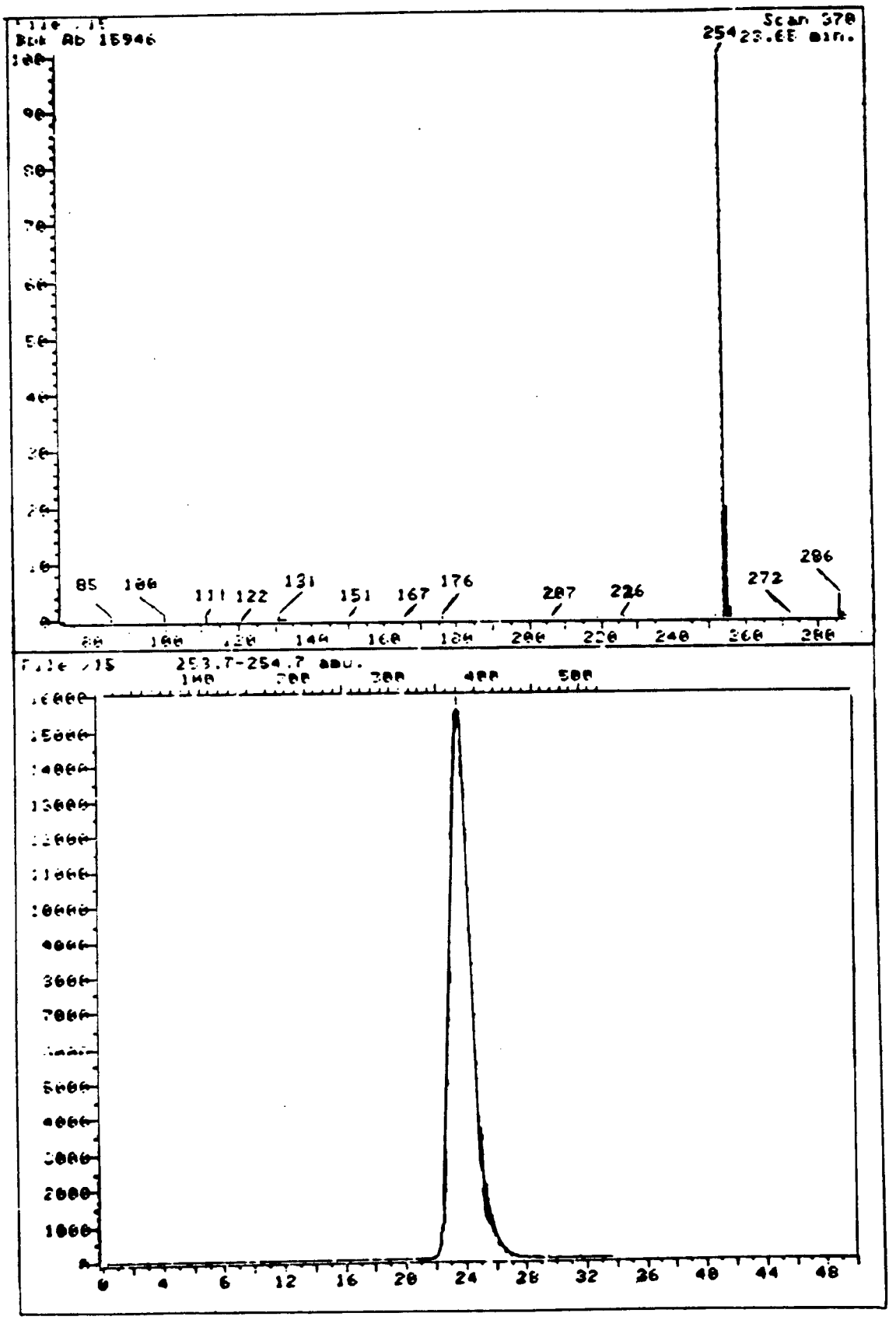


Figure 34. (b) LCMS of formamide (15))

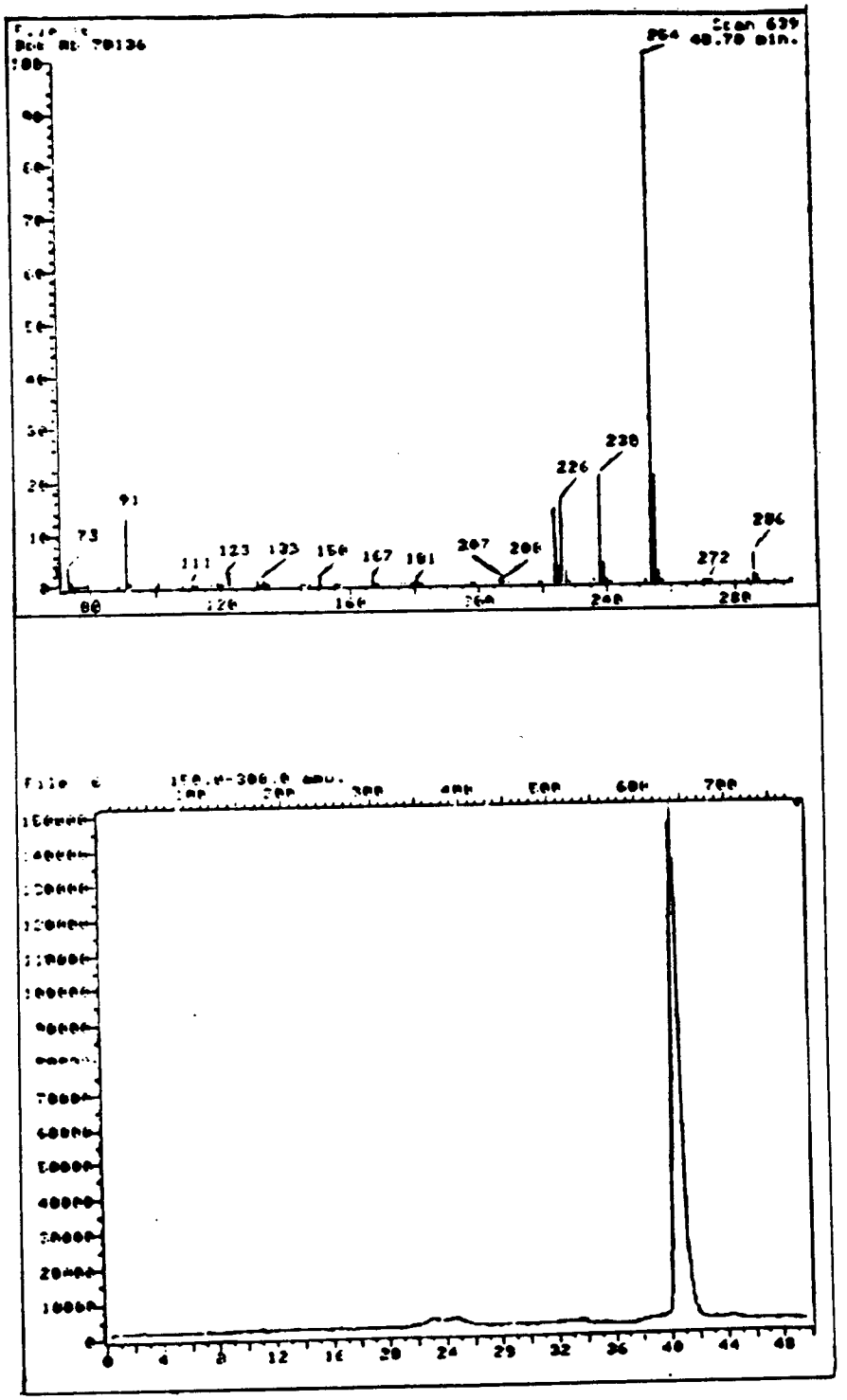
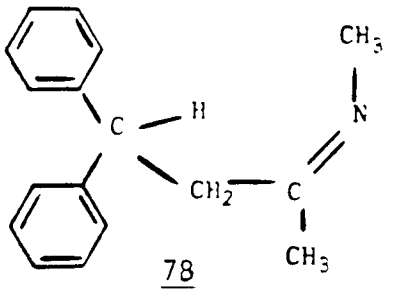


Figure 34. (c) LCMS of oxaziridine (14)

metabolites, the formamide and oxime peaks were present in roughly the right intensity, but the imine peak (formerly the largest) was virtually absent (Figure 35b). The imine (78) could account for the 7.4 minute peak in Figure 35b since the metabolite does not decompose to the same products as the synthetic methylene nitrone. The oxime (52) is either a metabolite of recipavrin or possibly a decomposition product of the endo nitrone (79). The inability to conclusively demonstrate the secondary hydroxylamine and the absence of the hydrolysis product, 1,1-diphenyl-3-butanone in the conjugated fraction does not favor the endo nitrone structure. Attempts to detect the tertiary amide (63) in the conjugated fraction were unsuccessful.



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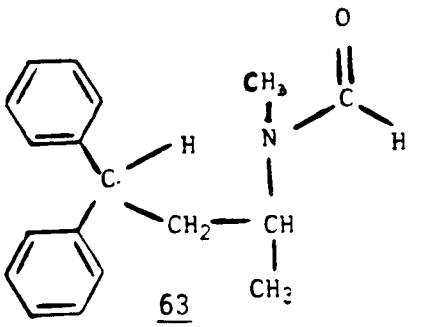

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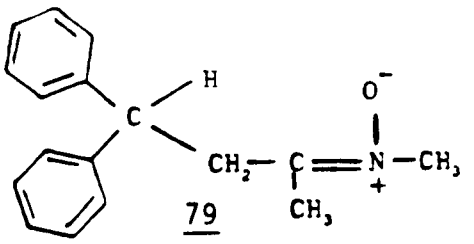

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The recipavrin metabolites from the conjugated bile fraction were derivatized with BSTFA and observed by GCMS as shown in Figure 36.

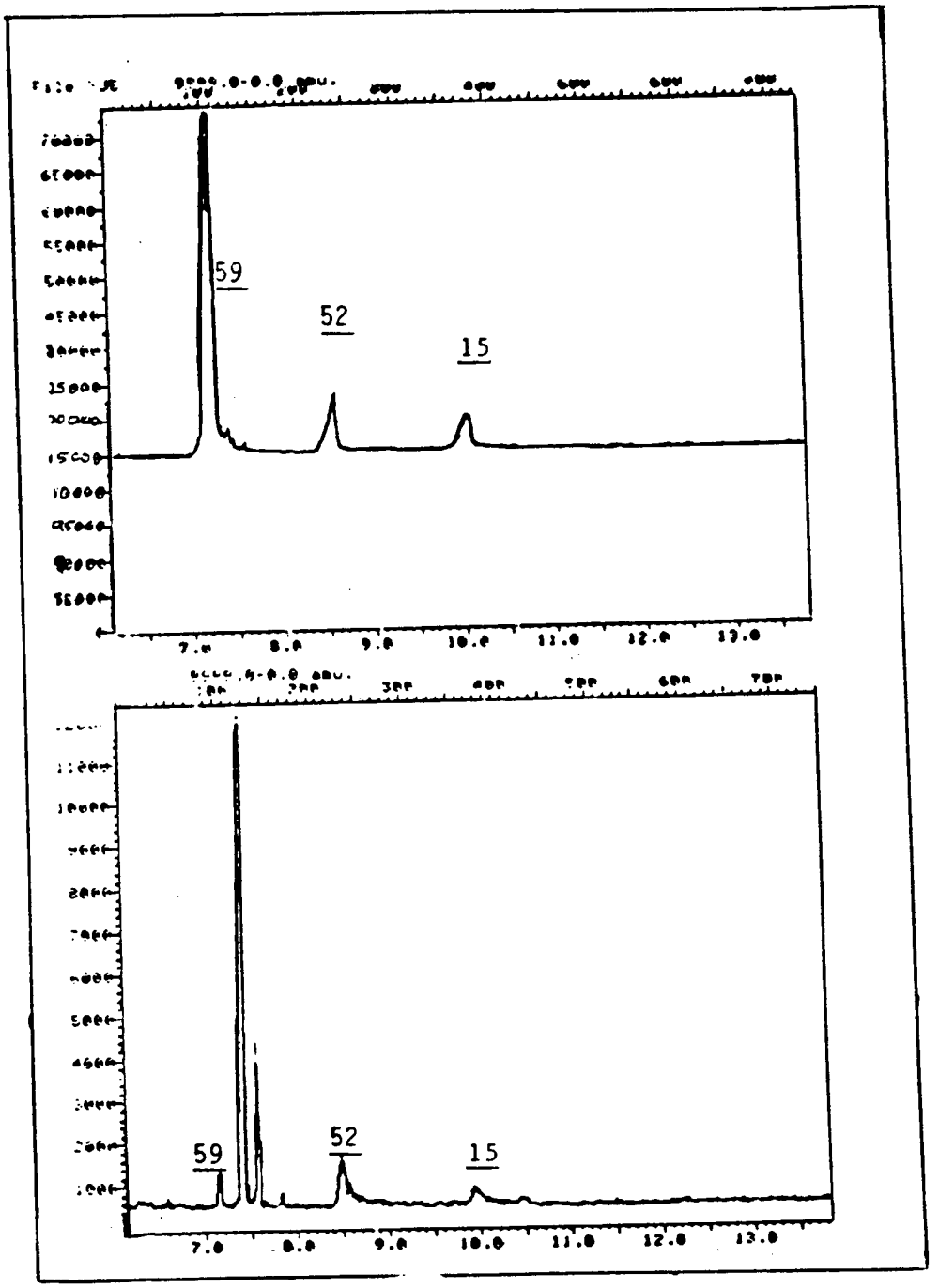


Figure 35(a) Capillary CI GCMS SIM of the recipavrin nitrone showing thermal breakdown products A (imine (59)), B (oxime (52)) and C (formamide (15)) (GC conditions fi)

(b) SIM of the same ions to detect recipavrin metabolites from the conjugated fraction of rat bile

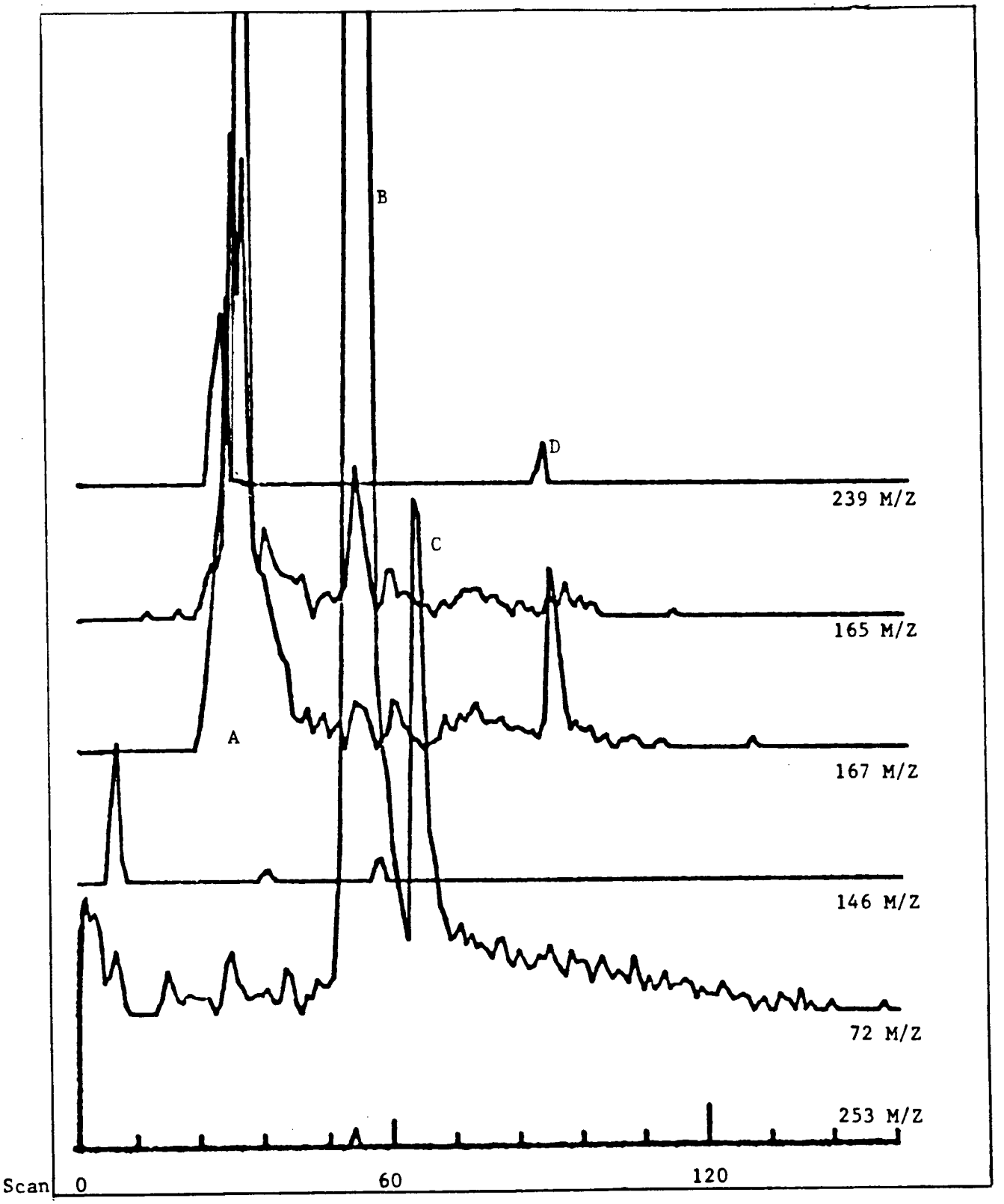


Figure 36. Mass chromatograms showing metabolites in the TMS derivatized conjugated fraction of bile from a recipavrin dosed rat (GC conditions e)

Metabolite A is likely a phenolic secondary amine, while B and C are the phenolic and catechol metabolites of recipavrin with base peaks at m/z 72.

2) Hydroxylamines as precursors of the observed recipavrin metabolite

Beckett has observed that secondary hydroxylamines are converted to nitrones in alkaline solution (54). To determine whether oxidation of the secondary hydroxylamine (57) during extraction from alkaline solution could account for the observed metabolite, a sample of blank rat bile was extracted and lyophilized by the normal procedure. After reconstitution with water, 10 mg of secondary hydroxylamine (57) (proven formamide and nitrone free by GCMS) was added prior to basification and extraction. GCMS of the extract revealed the formamide (m/z 73,253) at the correct retention time. Oxidation to the nitrone had occurred to a minor extent with the bulk of the underivatized hydroxylamine chromatographing as the secondary amine (54) (Figure 37, m/z 239).

The implication is that detectable amounts of precursor hydroxylamine should be identifiable in the rat bile extract. In the case of methadone, GCMS analysis of a methadone hydroxylamine should primarily give rise to EDDP or less probably a secondary amine (base peak m/z 58 with an intense 57 for the carbonyl fragment) neither of which were detected in the conjugated fraction.

Derivatization of recipavrin metabolites from the conjugated fraction with BSTFA did produce a compound with a m/z 146 peak (characteristic of a TMS secondary hydroxylamine) that was not observed in blank samples. However, the retention time was different by a small but significant amount in capillary and packed column GCMS, as shown in Figure 38. The experiment was performed by ion monitoring, thus no mass spectrum was available to compare to the synthetic sample.

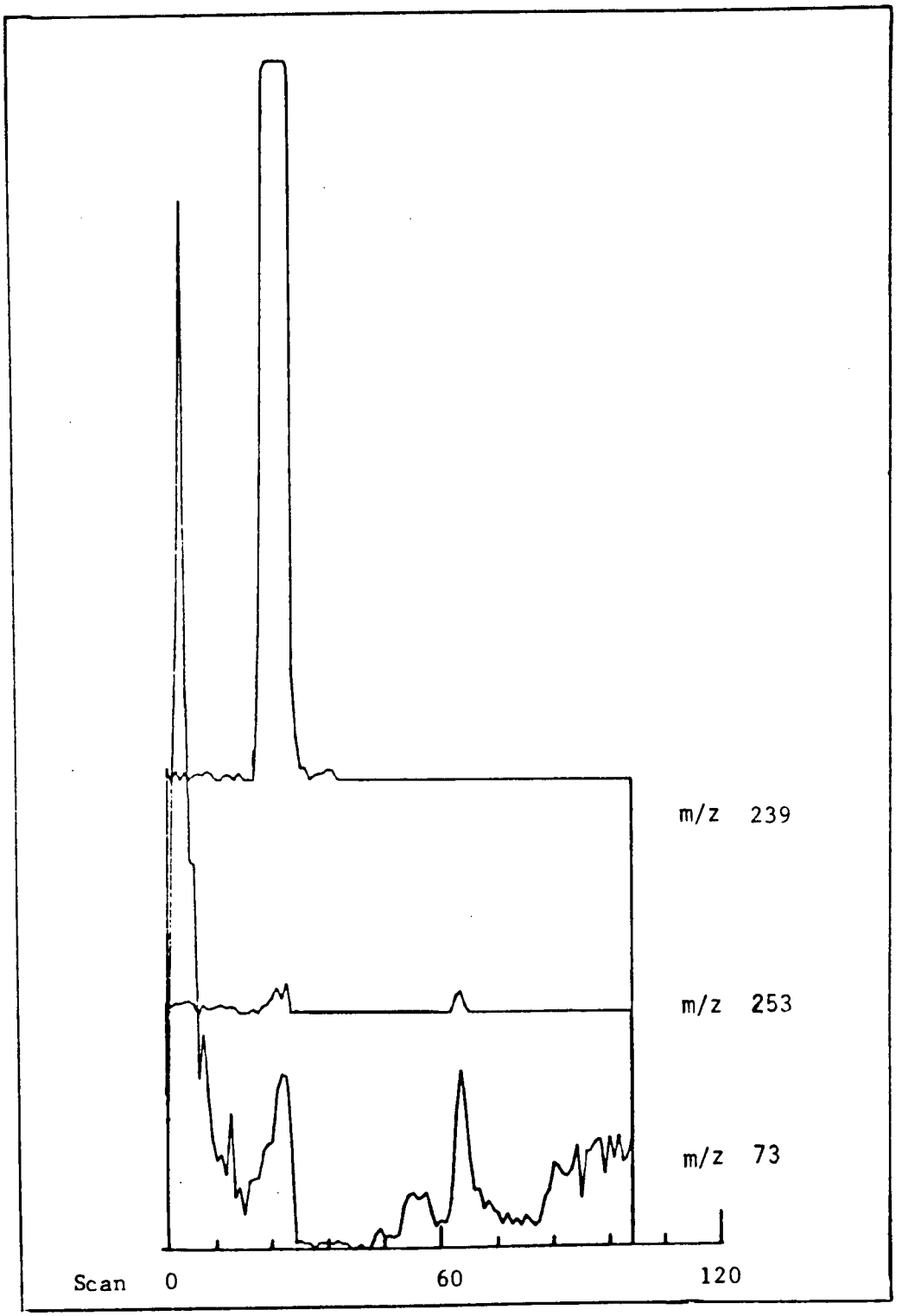


Figure 37. GCMS of the conjugated fraction of blank rat bile spiked with the secondary hydroxylamine (57). Formamide present at scan (63) (GC conditions (d))

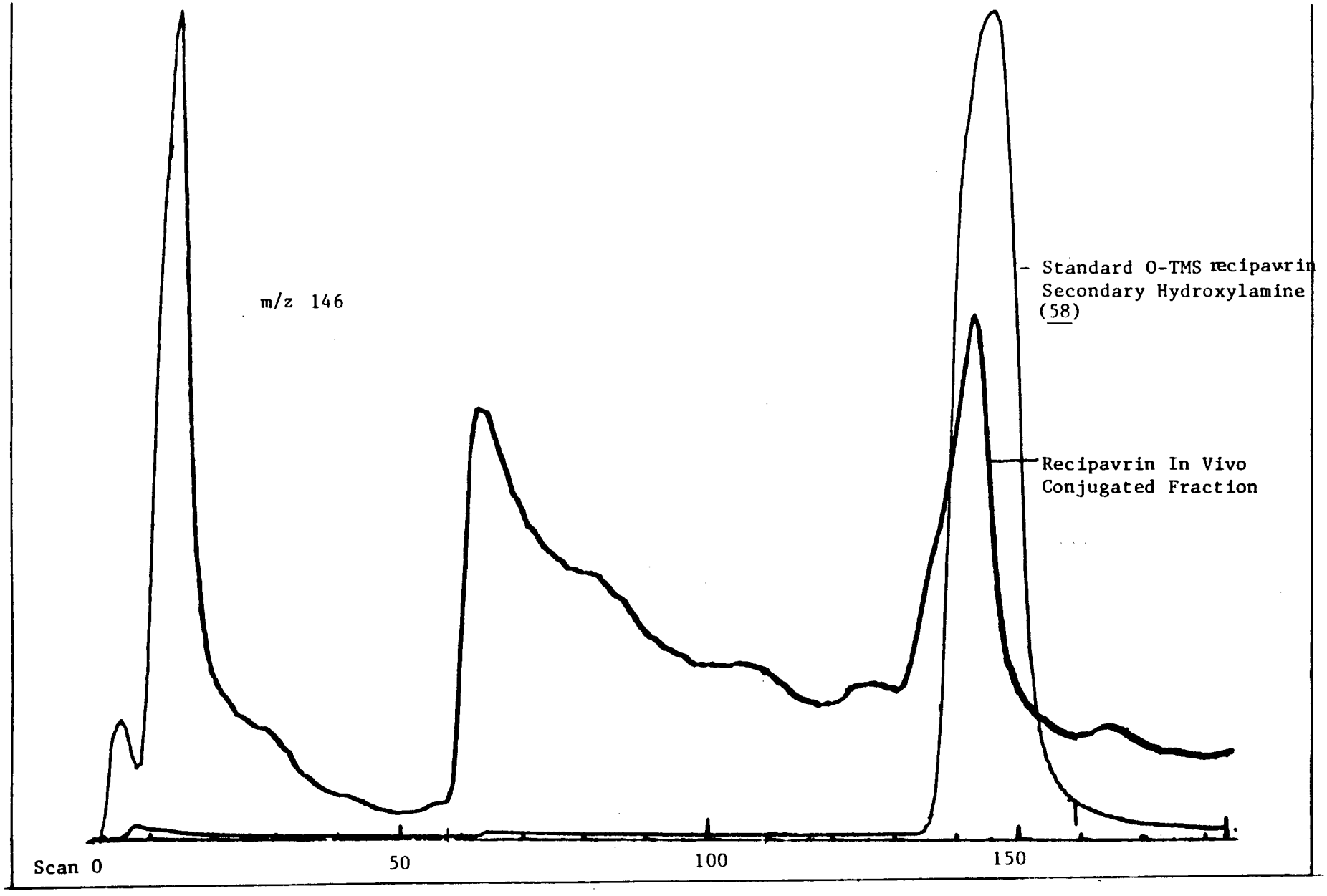


Figure 38. SIM of the m/z 146 peak of the recipavrin O-TMS secondary hydroxylamine (58) and the BSTFA derivatized recipavrin metabolites from the conjugated bile fraction, showing slightly different retention time (GC conditions(d), onescan/second)

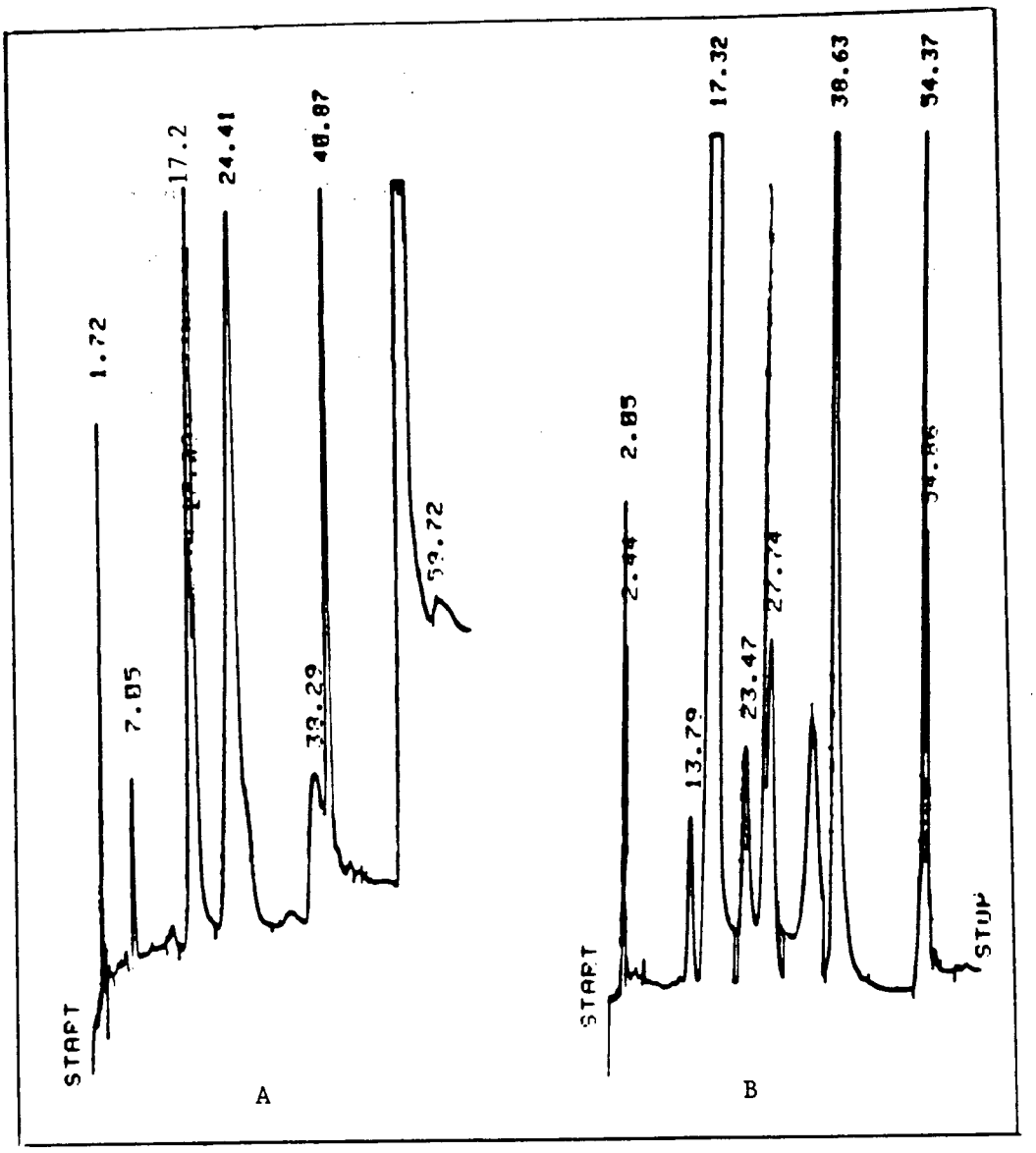


Figure 39(a) HPLC (UV detection) of a mixture of recipavrin nitrone (13) (17.2 min), formamide (15) (24.41 min) and oxaziridine (14) (40.87 min)

Figure 39(b) HPLC of secondary hydroxylamine (57) after treatment with alkali, showing nitrone (17.32 min) as a major product, with unreacted secondary hydroxylamine at 54.37 min (attenuated peak)

To ensure that the product of alkaline oxidation was the nitrone and not the formamide or oxaziridine, the secondary hydroxylamine was treated for one week with ethanolic NaOH, and then compared with HPLC reference standards of the recipavrin nitrone, formamide and oxazirdine. The chromatograms shown in Figure (39) indicate that the nitrone (t_R, 17.32 minutes) is the major oxidation product, in accord the results of amphetamine hydroxylamine oxidation performed by Beckett and Belanger (54).

C. NORRECIPAVIN METABOLISM

i) Conjugated metabolites

This experiment was performed to see whether the secondary amine was also a source of the formamide metabolite. Rats dosed with norrecipavrin also produced the formamide metabolite in the conjugated fraction, (t_R 11.4 minutes, GC conditions (fii)) as shown by the mass chromatogram and mass spectrum in Figure 40a, b: Compounds resembling a conjugated primary amine (80), and secondary amine (81) phenolic metabolites, the 2° amine catechol (82), a short retention time oxime like metabolite and two other unidentified compounds were also present. As in Noda's experiments (43), the formamide metabolite of the secondary amine was present only at the limit of detection. This result may be due to an error in the sample workup (the pH was incorrect during hydrolysis with β-glucuronidase) and the experiment should be repeated.

An alternative explanation for the decreased amount of formamide observed may be the rapid dealkylation of the secondary amine to dinorrecipavrin, or a concerted mechanism for the metabolism of the tertiary amine to the formamide.

The primary amine (53) was not administered to rats. The observations

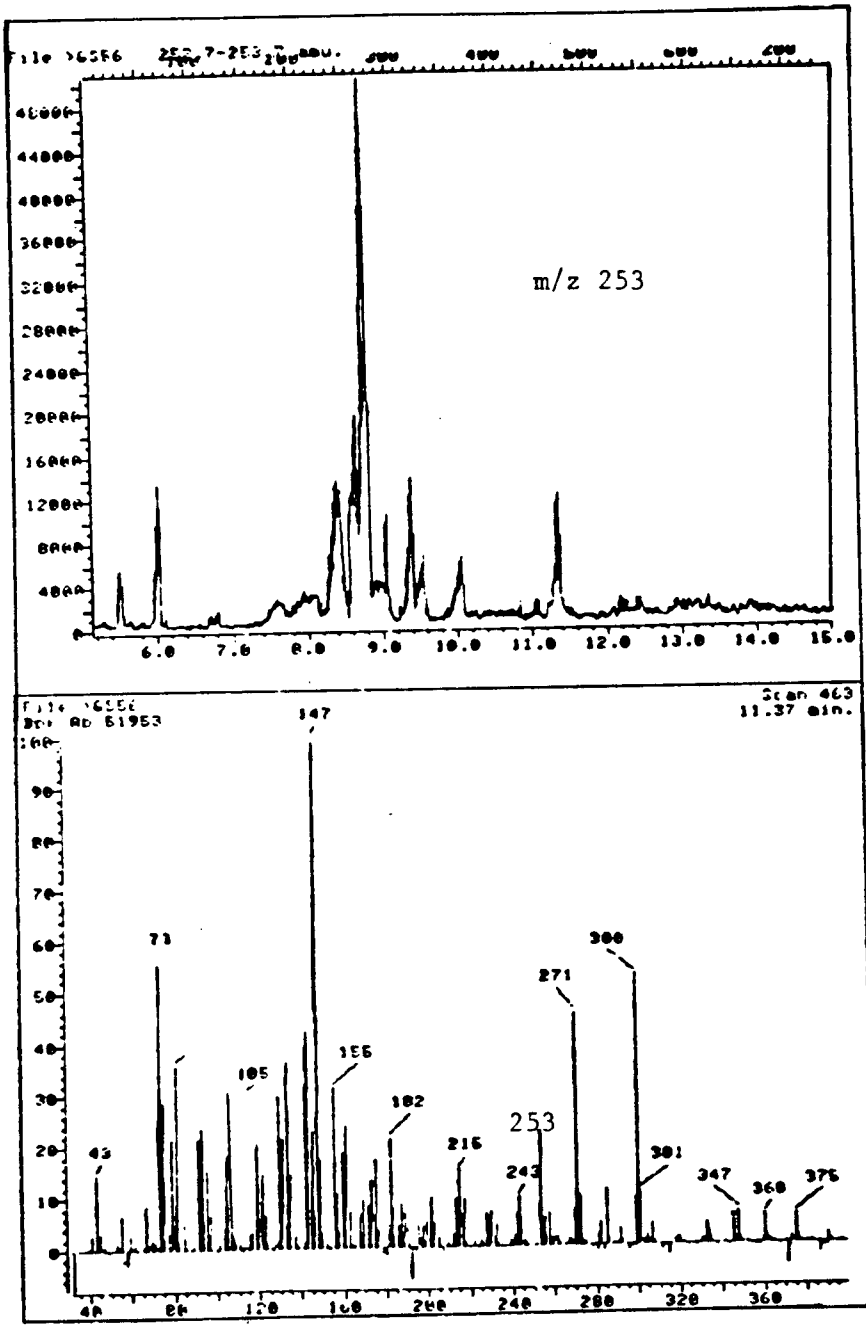


Figure 40(a) m/z 253 mass chromatogram and mass spectrum of the formamide metabolite from the conjugated fracton of bile from a norrecipavrin dosed rat (GC conditions fii)

of Noda et al (43) suggest that a formyl conjugation pathway is not a source of the formyl metabolites of aminopyrine. However, formyl metabolites are known to arise as artifacts during alkaline workup by reaction with solvent contaminants. Stilwell et al (52) have shown that a formyl metabolite of pethidine, arises from condensation of chloroform contaminants with norpethidine via a dichlorocarbene intermediate (Figure 40). Oxidation of the methyl group of pethidine was ruled out following the administration of $^{13}CH_3$-N and $^{2}H_3C$-N pethidine when the formyl metabolite did not retain the label. Oddly enough, toluene, benzene and ethyl acetate extraction resulted in lower but still detectable amounts of the same metabolite. The conjugated metabolite of methadone would have to hydrolyze to a primary amine for this pathway to be relevant. In our case the primary amine dinormethadone would cyclize spontaneously to EMDP in alkaline solution.

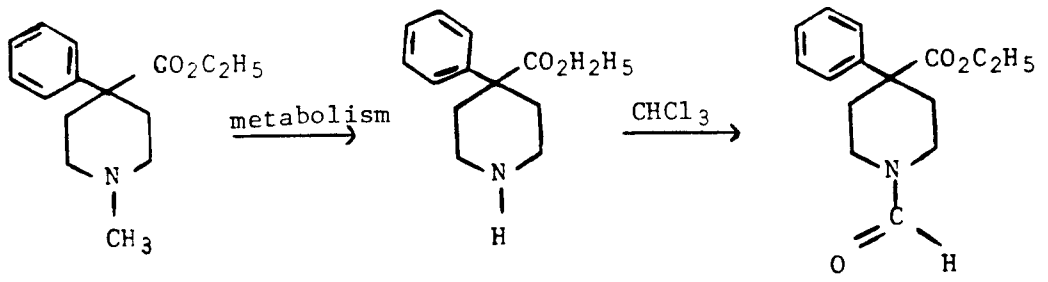


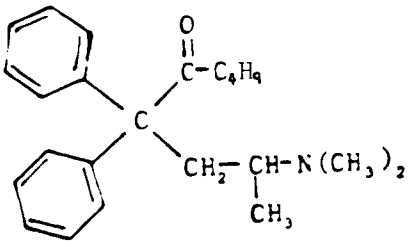
Figure 40. Pethidine, its metabolite norpethidine and a N-formyl compound which arises during workup by reaction with chloroform contaminants.

E. METABOLISM OF THE METHADONE ANALOGUE

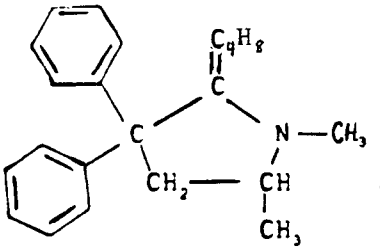
The nonanone analogue of methadone (86) was available in our lab, so attempts to detect the corresponding formamide metabolite in rat bile and in vitro were undertaken, but were not conclusive.

i) Non Conjugated Fraction

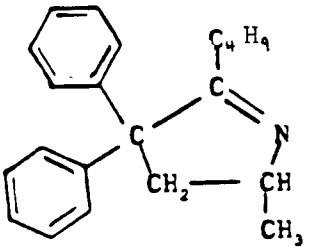
The major biliary metabolite, as in methadone, arises from N-demethylation and cyclization of the nor compound to 2-butylidene-1,5--3,3-dimethyldiphenyl pyrollidine (BDDP) (87) (peak C Figure 41), which is chemically or metabolically converted to DDP (12) and BMDP (88) (peak A) BMDP had an identical mass spectrum but different t_R than EMDP (11).



86



87



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BDDP has a strong molecular ion (m/z 305) (20%) and fragments via the m/z 275 pathway outlined in Figure 42a.

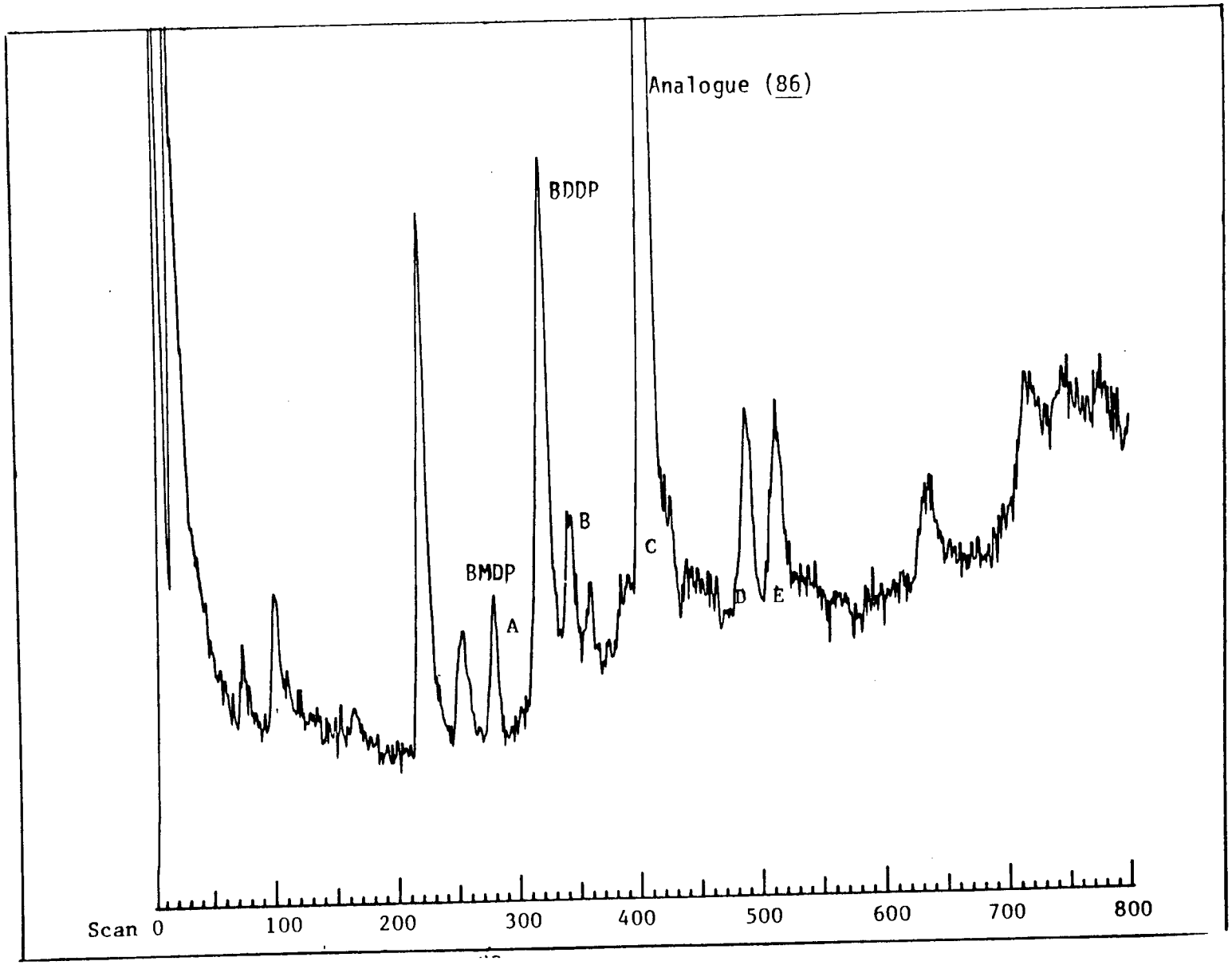


Figure 41. TIC of methadone analogue (86) non conjugated biliary metabolites (GC conditions g). A. BMDP (88), B. unknown M^+ 315, m/z 291, 262. C. BDDP (87) D. Unknown E. Unknown M^+ 315 (2%) m/z 72 (100).

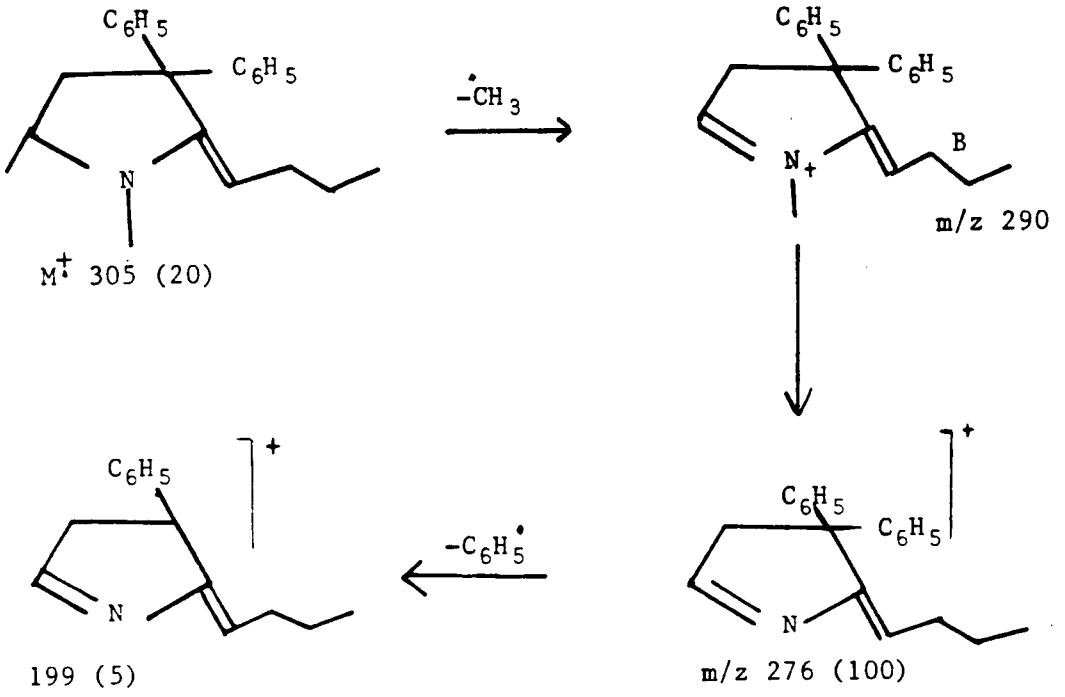


Figure 42a. Fragmentation of BDDP.

A tertiary amine metabolite had a mass spectrum and t_R identical to recipavrin (peak A Figure 41) but masses above m/z 73 are of low intensity and therefore unreliable. Two long retention time compounds, a tertiary amine (D) and a secondary amine (E) (m/z 72 and m/z 58 base peaks respectively) are possibly the result of metabolic reduction of the keto group to the corresponding alcohol since they have not cyclized. A novel metabolite (B) (similar to another observed in the in vitro metabolism of methadone) appears to fragment via a cyclopentenone pathway similar to that observed for (89).

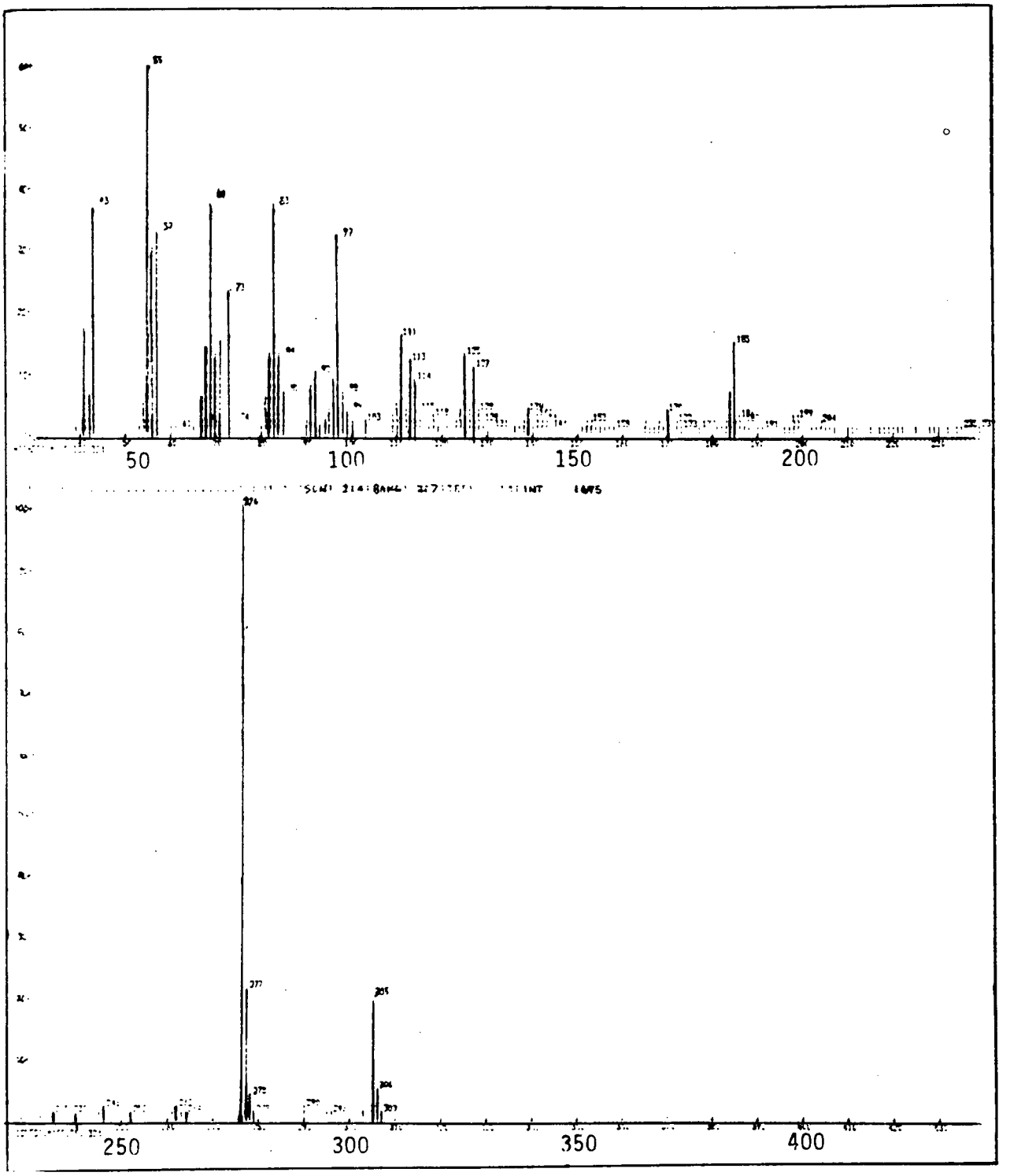
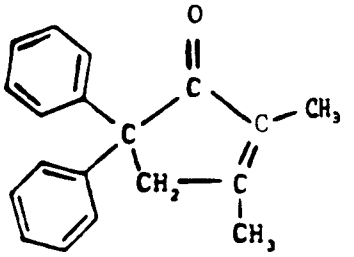


Figure 42(b) Mass spectrum (GCMS) of methadone analogue metabolite butylidene dimethyldiphenyl pyrollidine (BDDP) (87)



89

ii) Conjugated metabolites

In the conjugated bile fraction one compound at long retention time (peak E Figure 43) did have some mass ion fragments characteristic of the formamides of methadone and recipavrin ((ie) m/z 73, 57, 129, 207). The retention time was shorter than expected with the compound present in small quantities and no synthetic standards were available for comparison. Oddly enough, the other observed metabolites were not derived from BDDP since the mass spectra indicate intact tertiary and secondary amino groups, with few other intense peaks. The observed tertiary amine and secondary amines could be the phenolic metabolites.

iii) In vitro metabolism

The major metabolite in vitro was BDDP (peak A Figure 44a). Other observed compounds were DDP (peak C) BMDP and the N-oxide, which by GCMS appeared as the Cope elimination product, 4,4-diphenyl-5-non-2-enone at very short retention time (Figure 44b). No formamide type compounds were detected.

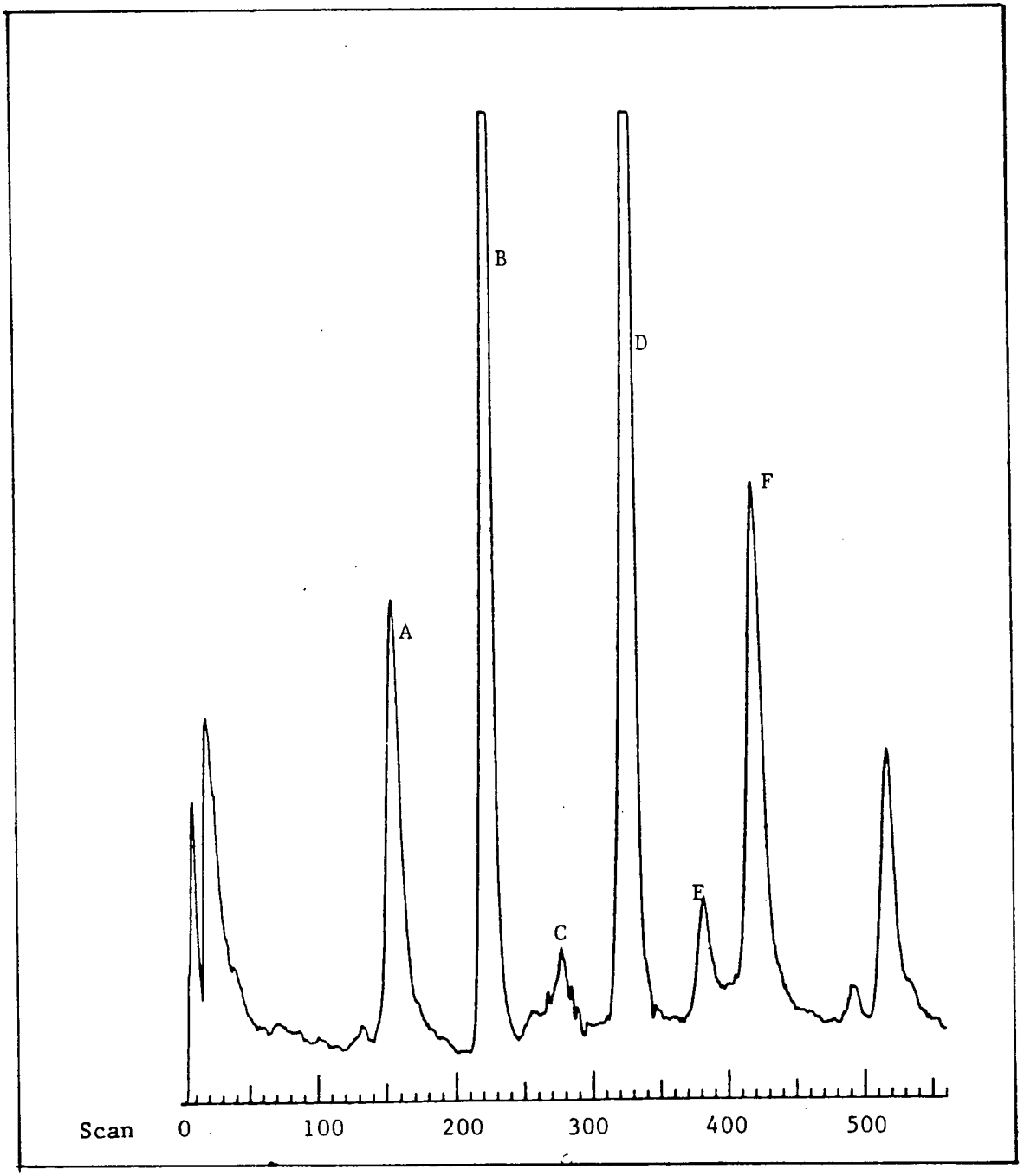


Figure 43(a) TIC of methadone analogue (86) conjugated biliary metabolites (GC conditions g) A. m/z 72 (100%) recipavrin like compound. B. 72 m/z (100%). C. Unidentified D. 72 m/z (100%). E. m/z 73, 129 compound. F. Secondary amine like (m/z 58 (100%)).

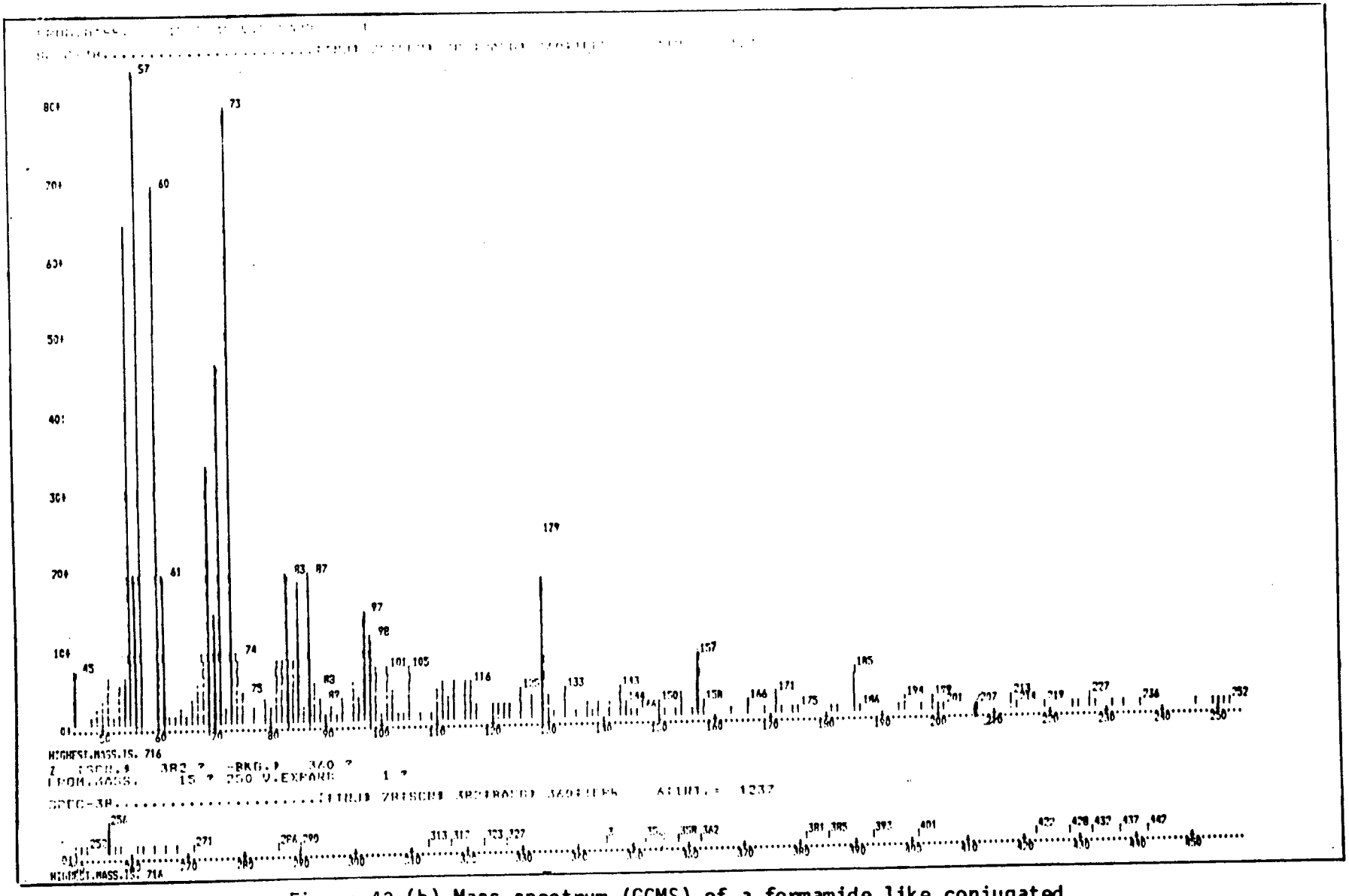


Figure 43 (b) Mass spectrum (GCMS) of a formamide like conjugated methadone analogue metabolite (E)

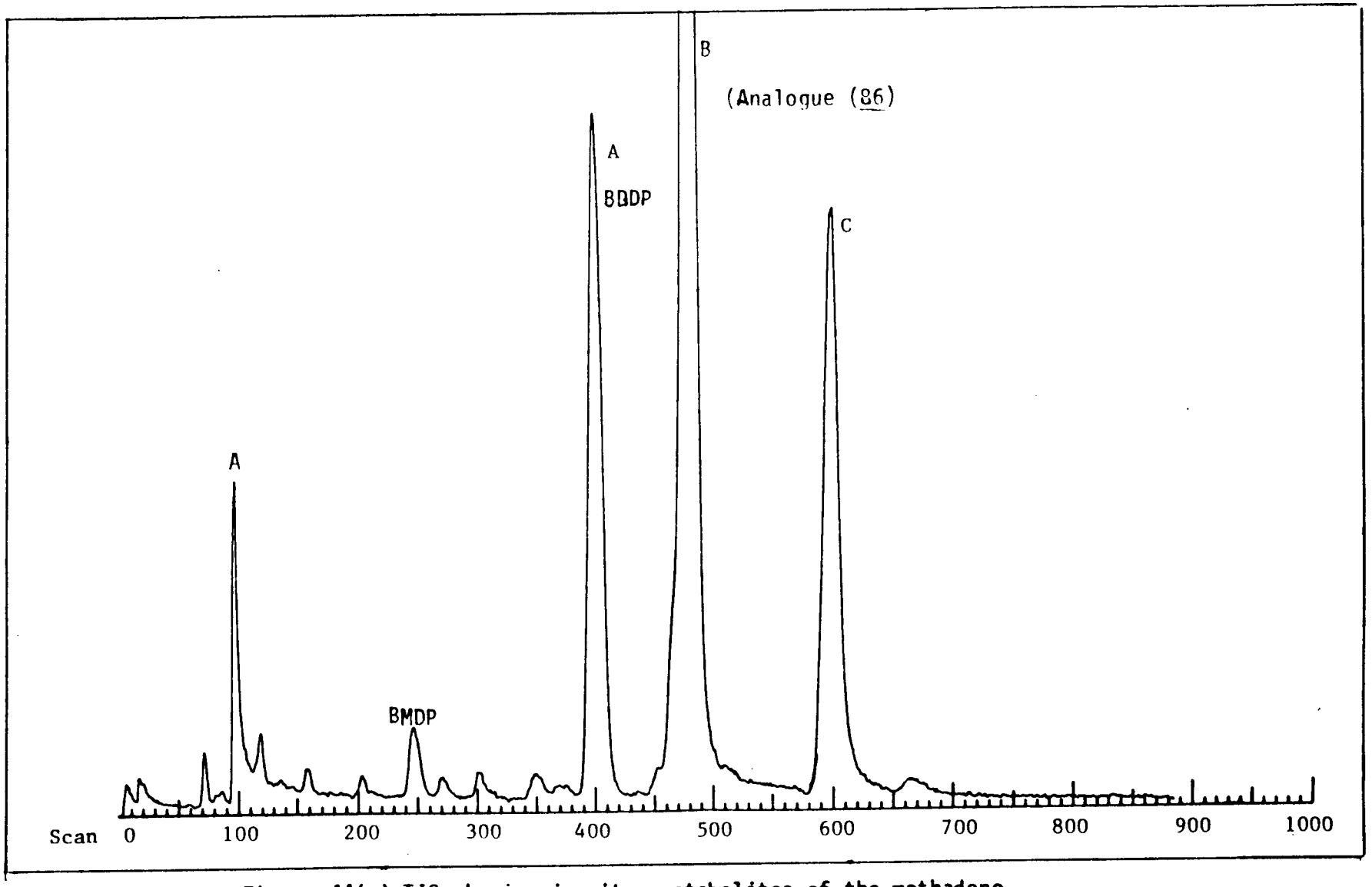


Figure 44(a) TIC showing in vitro metabolites of the methadone analogue (86)

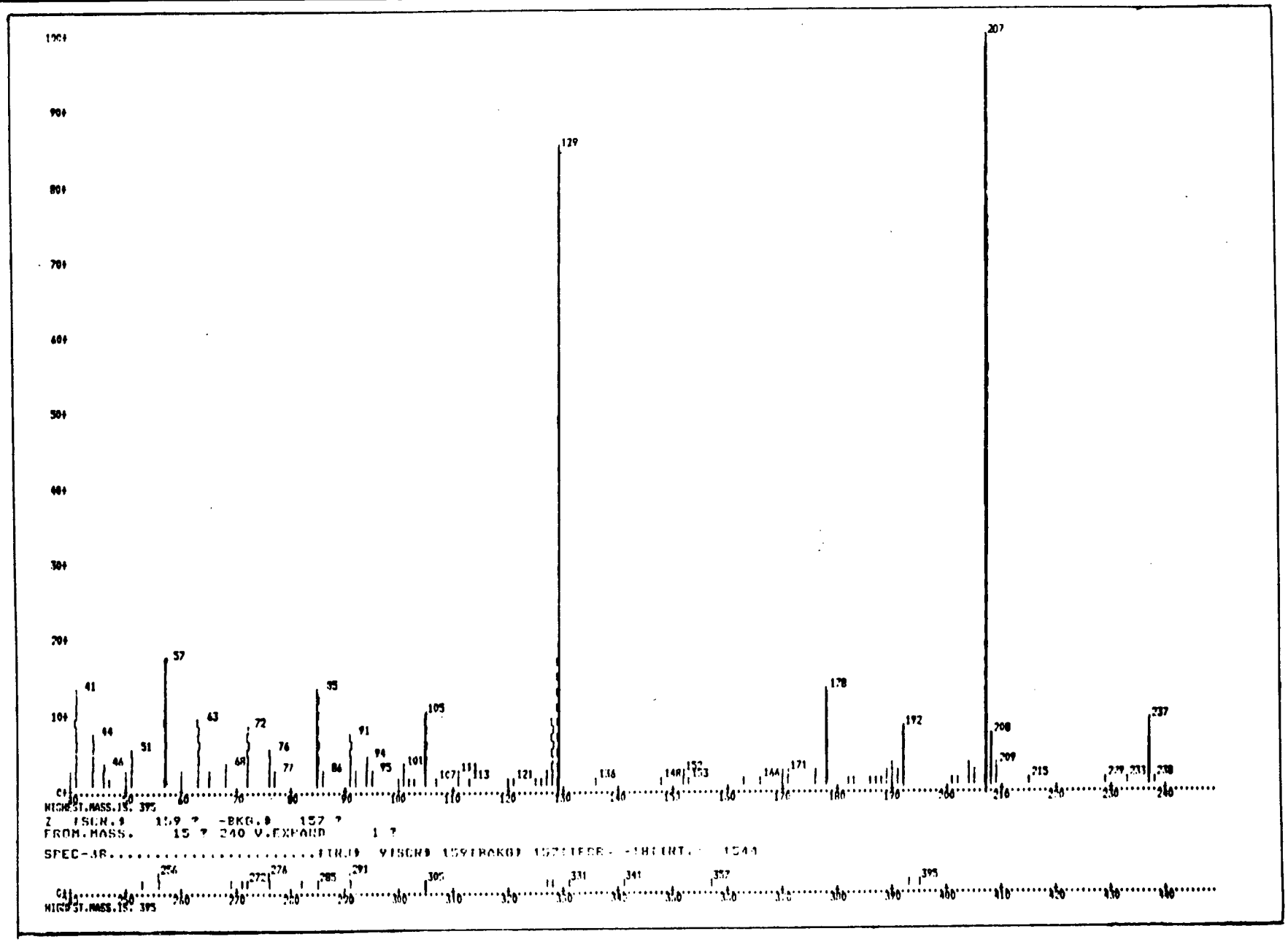


Figure 44 (b) Mass spectrum (GCMS) of the Cope elimination product of methadone analogue N-oxide from an in vitro metabolic extract

E. FINAL COMMENTS ON THE UNKNOWN METABOLITE

i) Problems in the analysis of thermolabile drug metabolites

The chemical results presented in this thesis have shown that the metabolite ultimately leaves the gas chromatograph as a formamide. Two compounds, the nitrone and oxaziridine in the case of recipavrin, and the oxaziridine in the case of methadone were shown to produce the corresponding formamide upon GCMS analysis. The actual structure of the metabolite or its glucuronide is still unknown.

Comparison of ^{13}C NMR peaks of all potentially thermolabile compounds with the spectrum of a metabolic extract of bile from a $(^{13}CH_3)_2$ N-recipavrin dosed rat should solve the problem. This experiment worked well for Noda et al in the study of aminopyrine metabolism (43) but its success depends on retention of the ^{13}C label. An alternative experiment involves HPLC analysis of bile, development of a successful preliminary sample cleanup and many rats, since the major problem here is the sensitivity of the HPLC and the minute quantities of these metabolites. GCMS analysis is of no use in elucidating the structure when so many thermolabile precursors are possible.

It should be stated that this is a minor metabolite. Successful detection by packed column GC requires about half of the extract of conjugated metabolites from one rat. In this study the recovery is variable, and the metabolite is best observed by selected ion monitoring. The $^2H_{10}$ metabolite required several experiments before it was observed in vivo, and attempts to demonstrate it in vitro failed entirely.

ii) Possible pathways to glucuronide conjugated N-formyl metabolites of methadone and recipavrin

If the observations of Noda et al (43) supporting a formamide metabolite of aminopyrine are coupled with those of Allen et al (44) where an amide carbinolamine glucuronide hydrolyzes and dealkylates to the amide (see introduction) , a mechanism for the formation of formamide metabolites of methadone and recipavrin may be postulated (Figure 45).

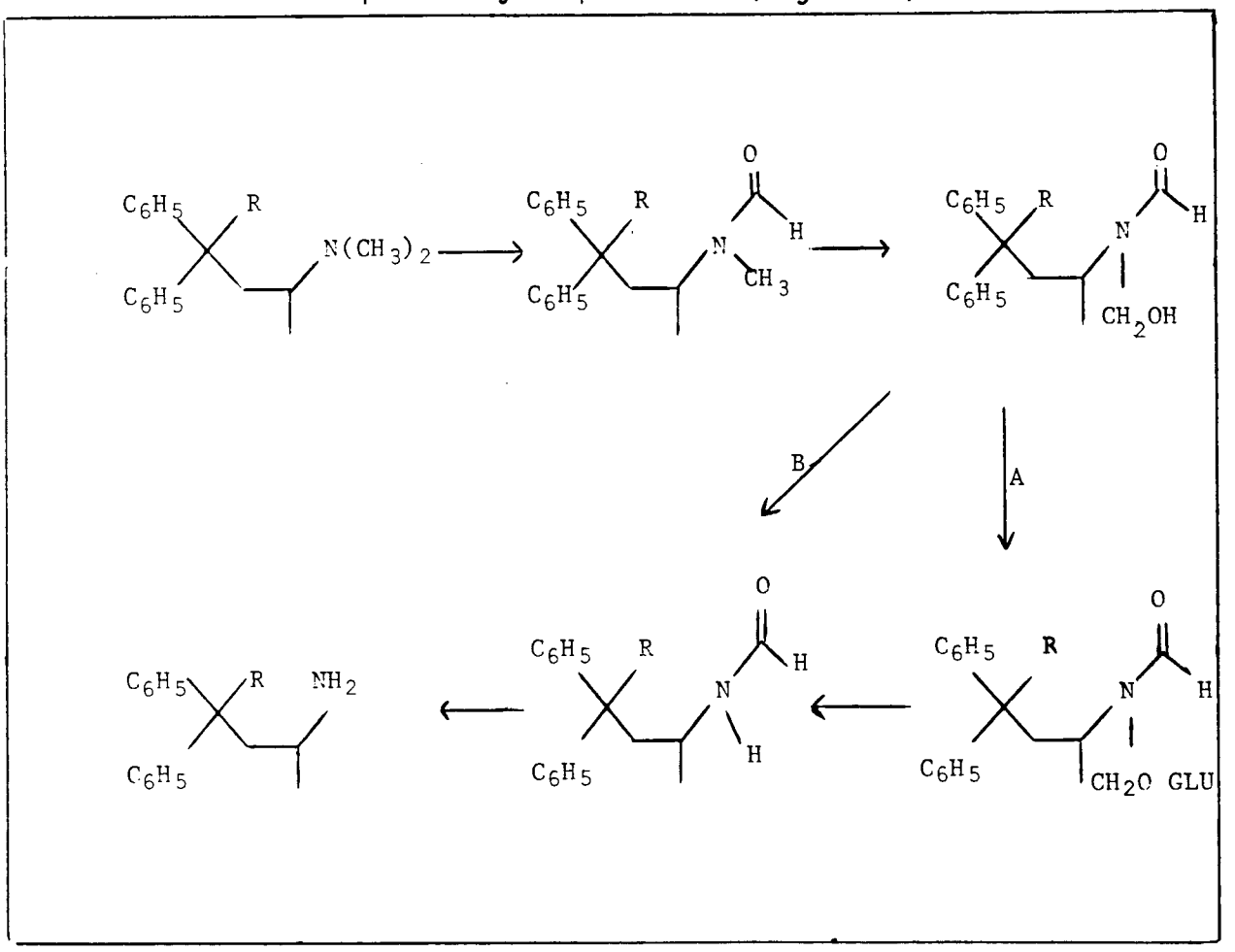


Figure 45. Possible mechanism for formation of formamide metabolites of methadone and recipavrin.

The initial step, oxidation by an unknown mechanism to a formamide, decreases the basicity of the nitrogen and stabilizes the subsequent

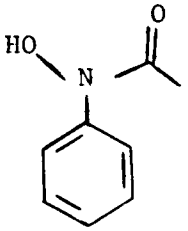
formation of a carbinolamine. This allows glucuronidation of the carbinolamine, which, during β-glucuronidase hydrolysis and workup, decomposes to the formamide plus formaldehyde (Pathway B). Since any basic desalkyl metabolite of methadone would cyclize spontaneously, a mechanism wherein the nitrogen lone pair is delocalized prior to N-dealkylation is necessary. The lack of formamide in the non conjugated fraction reflects either a concerted mechanism, or hydrolysis of non conjugated formamide to EMDP via dinormethadone. The facile deformylation under mildly alkaline conditions of the formyl caffeine metabolite observed by Tang et al (41) supports the latter proposal.

The existence of N-glucuronide conjugates of aliphatic amides such as sulfadimethoxine suggest that a formamide N-glucuronide (pathway C, Figure 8) is also a possible precursor of the observed metabolite.

iii) Other potential sources of a formamide metabolite

Hydroxamic acids such as (90) are well known as glucuronide conjugated metabolites of arylamines and amides (88) and have been implicated in the carcinogenicity of several compounds (89). The non basic nature of an aliphatic formamido nitrogen may make it amenable to N-hydroxylation and glucuronidation, but only one reference, without citation or structure to aliphatic hydroxamic acid metabolites was found in the literature (37).

None of the rearrangement products of tertiary amine N-oxides (ie. Polonovski, Meisenheimer and Cope Rearrangements) are formamides or their precursors (90).



90

IV. SUMMARY AND CONCLUSIONS

During the course of this thesis, the EDDP oxidation product was correctly assigned the oxaziridine structure (5) and was shown to thermally isomerize to the formamide (31). GCMS behavior of both compounds was identical to the biliary metabolite observed by Kang in rats.

The corresponding oxaziridine (14) and formamide (15) of recipavrin were synthesized and proven identical by GCMS to a conjugated biliary metabolite of recipavrin in rats. The possibility of a novel metabolic pathway of aliphatic tertiary amines was discussed in light of these observations. During GCMS analysis the recipavrin nitrone (13) decomposed to the imine (59) oxime (52) and a trace of formamide (16) in accord with literature reports. The decomposition product imine (51) was not present by GCMS of biliary extracts, suggesting that the nitrone is not the precursor of the observed formamide metabolite.

The diketone (45) described by Kang was synthesized in low yield and characterized by ^{1}H and ^{13}C NMR. It is a potential synthetic precursor of methadone and the N-oxidized congeners of the drug methadol.

The ^{13}C NMR results for the oxaziridines (5) and (14) and the nitrone (13) are the first reports of chemical shift values for methylene oxaziridines and nitrones which have α hydrogen atoms.

The synthesis and characterization of ten new compounds; the nitrone (13), oxaziridine (14), formamide (15), hydroxylamines (55) and (57), their TMS derivatives (56) and (58), the imine (59), nitroso compound (61) and oxime (64) as well as detailed characterization of the known amines (6), (53), (54), ketone (51) and oxime (52) provide the analytical basis for further investigation of a novel in vivo metabolic pathway.

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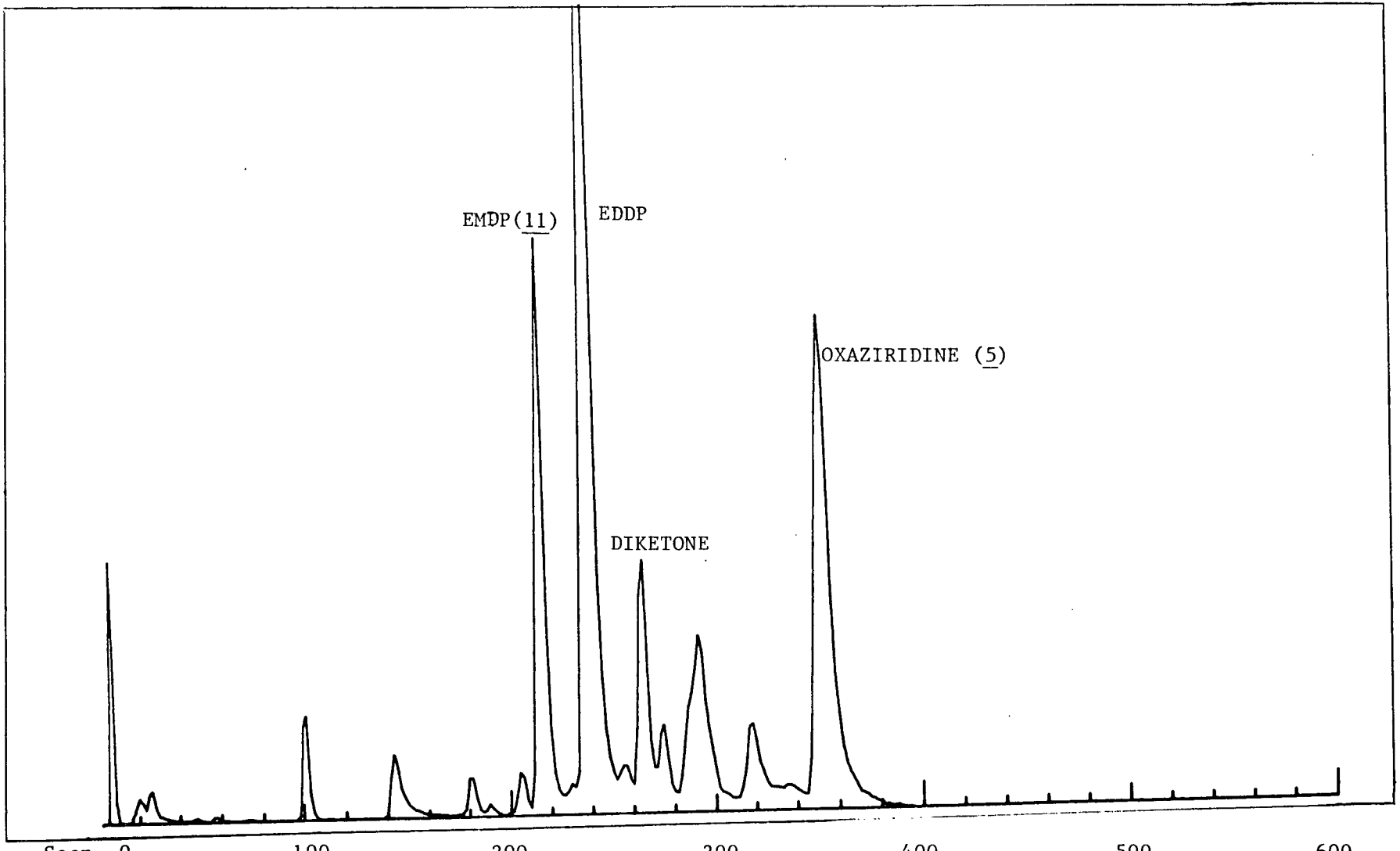
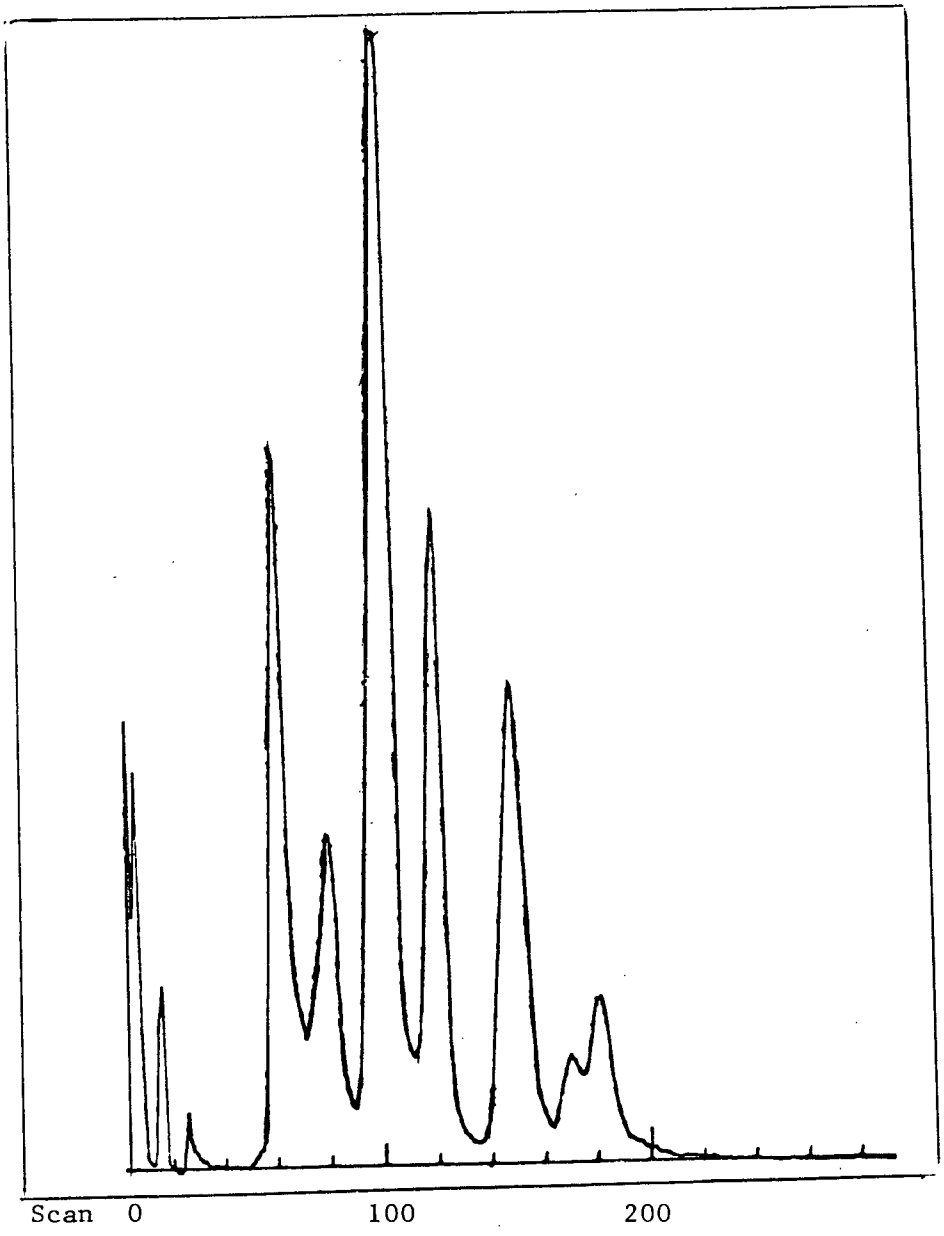
APPENDIX

GC TOTAL ION CURRENT TRACES



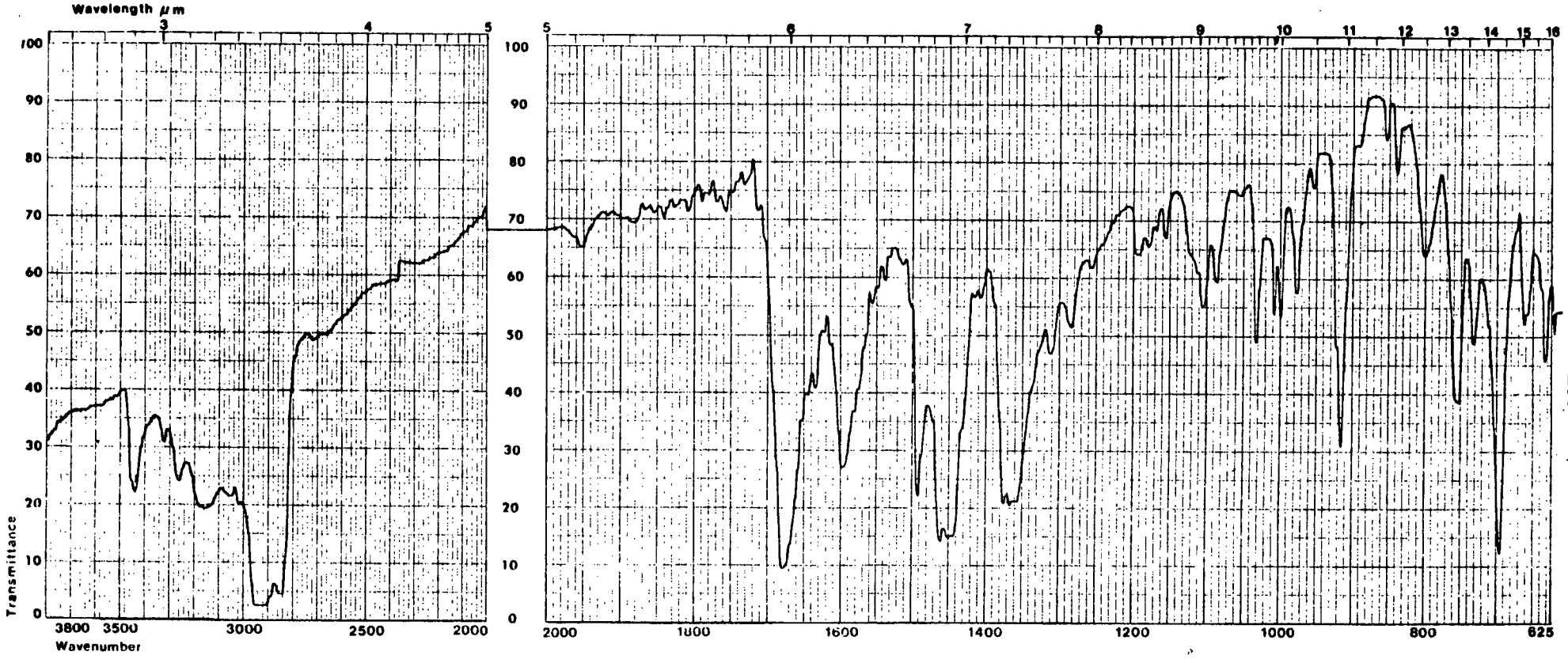
2. TIC of GCMS of EDDP oxidation products (GC conditions b)



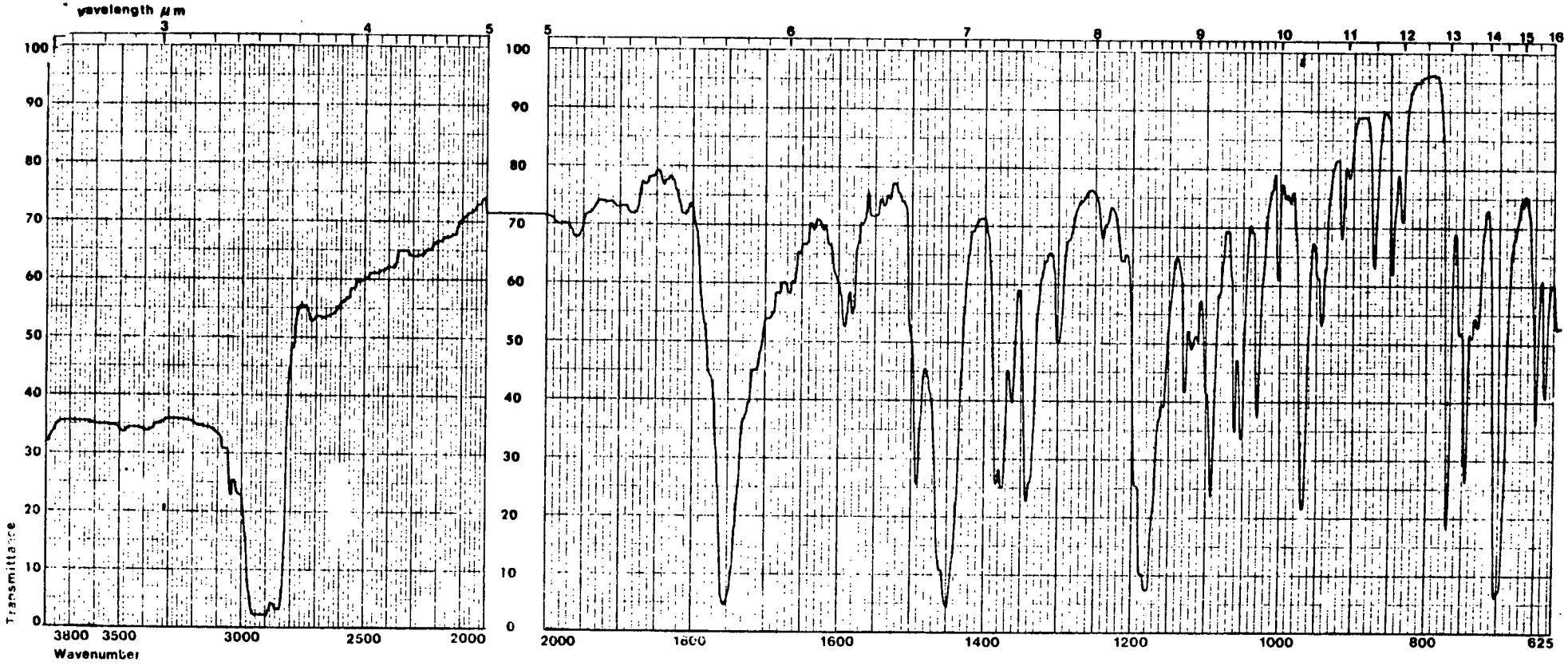
1. TIC of GCMS of EMDP oxidation products (GC conditions c)

APPENDIX

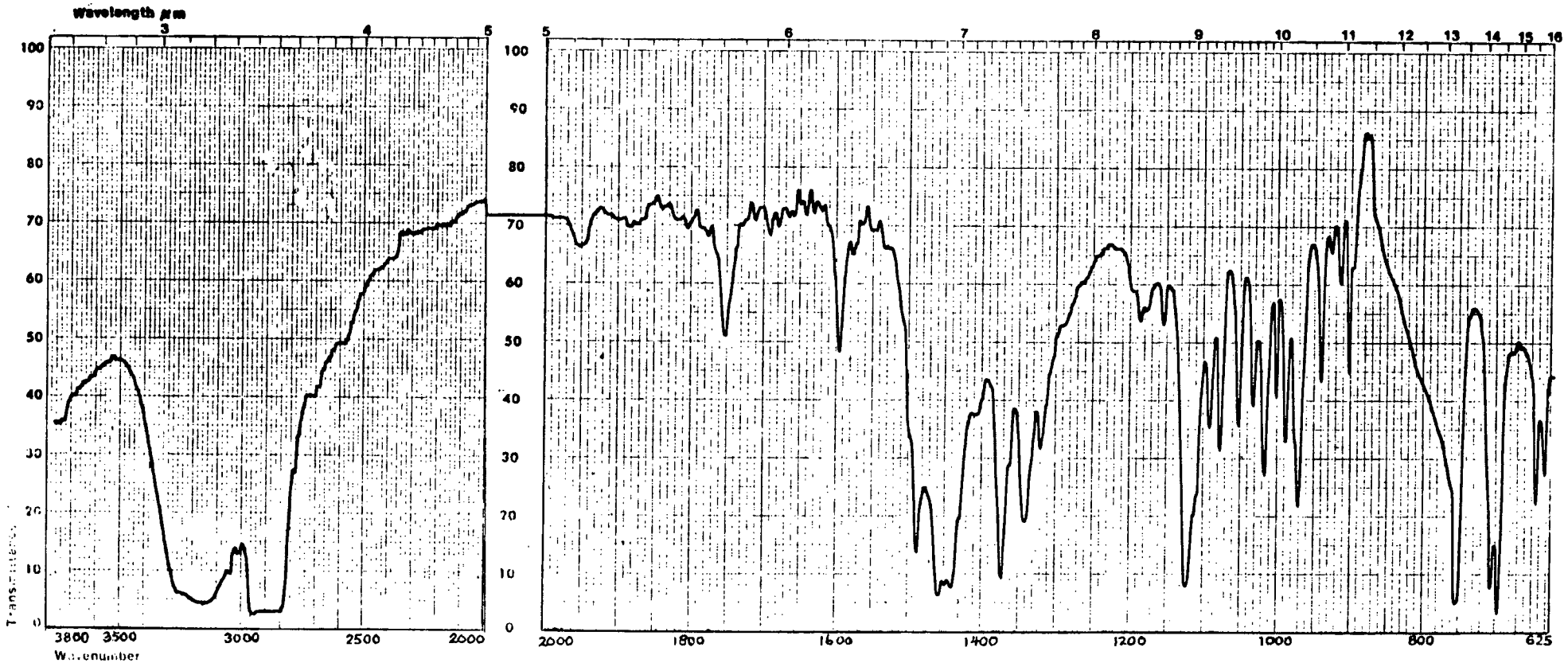
INFRARED SPECTRA



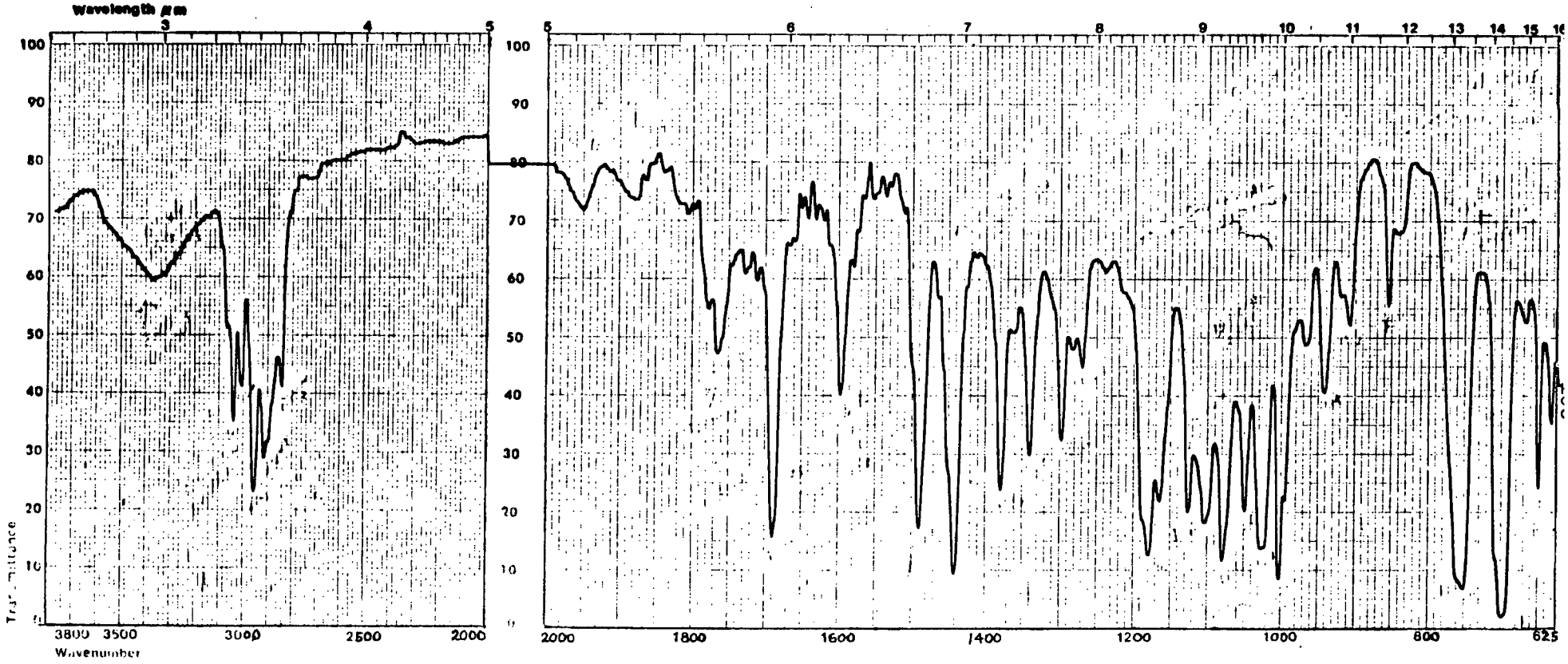
1. Infrared spectrum (nujol mull) of 3,3-diphenyl-5-methyl tetrahydro-2-furanone imine (40)



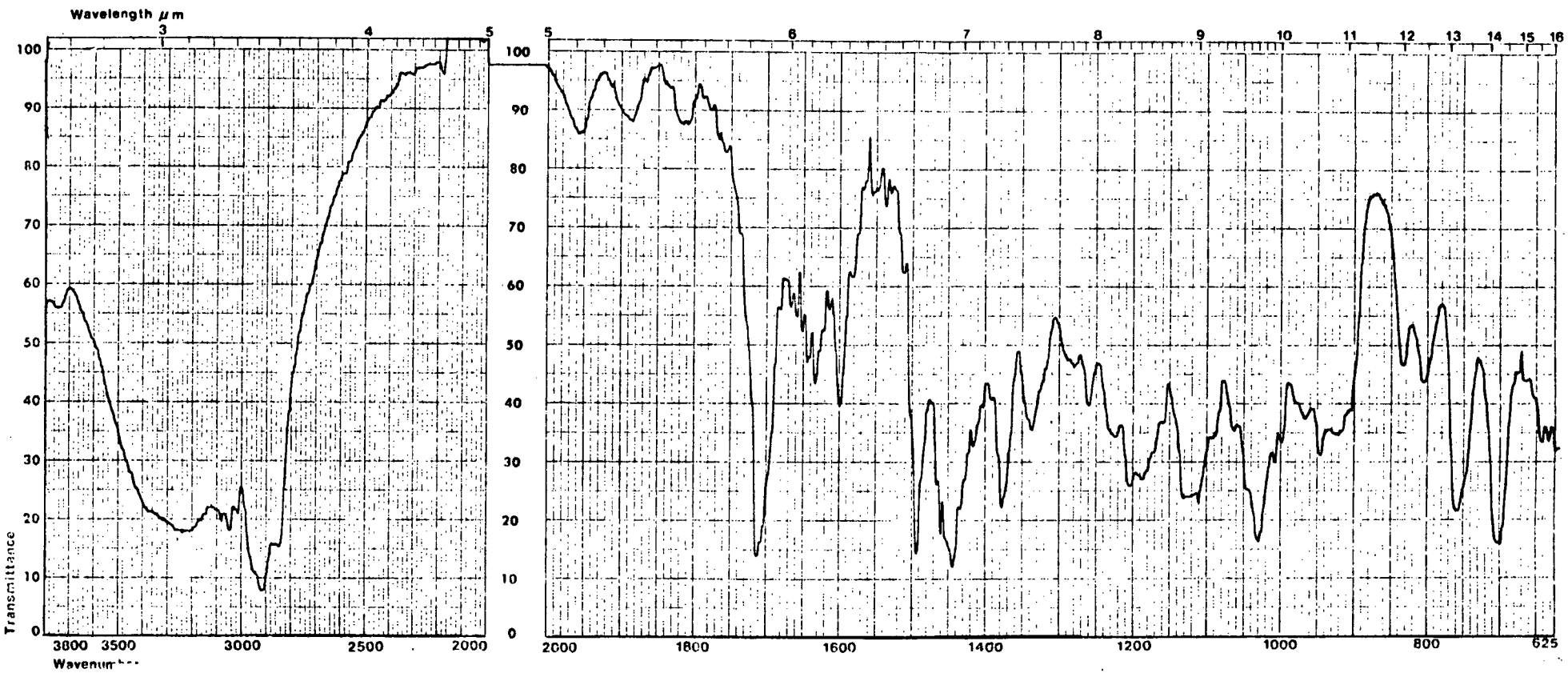
2. Infrared spectrum (nujol mull) of 2,2-diphenyl-4-valerolactone (41)



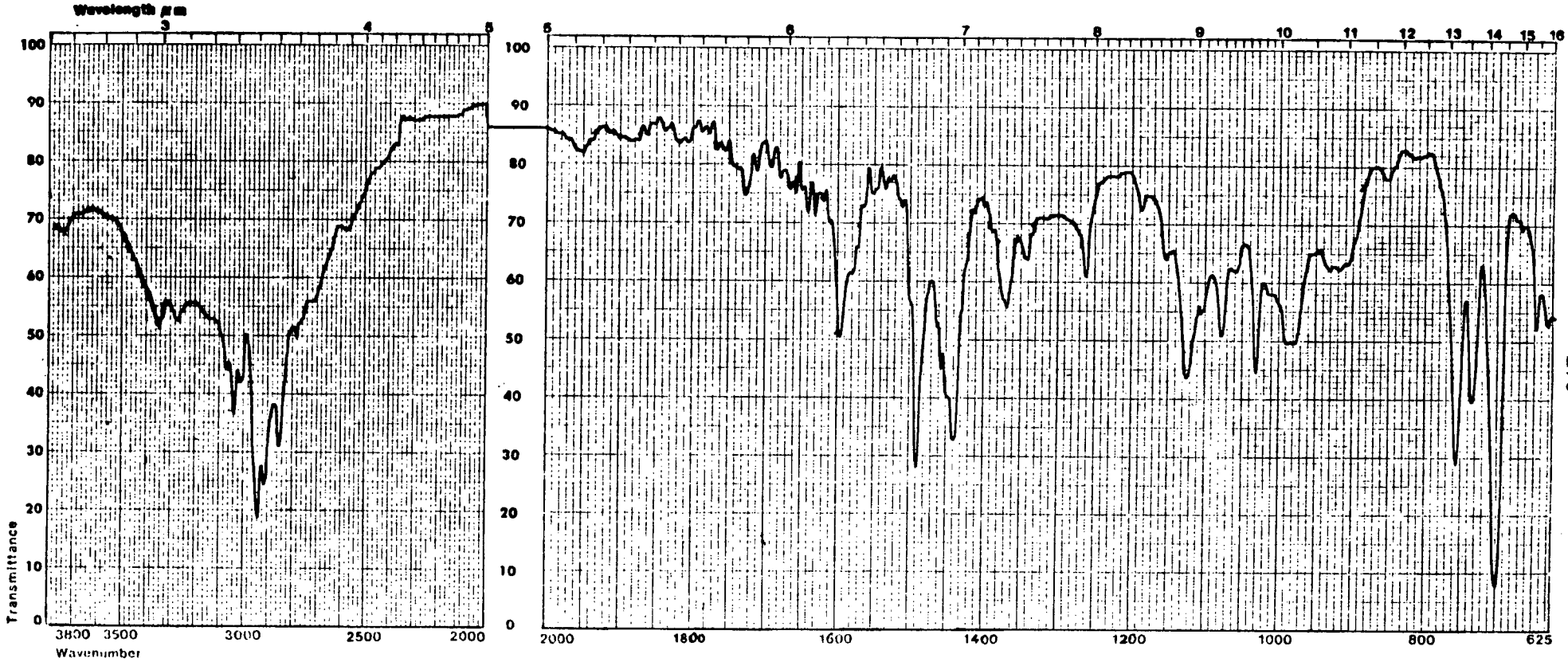
3. Infrared spectrum (nujol mull) of 4,4-diphenyl-2,5-heptane diol (43)



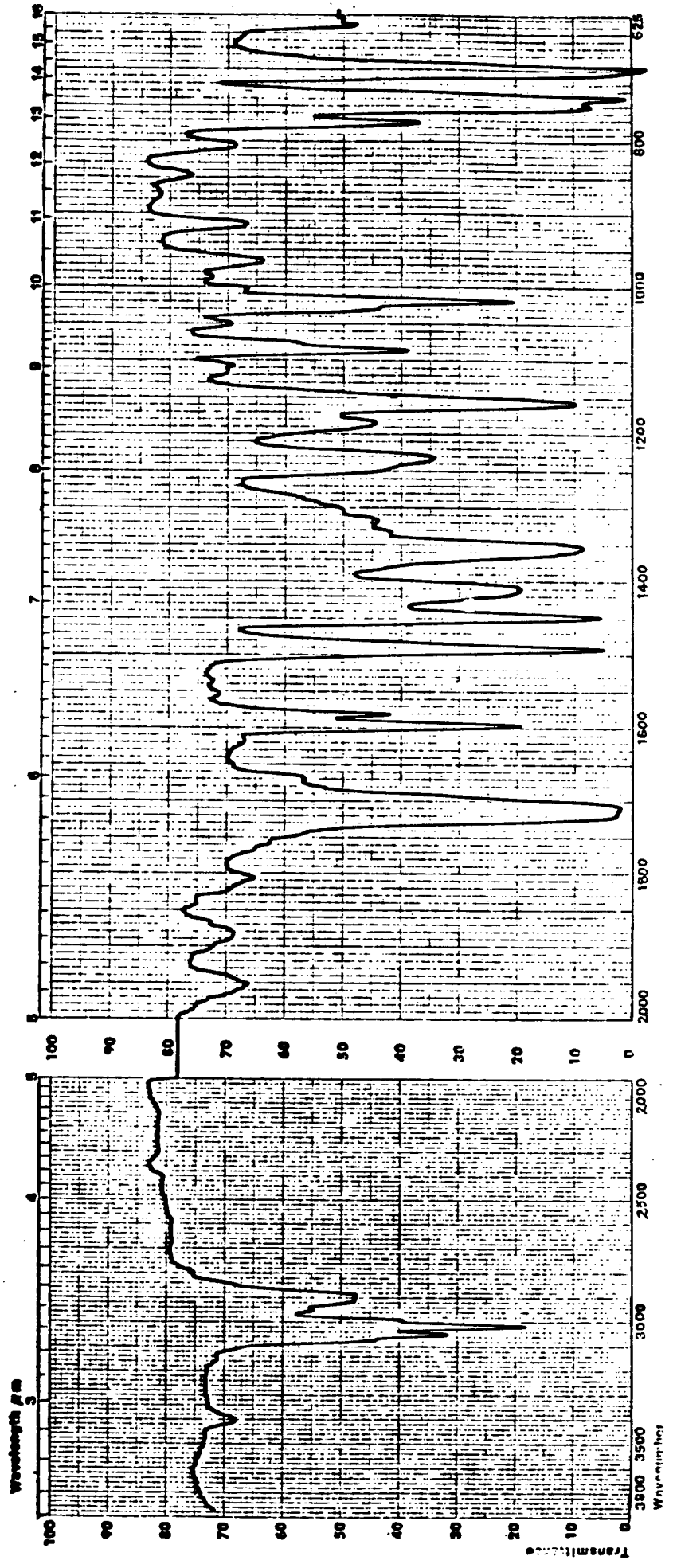
4. Infrared spectrum of 3,3-diphenyl-2-ethylidene-5-methyl-tetrahydrofuran (44)



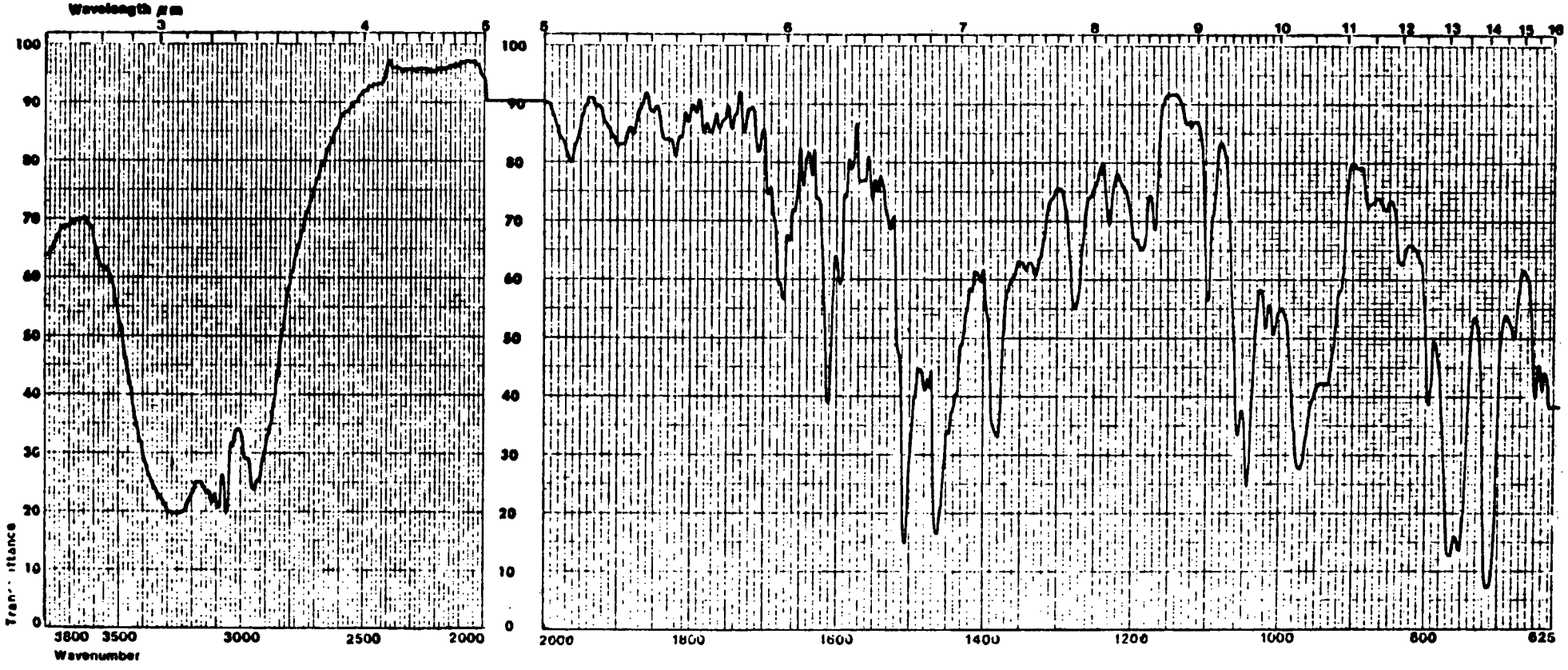
5. Infrared spectrum (film) of 4,4-diphenyl-2,5-heptanedione-2-oxime (46).



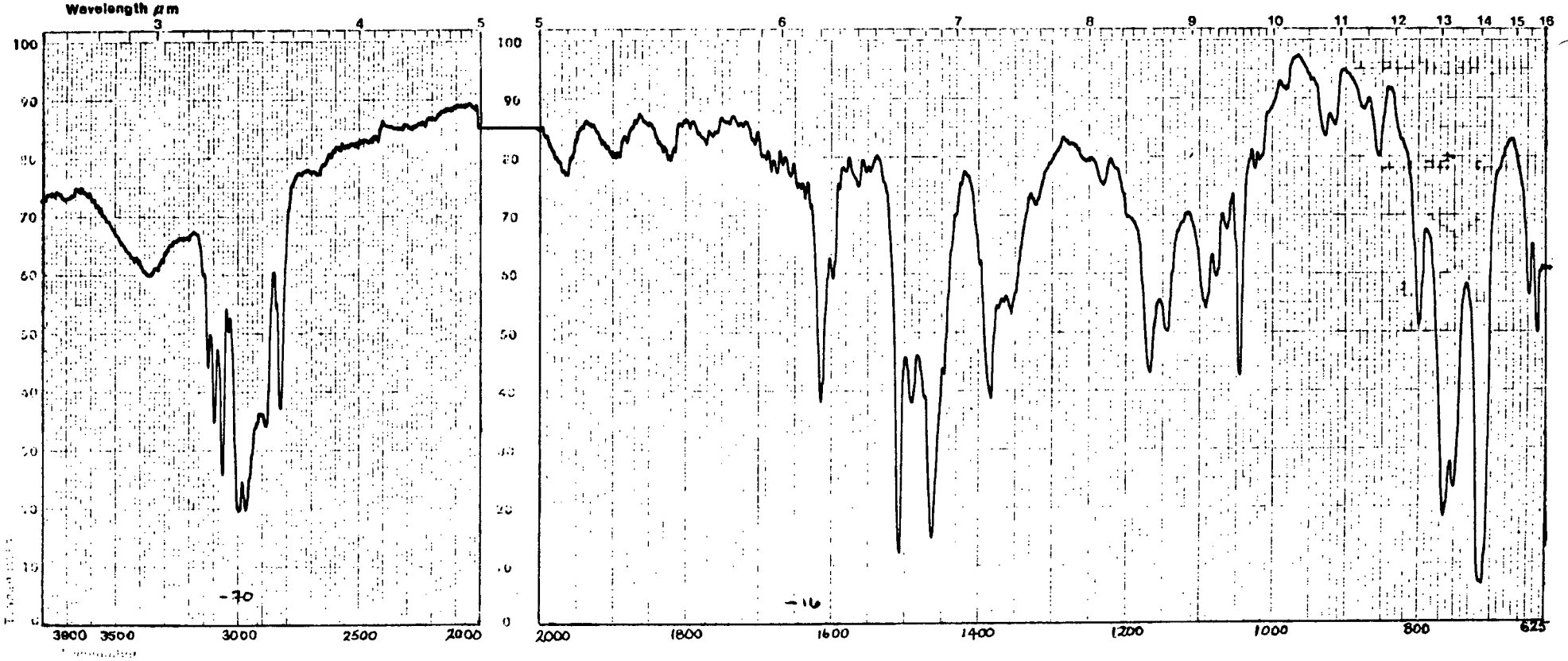
6. Infrared spectrum (film) of 4,4-diphenyl-5-hydroxy-2-amino-heptane (dinormethadol) (49)



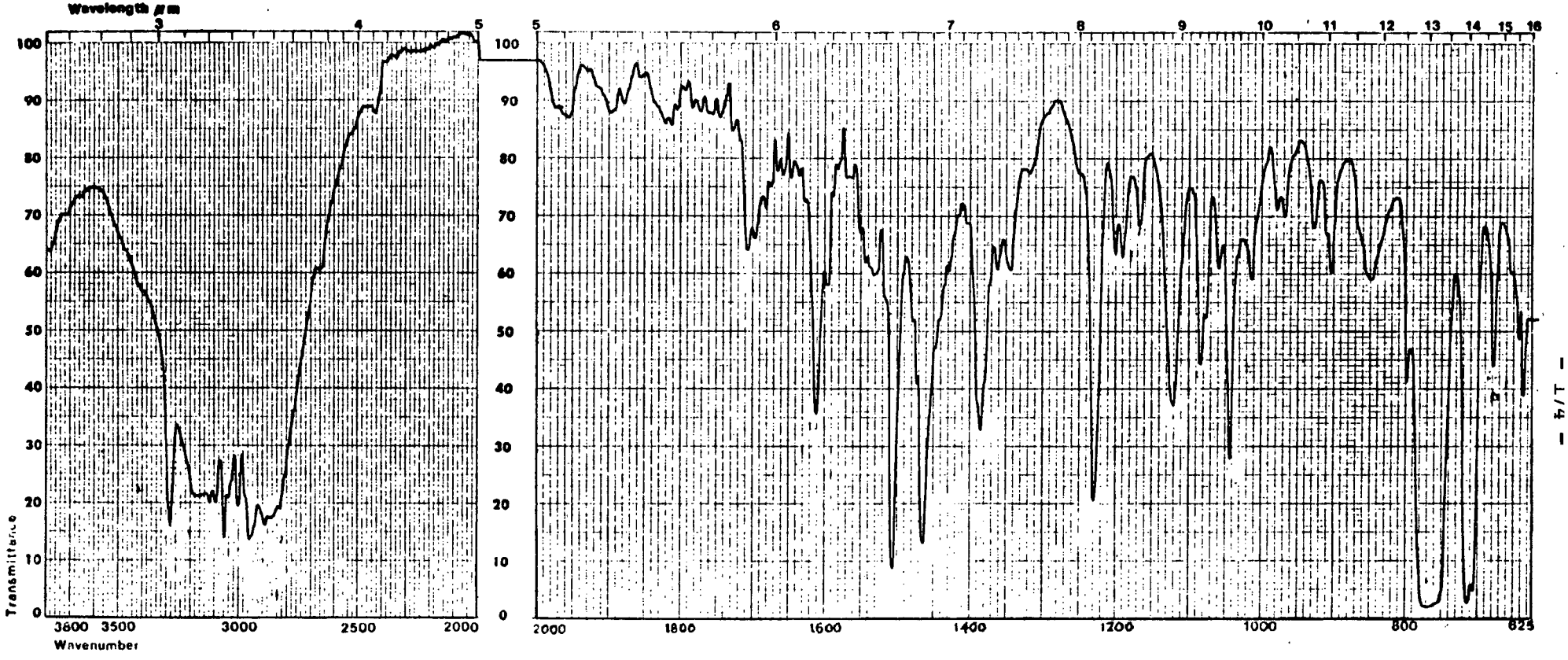
7. Infrared spectrum (nujol mull) of 1,1-diphenyl-3-butanone (51)



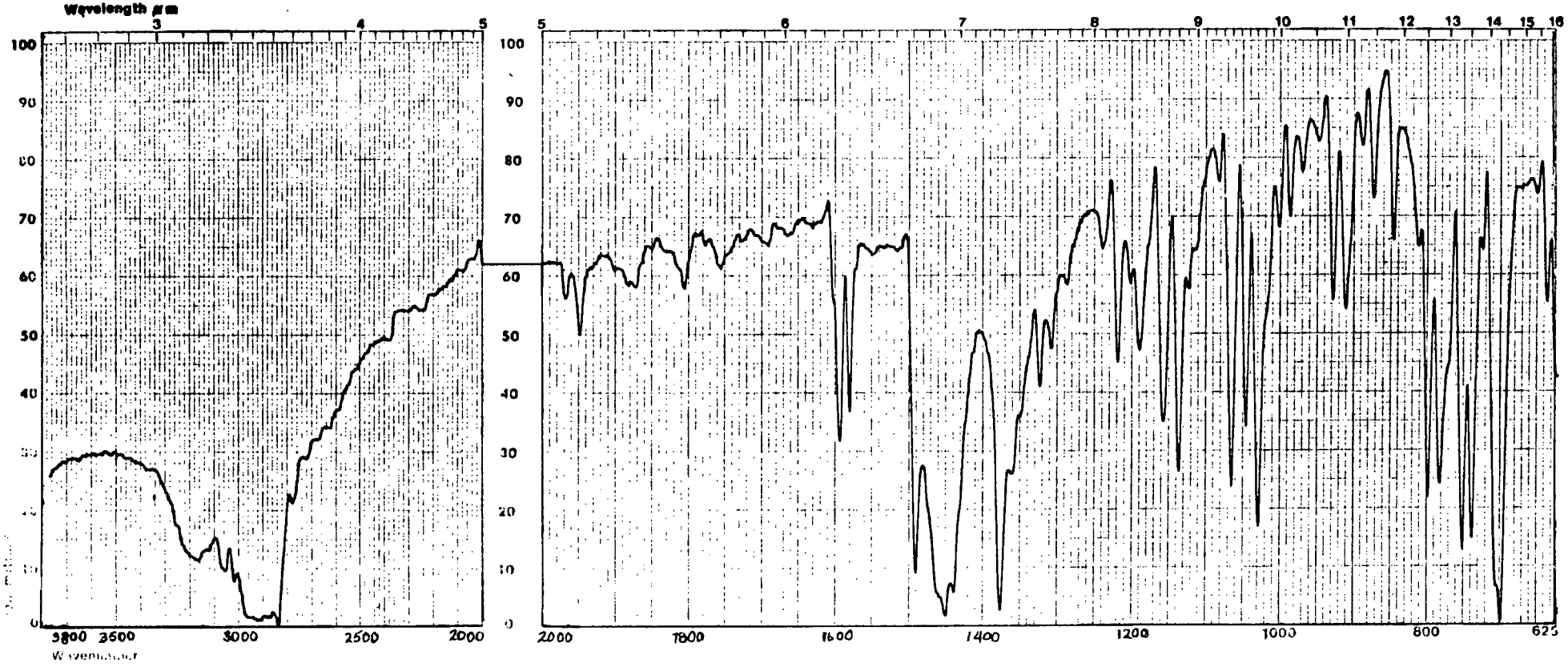
8. Infrared spectrum (film) of 1,1-diphenyl-3-butanone oxime (52)



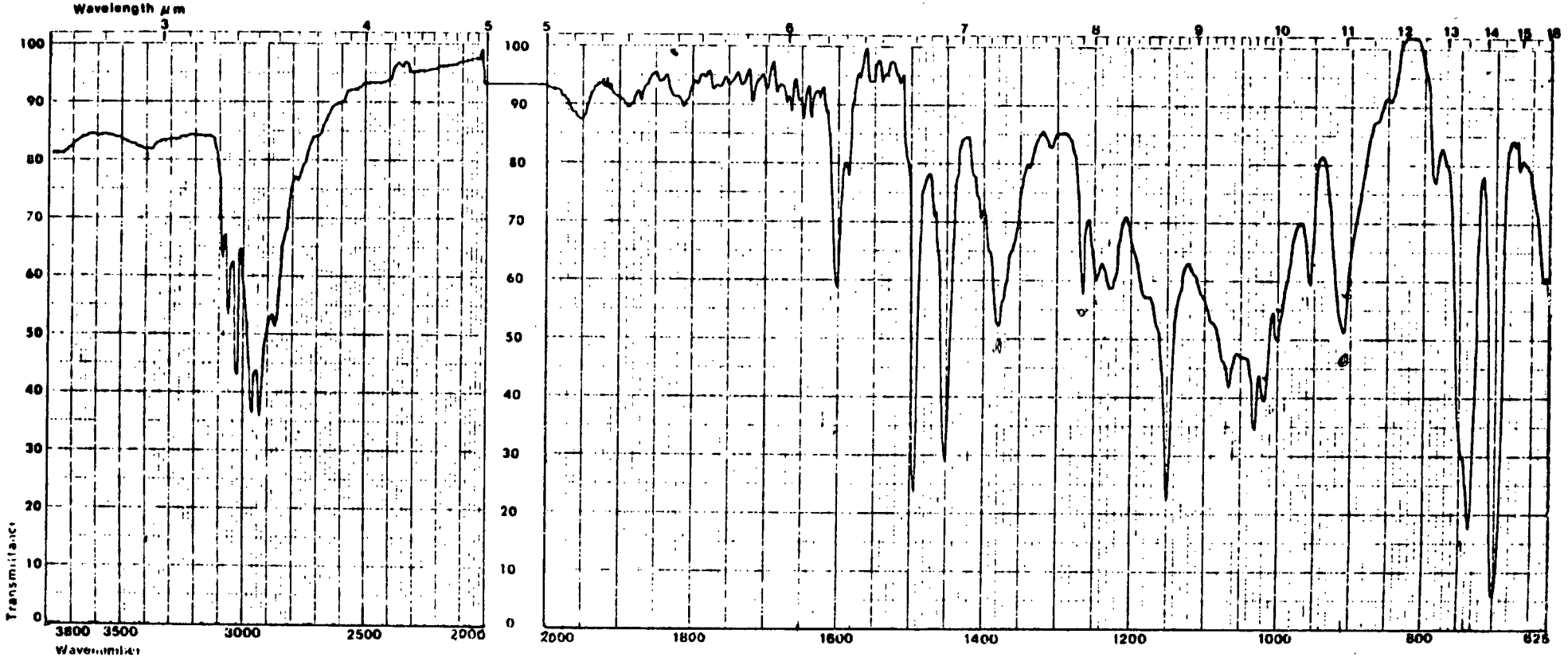
9. Infrared spectrum (film) of N,α-dimethyl-γ-phenyl-benzenepropanamine (54)



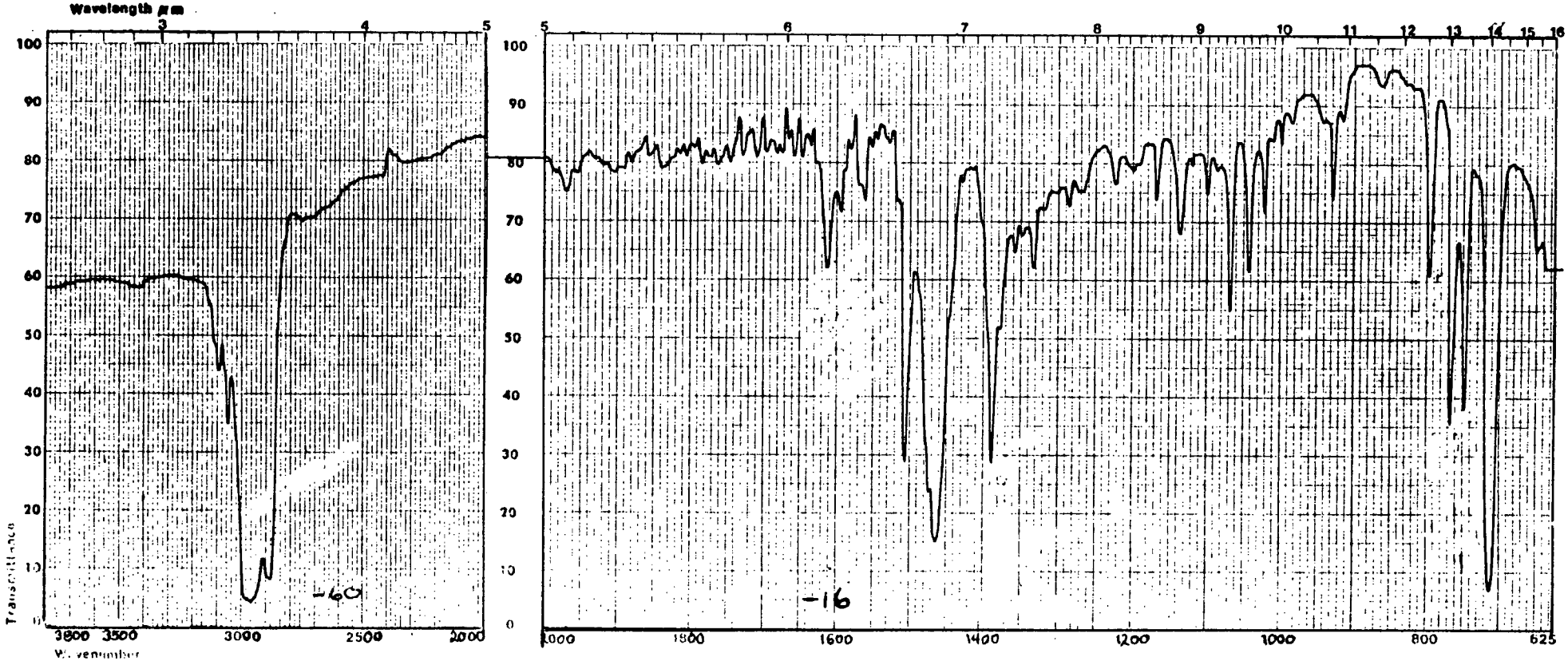
10. Infrared spectrum (nujol mull) of N-hydroxy-α-methyl-γ-phenyl benzenepropanamine (55)



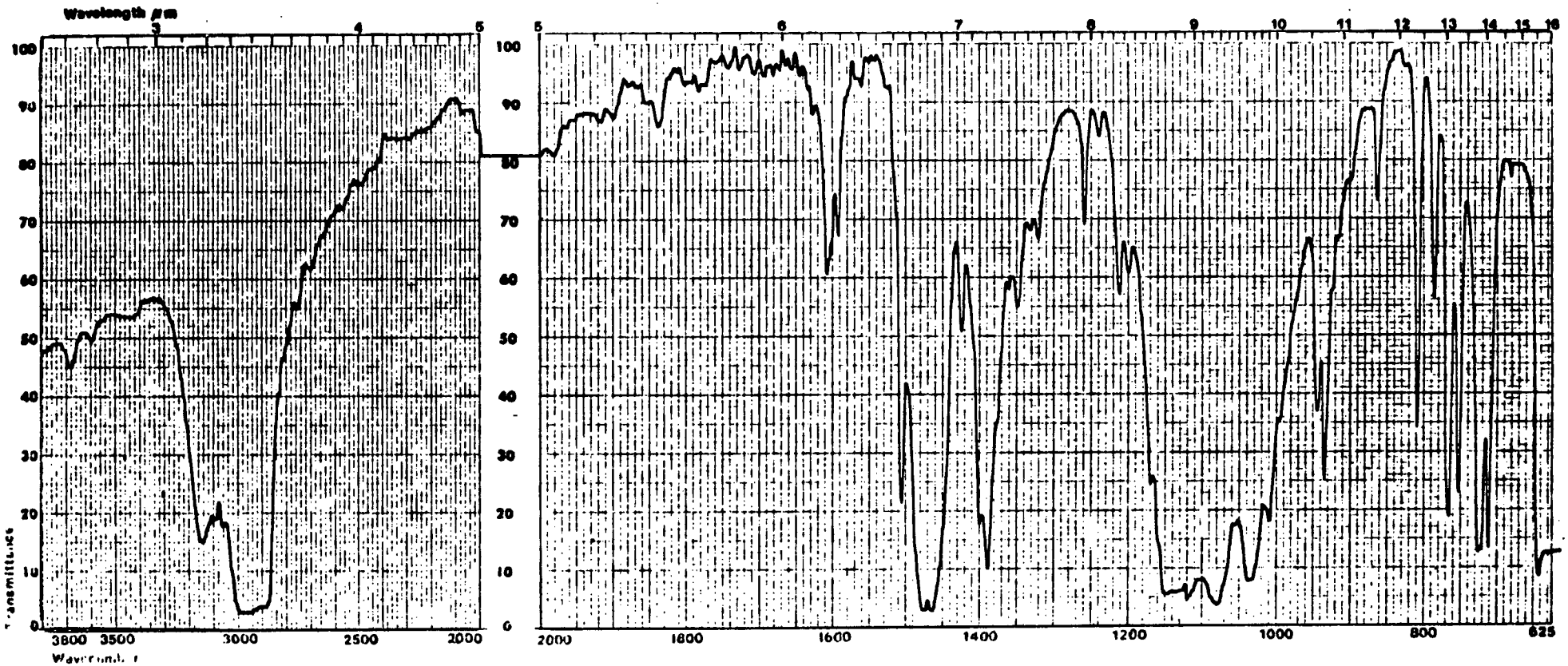
11. Infrared spectrum (nujol mull) of N,α-dimethyl-N-hydroxy-γ-phenyl-benzenepropanamine (57)



12. Infrared spectrum (film) of α-methyl-N-methylidene-γ-phenyl-benzenepropanamine (59)



13. Infrared spectrum (nujol mull) of 1,1-diphenyl-3-nitroso butane (61)



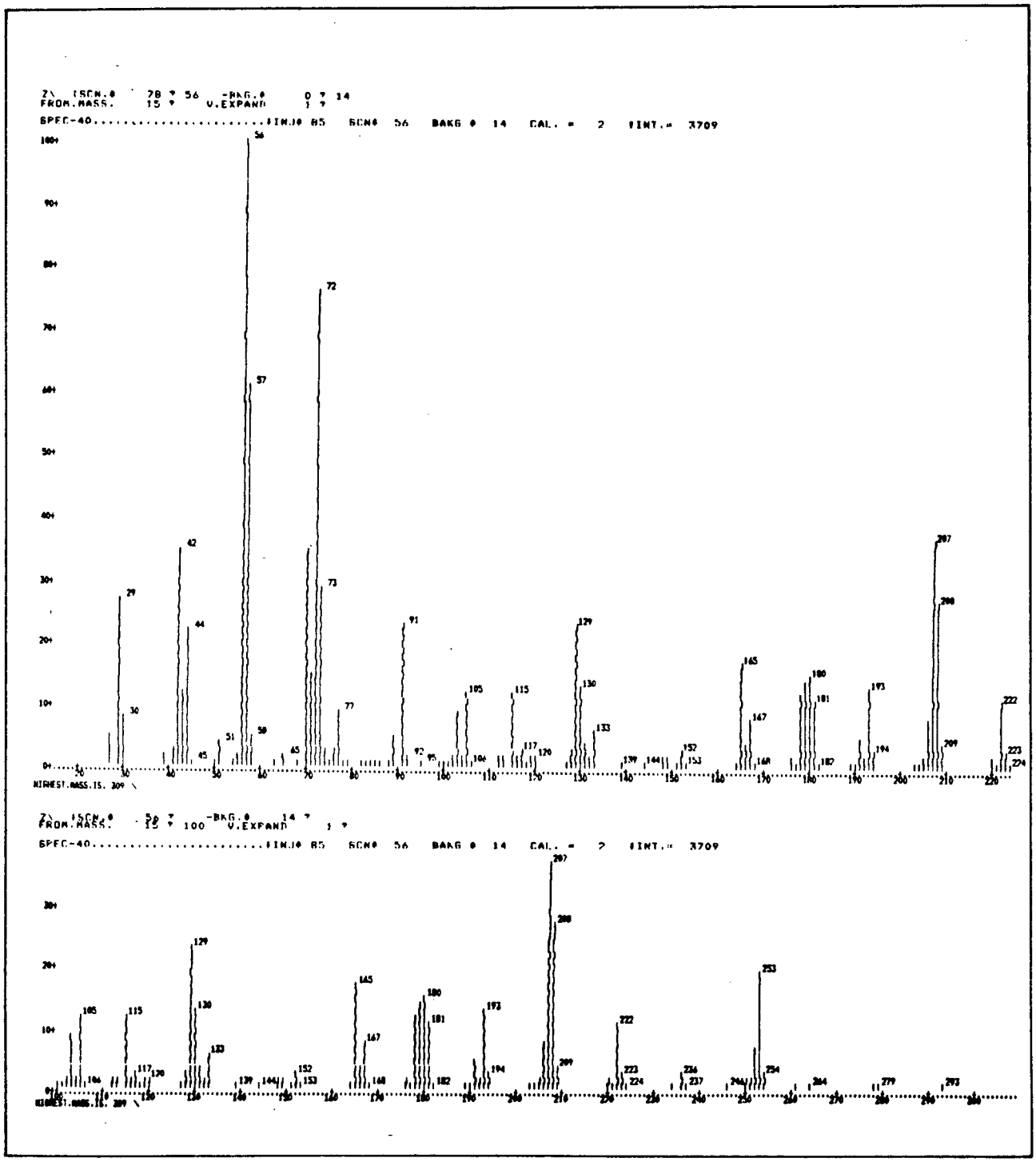
14. Infrared spectrum (nujol mull) of γ-phenyl-N,N,α-trimethyl benzenepropanamine perchlorate (6)

APPENDIX

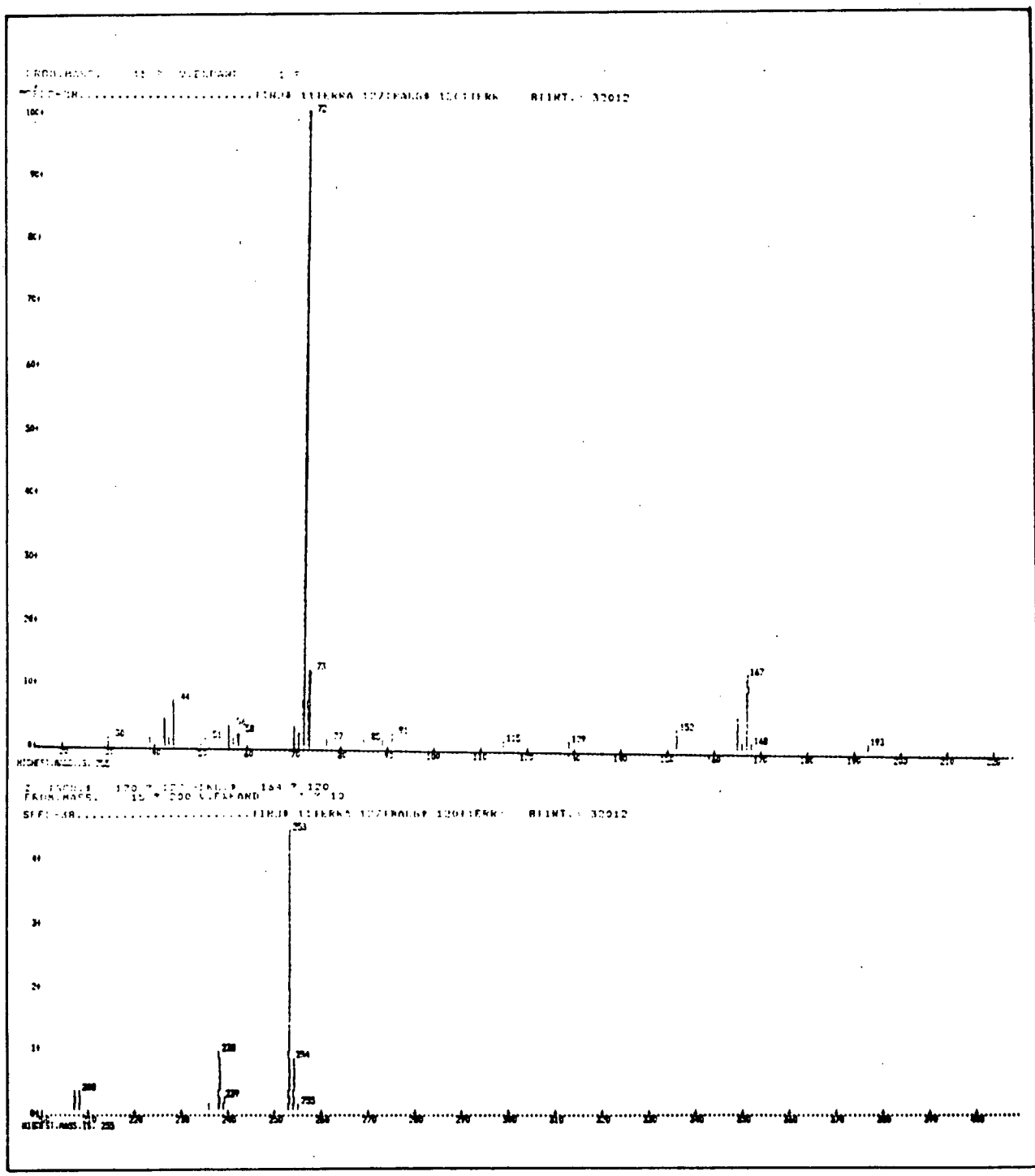
MASS SPECTRA

APPENDIX

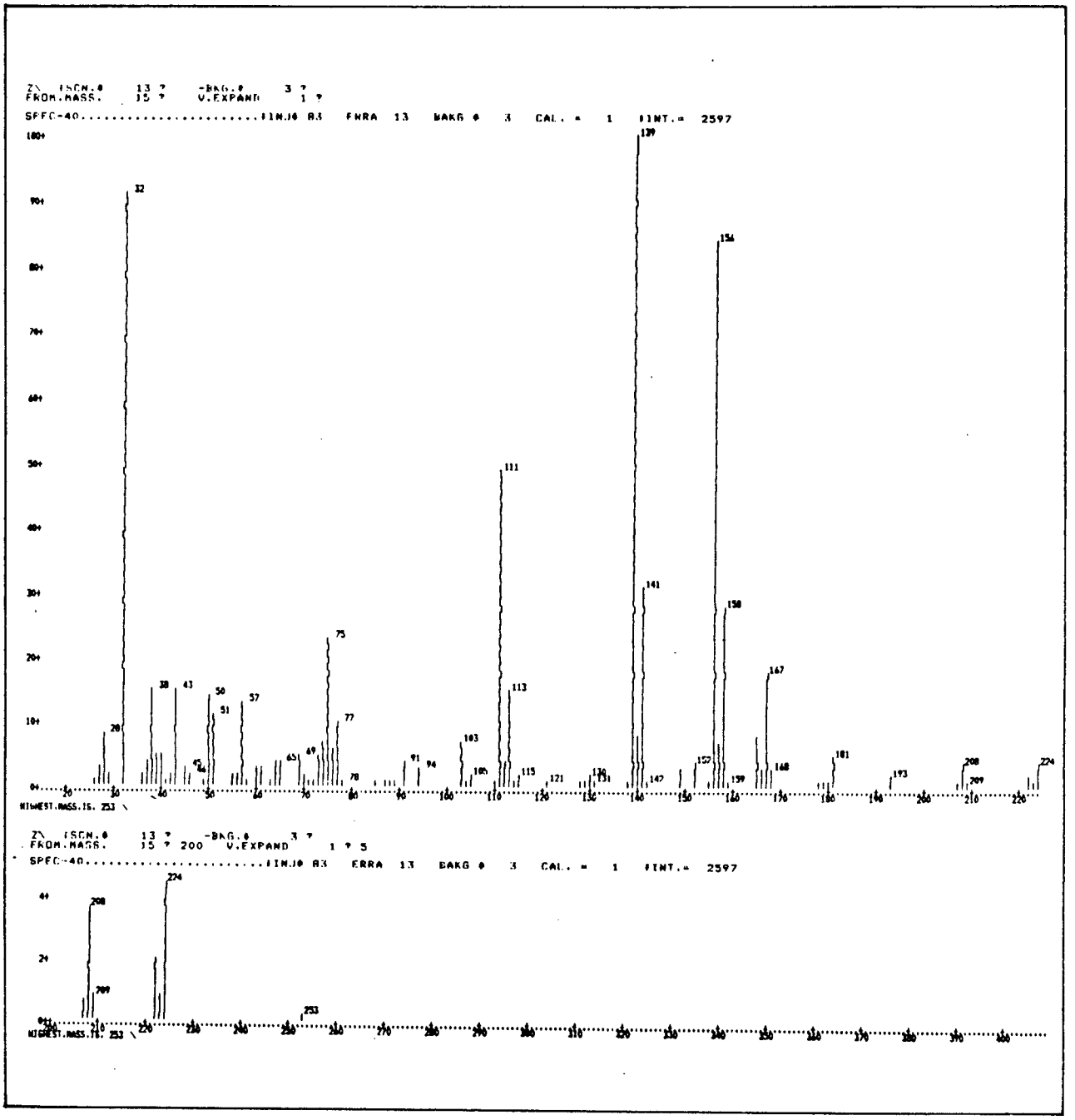
APPENDIX



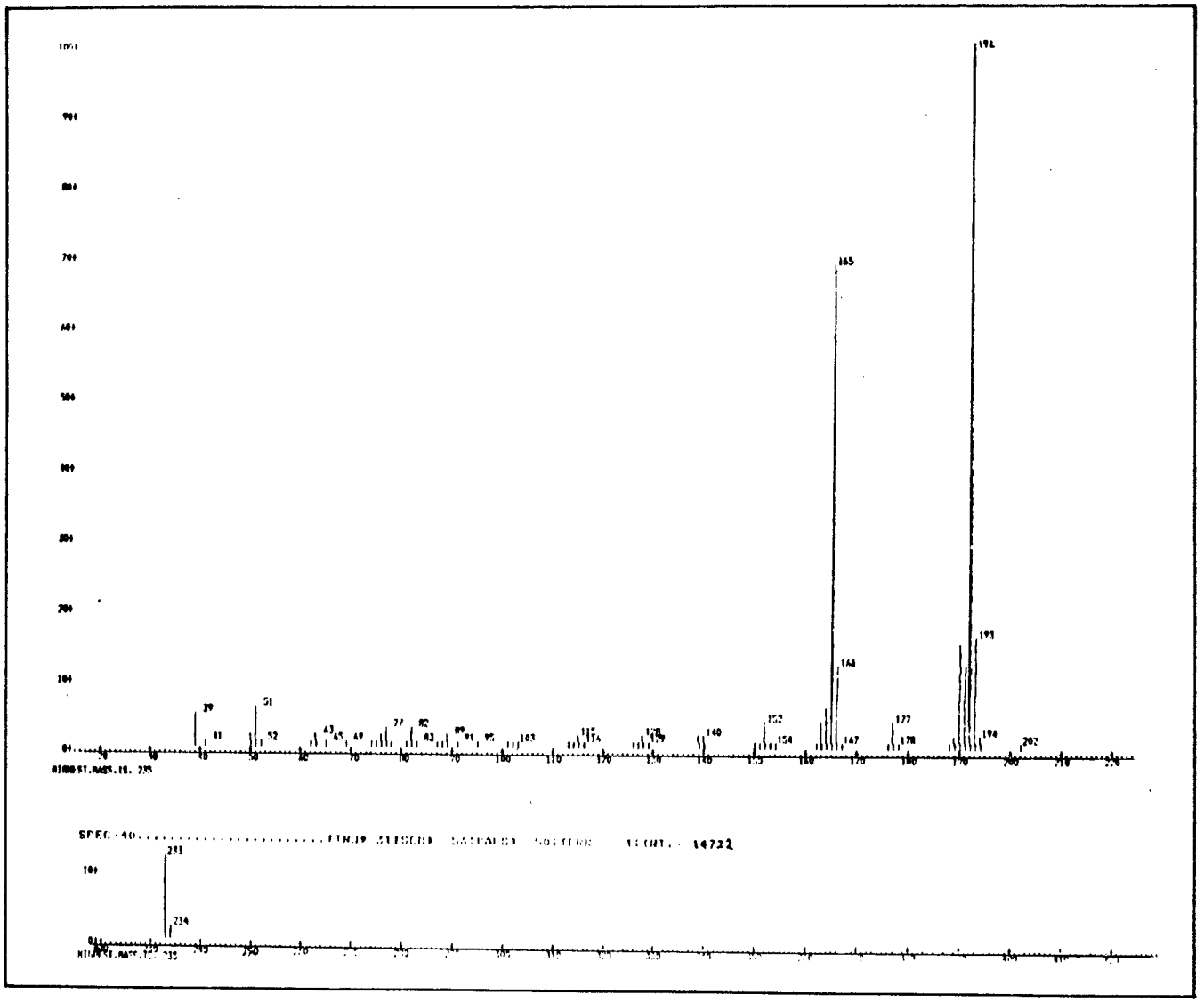
1. Mass spectrum (direct inlet) of 2-(4',4'-diphenylheptan-5'-one-2'-yl) oxaziridine (5)



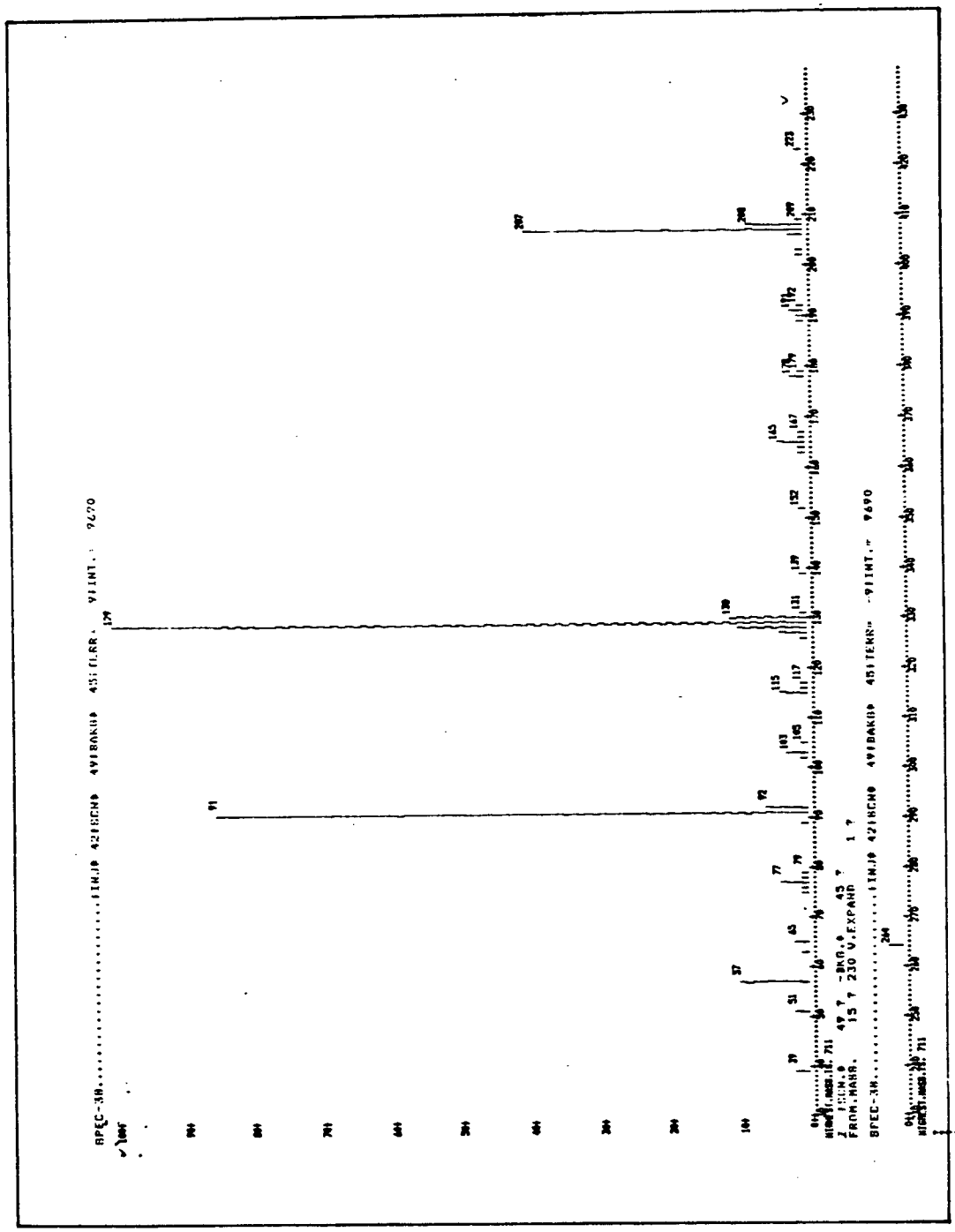
2. Mass spectrum (GCMS) of γ -phenyl-N,N-α-trimethyl benzenepropanamine (6)



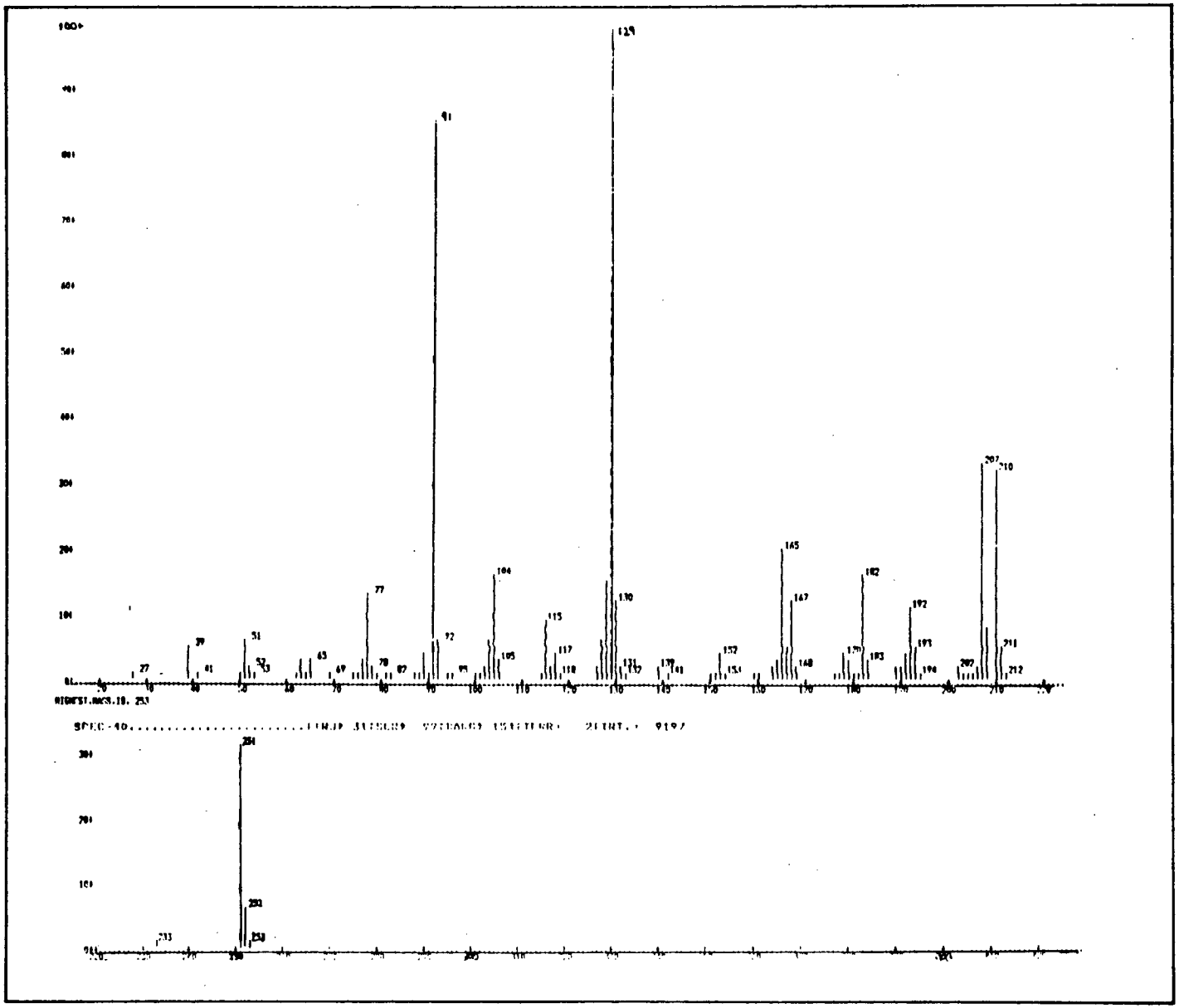
3. Mass spectrum (direct inlet) of 2-(4',4'-diphenyl-but-2'-yl) oxaziridine (14)



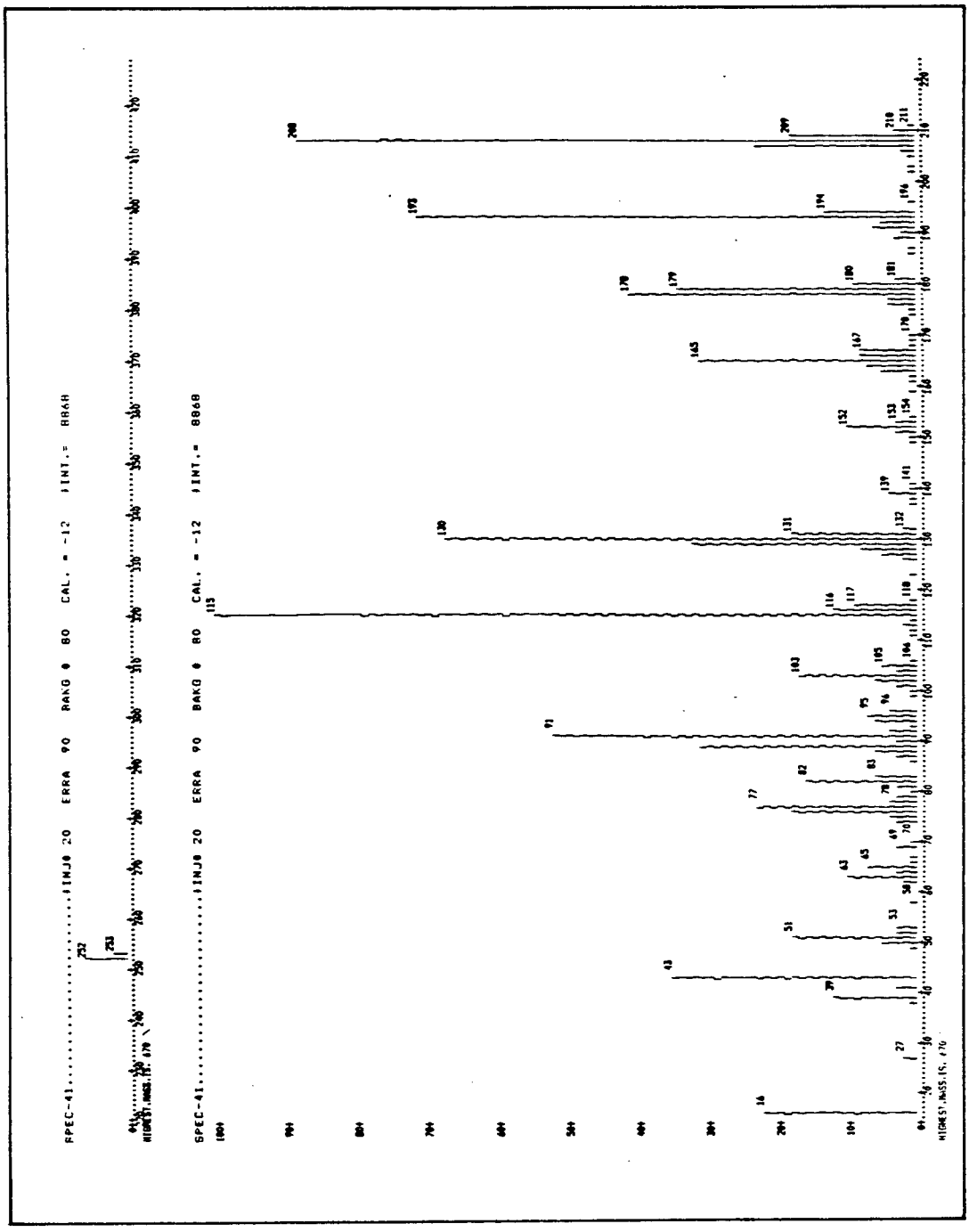
4. Mass spectrum (GCMS) of 2,2-diphenyl-pent-4-enenitrile (38)



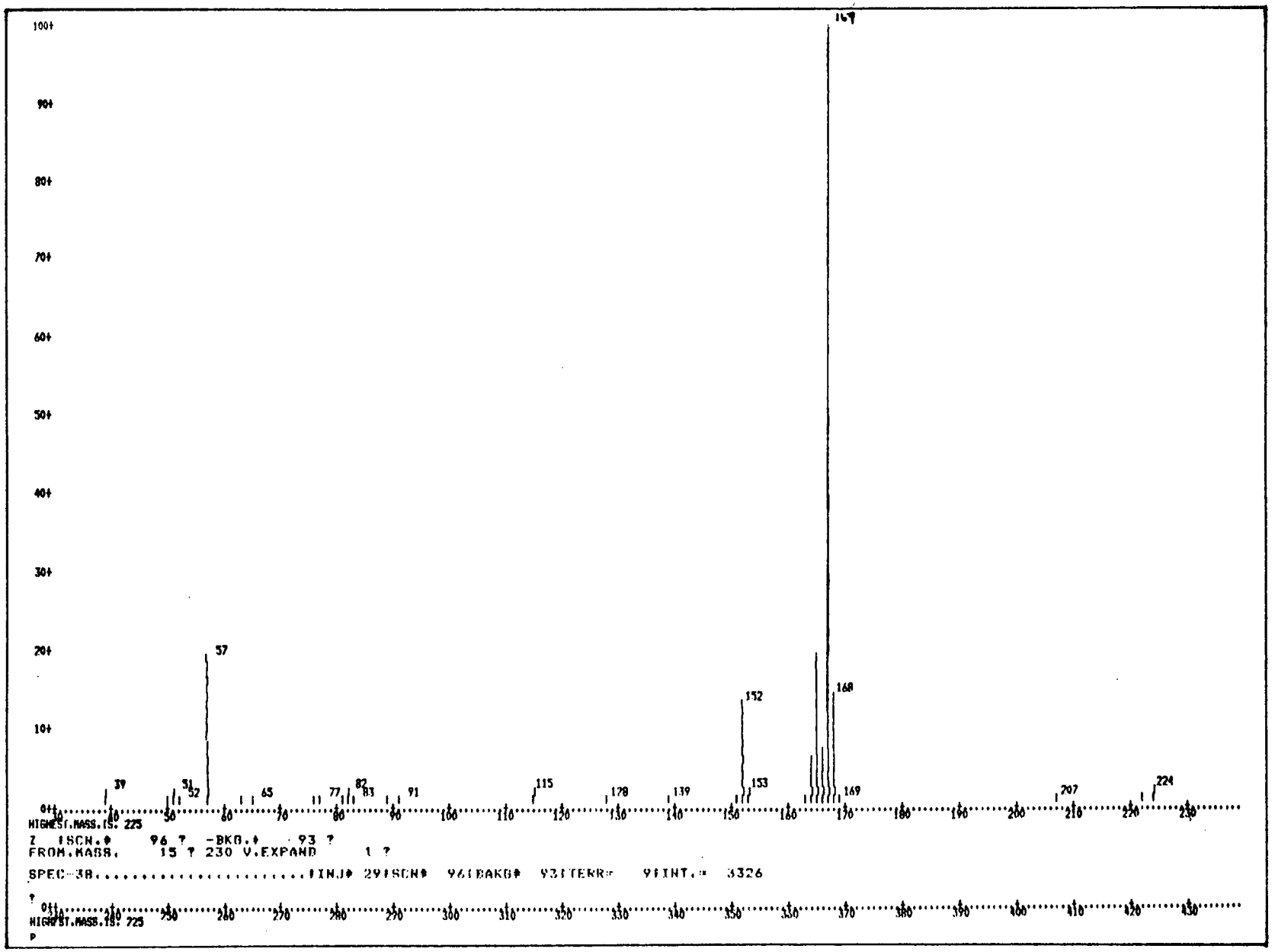
5. Mass spectrum (GCMS) of 4,4-diphenyl-hepten-5-one (39)



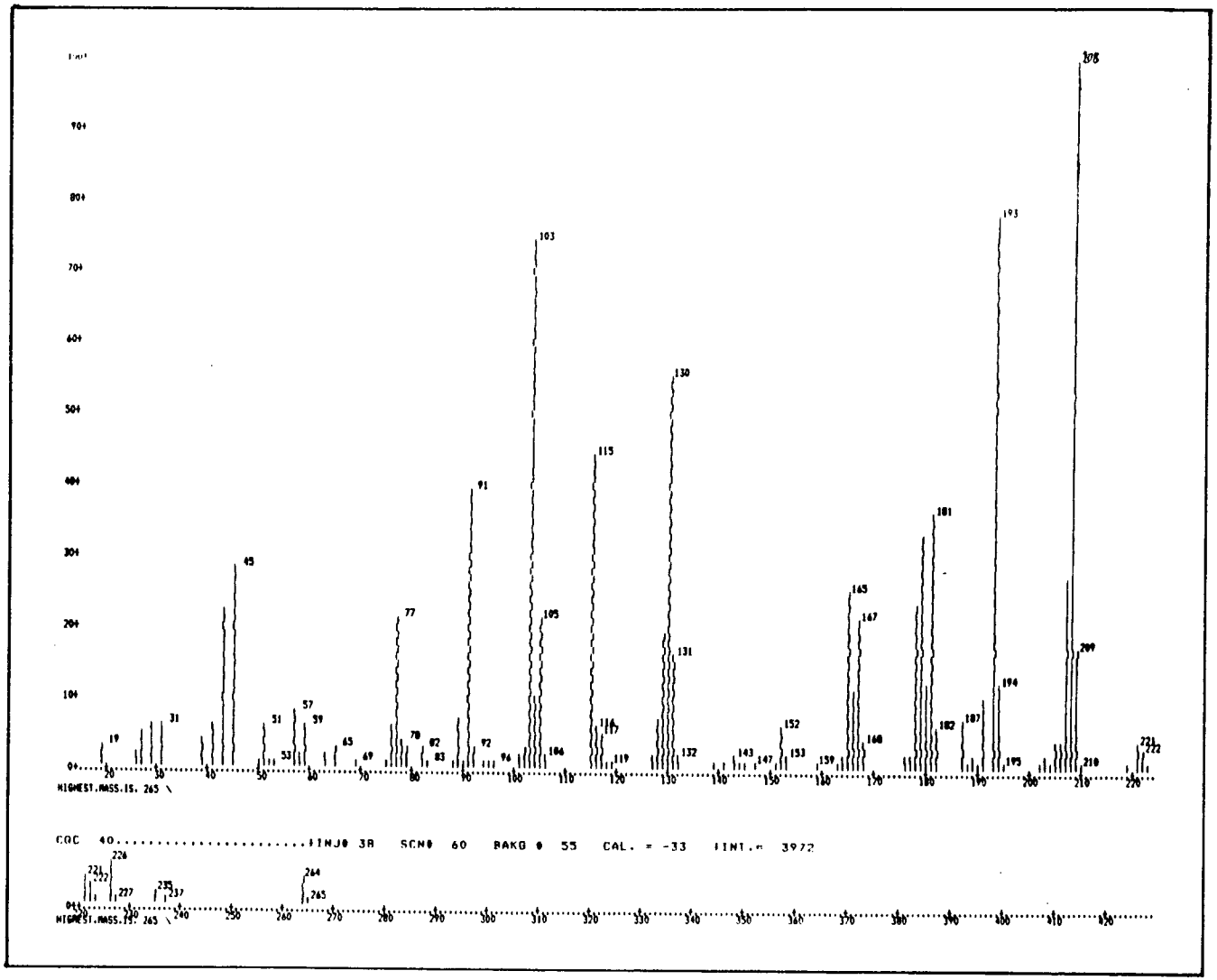
6. Mass spectrum (GCMS) of 3,3-diphenyl-5-methyl tetrahydrofuranone imine (40)



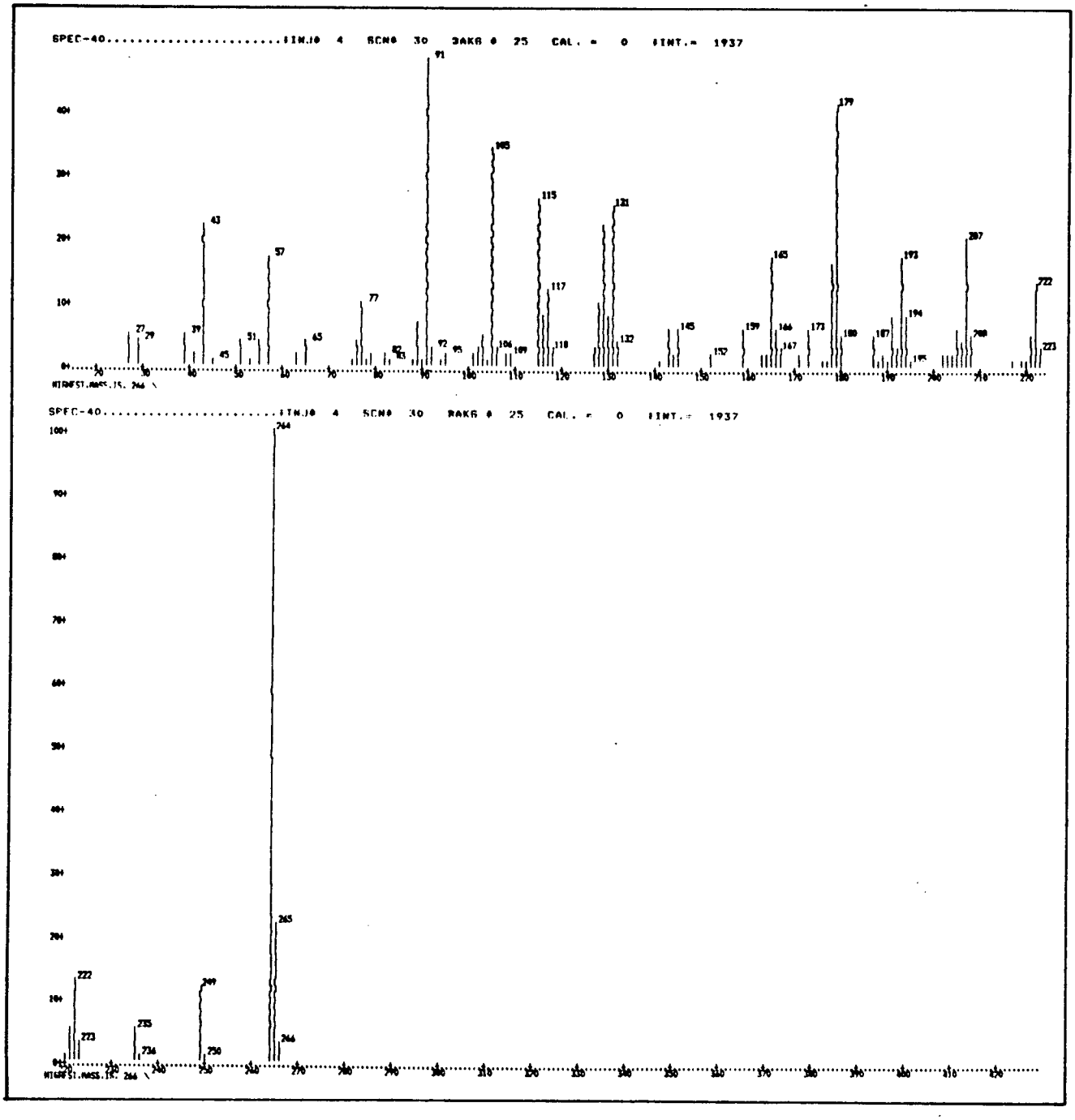
7. Mass spectrum (GCMS) of 2,2-diphenyl-4-valerolactone (41)



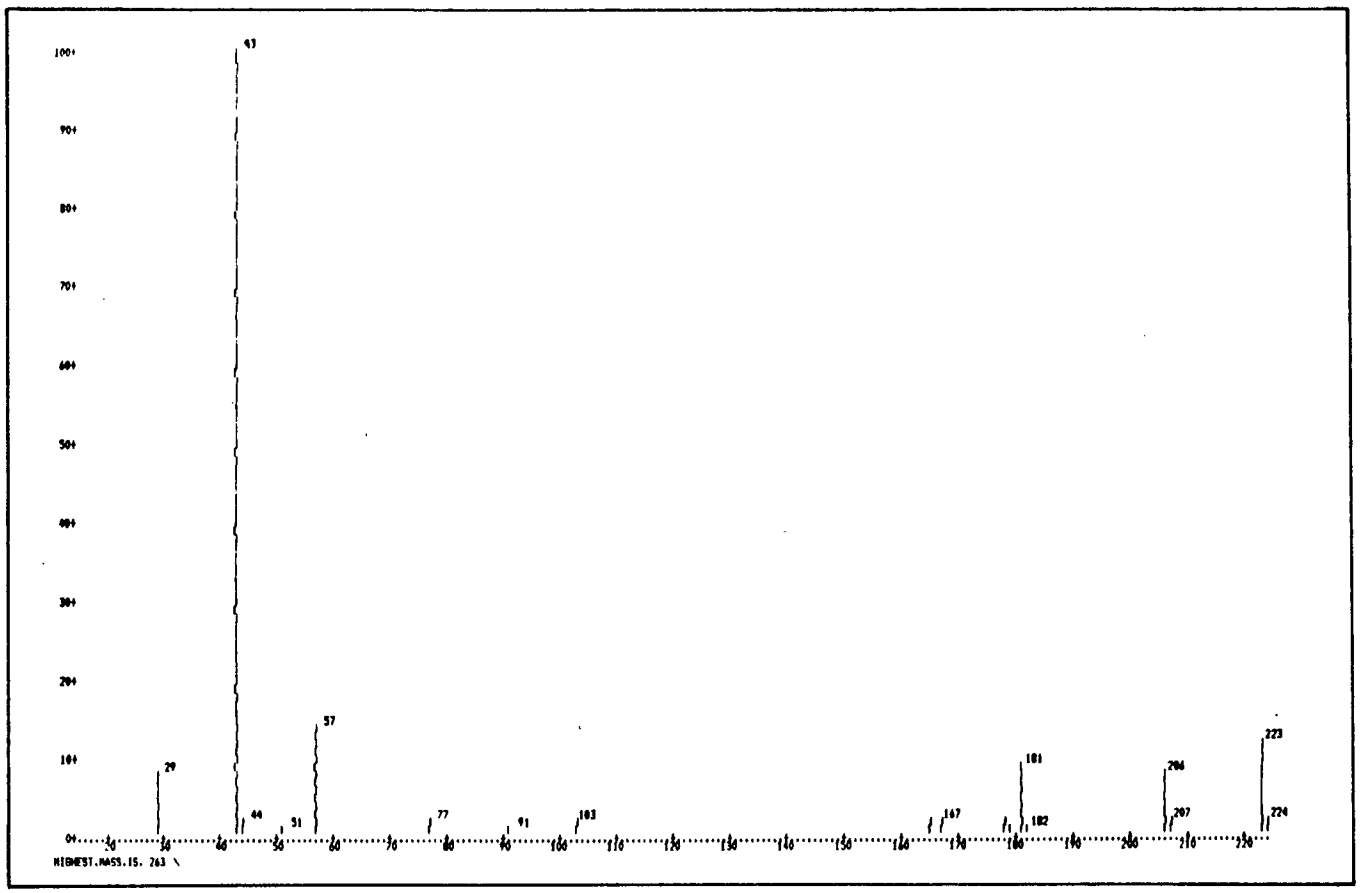
8. Mass spectrum (GCMS) of 1,1-diphenyl-2-butanone (42)



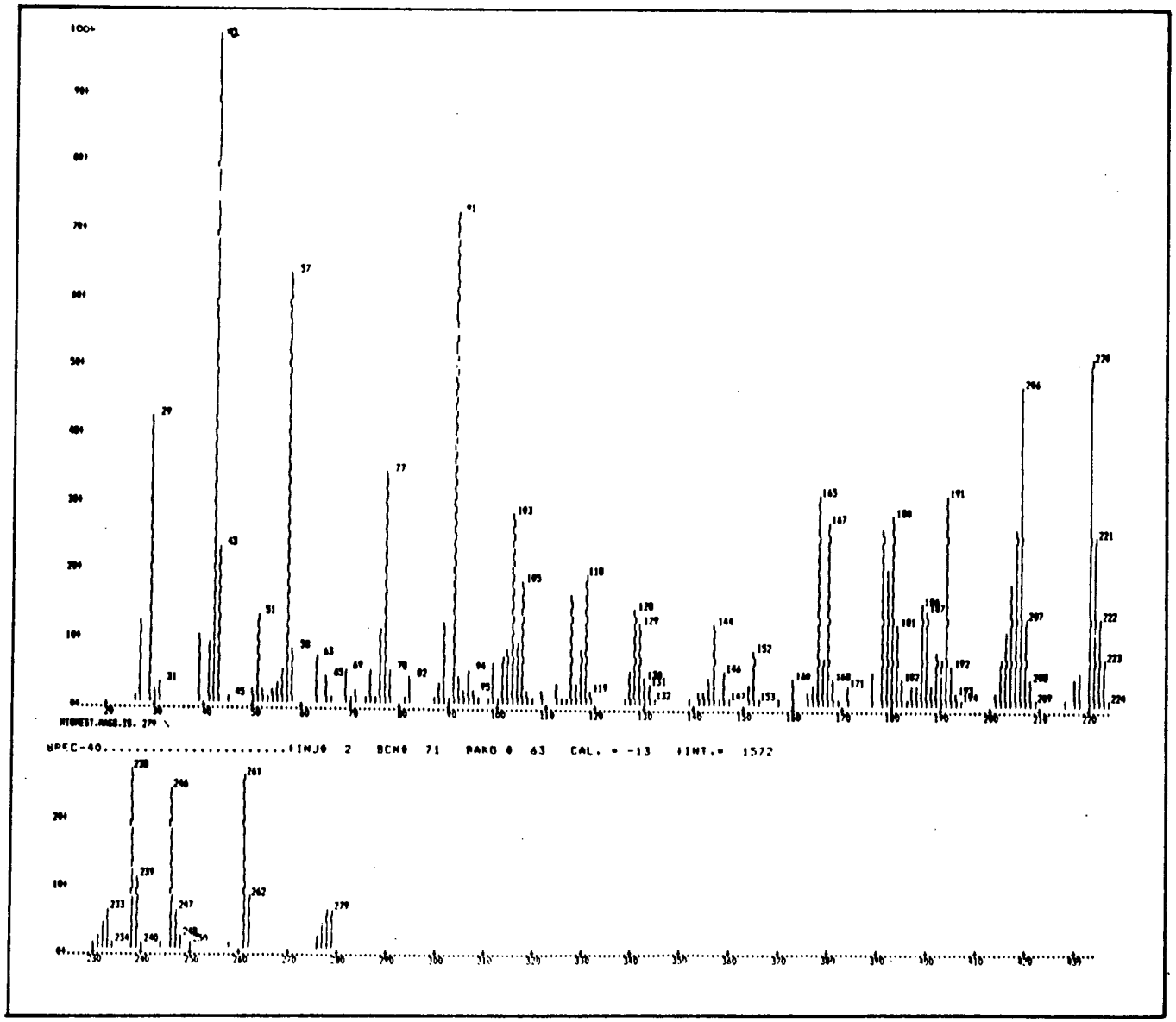
9. Mass spectrum (GCMS) of 4,4-diphenyl-2,5-heptane diol (43)



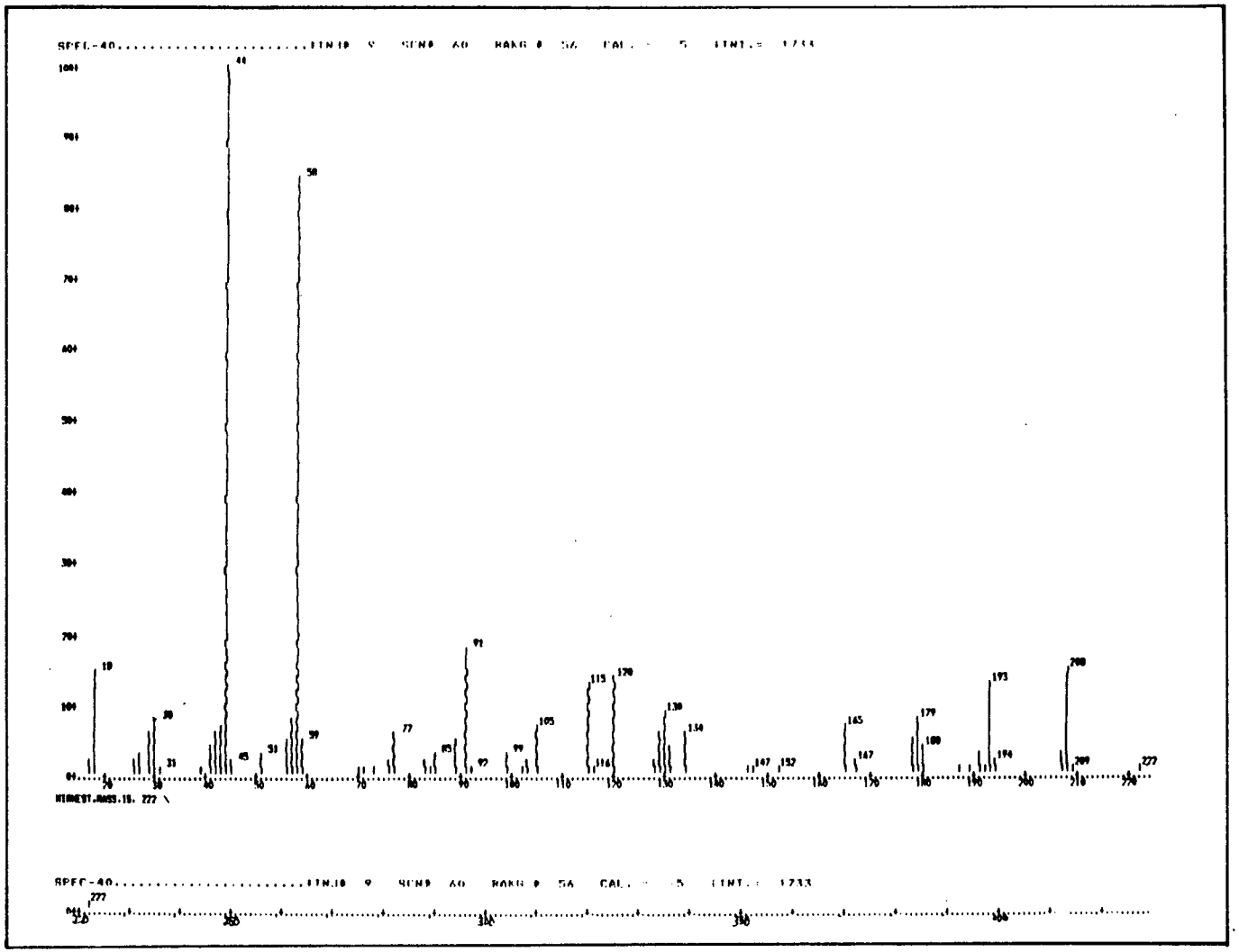
10. Mass spectrum (GCMS) of 3,3-diphenyl-2-ethylidene-5-methyl-tetrahydrofuran (44)



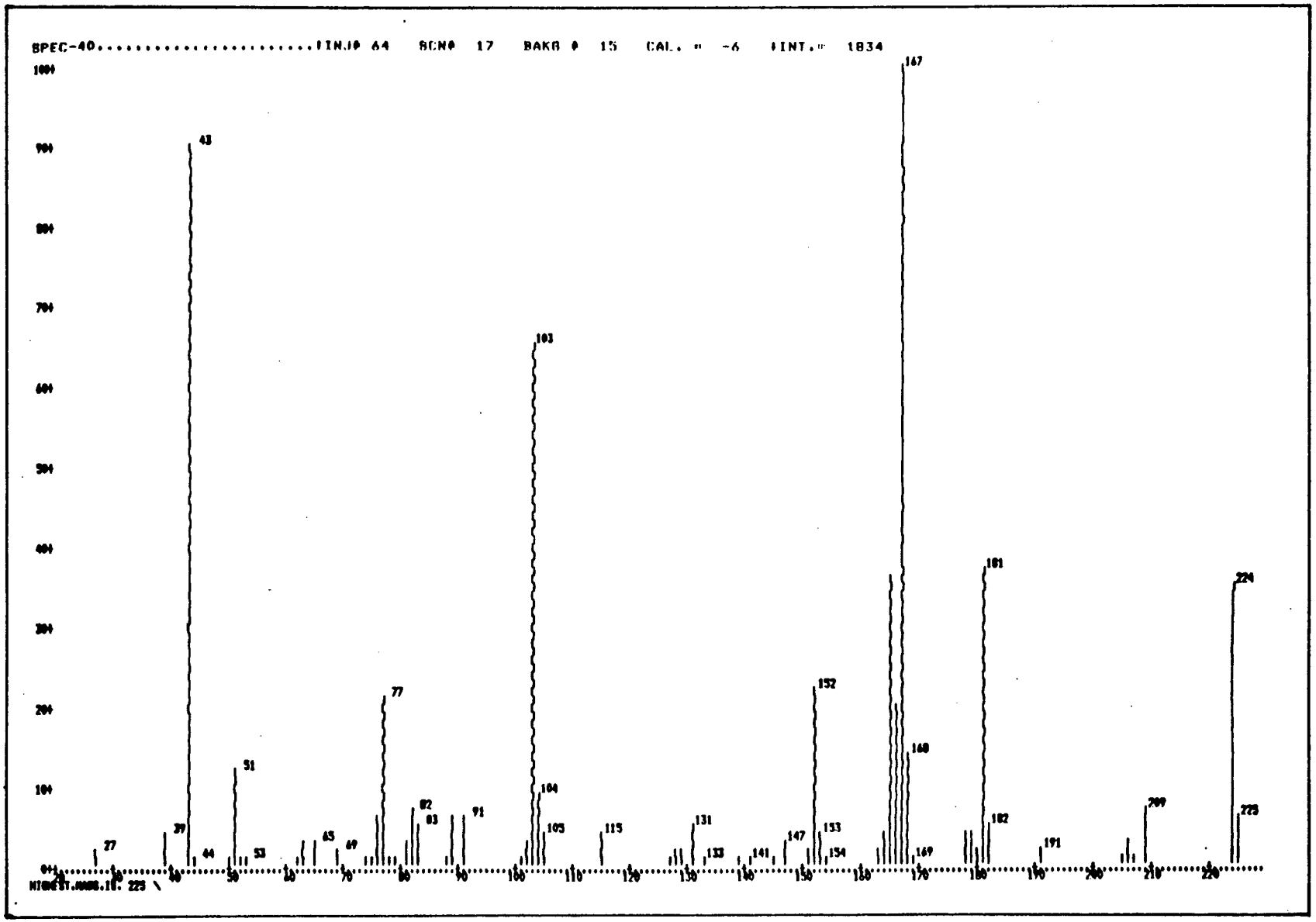
11. Mass spectrum (GCMS) of 4,4-diphenyl-2,5-heptanedione (45)



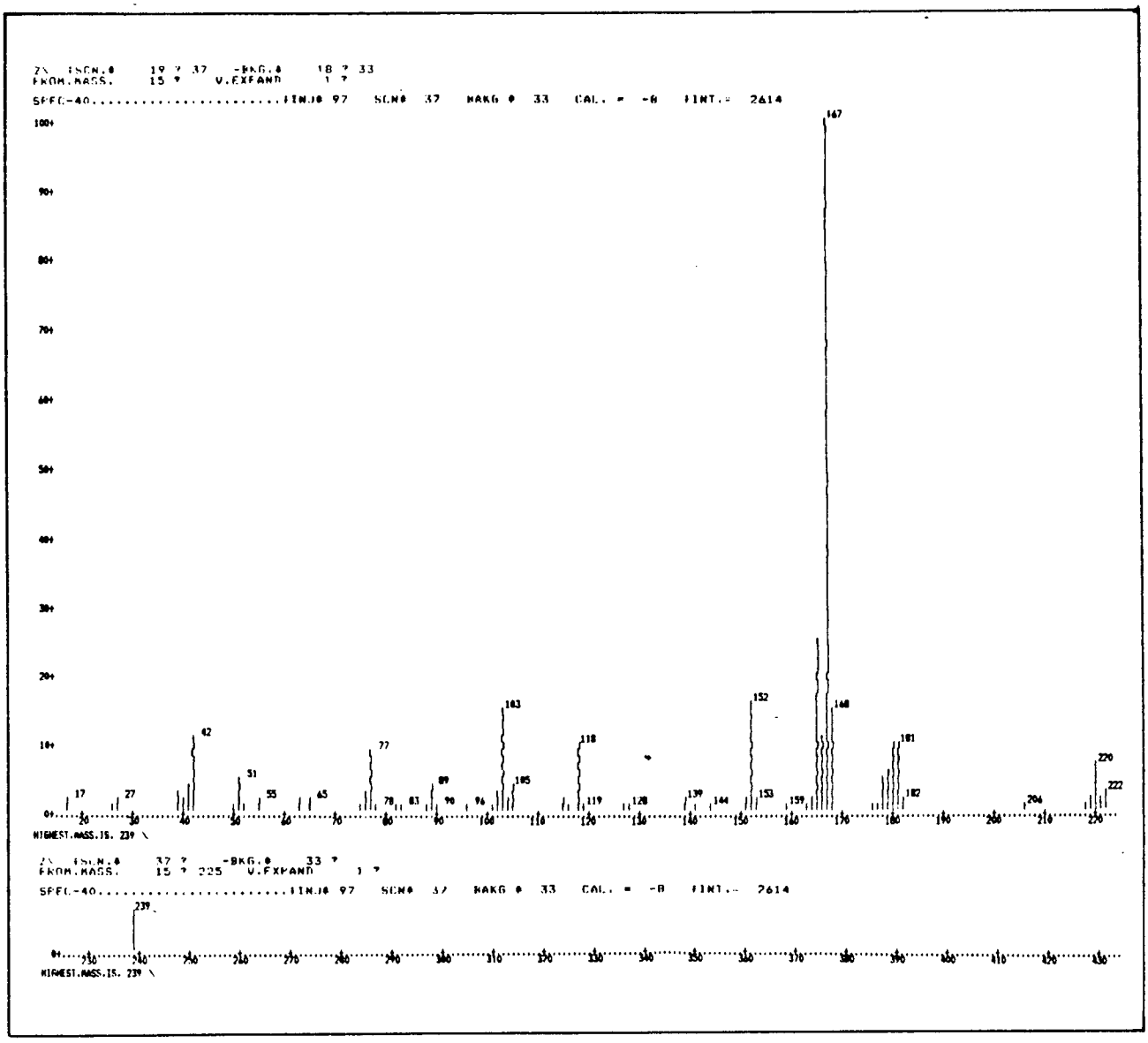
12. Mass spectrum (GCMS) of 4,4-diphenyl-2,5-heptanedione-2-oxime (46)



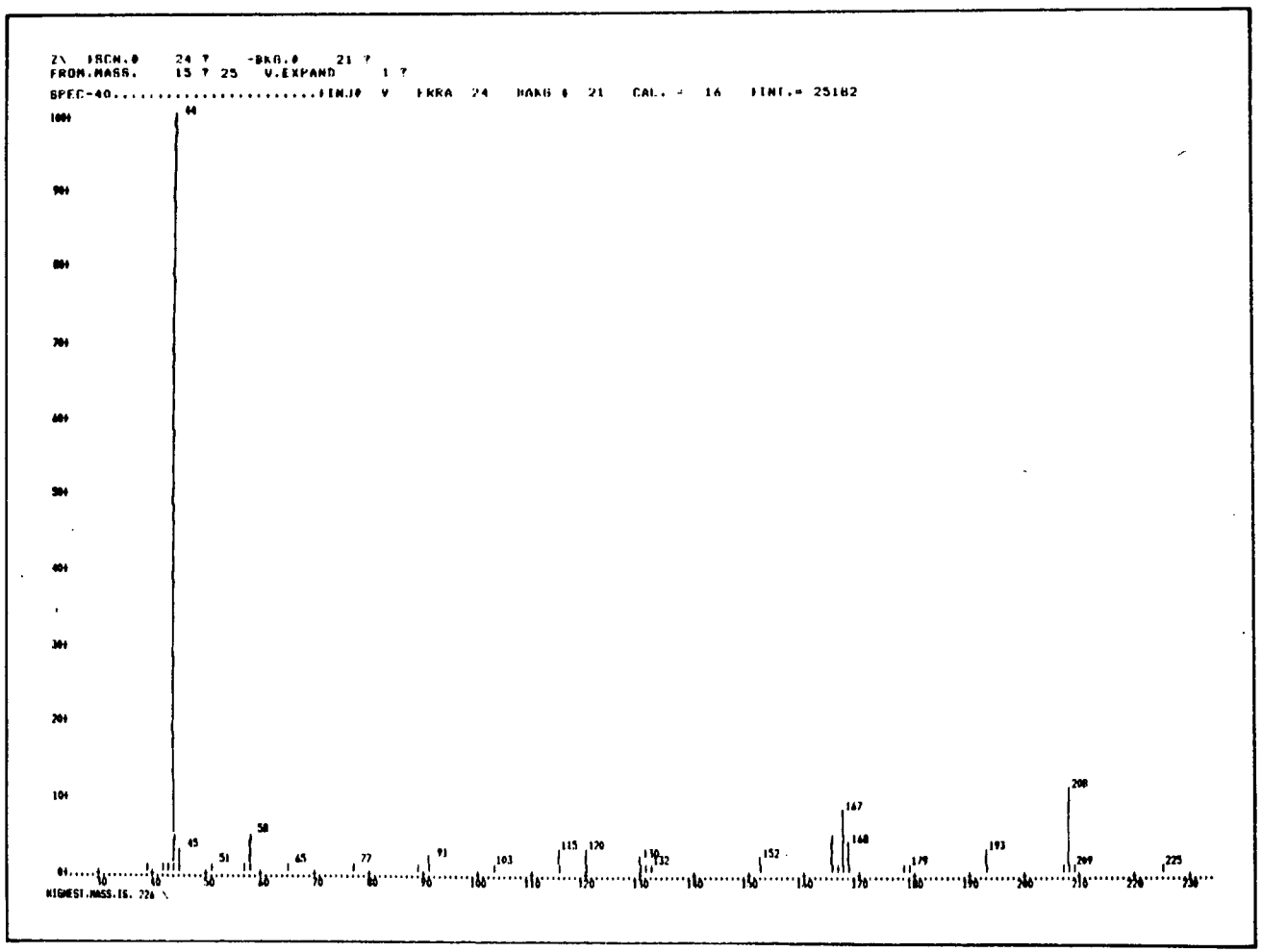
13. Mass spectrum (GCMS) of 4,4-diphenyl-5-hydroxy-2-amino heptane (dinormethadol) (49)



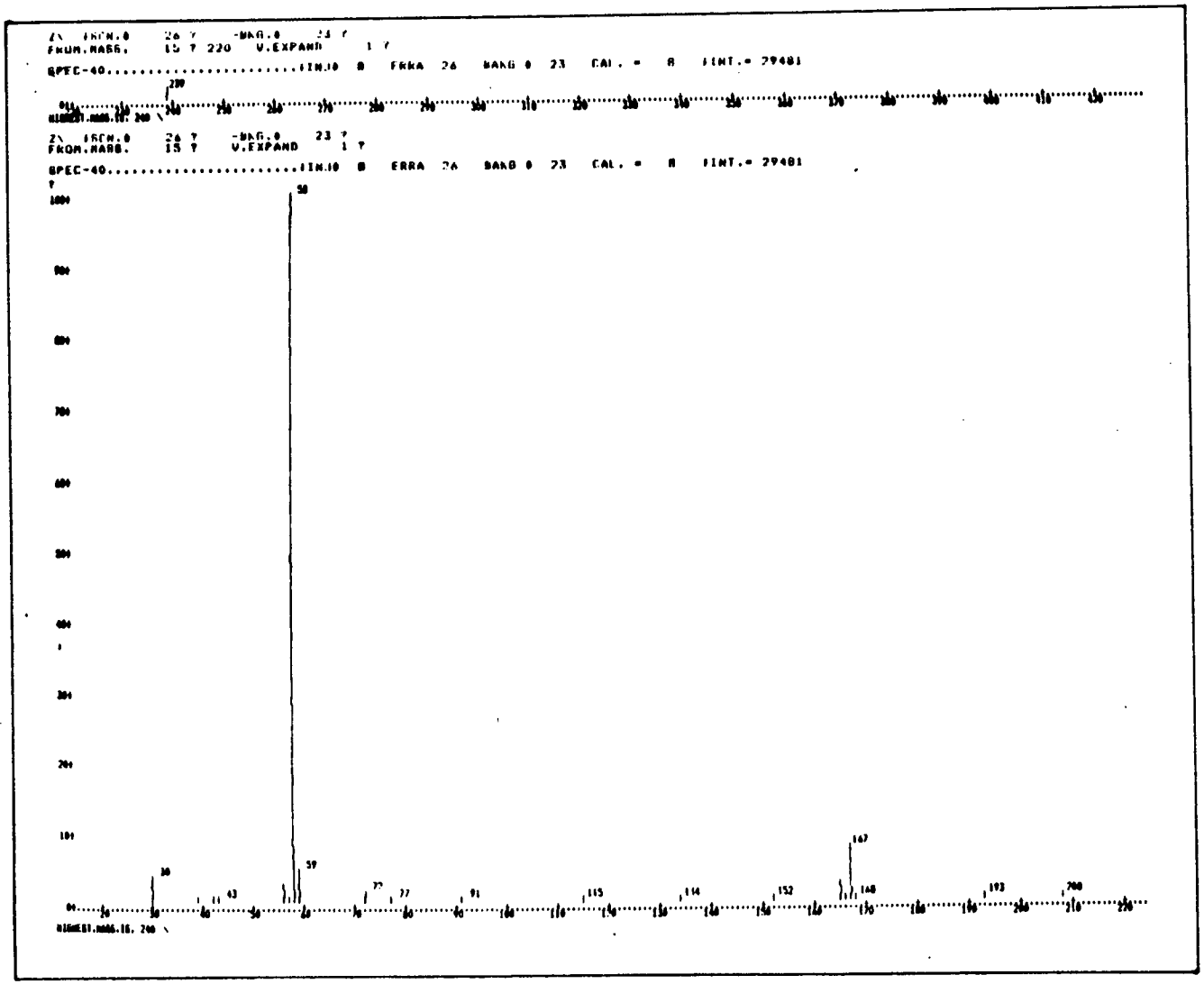
14. Mass spectrum (GCMS) of 1,1-diphenyl-3-butanone (51)



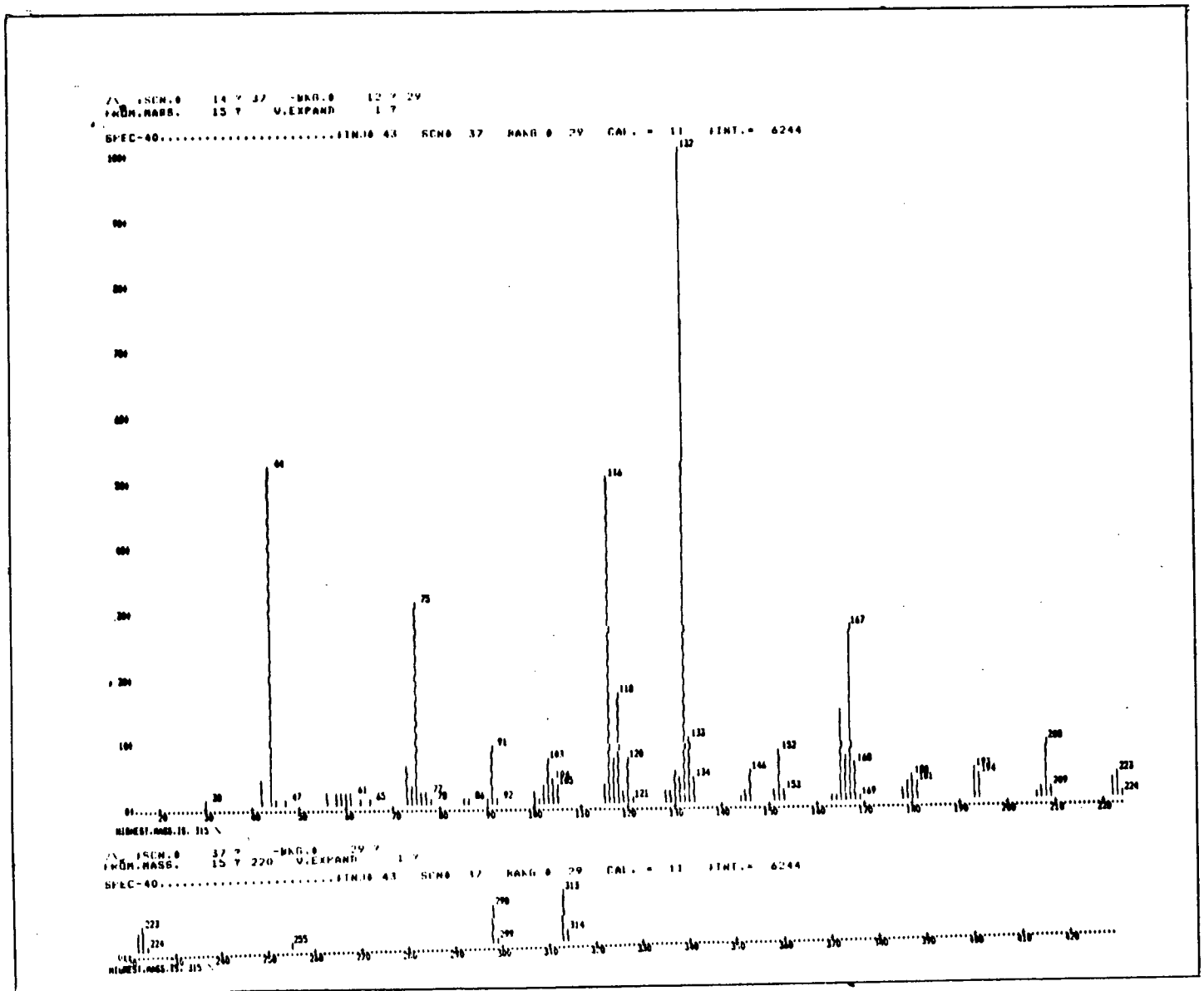
15. Mass spectrum (GCMS) of 1,1-diphenyl-3-butanone oxime (52)



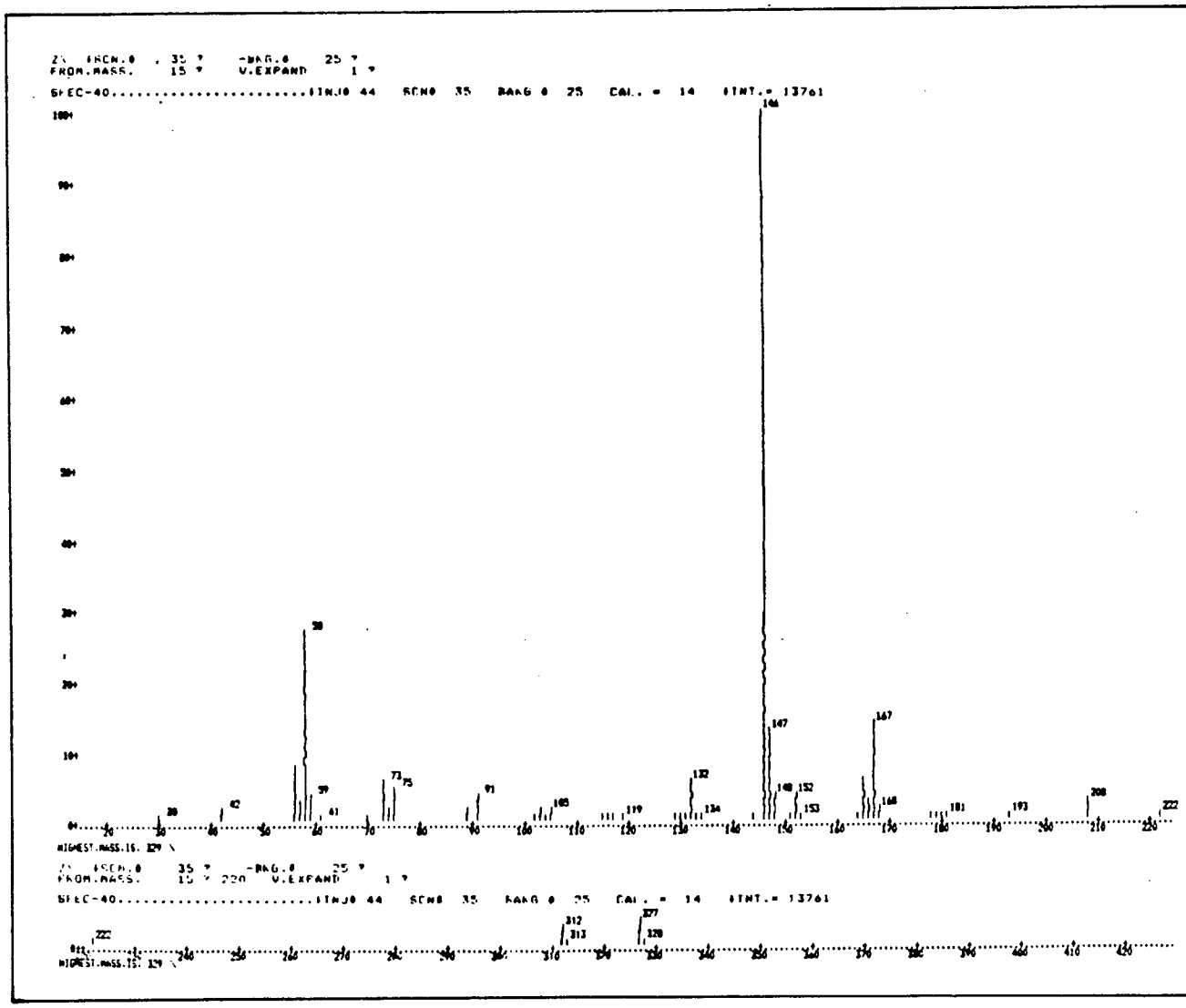
16. Mass spectrum (GCMS) of α-methyl-γ-phenyl-benzenepropanamine (53)



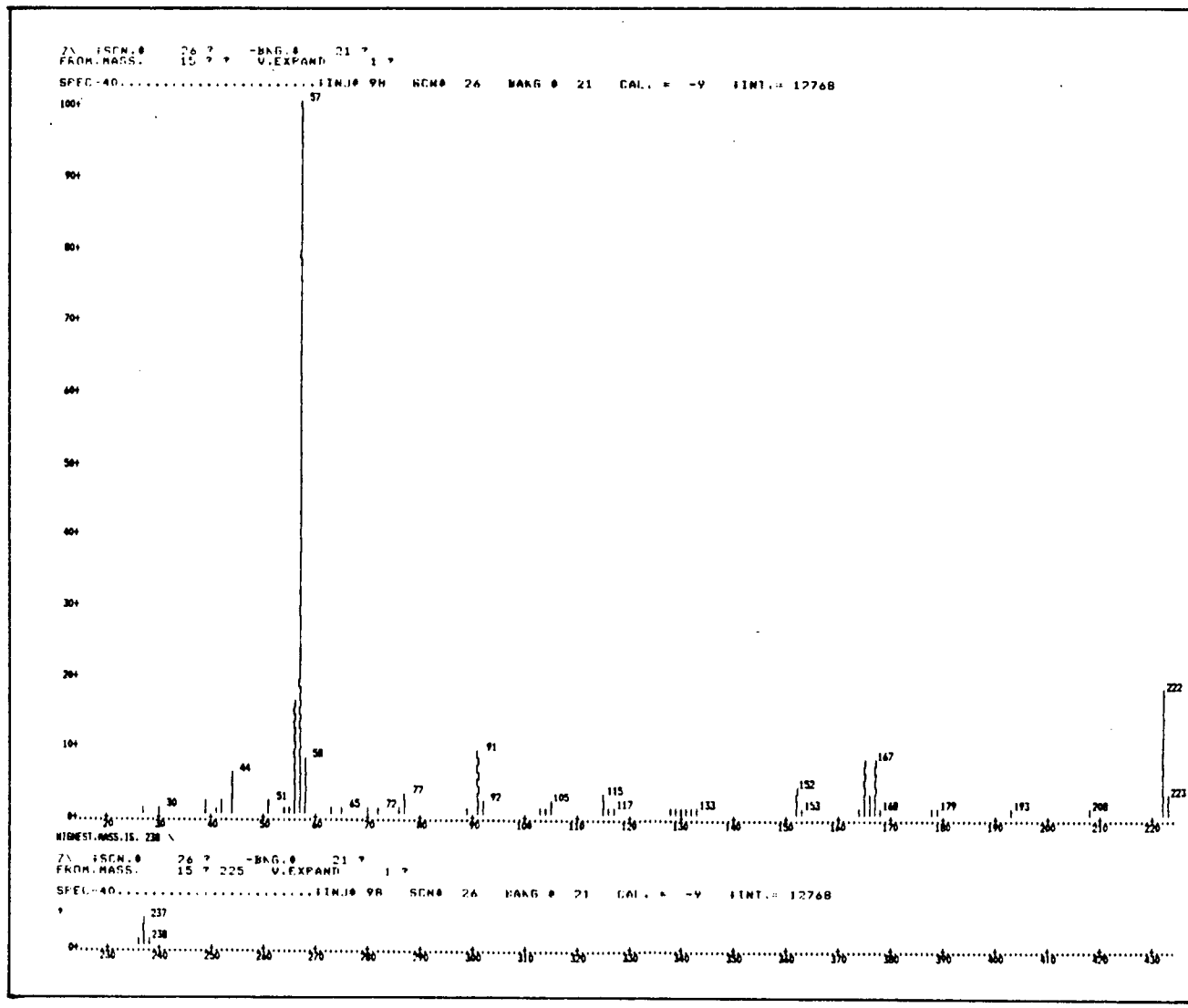
17. Mass spectrum (GCMS) of -N, α -dimethyl-γ-phenyl benzenepropanamine (54)



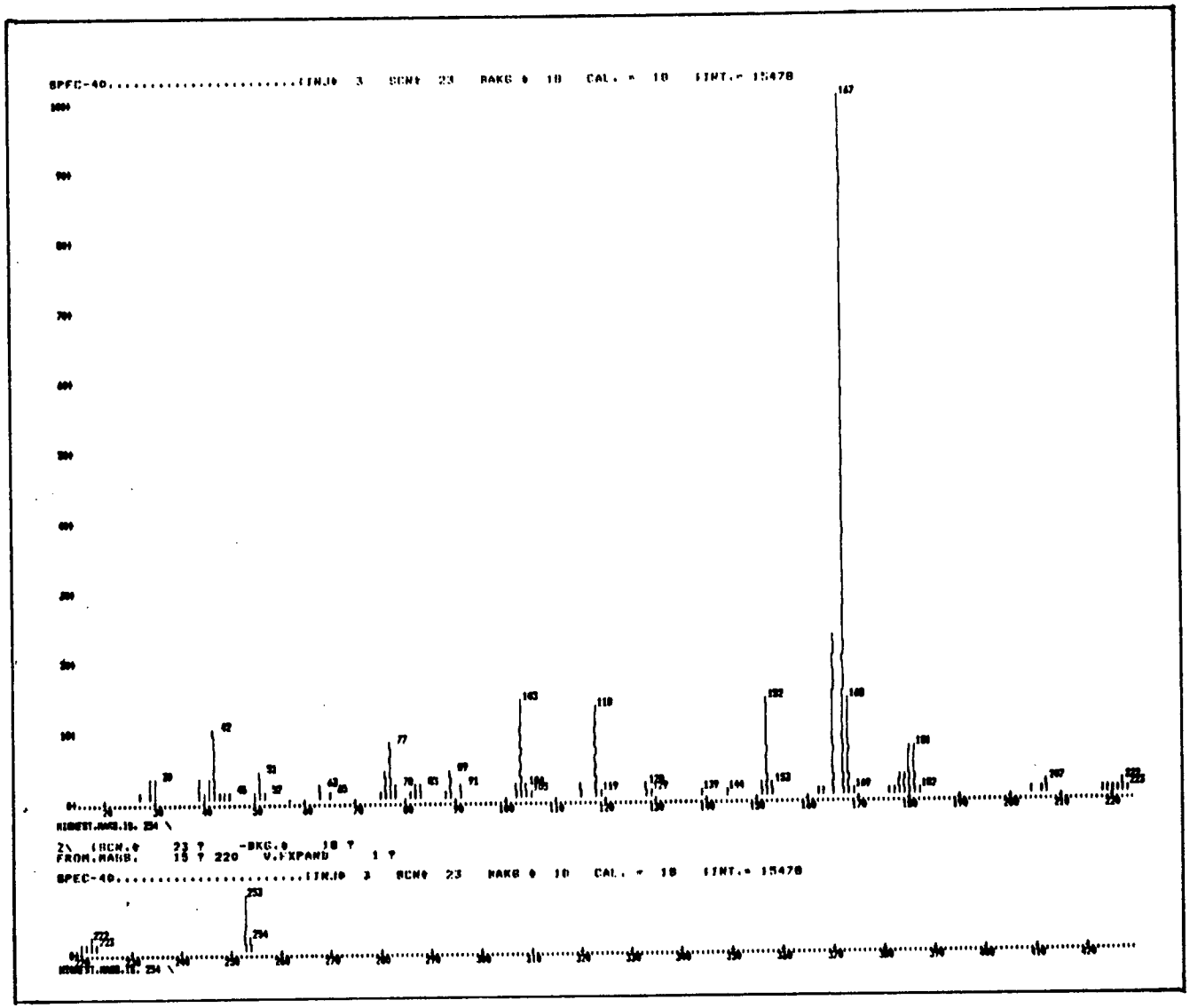
18. Mass spectrum (GCMS) of N-hydroxy-α-methyl-γ-phenyl benzenepropanamine-TMS-ether (56)



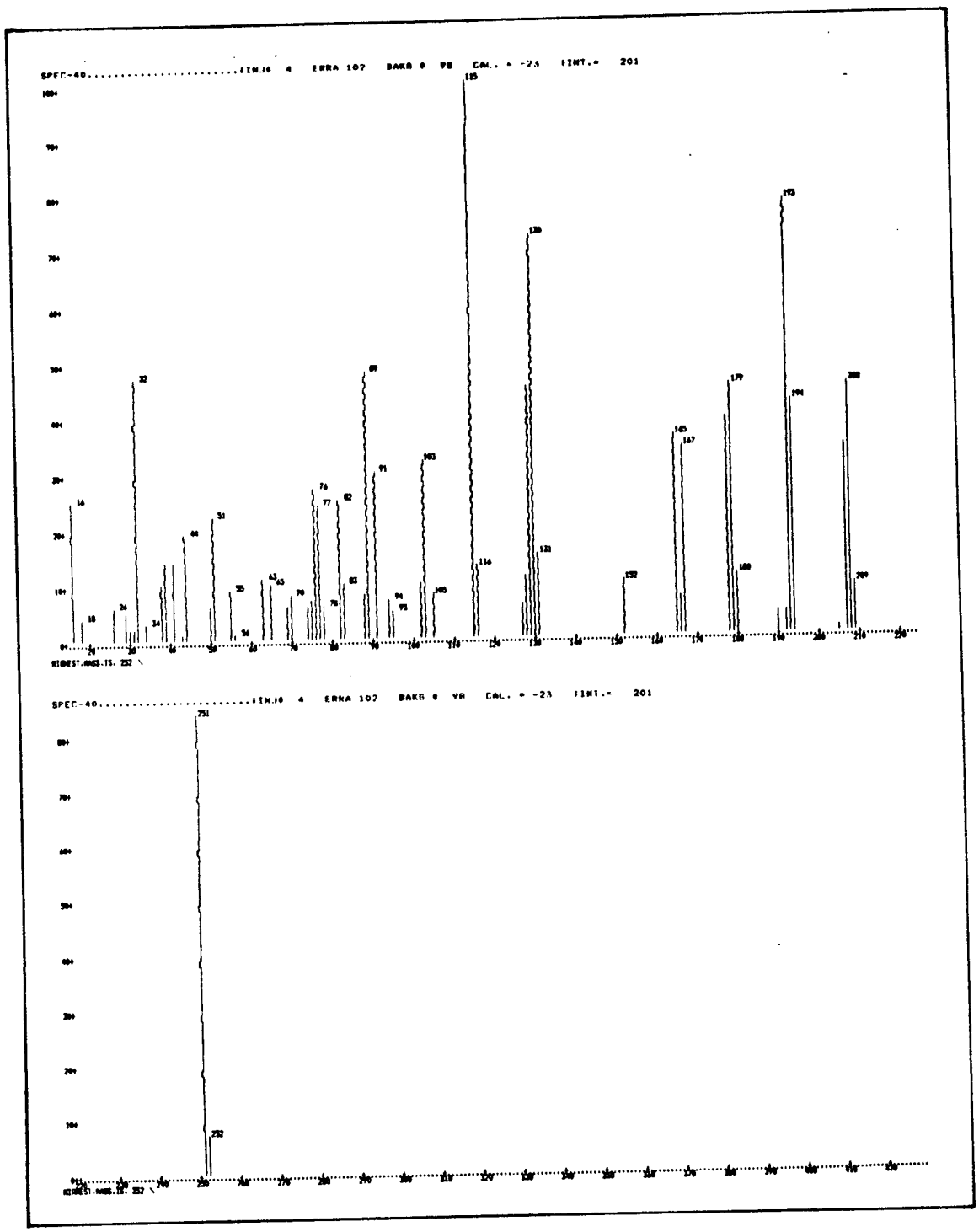
19. Mass spectrum (GCMS) of N,α-dimethyl-N-hydroxy-γ- phenyl benzenepropanamine-TMS-ether (58)



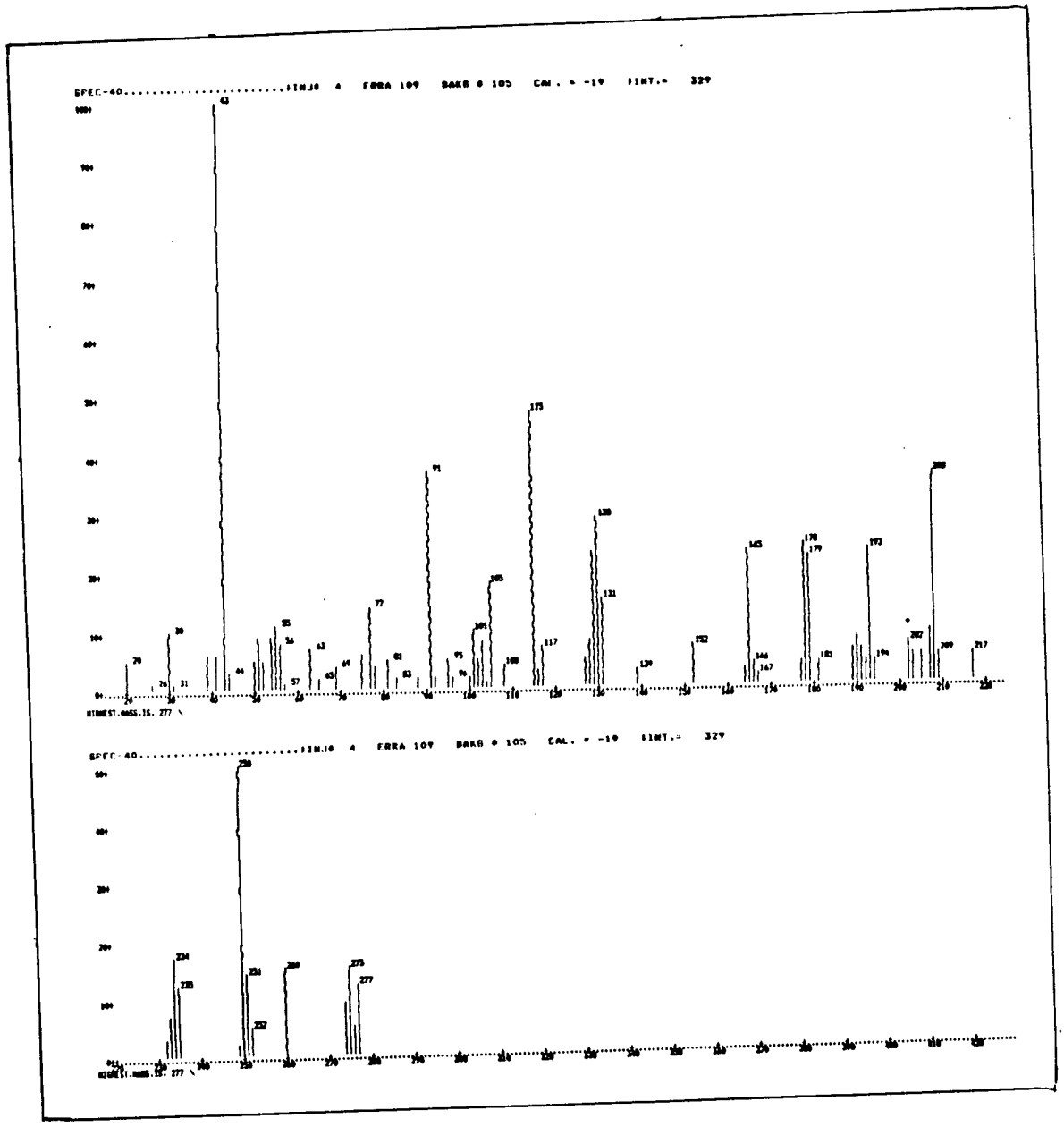
20. Mass spectrum (GCMS) of α-methyl-N-methylidene-γ-phenyl-benzenepropanamine (trimer) (59)



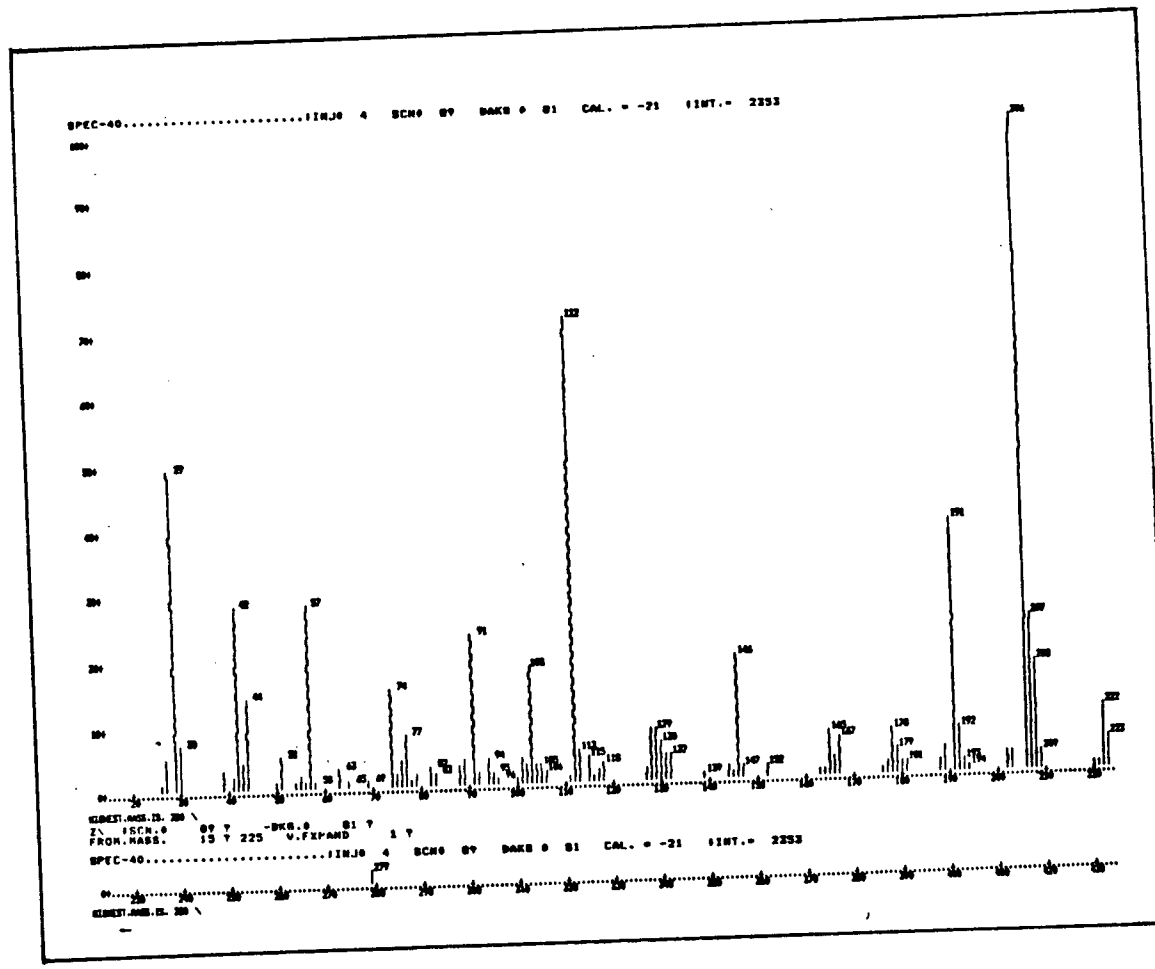
21. Mass spectrum (GCMS) of 1,1-diphenyl-3-butanone oxime-O-methyl ether (64)



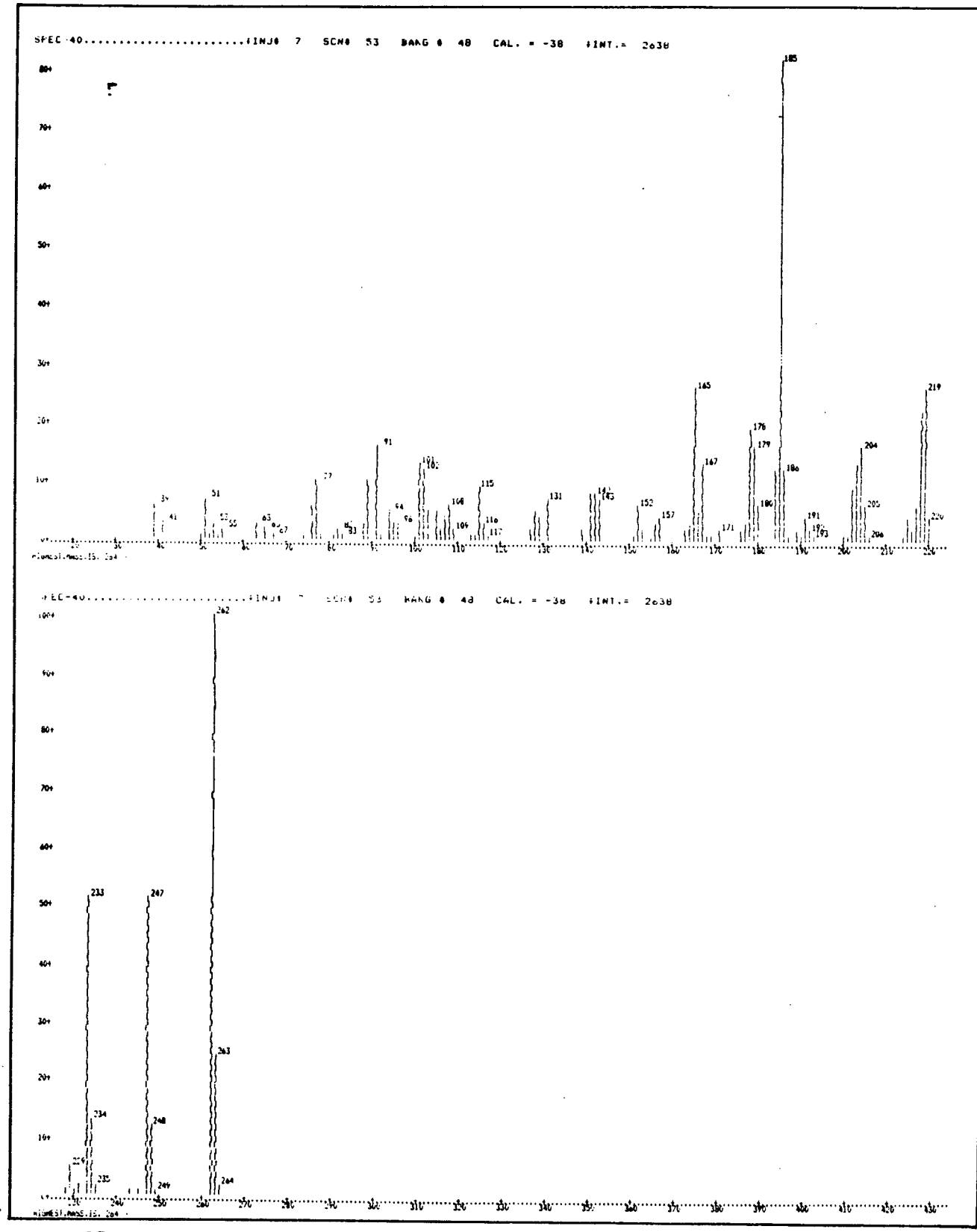
22. Mass spectrum (GCMS) of EMDP oxidation product MDP (71)



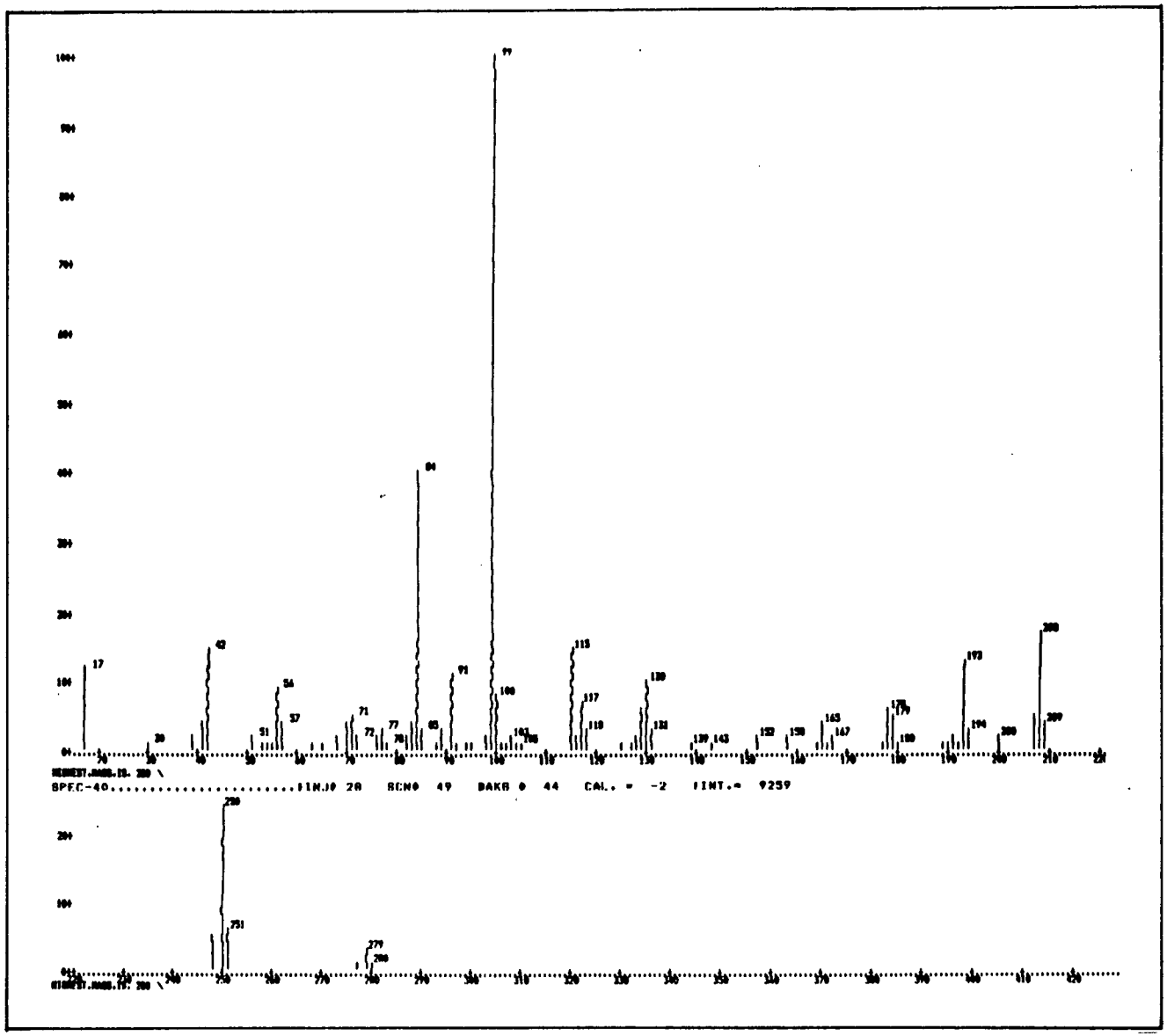
23. Mass spectrum (GCMS) of EMDP oxidation product G (72)



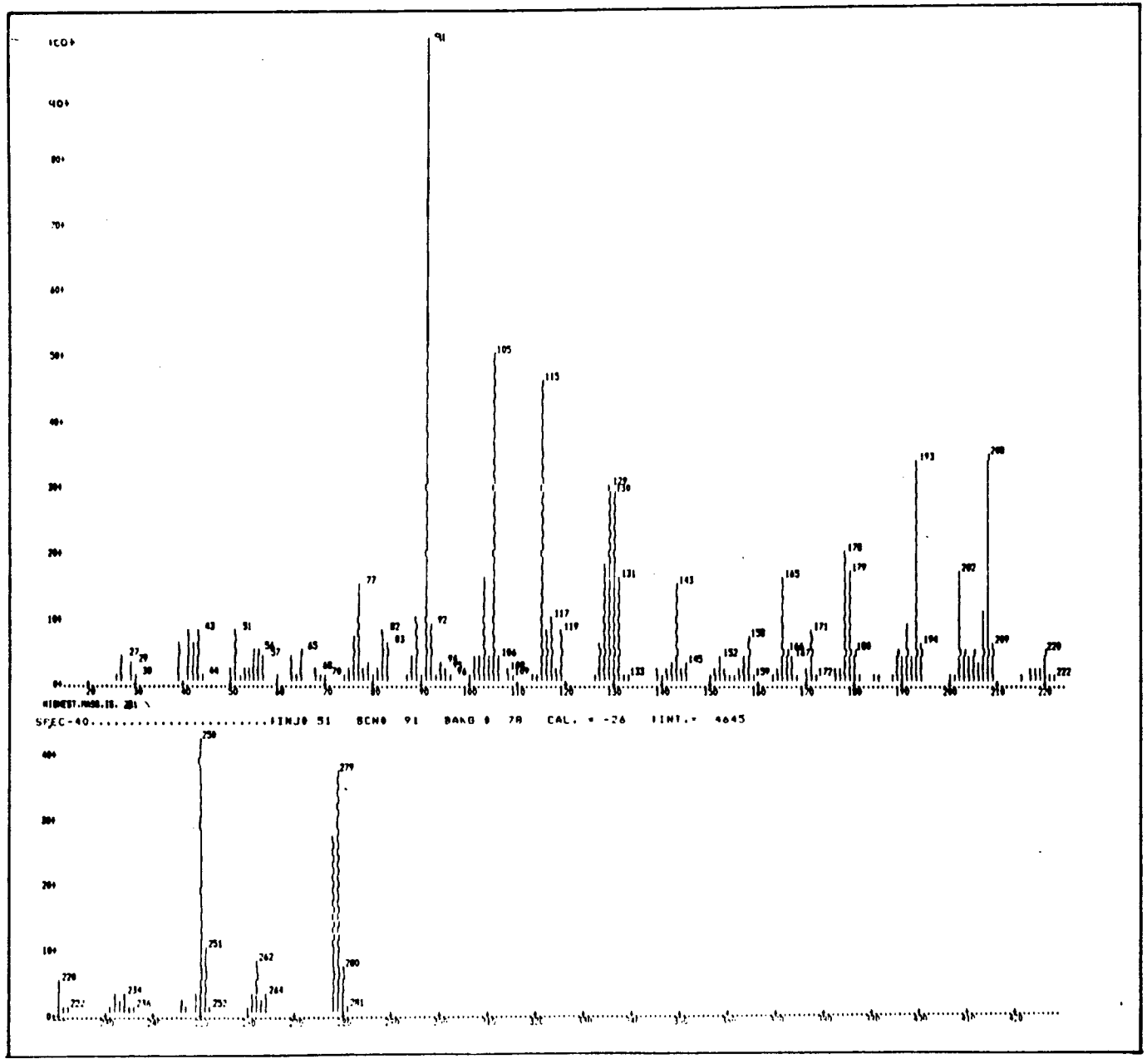
24. Mass spectrum (GCMS) of EMDP oxidation product E (72)



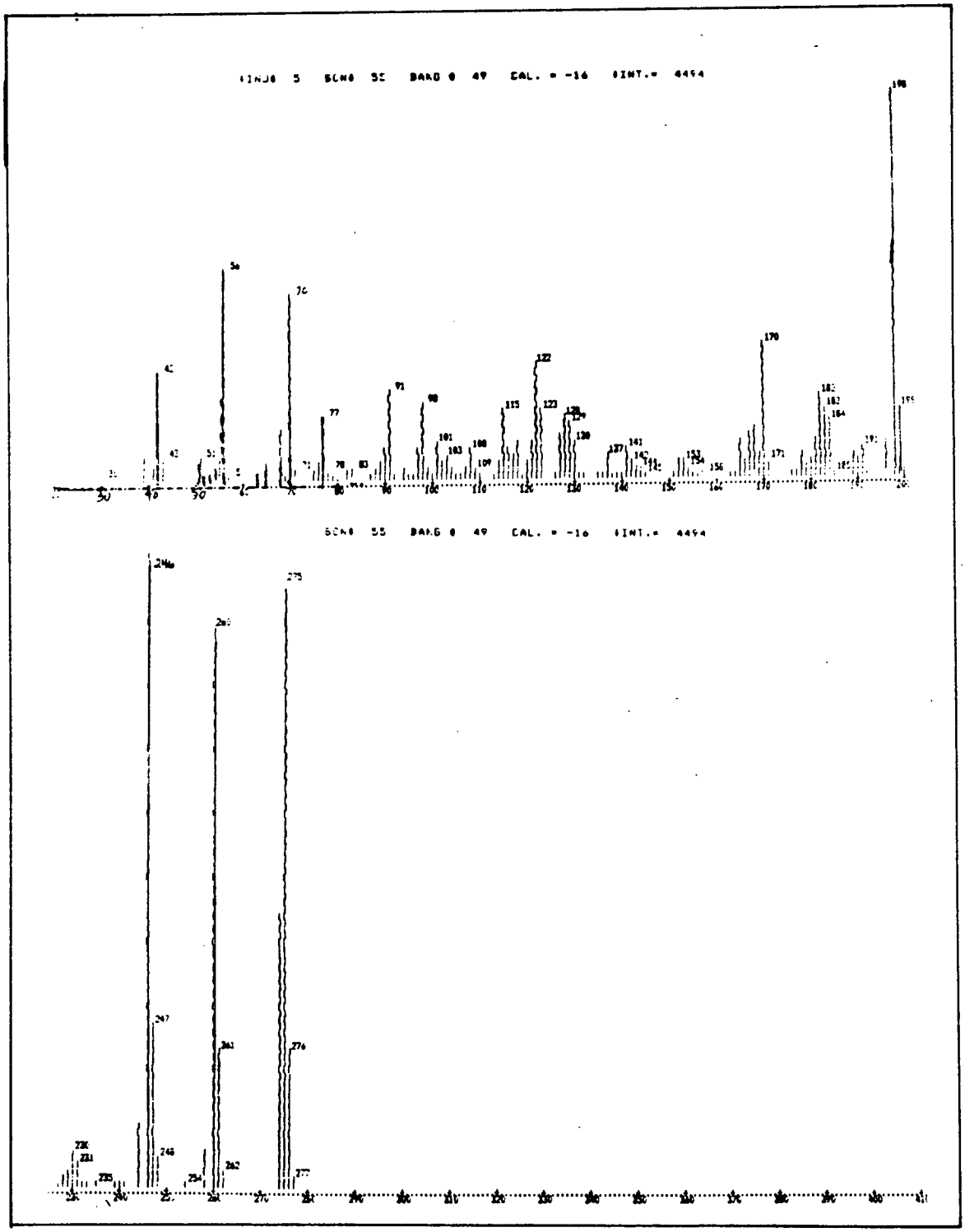
25. Mass spectrum of cyclopentenone product of alkaline treatment of diketone (89)



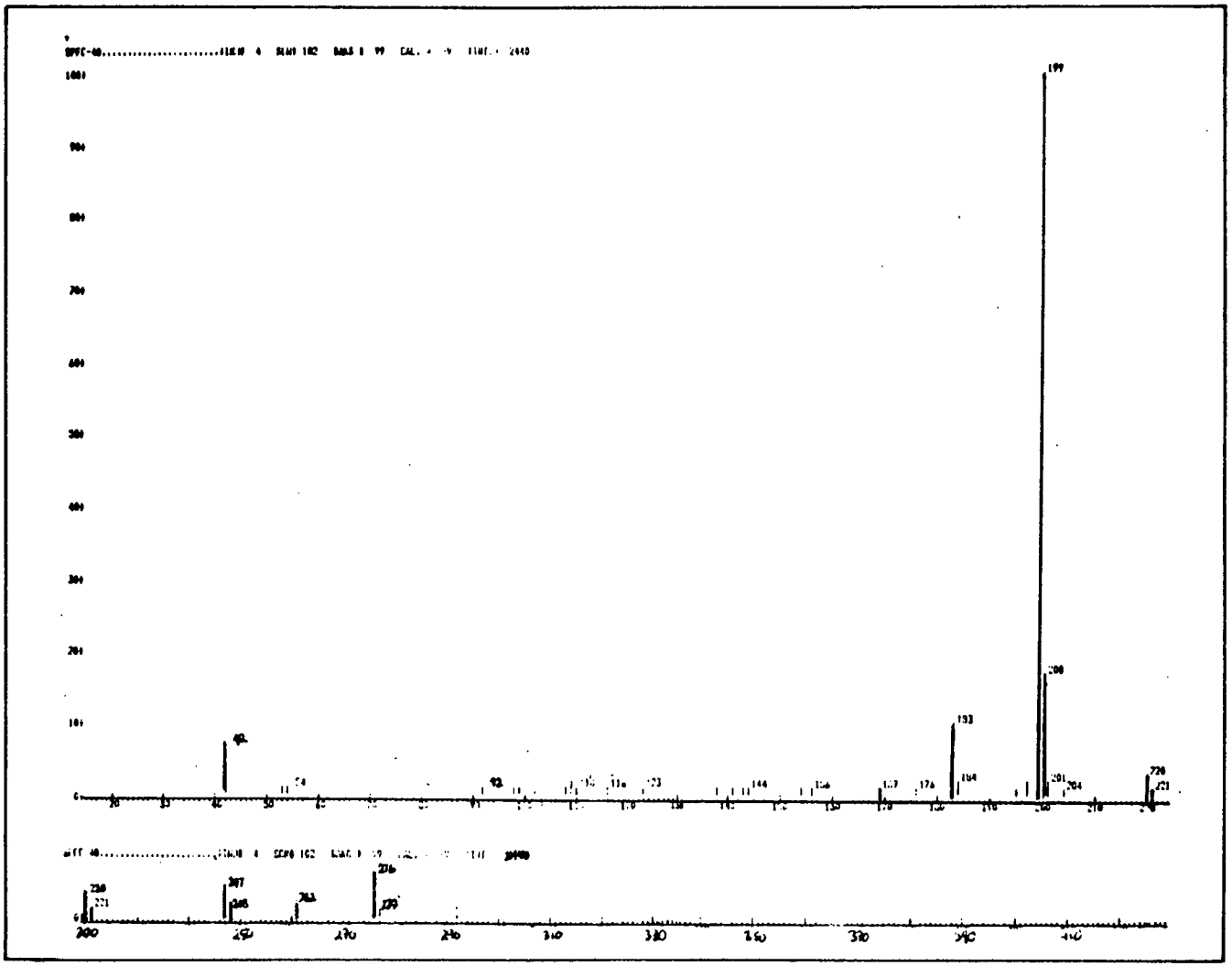
26. Mass spectrum of N_aBH_3CN reduction product of the methadone oxaziridine (5)



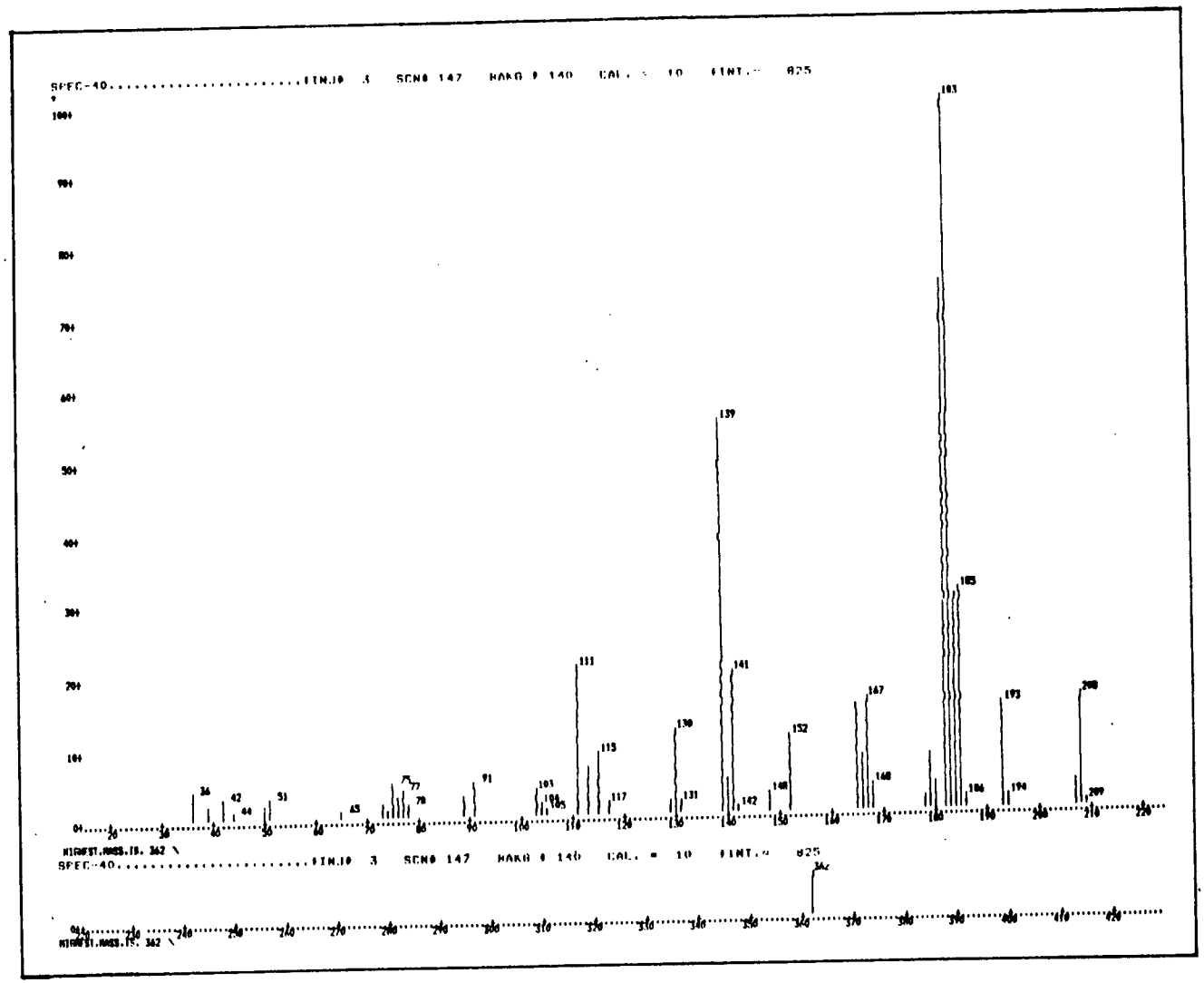
27. Mass spectrum (GCMS) of ethyl methyl diphenyl pyrollidine like reductive hydroxylamination product of diketone



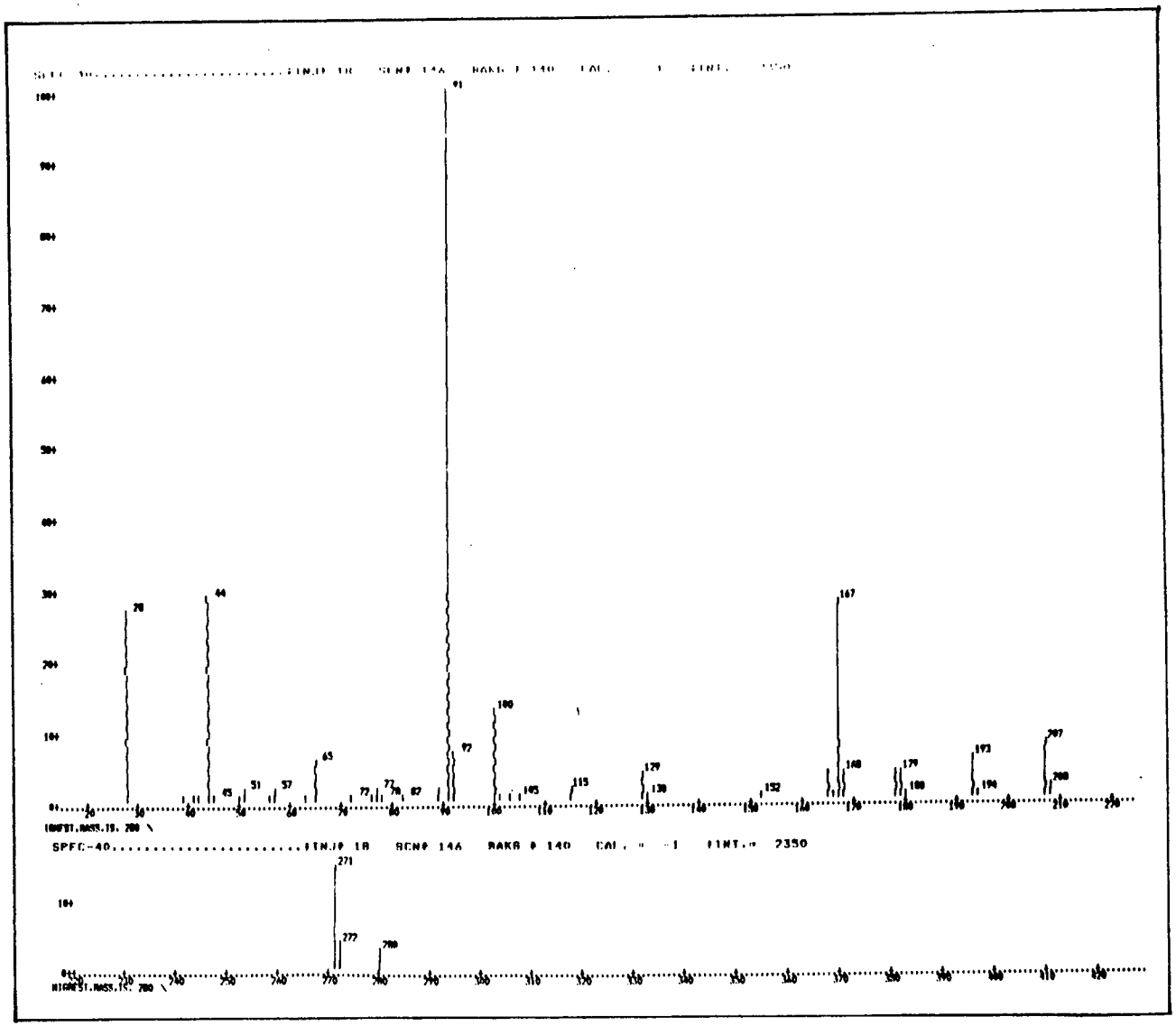
28. Mass spectrum (GCMS) of product of reaction of diketone and methylamine (Dimethyl diphenyl ethylidene 2,3-dihydro pyrrole



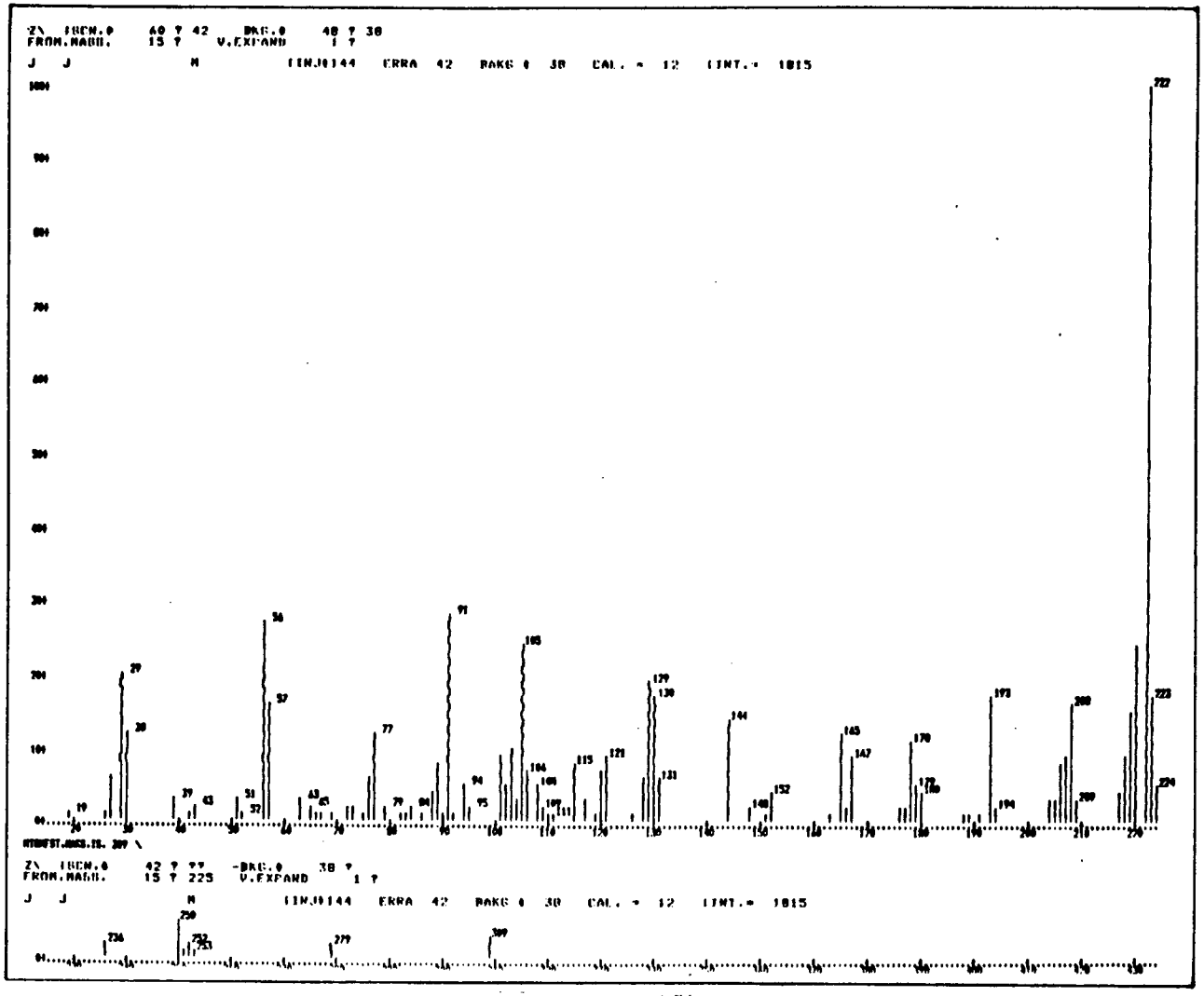
29. Mass spectrum (GCMS) of EDDP like major product reductive N-methyl hydroxylamination of diketone



30. Mass spectrum (GCMS) of long retention time product of recipavrin nitrone and methyl acrylate.



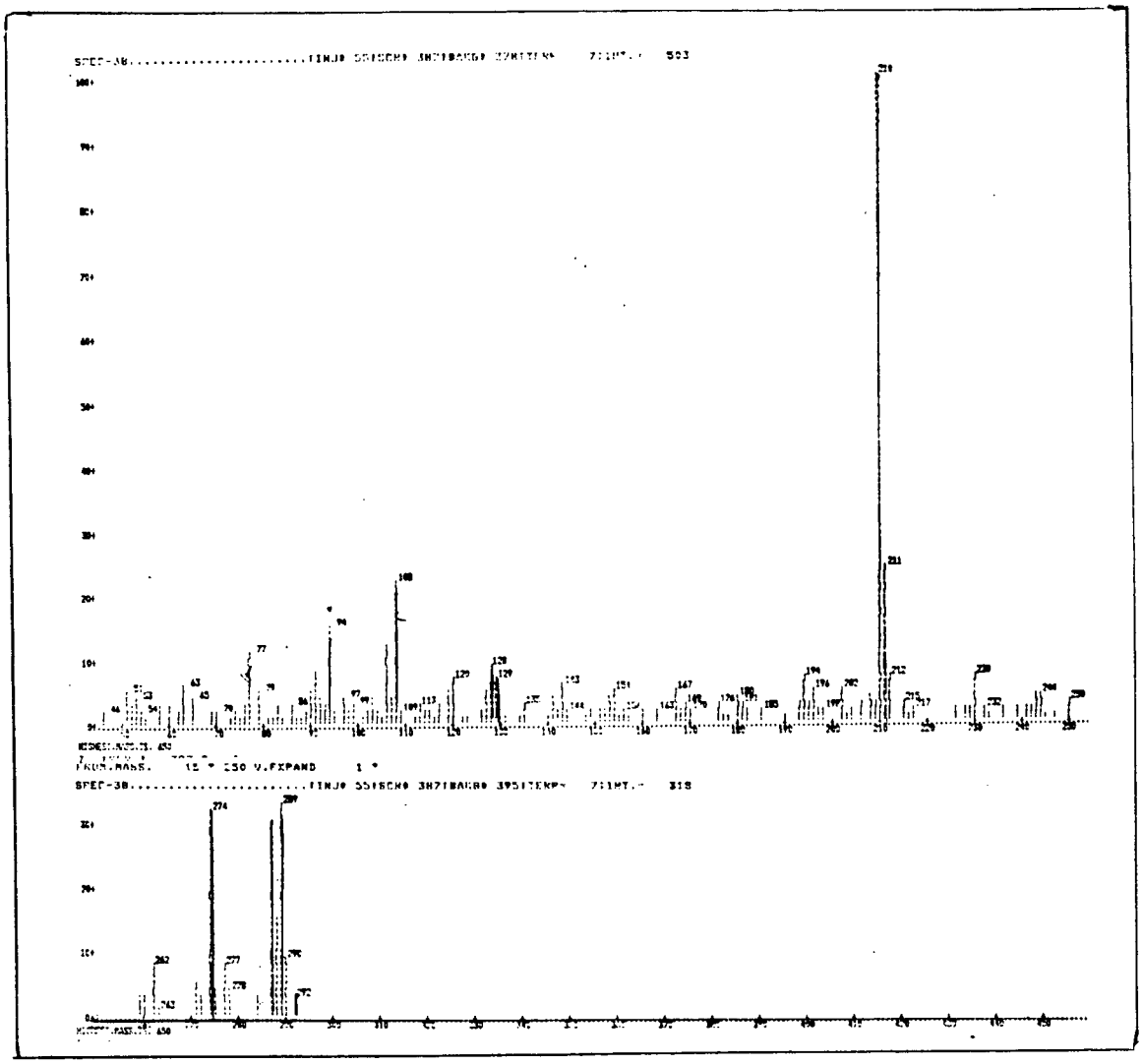
31. Mass spectrum (GCMS) of long retention time product of reaction with thermally isomerized (120°) methadone oxaziridine



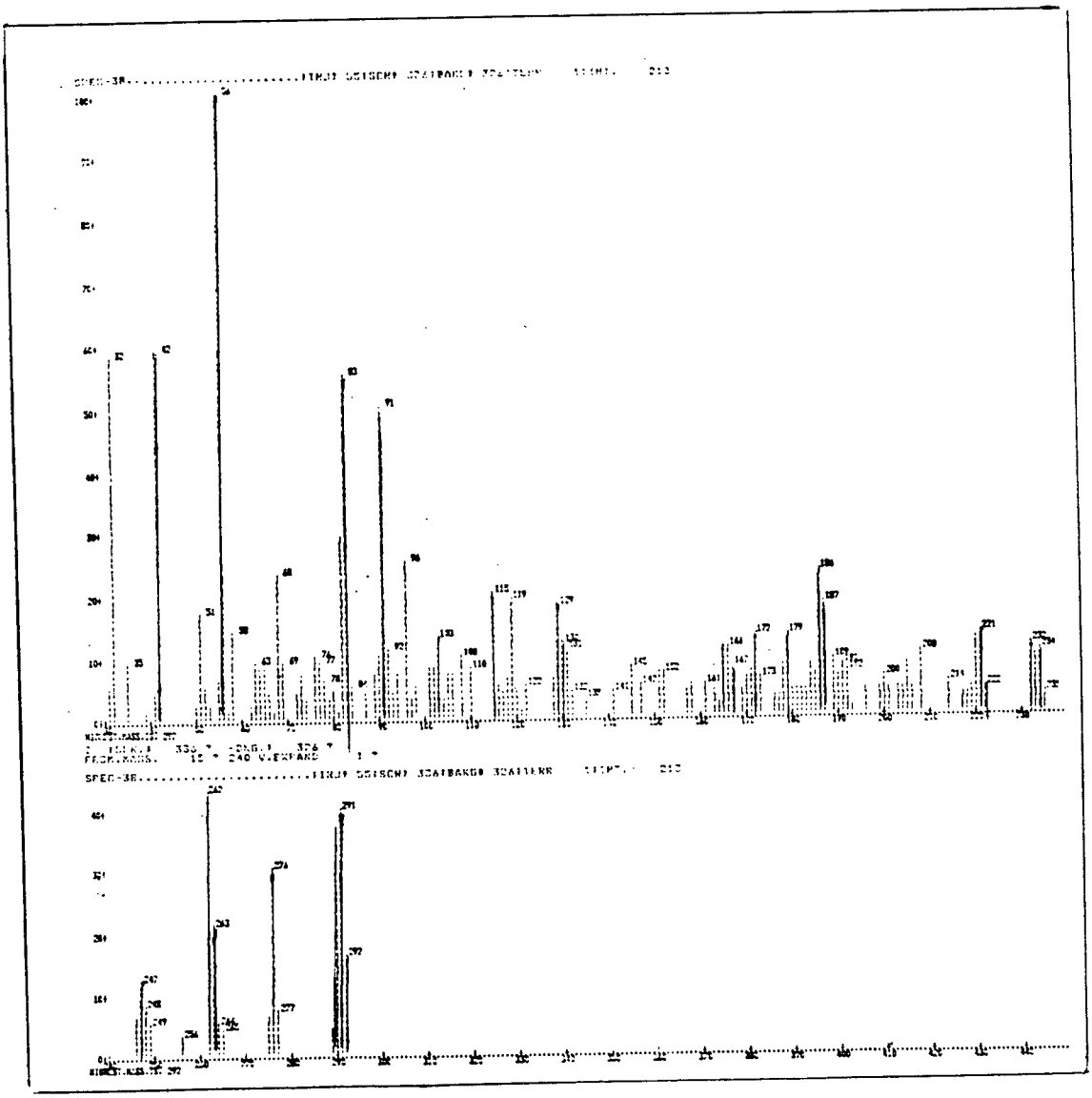
32. Mass spectrum (GCMS) of the by product of thermal isomerization of methadone oxaziridine (5)

APPENDIX

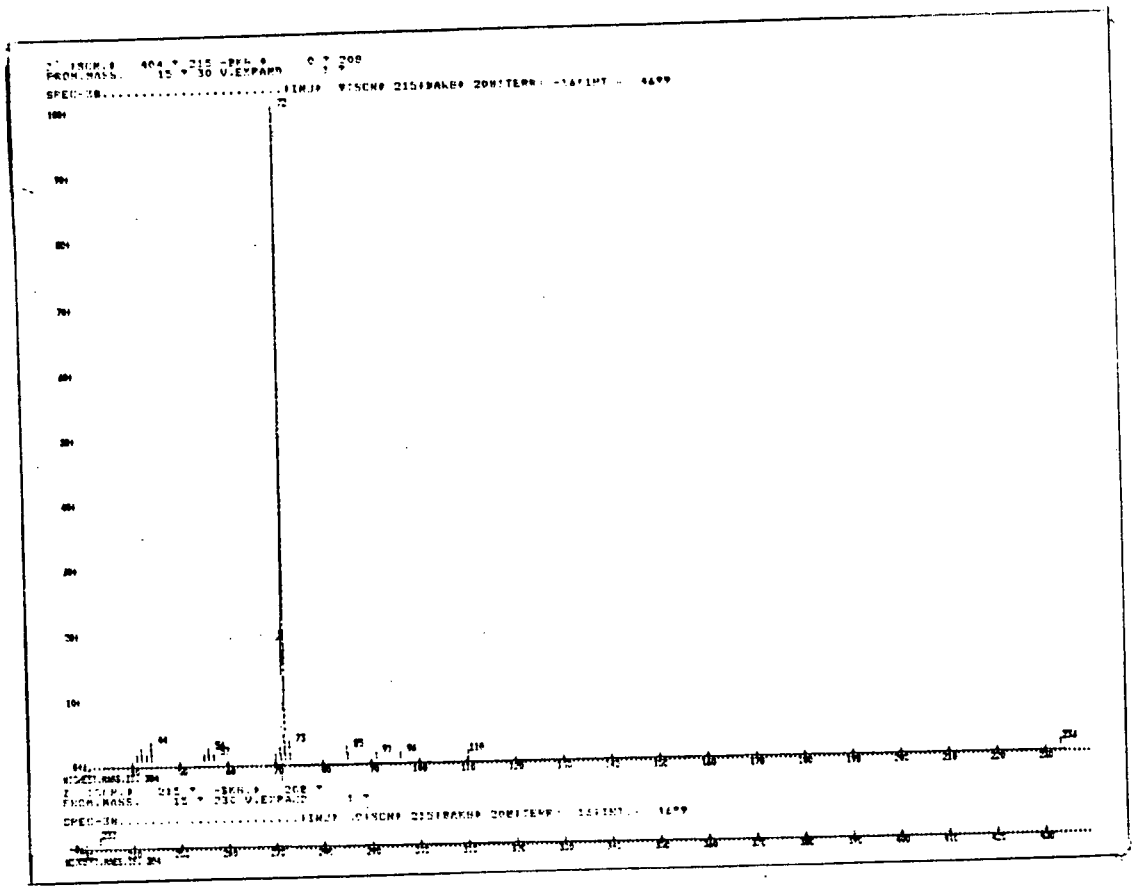
B. METABOLITES



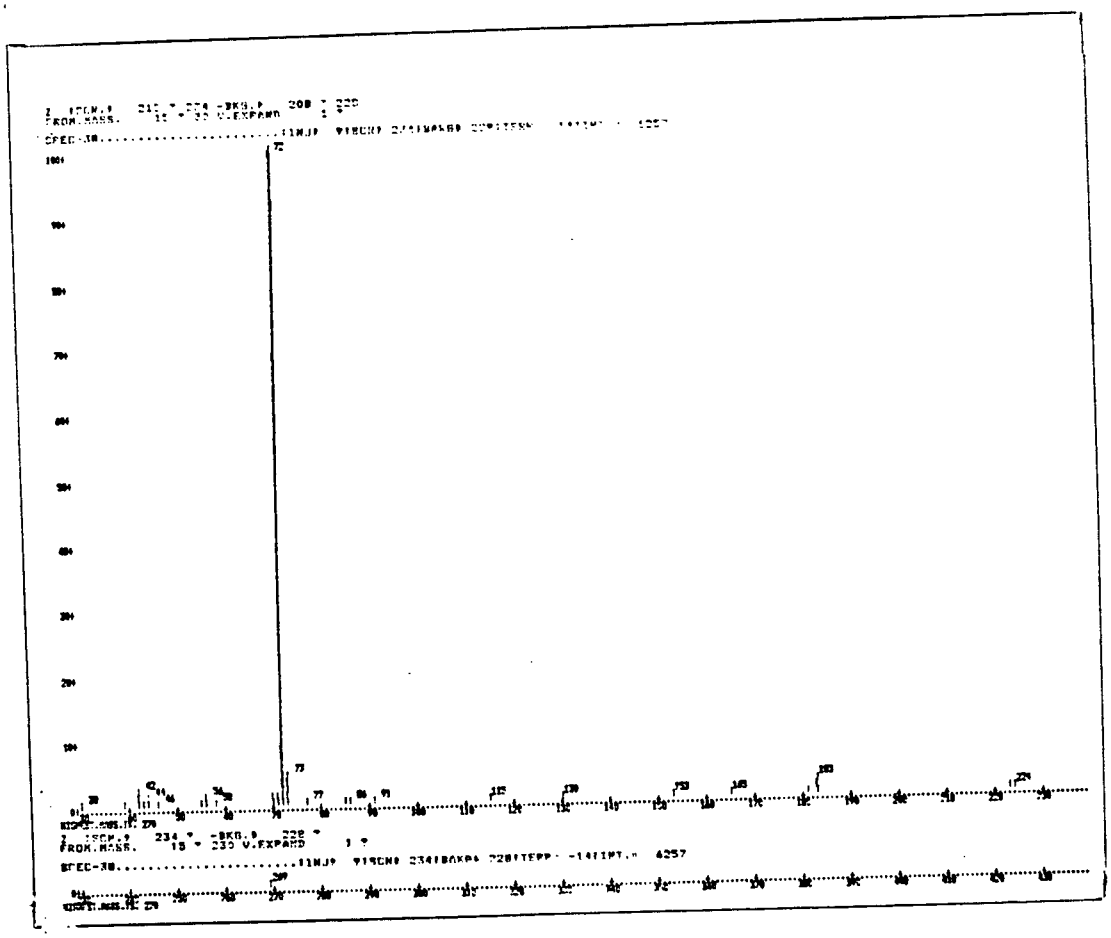
1. Mass spectrum (GCMS) of a novel EDDP like in vitro methadone metabolite



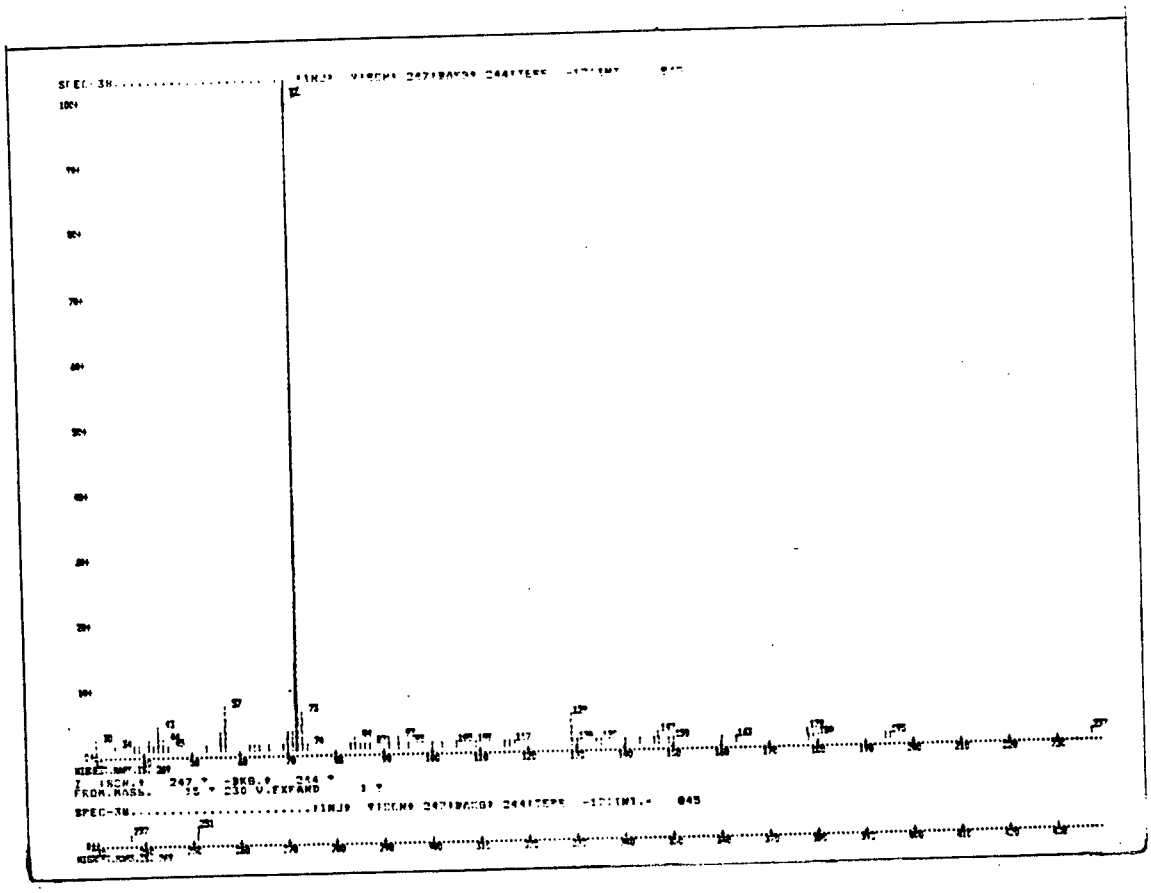
2. Mass spectrum (GCMS) of a novel in vitro methadone metabolite, also found as a non conjugated methadone analogue metabolite



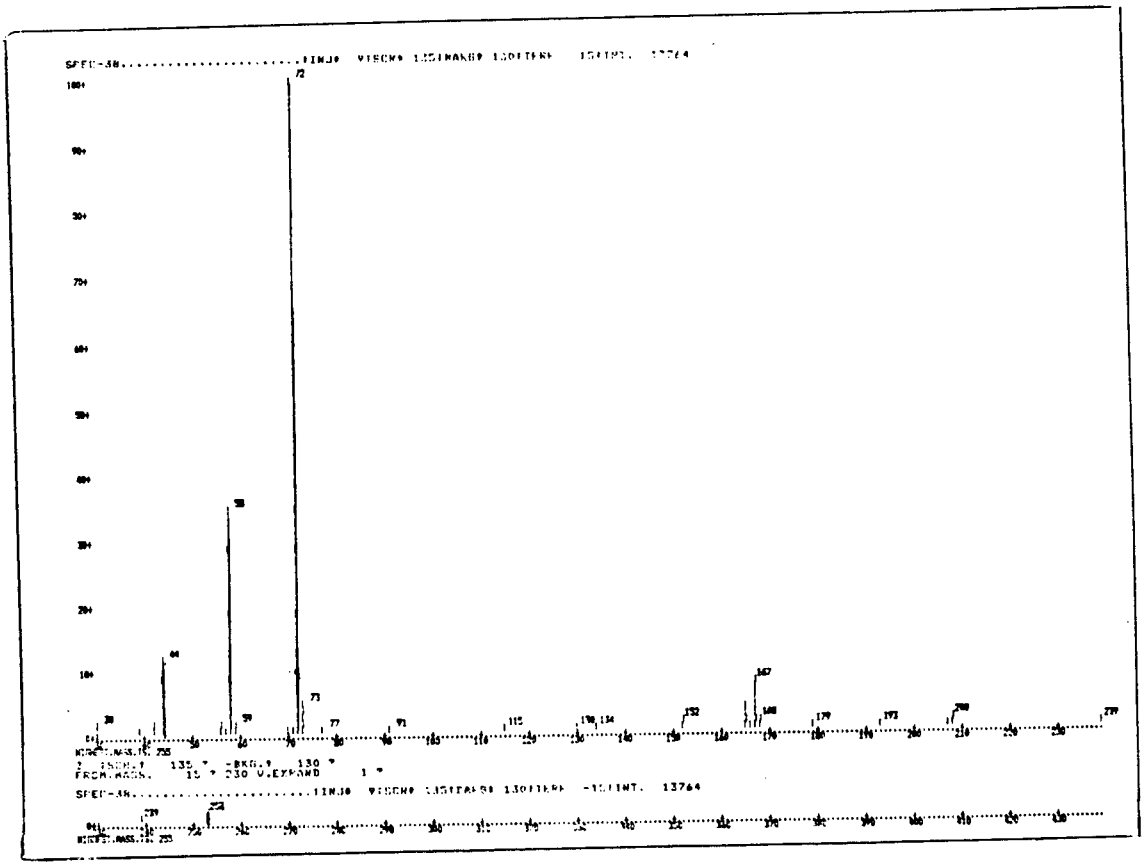
3. Mass spectrum (GCMS) of in vitro recipavrin metabolite (76)



Mass spectrum of tertiary amine in vitro recipavrin metabolite (77)



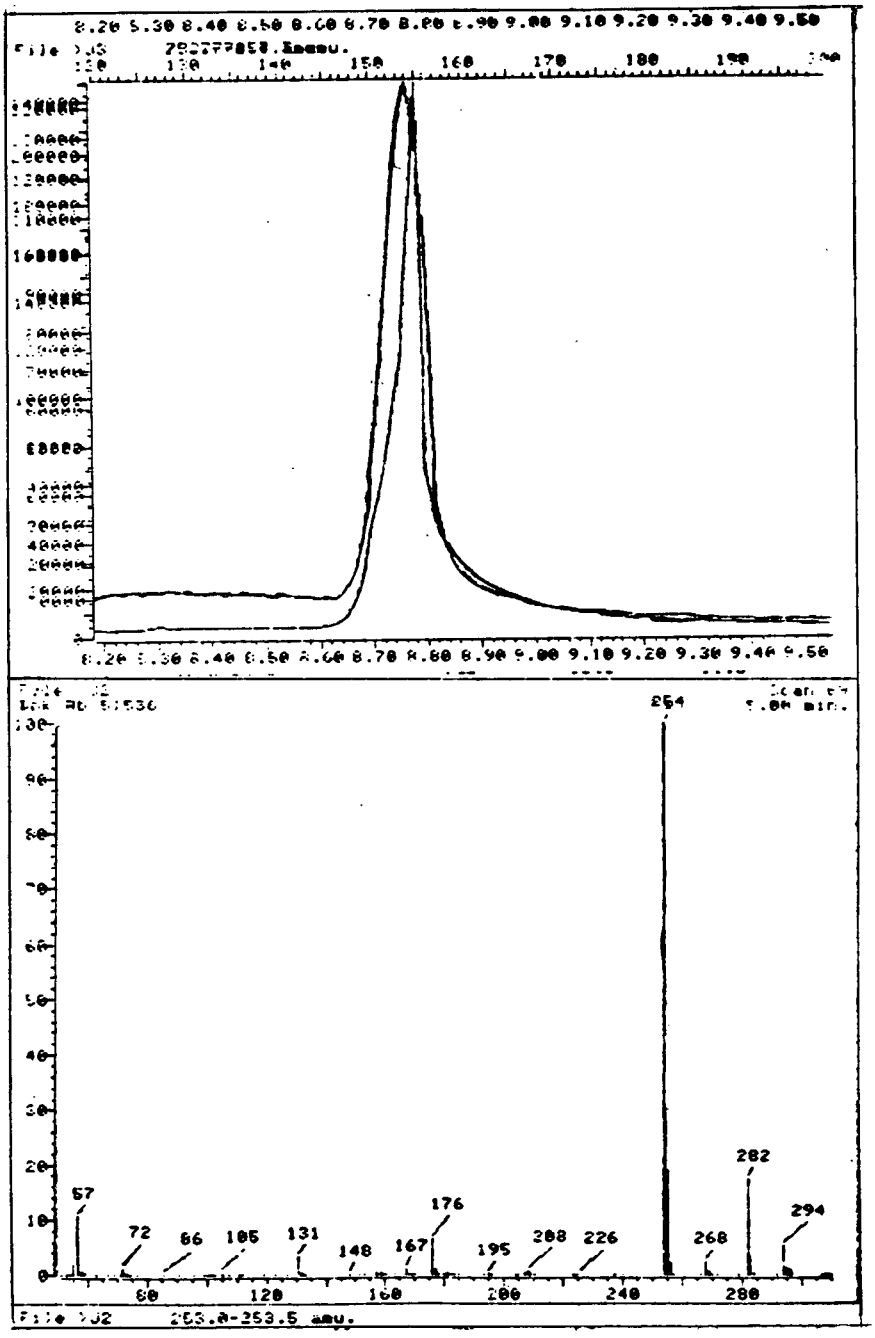
5. Mass spectrum of tertiary amine in vitro recipavrin metabolite (78)



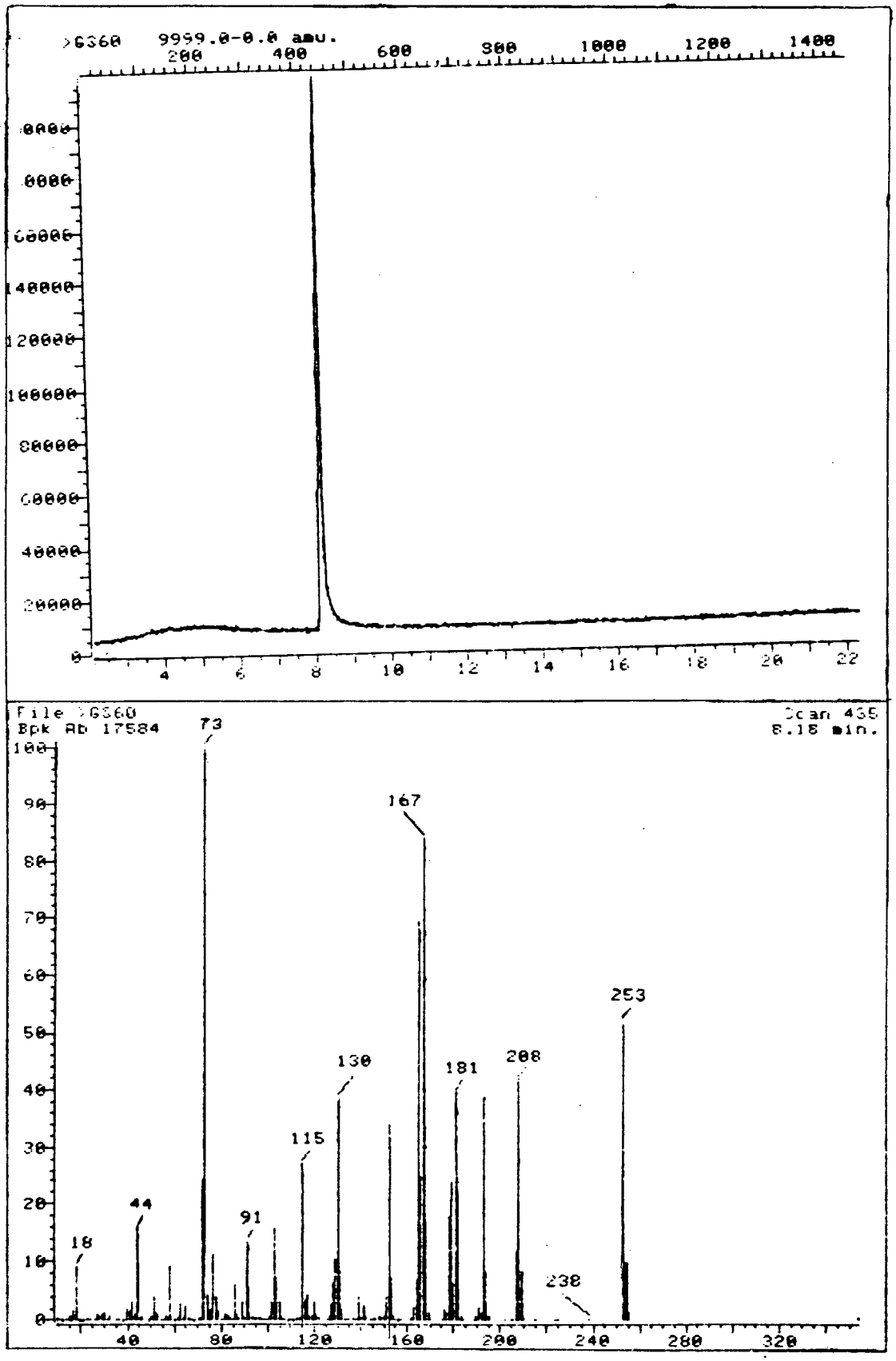
6. Mass spectrum of overlapping dealkylated recipavrin in vitro metabolites (Nor (81), and Dinor (80) Recipavrin)

APPENDIX

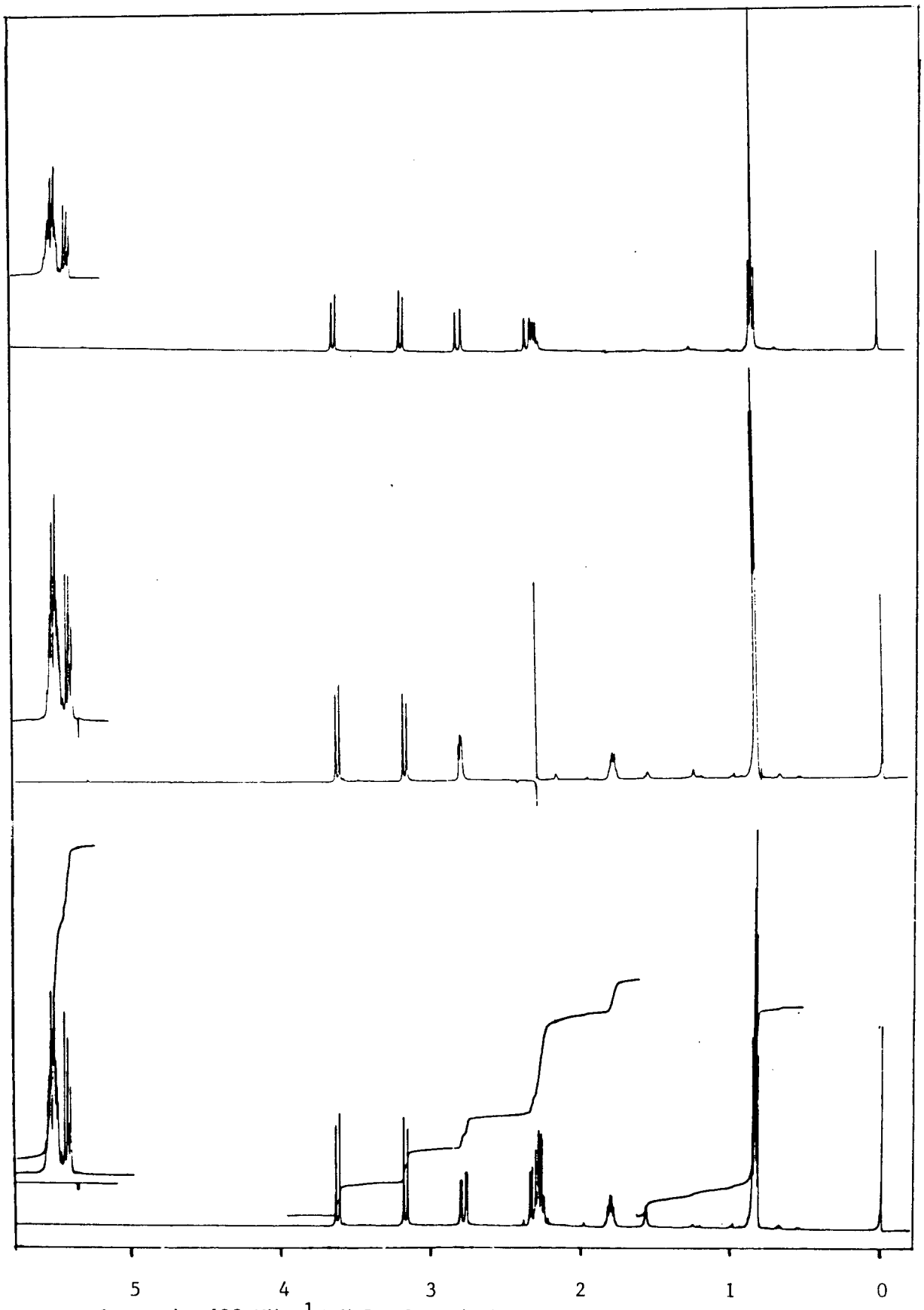
APPENDIX



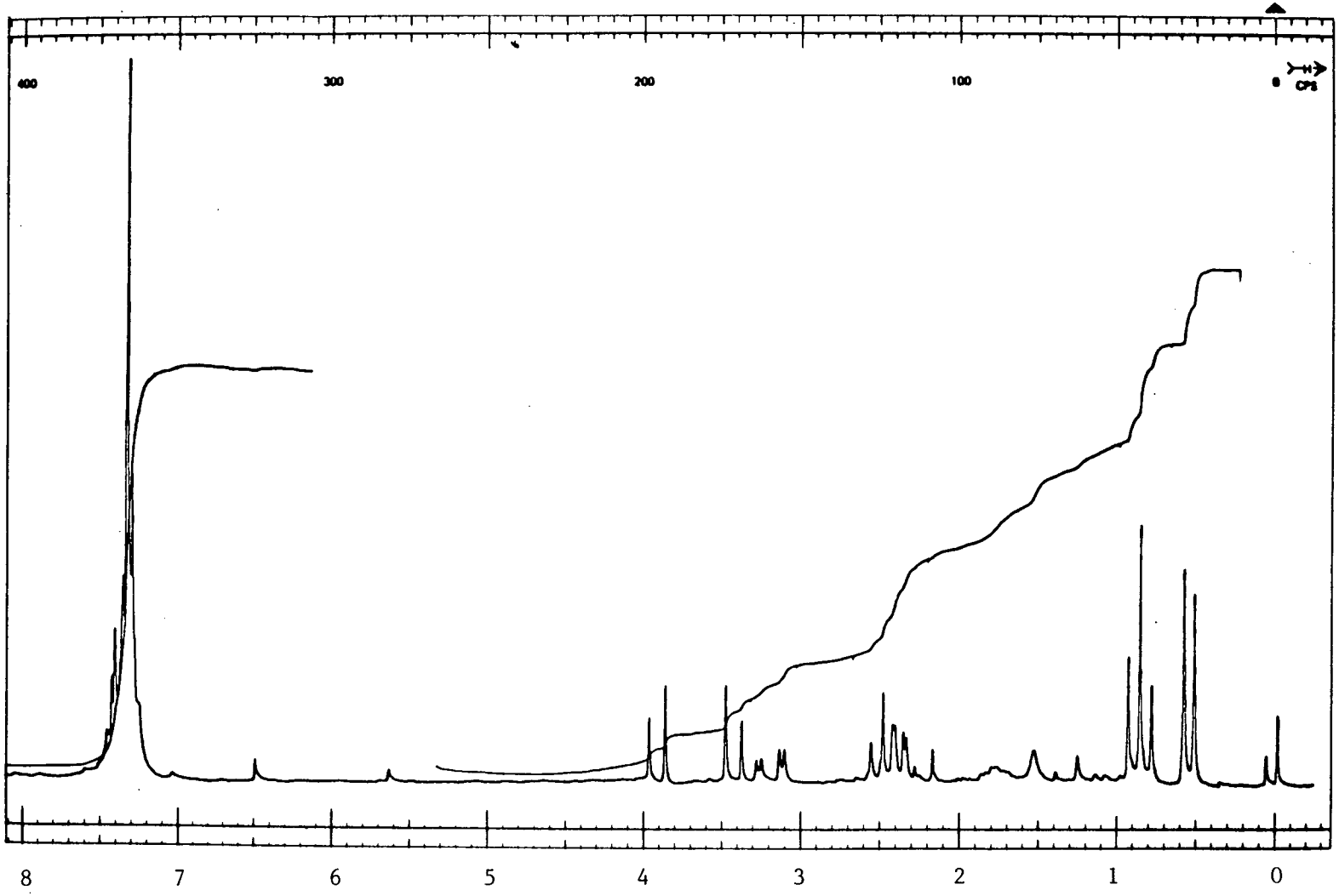
1. Capillary CI GCMS of recipavrin formamide (15)



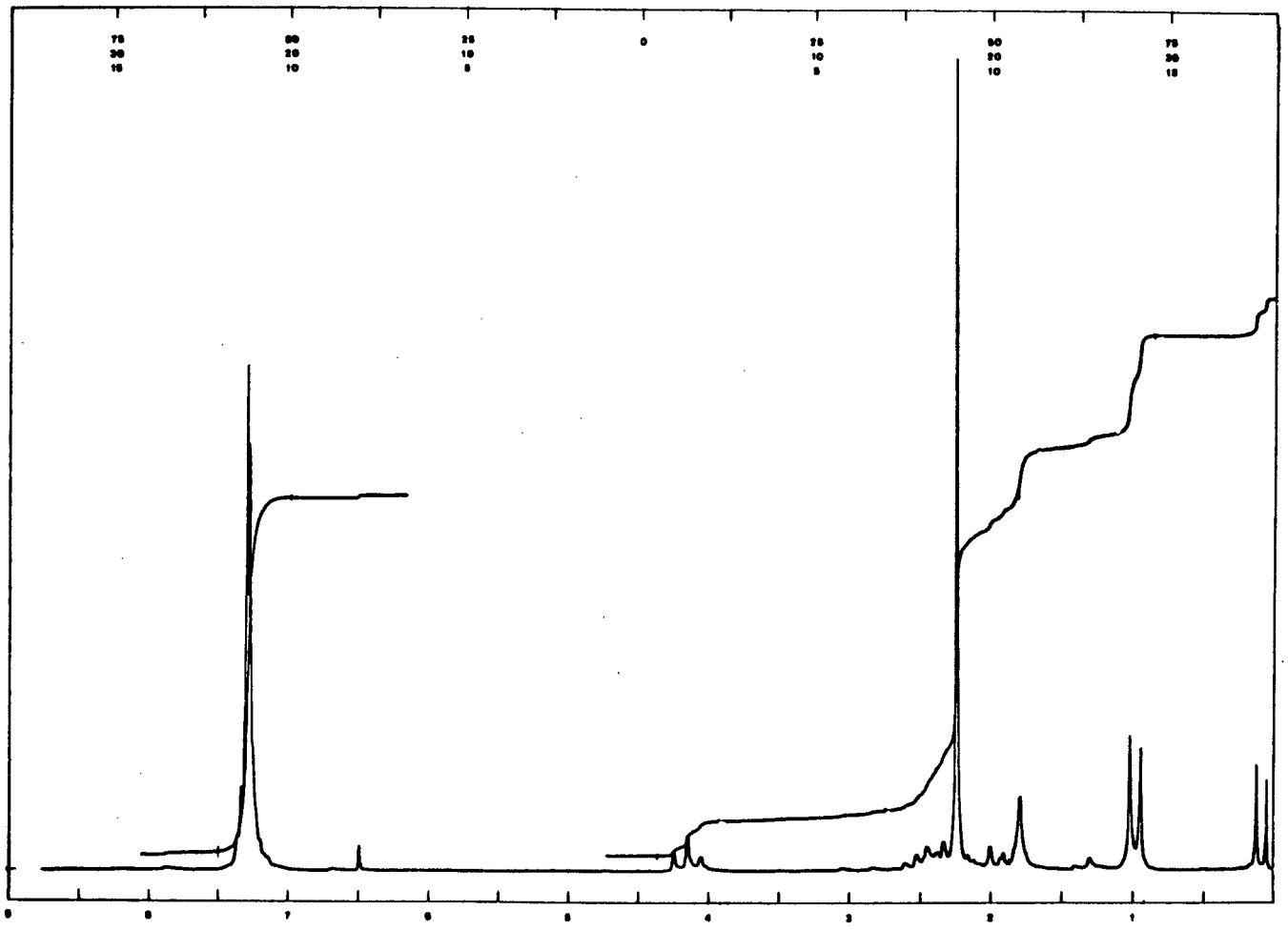
Capillary GC and mass spectrum of the recipavrin formamide (15)



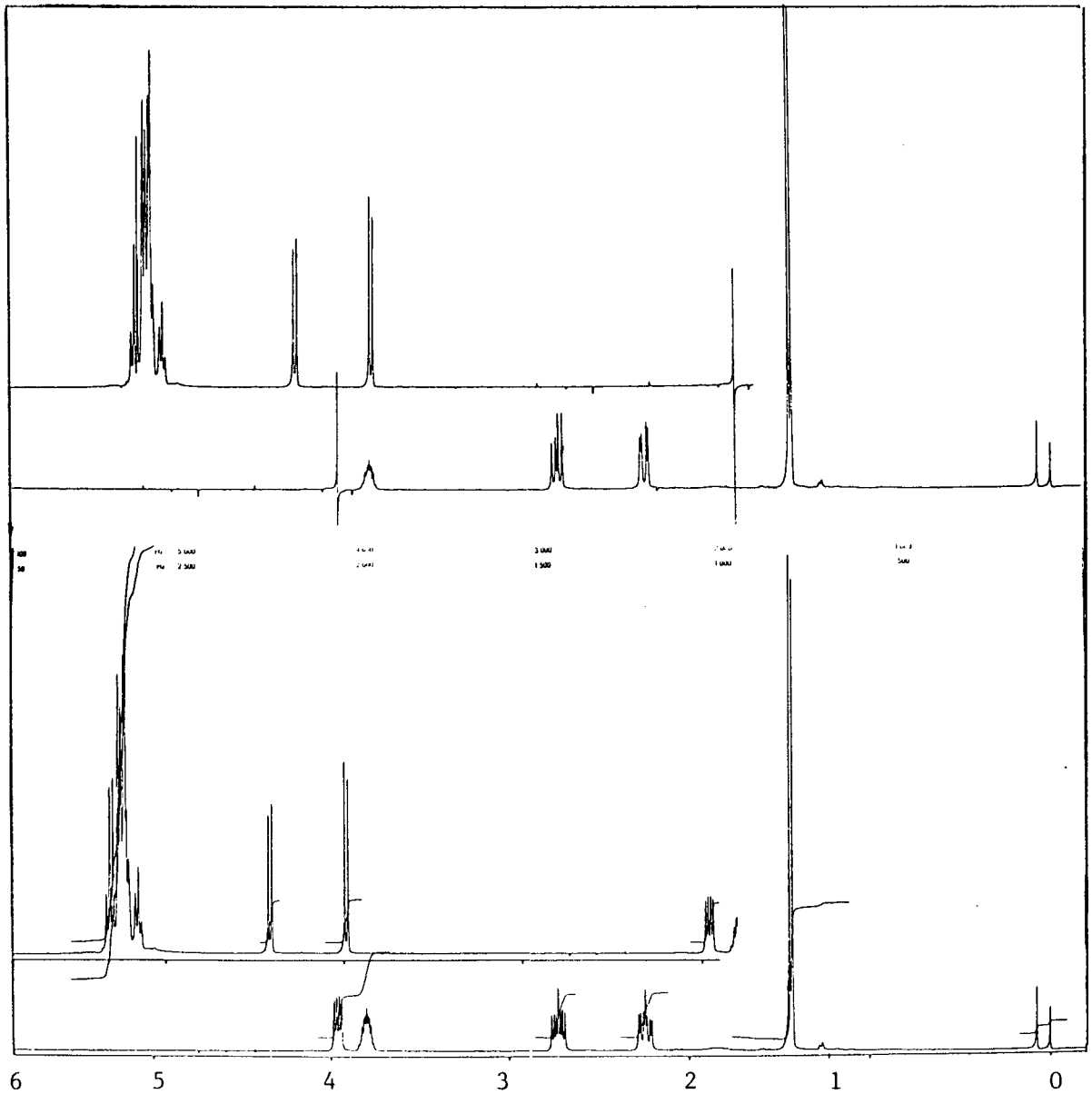
1 a) 400 MHz ^{1}H NMR of methadone oxaziridine (5)
b) Decoupled at 2.3 ppm
c) Decoupled at 1.8 ppm



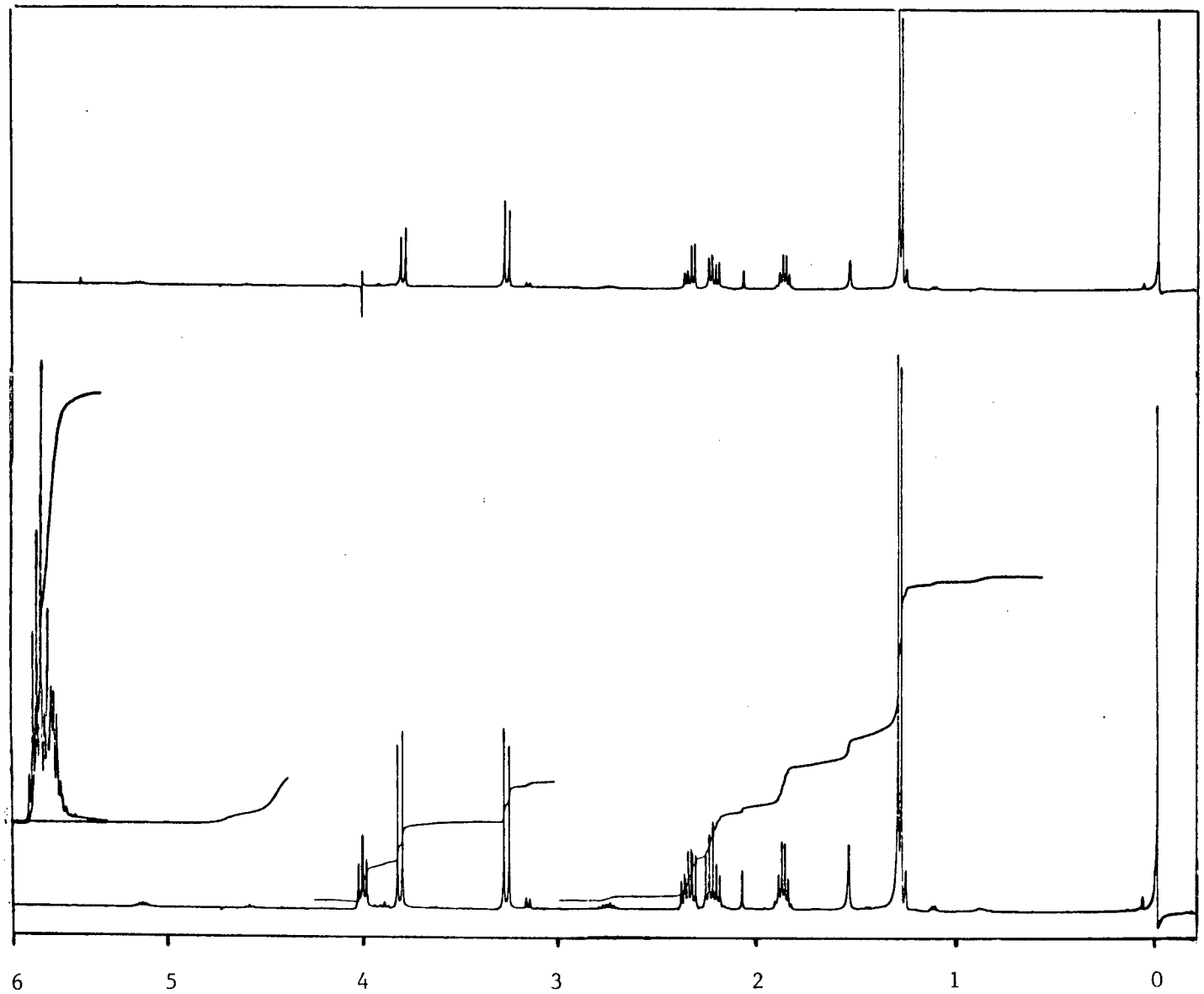
2. 100 MHz ^{1}H NMR of 2-(4',4'-diphenylheptan-5'-one-2'-yl) oxaziridine (5) minor diastereomer



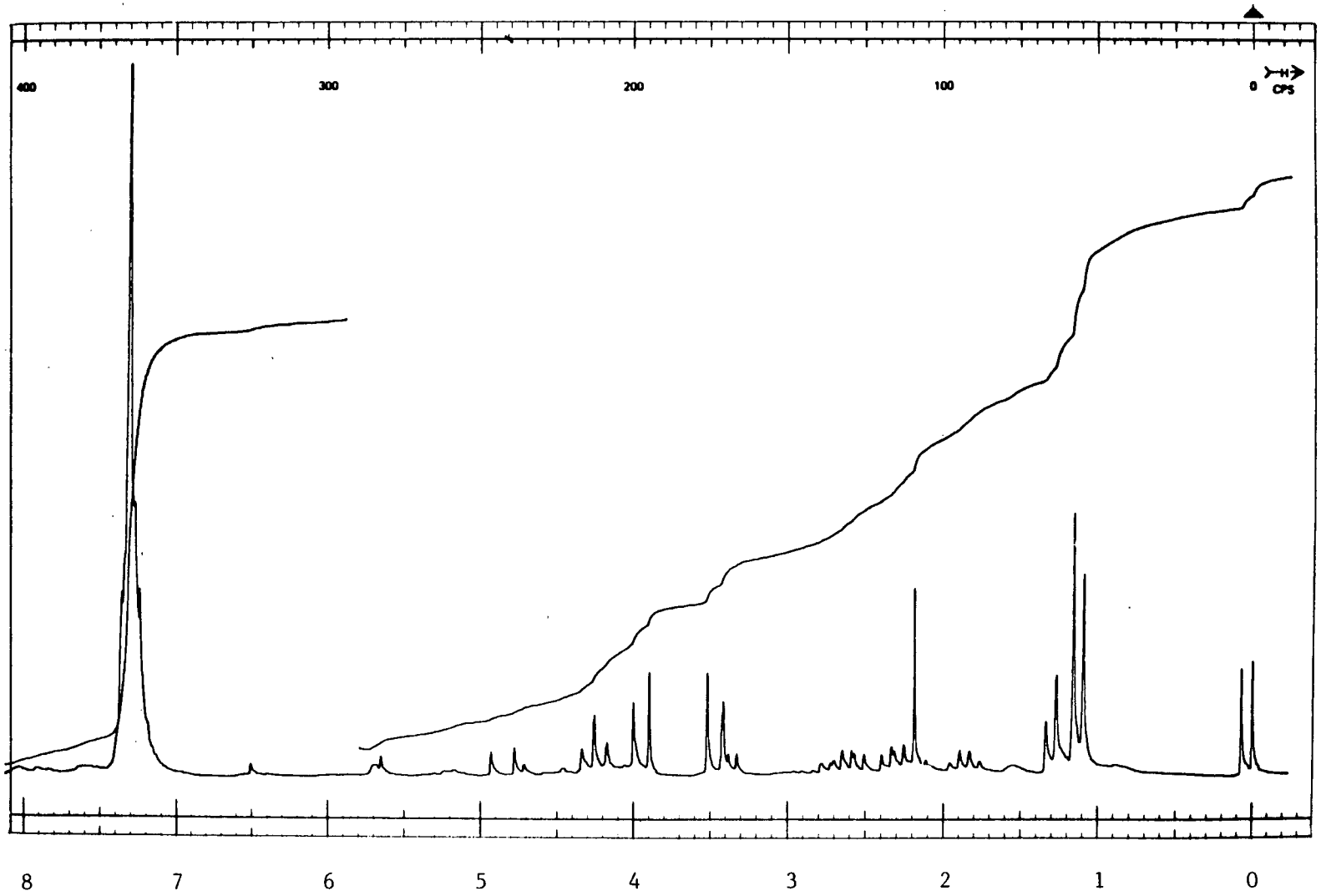
3. 100 MHz ^{1}H NMR of γ-phenyl-N,N, α-trimethyl-benzene-propanamine (Recipavrin) (6)



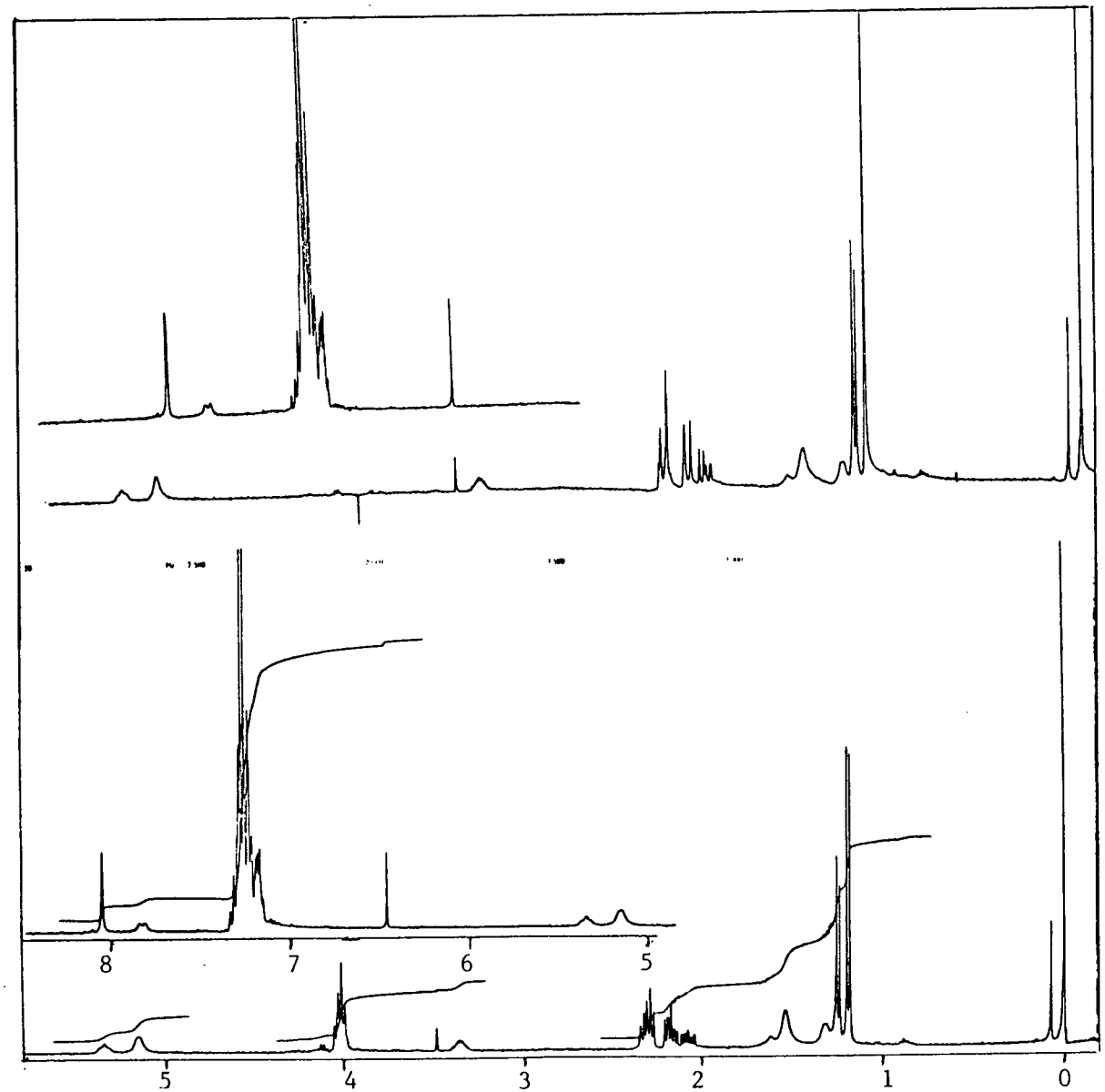
4. a) 400 MHz ^{1}H NMR of Recipavrin nitrone (13)
   b) Decoupled at 3.96 ppm



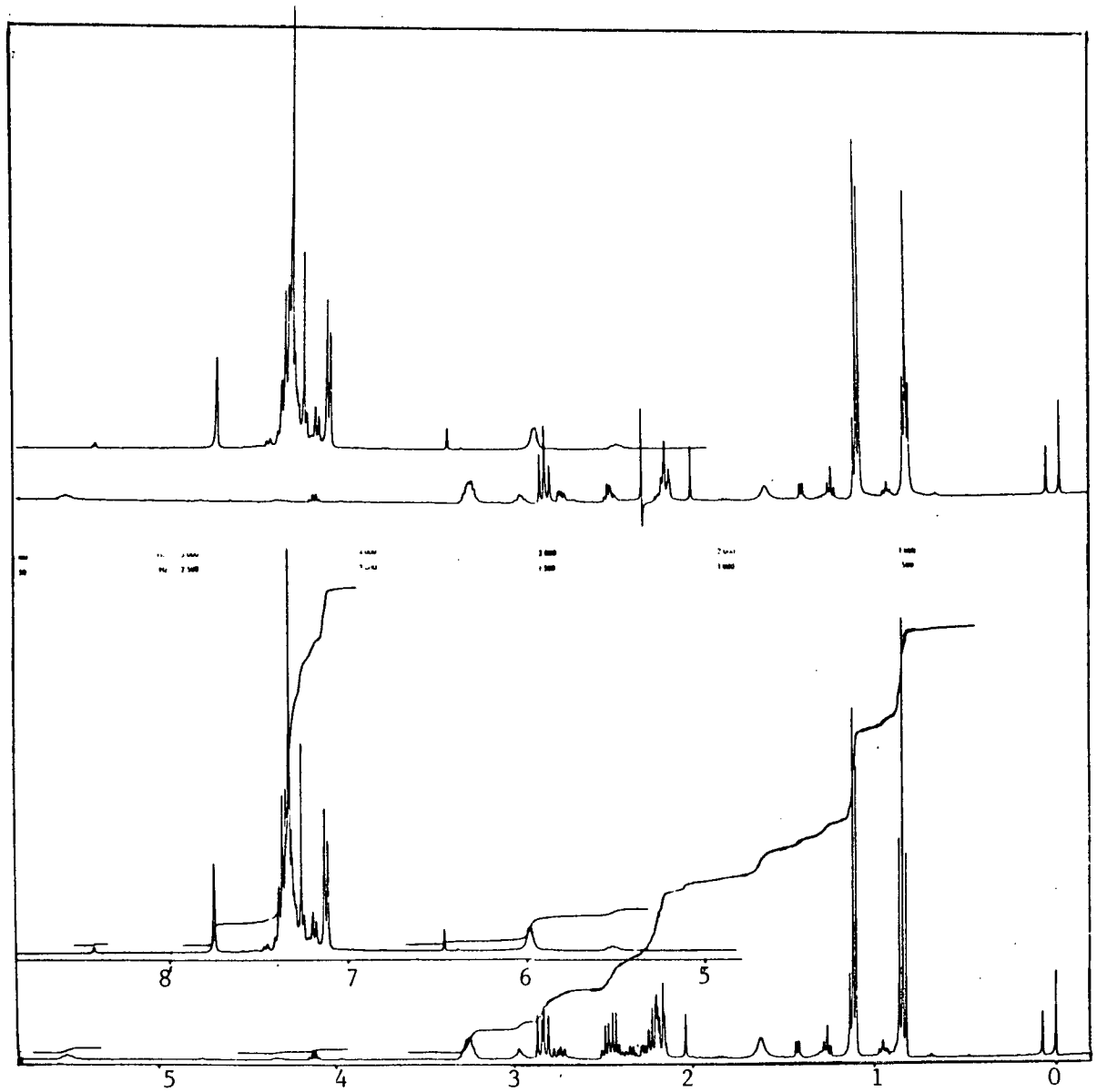
5. a) 400 MHz ^{1}H of 2-(4',4'-diphenyl-but-2'-yl) oxaziridine (major diastereomer) (14)
   b) Decoupled at 4.01 ppm



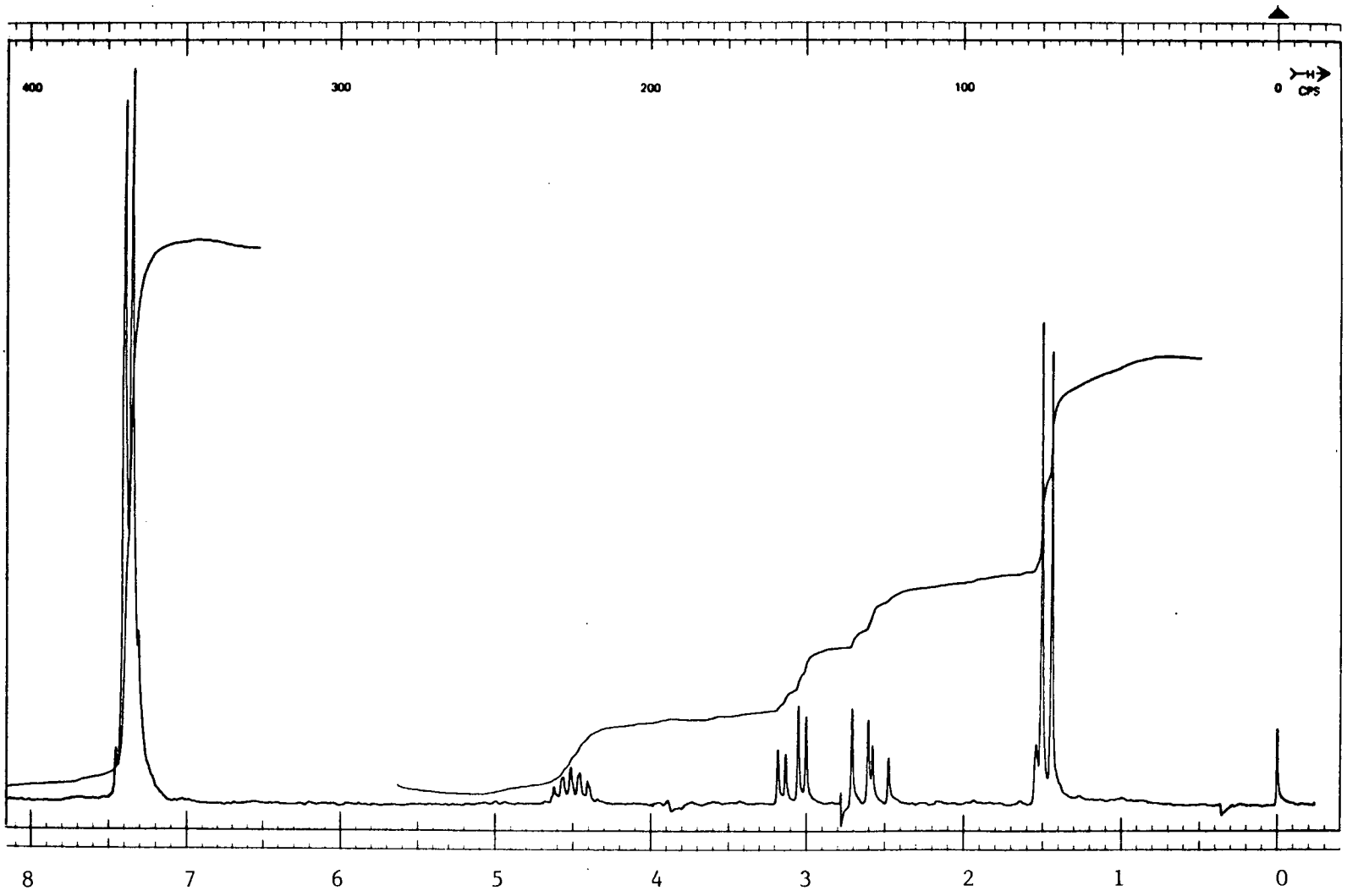
6. 100 MHz ^{1}H NMR of 2-(4',4'-diphenyl-but-2'-yl) oxaziridine (minor diastereomer) (14)



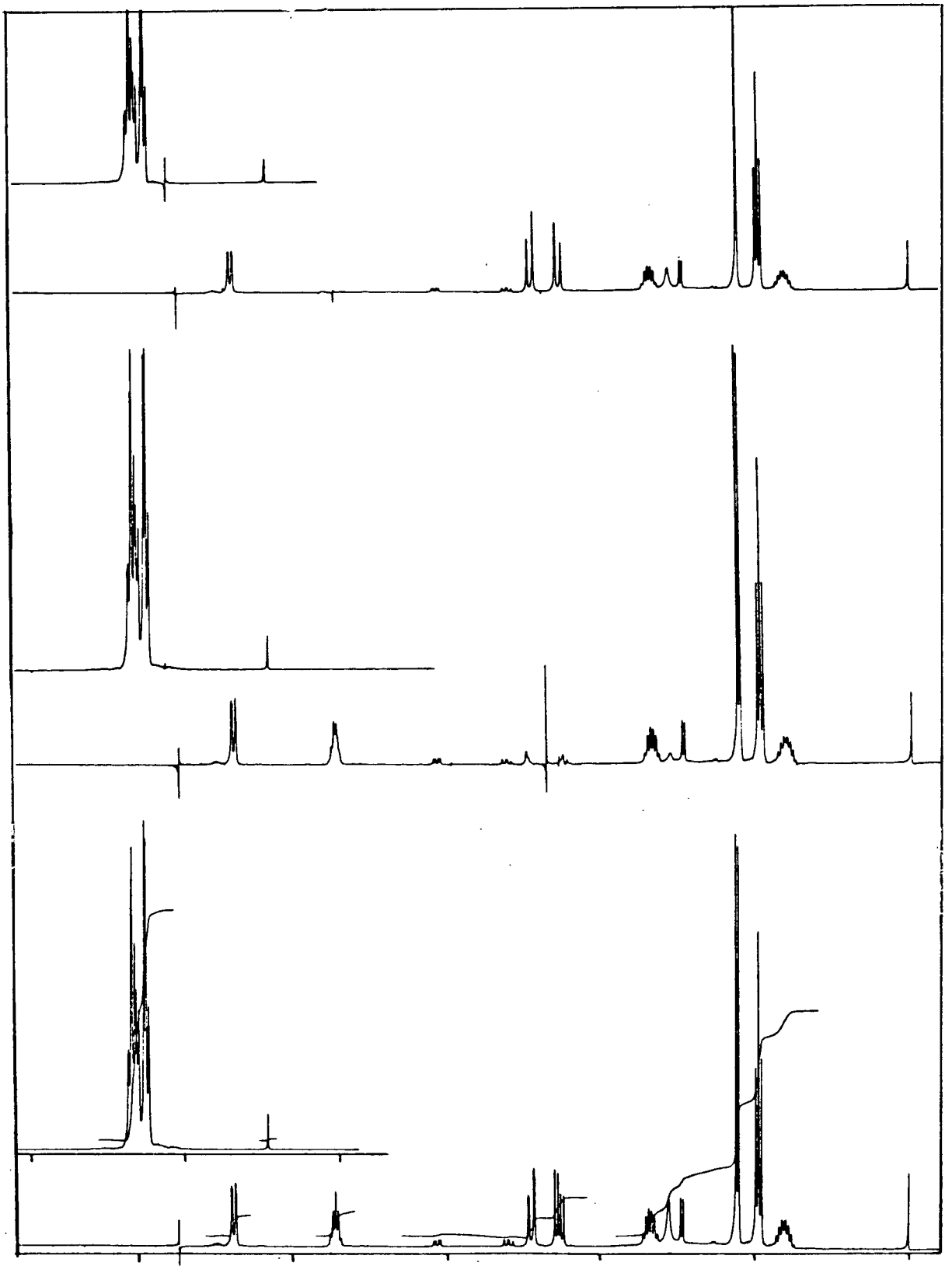
7. a) 400 MHz ^{1}H NMR of N-formyl-α-methyl-γ-phenyl-benzene propanamine (15)
   b) Decoupled at 4.1 ppm



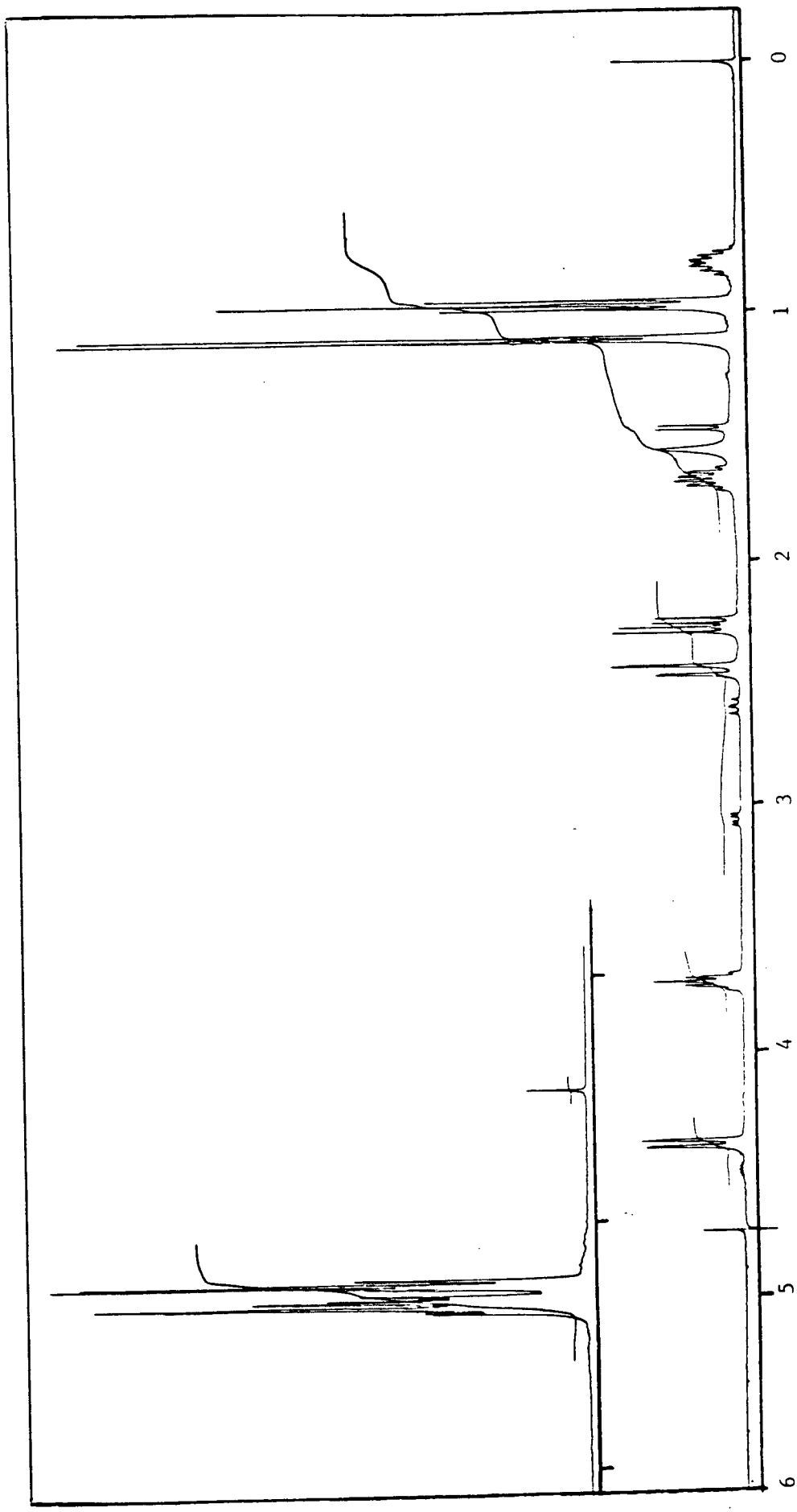
8. a) 400 MHz ^{1}H NMR of 2-(N-formyl)-4,4-diphenyl-5-heptanone (31)
   b) Decoupled at 2.3 ppm



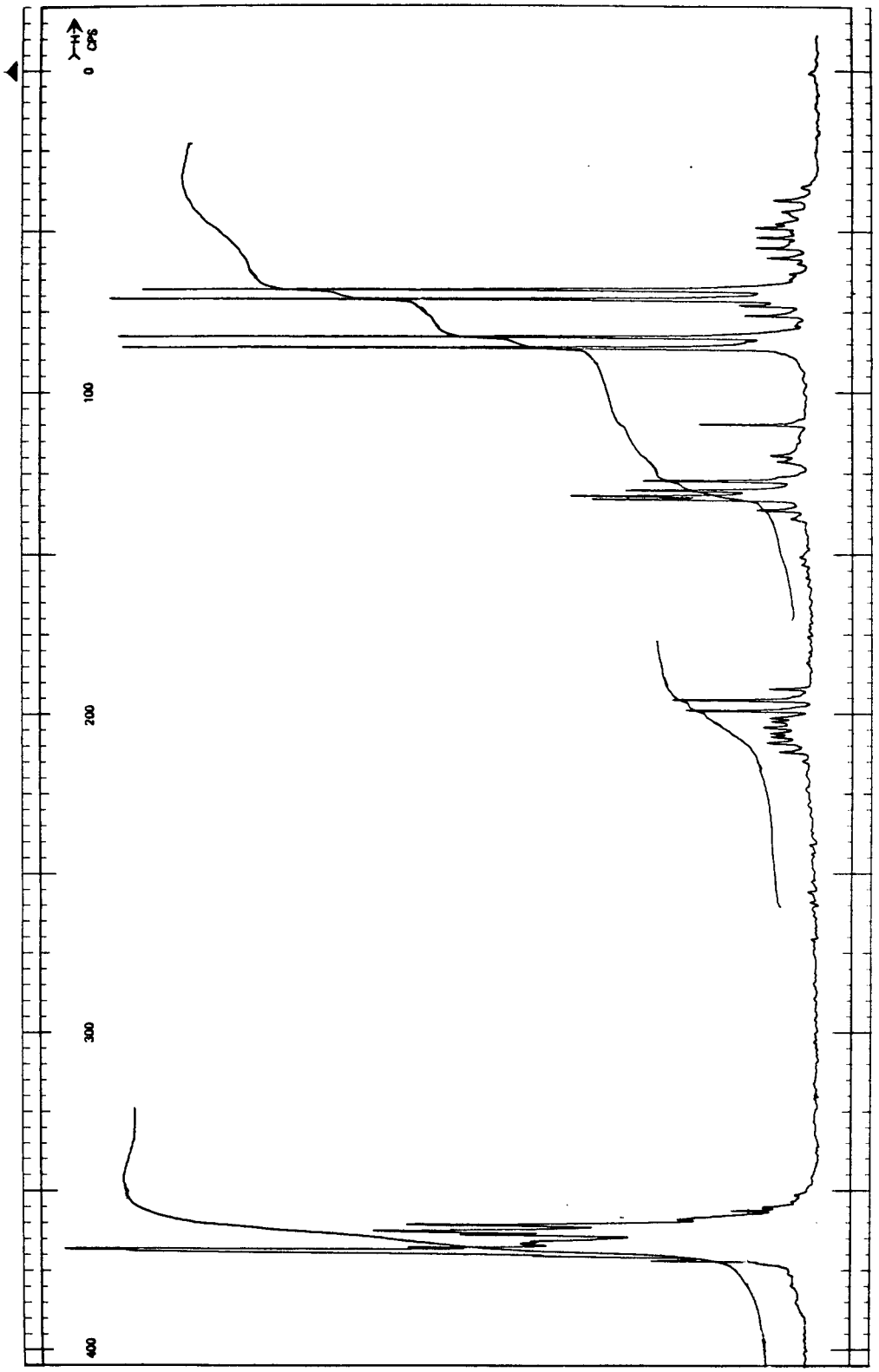
9. 100 MHz ^{1}H NMR of 2,2-diphenyl-4-valerolactone (41)



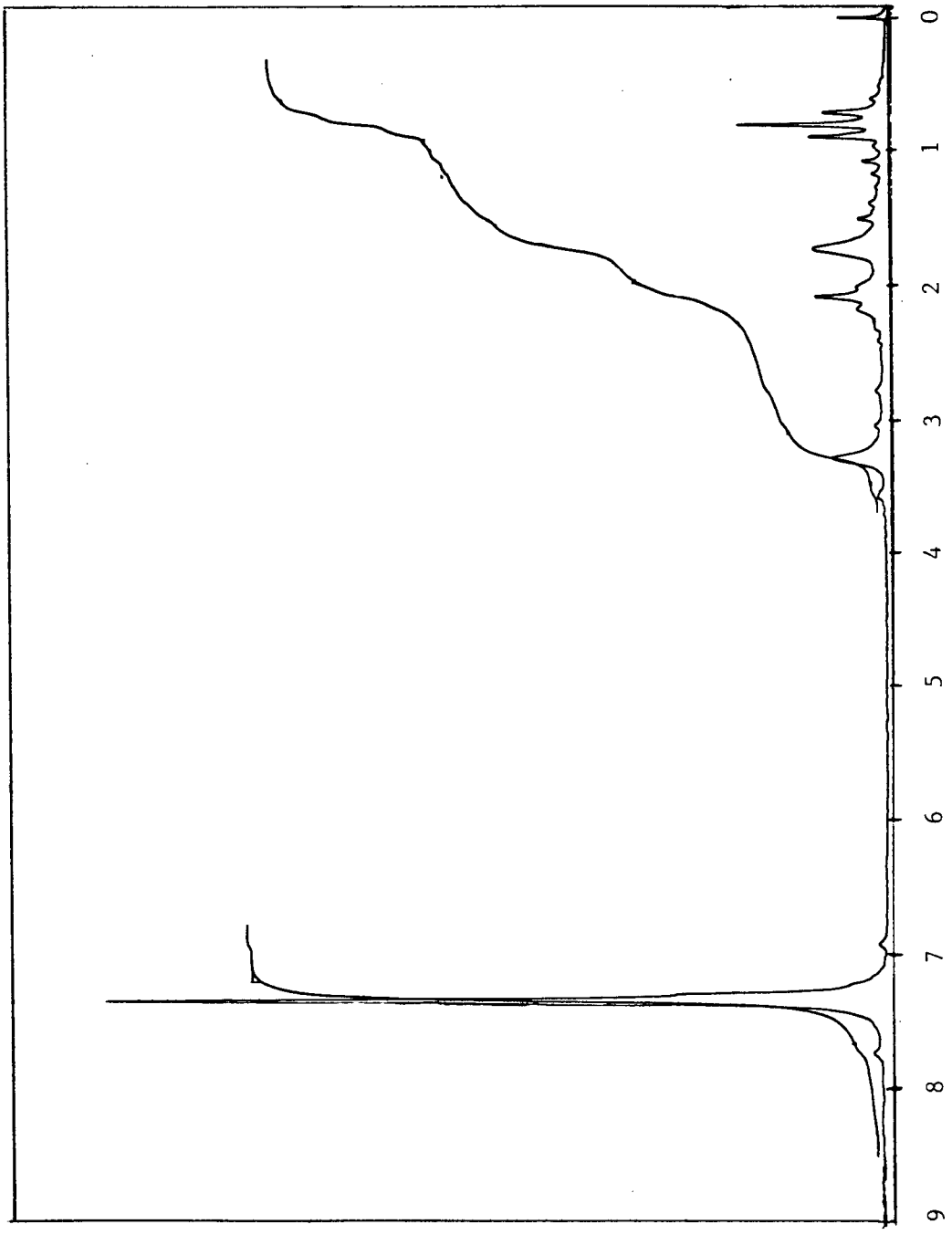
10. a) 400 MHz ^{1}H NMR of 4,4-diphenyl-2,5-heptanediol (43)
    b) Decoupled at 2.44 and 2.27 ppm
    c) Decoupled at 3.72 ppm



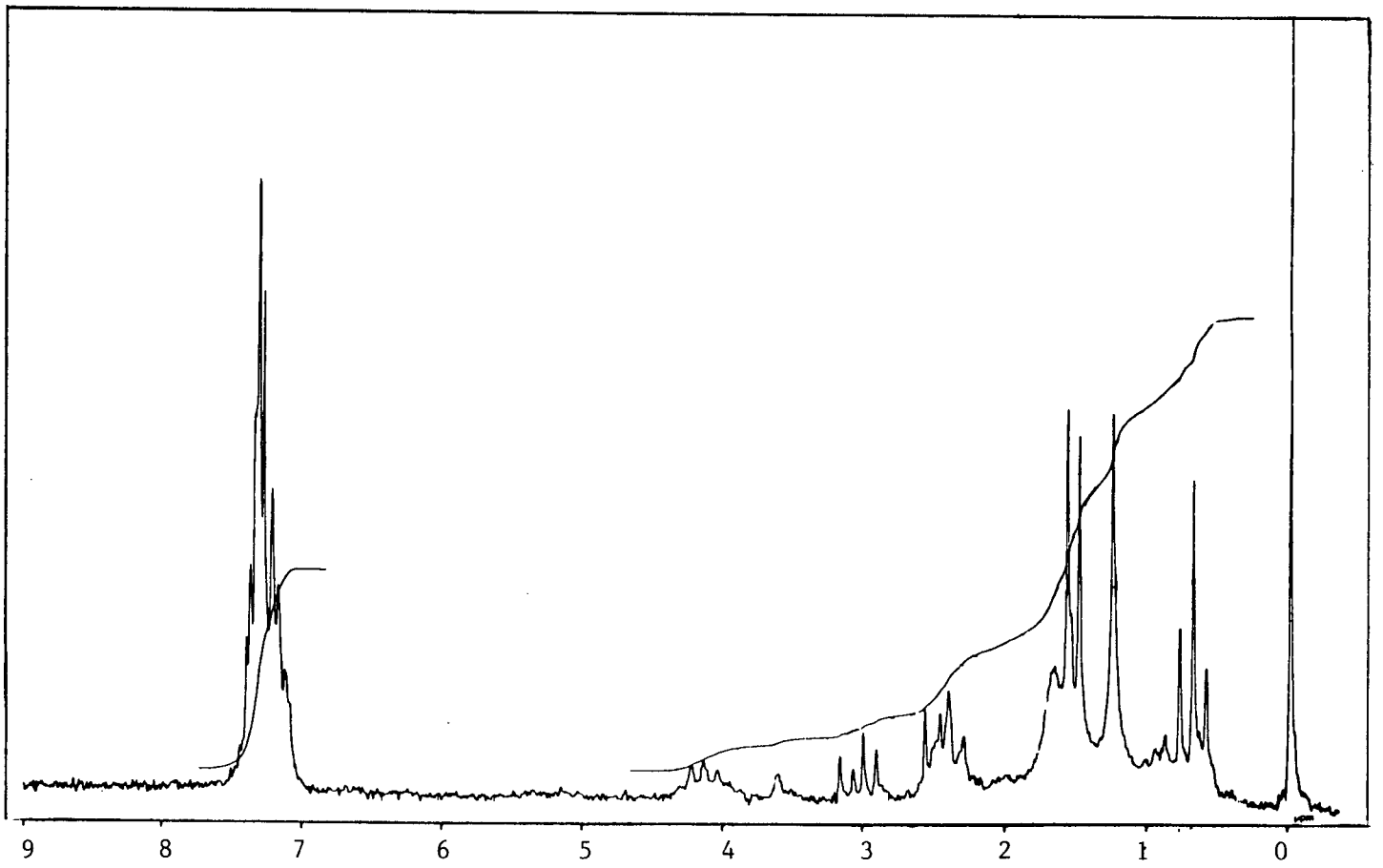
11. 400 MHz ^{1}H NMR of 4,4-diphenyl-2,5-heptanediol (43)



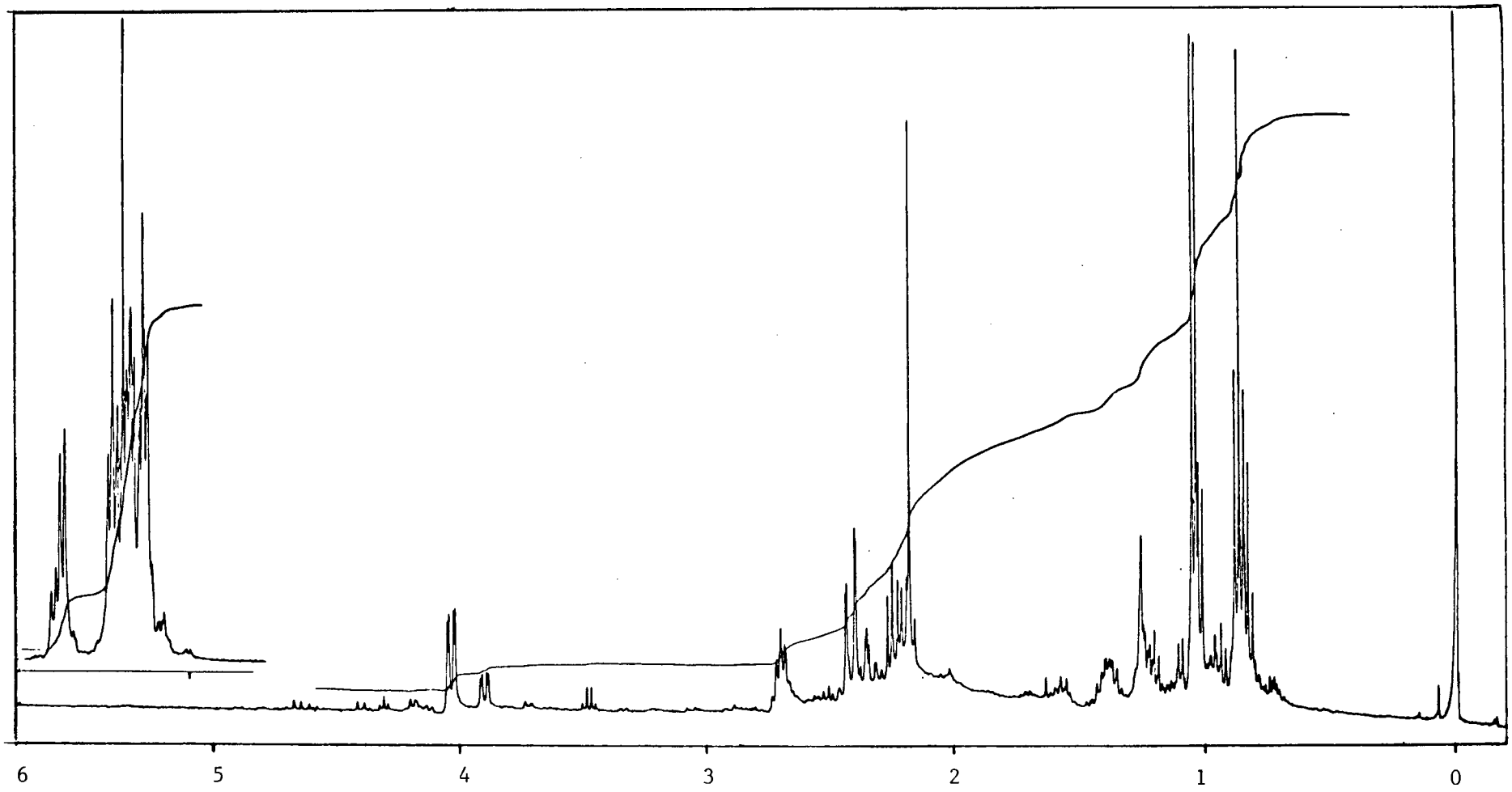
12. 100 MHz ^{1}H NMR of 3,3-diphenyl-5-methyl-2-ethylidene tetrahydrofuran (44)



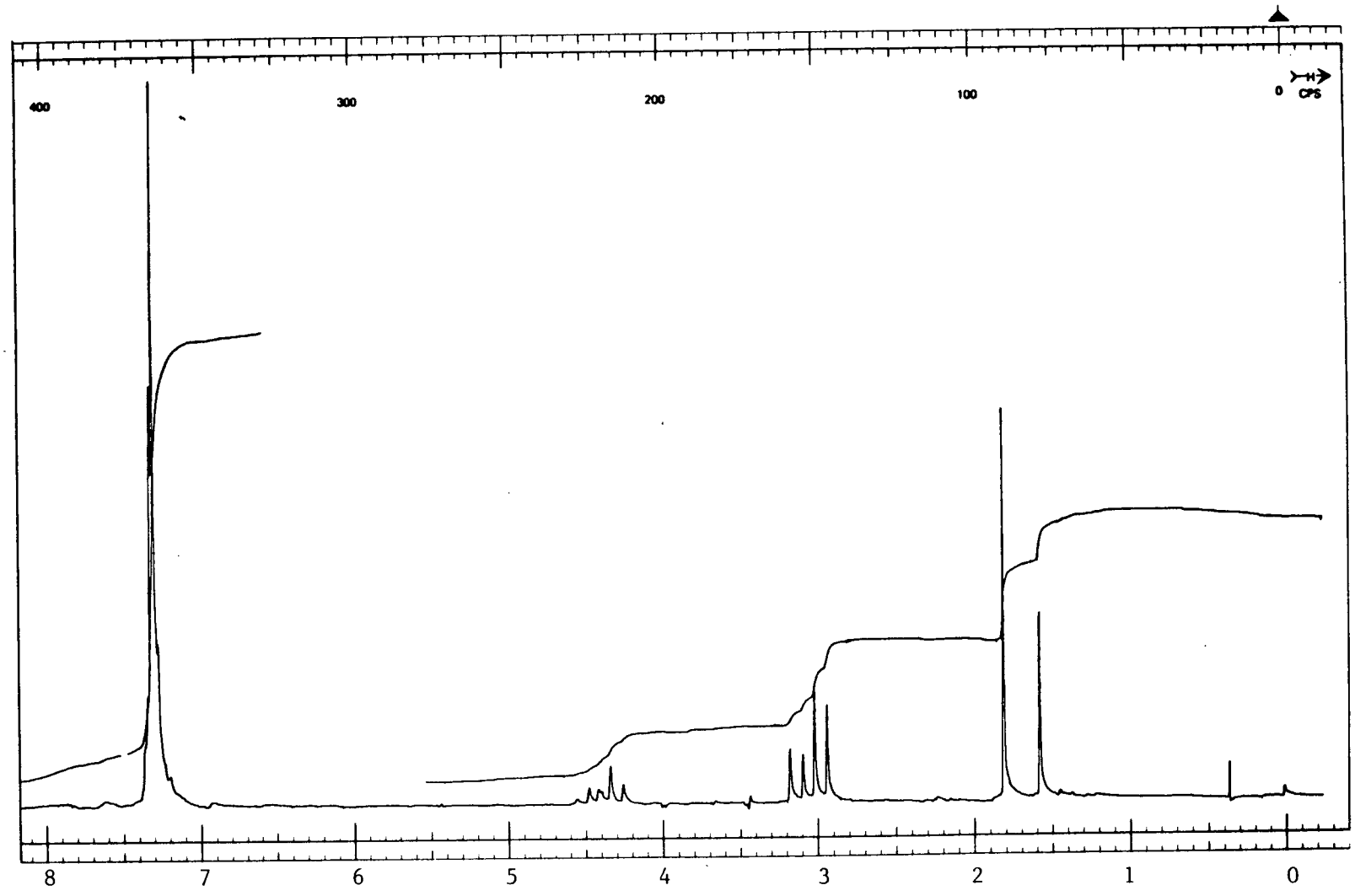
13. 80 MHz ^{1}H NMR of 4,4-diphenyl-2,5-heptanedione-2-oxime (46)



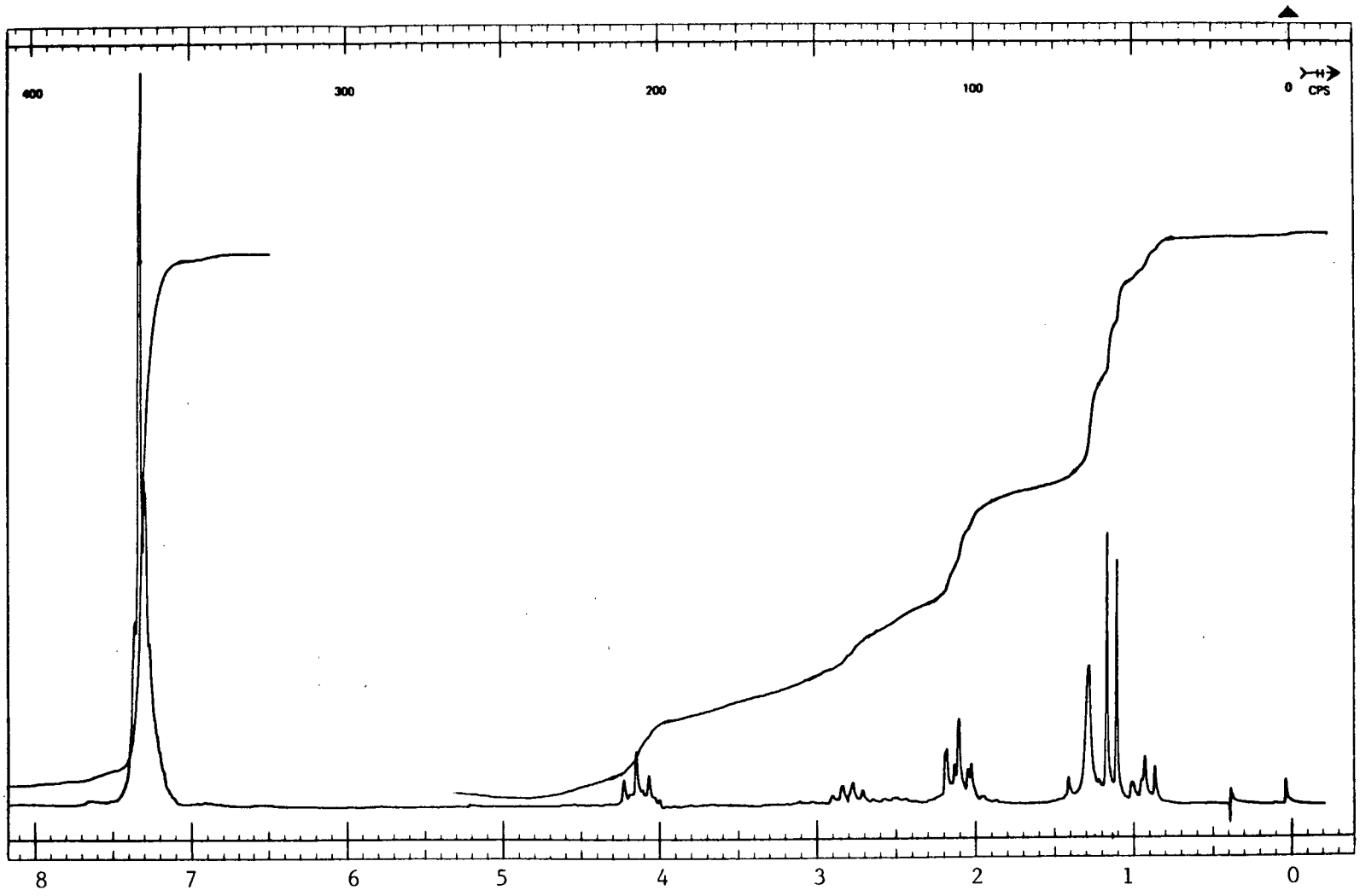
14. 80 MHz ^{1}H NMR of keto oxime (46) reduction product



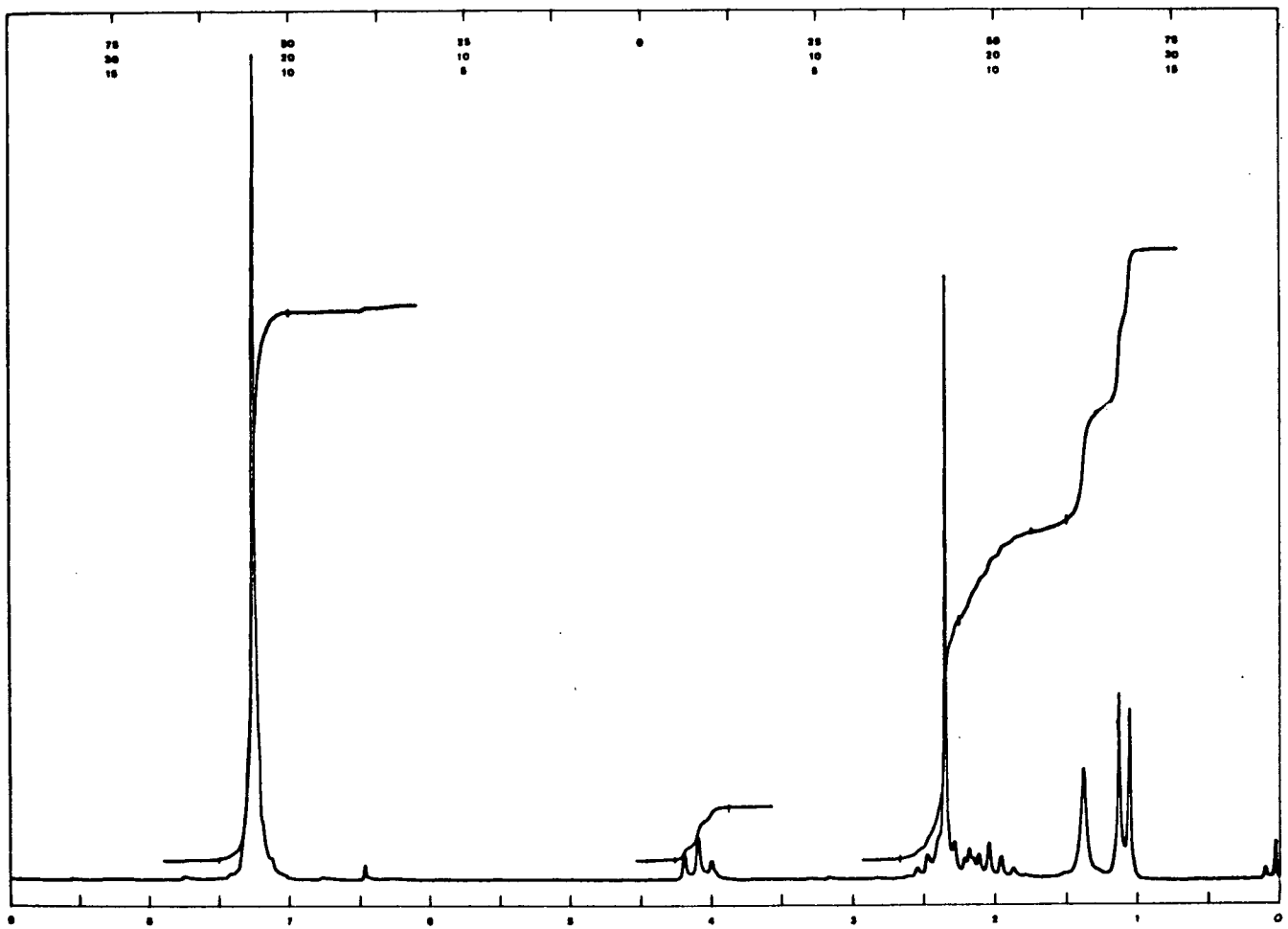
15. 400 MHz ^{1}H NMR of 4,4-diphenyl-2-amino-5-heptanol (49)



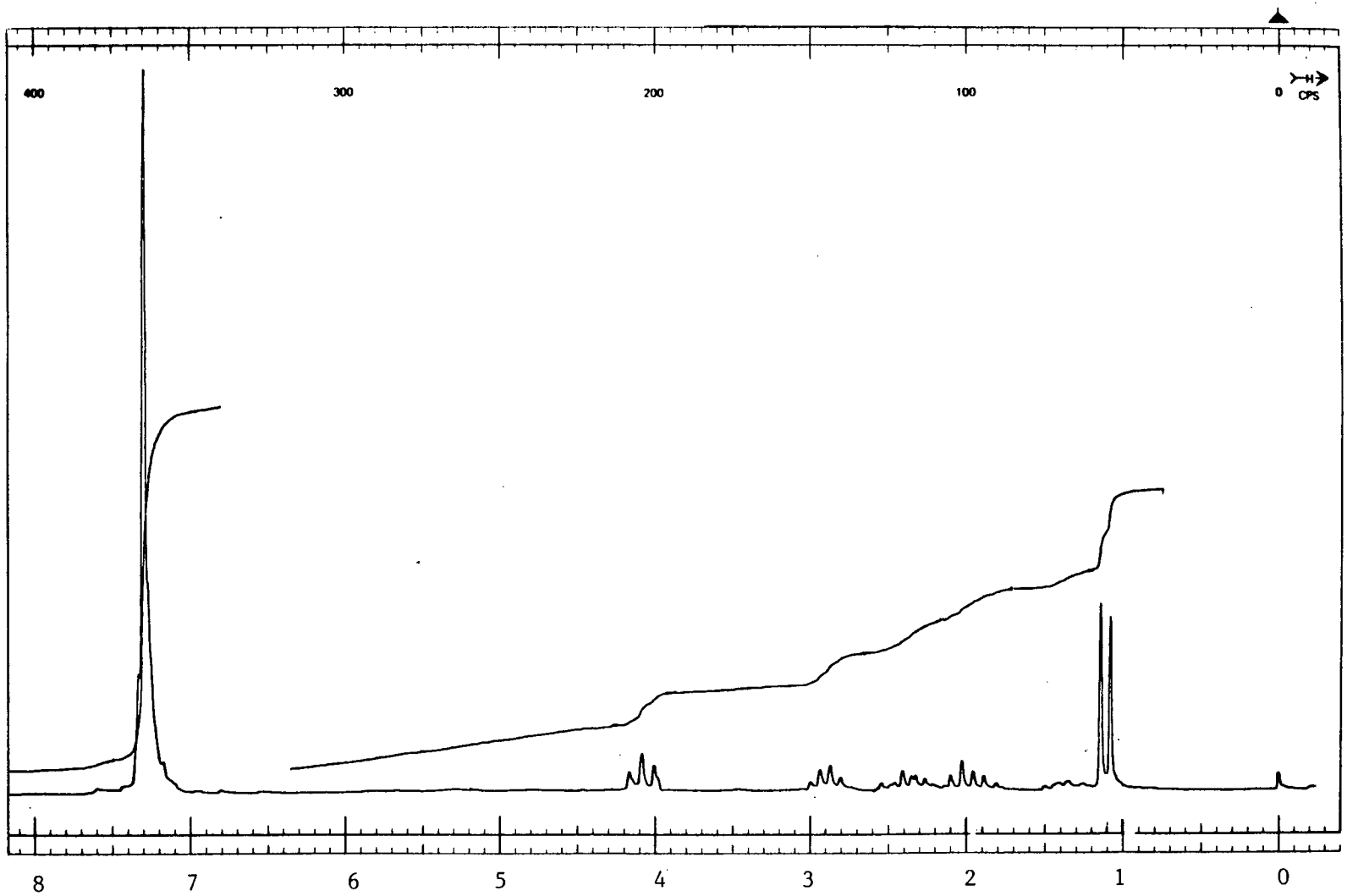
16. 100 MHz ^{1}H NMR of syn and anti 1,1-diphenyl-3-butanone oximes



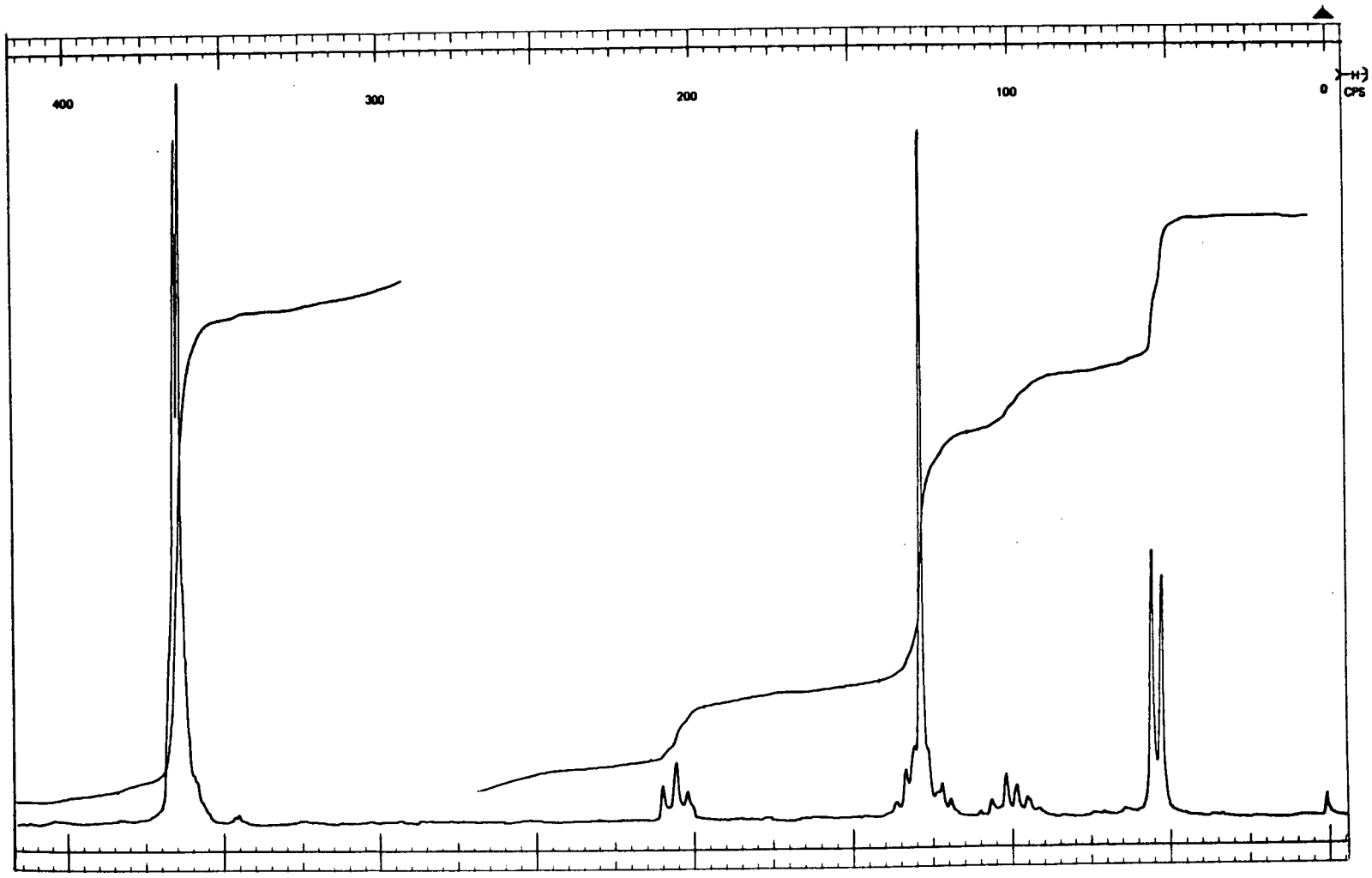
17. 100 MHz ^{1}H NMR of α-methyl-γ-phenyl-benzenepropanamine (Dinorrecipavrin) (53)



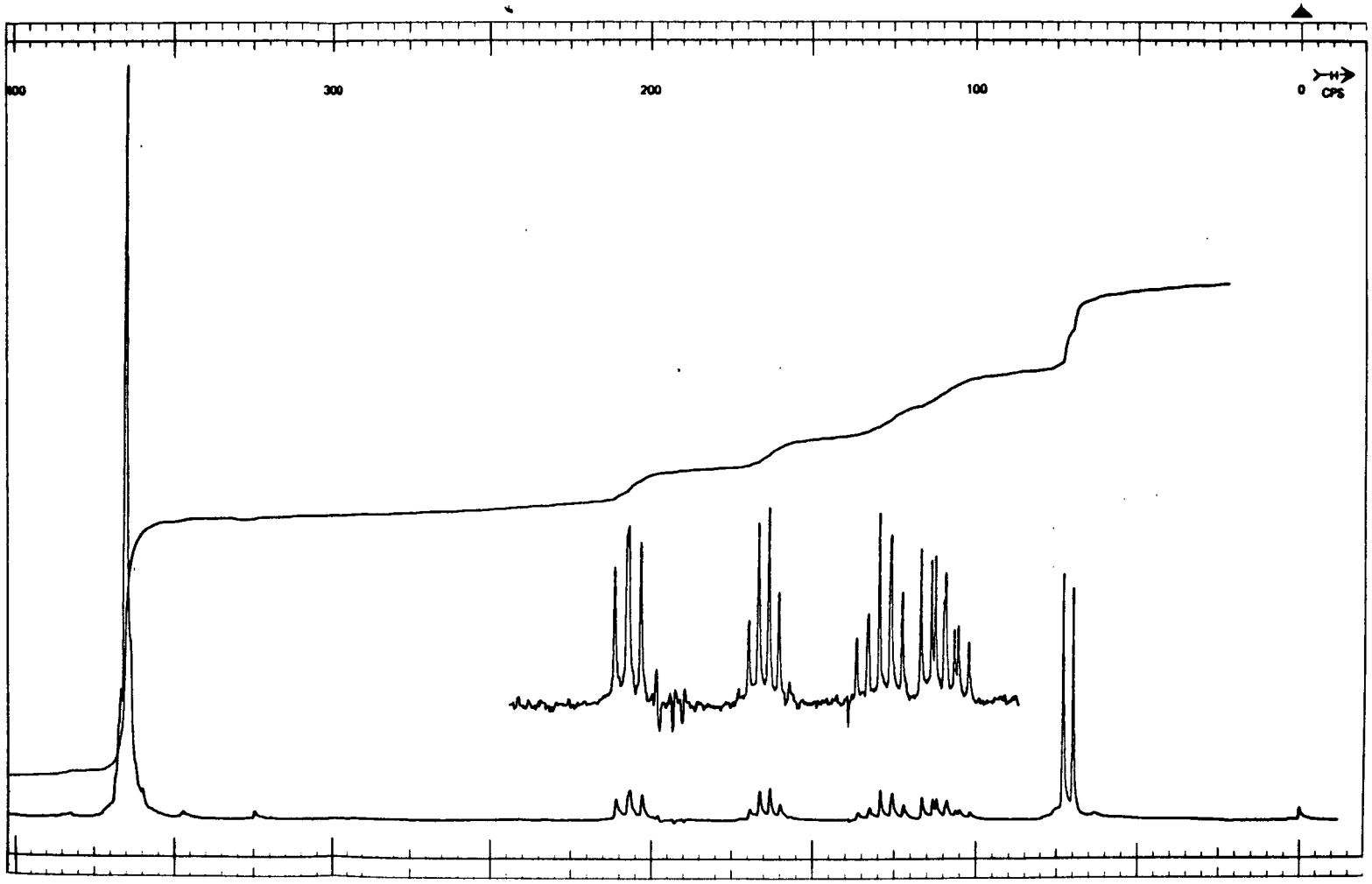
18. 100 MHz ^{1}H NMR of N, α-dimethyl-γ-phenyl-benzene-propanamine (Norrecipavrin) (54)



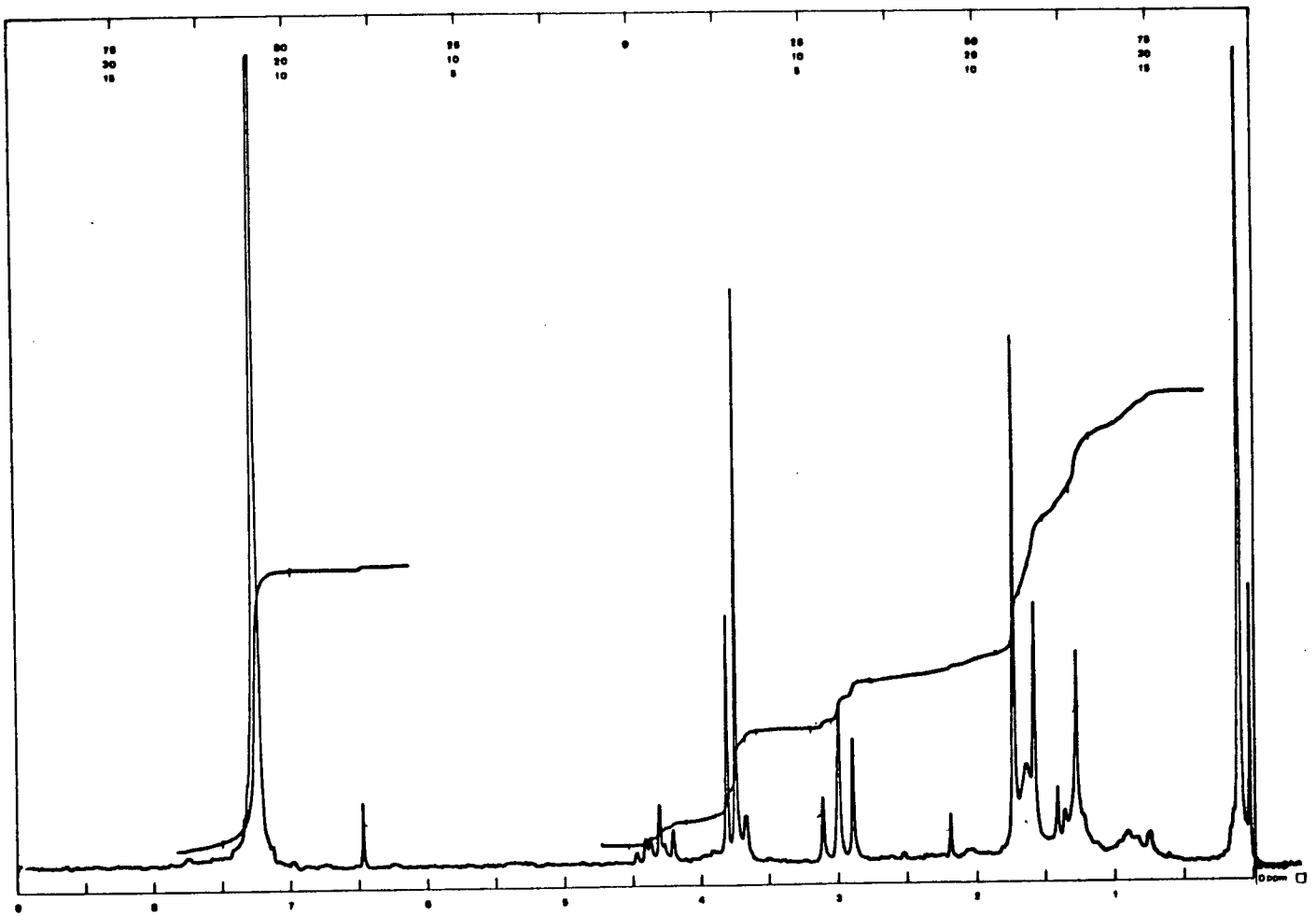
19. 100 MHz ^{1}H NMR of -N-hydroxy-α-methyl-γ-phenyl-benzene-propanamine (55)



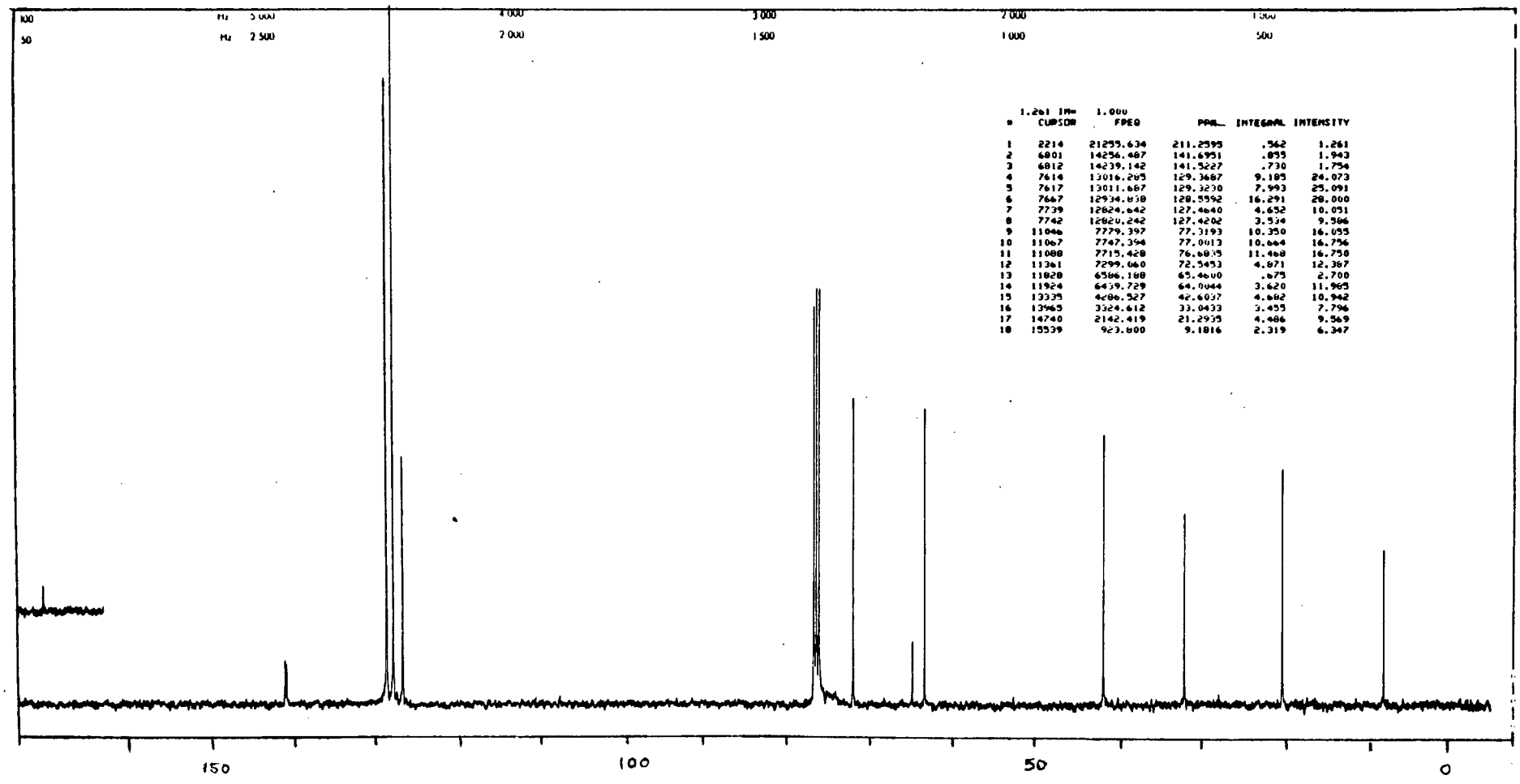
20. 100 MHz ^{1}H NMR of N, α-dimethyl-N-hydroxy-γ-phenyl-benzenepropanamine (57)



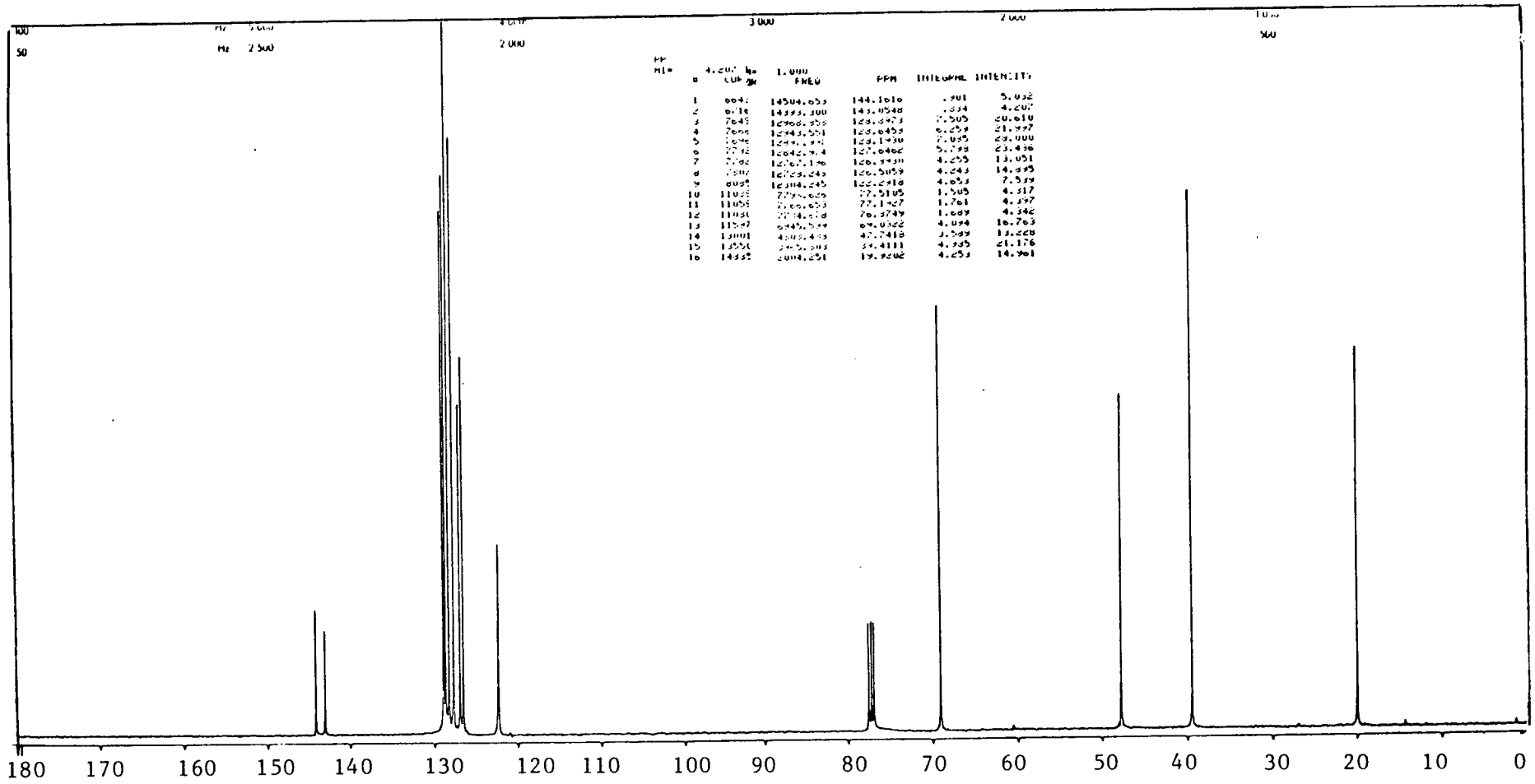
21. 100 MHz ^{1}H NMR of 1,1-diphenyl-3-nitrosobutane-dimer (61)



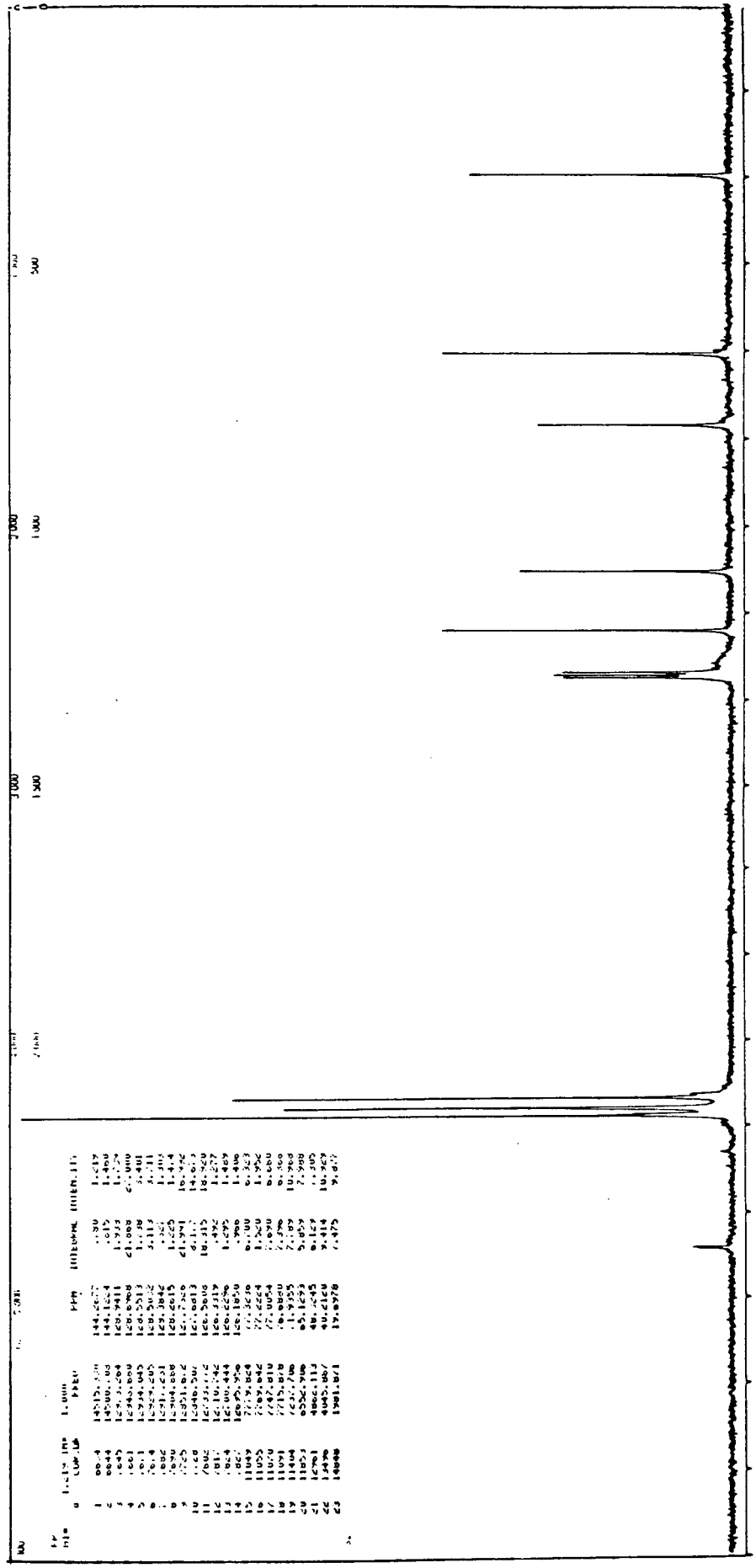
22. a) 80 MHz ^{1}H NMR of syn and anti 1,1-diphenyl-3-butanone-O-methyl oxime (64)



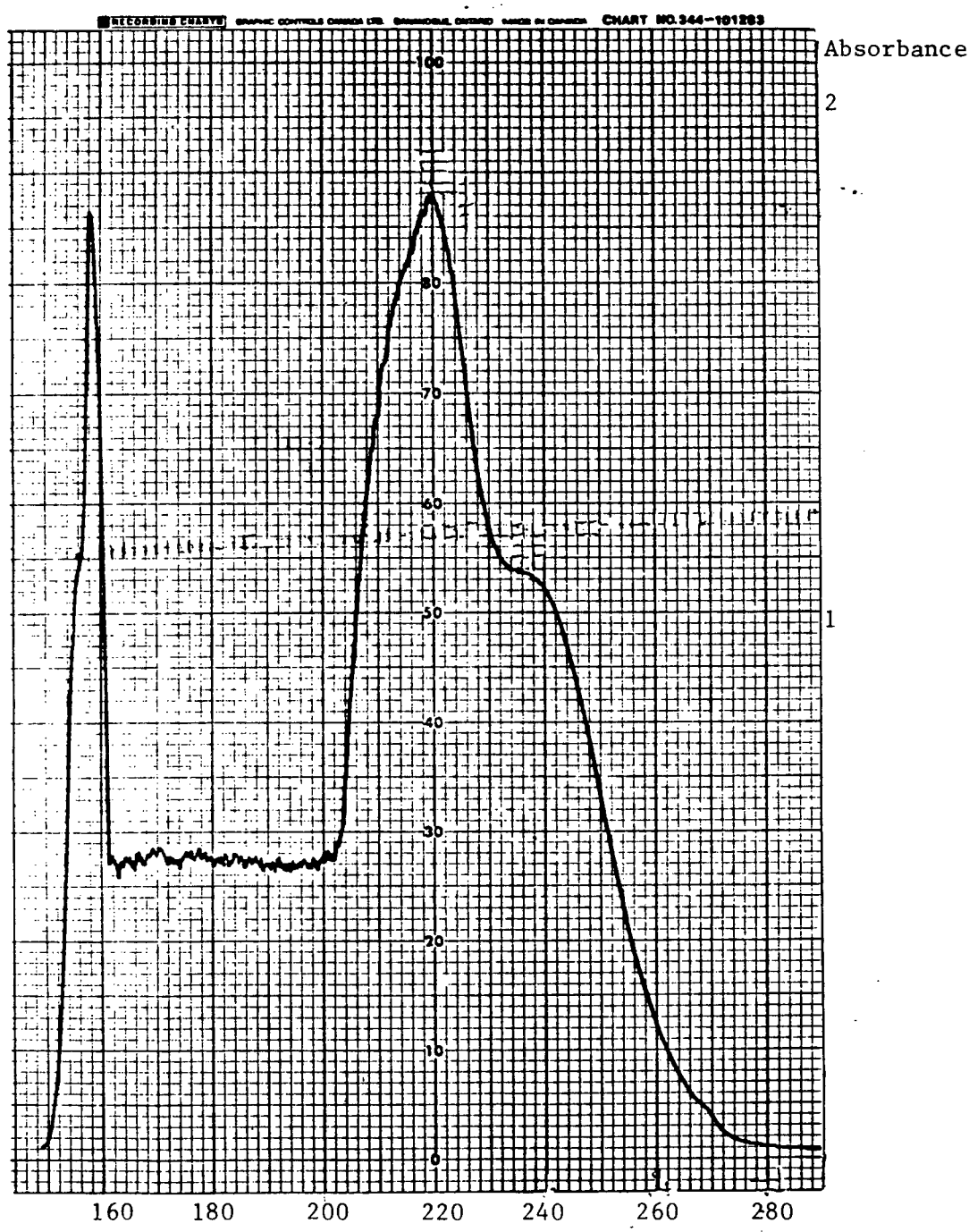
23. Broad band decoupled 400 MHz ^{13}C NMR of 2-(4',4'-diphenyl heptan-5'-one-2'-yl) oxaziridine (major diastereomer) (5)



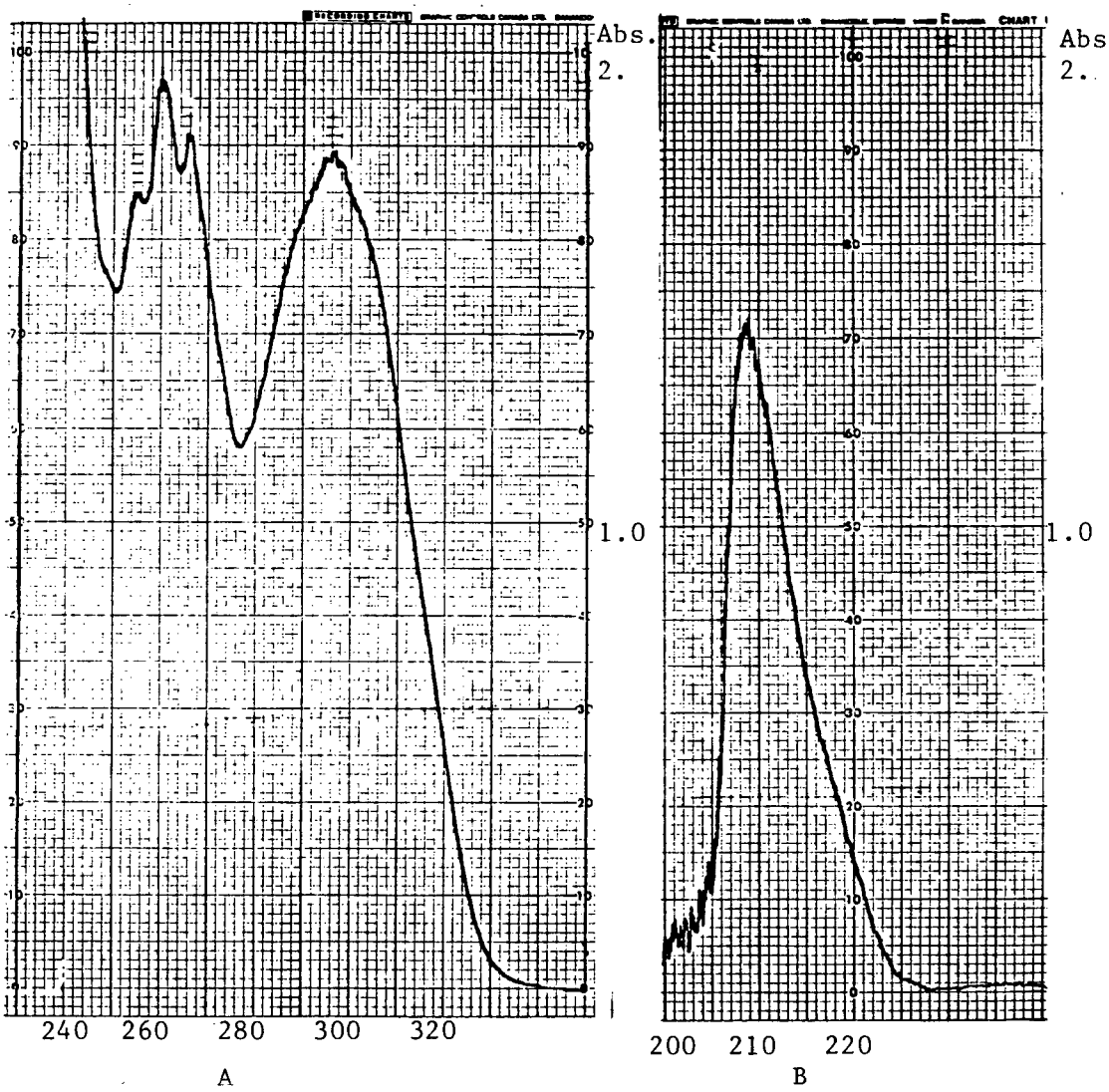
24. Broad band decoupled 400 MHz ^{13}C NMR of α-methyl-(N-methylene)-γ-phenyl-benzenepropanamine N-oxide (13)



25. Broad band decoupled 400 MHz ^{13}C NMR of 2-(1',1'-diphenyl-but-2'-yl) oxaziridine (major diastereomer) (14)



1. Ultraviolet spectrum of Recipavrin nitrone (13) in methanol



2. Ultraviolet spectrum of methadone oxaziridine (5) in methanol (A) 3.54×10^{-3} M (B) 3.54×10^{-5} M