SYNTHETIC STUDIES ON SLAFRAMINE

by

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ABSTRACT

This thesis is concerned with the preliminary studies in the synthesis of an indolizine alkaloid, slaframine.

A Wittig reaction of 3-carbethoxypropyltriphenyl-phosophonium iodide (43) with 4-pentenal (44) gave ethyl-(Z)-4,8-nonadienoate (45). Hydrolysis of this product gave the unsaturated acid (46) which was then converted into acid chloride (47), acylazide (48), isocyanate (49) and finally 1-amino-3,7-octadiene (50). The primary amino group of (50) was protected by forming trifluoroacetate derivative (51). 1-Amino-3,7-octadiene was converted in N-(Benzyloxycarbonyl)-3,7-octadiene (52) which was finally converted into epoxide (53).

Other related studies involved: (1) preparation of 1,2-oxidocyclohex-4-ene (54) which was converted into trans-2-azido-cyclohex-4-enol (55). The secondary alcohol group was protected by forming acetate derivative (56). A tetrahydropyranyl derivative (57) was also prepared.

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INTRODUCTION AND HISTORICAL

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In 1959 O'Dell, Regan and Beach [1] in Missouri reported a widespread toxicity problem associated with second-cutting red clover hay. Cattle and sheep which ate such hay slobbered excessively, went off feed, developed diarrohoea, bloat, stiff joints and sometimes died. The toxic principle was organic in nature, slowly lost activity in solution and was soluble in water, ethyl alcohol and chloroform. Bayers and Broquist [2,3] investigating similar cases in Illinois, suggested that an alkaloid was involved, but did not identify the causal agent.

Smalley et al [4] and Crump et al [5] investigated similar disorder in ruminants in Wisconsin. They observed that samples of red clover forage associated with excessive salivation in diary cattle were infested with a dark-brown coloured fungus identified as Rhizoctonia leguminicola Gough and Elliott, the cause of black patch disease of red clover.

The refusal of the cattle to consume "slobber forage" constituted an economic loss to the farmer, including a loss of milk production and the necessacity of purchasing replacement forage.

The isolation and purification of the active agent in

slobber forage from R. leguminicola was reported in 1965 independently by two research groups.[6,7]

D. P. Rainey et al[6] isolated R. leguminicola from second-cutting red clover hay from Galesville, Wisconsin, by culturing on a medium containing 20 g soybean meal, 20 g dextrose, 5 g $CaCO_3$ and 5 g corn steep liquor per litre distilled water. This medium yielded consistent and reproducible production of the active principle.

For bioassay of the salivation factor guinea-pigs were used. Each animal was rated for the degree of response to the stomach feeding trial. Numerical ratings were assigned as follows:

1 = no response

2 = slight salivation

3 = excessive salivation, lacrimation and defecation

4 = severe depression, salivation, lacrimation, defecation and dyspnoea

5 = death

This rating provided a semiquantitative measure of the activity of the preparation tested. D. P. Rainey et al[6] concluded from the general chemical behaviour that the toxic substance from the R. leguminicola was an alkaloid.

S. D. Aust et al[7] obtained R. leguminicola from Dr. J. W. Gerdenmann of the University of Illinois, who had isolated

a pure culture of this fungus from red clover. From the parasympathomimetic action of salivation factor ,S.D.Aust et al[7] suggested that it may be an anticholinesterase or an acetyl-choline-like substance which was not true.

In 1966, S.D.Aust et al[8] assigned the structure of the slobber factor. They assigned structure* (1) to the alkaloid and proposed the name slaframine (slafra: to slaver). The structure (1) was incorrect and the correct structure was proposed by them later. They also showed that this compound was not a cholinesterase inhibitor. It did not stimulate cholinergic fibers directly, it appeared to hypersensitize smooth muscle preparations to acetylcholine.

S.D. Aust et al[8] isolated slaframine ($C_{10}H_{18}N_2O_2$) from the mycelium of R. leguminicola as its amorphous hygroscopic dihydrochloride, and characterized as its crystalline dipicrate, m.p. 183-184° , $C_{10}H_{18}N_2O_2$, $2C_6H_3N_3O_7$.

Slaframine hydrochloride contains a secondary acetate group (n.m.r. in D_2O, three proton singlet at δ 2.15, one proton multiplet at δ 5.55). Exposure of slaframine or impure 'salivation factor' to mild alkali (e.g. pH>10 for several hours at 25°) resulted in loss of physiological activity and treatment of slaframine for two minutes with boiling 1N sodium hydroxide yielded crystalline Dragendorff-positive deacetylslaframine (2) $C_8H_{16}N_2O$ which was devoid of biological activity.

* See plate 1

In the n.m.r. spectrum (D_2O) of the hydrochloride of this compound, the secondary carbinol proton appeared at δ 4.60.

A primary amino group in slaframine was indicated by a purple ninhydrin test and by Van Slyke analysis on the hydrochloride. Treatment of the slaframine free base with acetic anhydride at 95° gave crystalline N-acetylslaframine (3) $C_{12}H_{20}N_2O_3$ m.p. 140-142° , $[\alpha]_D^{25°} = -15.9°$, whose infrared spectrum ($CHCl_3$) contained bands at 3420 cm^{-1} (amide N-H stretch), 1665 cm^{-1} and 1510 cm^{-1} (amide I and amide II respectively).

Slaframine, with neither C=C nor C=N (infrared) and no n.m.r. methyl signal other than that of the acetyl group, must be a bicyclic tertiary amine since the remaining basic nitrogen (bridgehead) was not acetylatable and gave positive citric acid-acetic anhydride and positive Dragendorff's tests.

Treatment of N-acetylslaframine with cyanogen bromide gave the ring-opened product (4) $C_{13}H_{20}BrN_3O_3$ (N-C≡N band at 2210 cm^{-1}) which when treated with sodium iodide followed by lithium aluminium hydride gave (5) $C_{10}H_{22}N_2O$.

The latter was methylated with formaldehyde-formic acid to give (6) $C_{12}H_{26}N_2O$ and was also acetylated with acetic anhydride to give (7) $C_{16}H_{28}N_2O_4$.

The n.m.r. spectrum of (5) showed in addition to the expected N-ethyl group ($N-CH_2CH_3$, δ 3.21 m,1.10 t) a $C-CH_2CH_3$ group (δ 1.55 m,1.00 t) established as part of a $-CHOHCH_2CH_3$ group by the loss of 59 mass units (C_3H_7O) from the parent ions of (5) and (6) and the loss of 101 mass units from the parent ion of (7) a fragmentation not found for slaframine and its derivatives (2) and (3). Thus ,the partial formula $>N-CH_2CH_2CHOH-$ was established for (1).

The similar mass spectra of slaframine (1) and deacetylslaframine (2) showed major peaks, independent of the oxygen atom, for losses of 56 and 43 mass units.The peak at M-43 (due to loss of C_3H_7) shifted on deuterium exchange of slaframine hydrochloride,that at M-56 (due to loss of C_3H_6N) did not.

The former indicated a $-CH_2CH_2CH_2-$ unit, the latter suggested the unit $-CH(NH_2)CH_2CH_2-$; the two were then combined as $-CH(NH_2)CH_2CH_2CH_2-$,and the structure of slaframine was assigned (incorrectly) as (1), 1-acetoxy-8-aminooctahydroindolizine.

The fragmentation pathways referred to above are summarized in Scheme-1* for the unacetylated compounds (2) and (5).

S.D. Aust et al[8] suggested the stereochemistry of the molecule to be that of (1a) from the low frequency (1700 cm^{-1}) of

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*See plate II

the ketone formed on chromic acid oxidation of N-acetyldeacetylslaframine and the instability of slaframine free base.

In the same year Barbara J. Whitlock et al[9] proposed an identical structure for slaframine (1).

In 1968 Robert A. Gardiner et al[10] proposed the revised structure* (8) for slaframine and assigned the stereochemistry as (1S, 6S,8aS)-1-acetoxy-6-aminooctahydroindolizine (8a).

Robert A. Gardiner et al[10] performed spin-decoupling experiments on N-acetylslaframine hydrochloride (D_2O solution) and showed that H-8a (δ = 3.41) coupled to H-1 (δ = 5.49, J = 6.5 Hz) was also coupled to one or more protons at δ = 2.1 (H-8) rather than to the -CHNAc proton. That proton (H-6, multiplet, δ = 4.15) was also coupled to H-5 axial (quartet, δ = 3.1, $J_{5a,6}$ = 2.8 Hz) which in turn was coupled only to H-5 equatorial (doublet, δ = 3.90, $J_{5a,5e}$ = 13.0 Hz) establishing its location. The half-band width of H-6 was 7 Hz consistent only with its equatorial nature.

The relative configuration at C-1 and C-6 was assigned by comparison of the n.m.r. spectrum ($CDCl_3$ solution) of N-acetylslaframine to those of isomeric 1-acetoxyoctahydroindolizine (9) prepared from the corresponding isomeric 1-hydroxyoctahydroindolizine (10). The carbinyl

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*See plate III

acetate proton of N-acetylslaframine appeared at δ = 5.24 with a half-band width of 13 Hz while the carbinyl acetate proton of cis-(9) (H_1,H_{8a} cis) appeared at δ = 5.21 with a half-band width of 13 Hz. The carbinyl acetate proton of trans-(9) appeared at δ = 4.76 with a half-band width of 21 Hz. Moreover,the general shape of the spectrum of N-acetylslaframine was nearly identical with that of cis-(9) but quite different from that of trans-(9). In particular, the splitting patterns for the carbinyl acetate protons were superimposable.

Treatment of N-acetyl-O-deacetylslaframine with α-phenylbutyric anhydride gave residual α-phenylbutyric acid of (+) rotation $[\alpha]_D^{25^\circ} = -0.48^\circ$, thus assigning C-1 the S absolute configuration. The mass spectrum agreed with the structure (8) assigned to the slaframine.

Studies on slaframine have indicated that it has potential value both as a research tool, for the isolation of the acetylcholine receptor site and as a medicinal agent for the treatment of cystic fibrosis syndrome, since it stimulates pancreatic secretion. However, slaframine has been produced in low yield by surface cultures of R. leguminicola and it is very difficult to obtain the metabolite from its natural source and the chemical synthesis of slaframine is an attractive prospect.

The synthesis of slaframine was reported in 0.12% overall yield by Cartwright et al [11] in 1970 by route shown* in the scheme-II.

The route chosen for the synthesis proceeds from 2-bromo-5-nitropyridine (12) (prepared from 2-hydroxy-5-nitropyridine) (11) by Binz and Schick method [12]). Fusion of (12) with cuprous cyanide and acidic hydrolysis of the resulting nitrile gave the carboxylic acid (13) m.p. 211-212° which was esterified in ethanol-sulfuric acid to (14) m.p. 107-108° in 11% overall yield. Reduction of (14) over platinum oxide in ethanol gave the amino ester (15) m.p. 129-130° (98% yield); acetylation of (15) with acetic anhydride gave the acetyl derivative (16) which was crystallized from ethylacetate as white needles m.p. 156-157°. Hydrogenation of the pyridine ring at 50 psi converted (16) to (17) a clear oil b.p. 187-200° (0.05 mm). Cyclization of (18) using potassium-tert-butoxide in toluene at 0° gave the unstable β-ketoester (19) in 75% yield. The β-ketoester (19) was hydrolysed and decarboxylated by heating with 8N hydrochloric acid at 100° for 5.5 hours to give the ketone which on reduction with sodiumborohydride gave the important amino alcohol intermediate (20) (a mixture of stereoisomers). The mixture of amino alcohols was acetylated with acetic anhydride to give 1-acetoxy-6-acetamidoindolizidine (21) in 81% yield (a mixture of stereoisomers). Careful chromatography on alumina separated the four stereoisomers, labeled 21a, 21b, 21c and 21d in order of their elution with chloroform;

*See plate IV

the approximate ratio of the isomers isolated was 1:1.4:4:5 (21a:21b:21c:21d) isomer 21c was identical with N-acetylslaframine. Deacetylation of (21c) was achieved by boiling with hydrazine hydrate for 3 days. The product deacetylslaframine (22) was converted with benzylchloroformate to N-carbobenzoxy-slaframine (23) which was purified by chromatography on alumina and crystallized from ether as white needles, m.p. 154-156°. Acetylation of (23) with acetic anhydride gave the acetate (24) (93% yield) which was hydrolyzed to slaframine (8) in 98% yield by stirring with 30% hydrobromic acid in acetic acid for 1 hour.

More recently, Gensler and Hu[13] accomplished the synthesis of slaframine in 0.32% overall yield by the route* shown in the scheme-III.

The starting compound was ethyl-N-(β-carbethoxy-ethyl)-5-oxopyrrolidine-2-carboxylate (26) which can be prepared conveniently from L-(+)-glutamic acid (25) and acrylonitrile. Cylization with sodium ethoxide produced ethyl-1,5-dioxopyrrolizidine-2-carboxylate (27). Decarboxylation of the pyrrolizidine (27) in hot hydrochloric acid was accompanied by lactam ring hydrolysis, so the product was the 3-oxopyrrolizidine acid (28). The corresponding alcohol methyl ester (29) was obtained by hydrogenating the keto group over a platinum catalyst in methanol solvent.

To attach the fused six-membered rings as in slaframine (8)

* See plate VI

the sequence continued by alkylating hydroxypyrrolidine (29) on the nitrogen with methylbromoacetate. The expected diester (30) was obtained mixed with equally useful lactone (31). The relation between the two products was established by allowing lactone (31) to methanolize, whereupon dimethyl ester (30) was produced.

Dieckmann cyclization of the mixture of diester (30) and ester lactone (31) gave rise to indolizidine (32). The unstable Dieckmann product (32) was decarboxylated with acid to give 1-hydroxy-6-oxoindolizidinehydrochloride (33) which was first acetylated to (34) and then converted into the relatively stable oxime (35). The oxime (35) emerged as a mixture of syn and anti forms, which could be separated and characterized. The last step proceeded by hydrogenating the mixed oximes to the final products DL-slaframine (36).

The biosynthesis of slaframine (8) which has been studied by Broquist et al [14,15] proceeds from Lysine (37) through pipecolic acid (38), other intermediates* (39-42) are shown in the scheme-IV. (1-^{14}C) DL-Lysine and (6-^{14}C) DL-Lysine were equally incorporated into slaframine and this incorporation was effectively blocked by pipecolic acid. Both ring-labeled and carboxyl-labeled pipecolic acid were equally incorporated into slaframine and were 1,000 times more effective than Lysine. Tritiated 1-ketoindolizidine (39), 1-hydroxylindolizidine (40) and 1,6-dihydroxy-indolizidine (41) were all utilized in slaframine biosynthesis.

*See plate VIII

Slaframine possesses extensive cholingeric properties due to its ability to bind strongly to the acetylcholine receptor site. It has also been demonstrated that slaframine is a specific and potent stimulator of exocrine glands.[16] This, in conjunction with its long duration of action and low toxicity, suggests that slaframine may have potential value as a research tool or as a medicinal agent for example in the study or treatment of cystic fibrosis.

Slaframine has been shown to be inactive in its native form, but to be converted to an active metabolite by the drug metabolizing enzymes of the liver.[16] Some recent efforts have been directed toward determining the structure of the active factor resulting from the action of liver microsomes on slaframine. It has been demonstrated that flavin mononucleotide (FMN) in the presence of light mimiced the liver oxidase system by converting slaframine to the same active factor.[17]

Despite the existing syntheses there is a need for new and better routes to slaframine, its derivative and to related compounds which may possibly be converted to the same active factor by the liver microsomes. The author, under the direct supervision of Dr. D. E. Orr, attempted the synthesis of slaframine.

In the partial synthesis of slaframine presented in this thesis, 3-carbethoxypropyltriphenylphosphonism iodide* (43) was

*See plate IX

reacted with 4-pentenal (44) to give ethyl-(Z)-4,8-nonadienoate (45). Alkaline hydrolysis of the ester gave the corresponding acid (46). The acid was converted into the acid chloride (47) acylazide* (48), isocyanate (49) and then into amine (50).

The primary amino group thus produced was protected by forming N-benzyloxycarbonyl derivative (52). This derivative was then converted into epoxide (53).

The compound (53) was felt to represent a key intermediate in the slaframine synthesis. All the necessary atoms in the basic skeleton of slaframine appear to be present in this compound. This compound contains the required molecular skeleton with appropriate functionality for conversion into slaframine.

An alternative approach was also attempted. Cyclohexadiene was converted into 1,2-oxidocyclohex-4-ene** (54) with m-chloroperoxybenzoic acid. This compound was converted into trans-2-azidocyclohex-4-enol (55). Attempts at ozonolysis followed by catalytic hydrogenation of this compound to the desired dialdehyde for slaframine, under a variety of experimental conditions, were not successful.

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*See plate X
** See plate XI

DISCUSSION

DISCUSSION

Our early thinking connected with the synthesis of slaframine involved the initial formation of the five-membered heterocylic ring. This ring closure would have to occur in such a way as to produce a secondary alcohol which is trans to the bridge head hydrogen. It is well known that oxiranes open in a stereospecific fashion, so we reasoned that if the synthesis of the appropriate Z-olefin could be achieved, we might test this hypothesis which is outlined in scheme-V (plate XII).

The nitrogen would have to be suitably protected and the R. group should contain the required functionality for construction of the six-membered heterocyclic ring and introduction of the primary amine group. Due to the ready availability of starting material we decided to use a molecule in which the R. group contained a terminal carbon-carbon double bond.

We anticipated that the Z-olefin could be constructed by using appropriate Wittig reactions. L. D. Bergelson et al[30] has shown the stereochemistry of the Wittig reaction between an aldehyde and a non-stablized ylid is influenced by a number of factors. In particular, L. D. Bergelson[31] has shown that Z-olefin results from the reactions in DMF of the ylid derived from ω-alkoxycarbonylalkylidinetriphenylphosphonium

iodide and an aldehyde. M. Schmid and R. Barner[32] describe a similar reaction involving a substituted butanoate to produce the Z-olefin and in our own case we have applied the reaction to the synthesis of ethyl-(Z)-4,8-nonadienoate.

In the initial step of the reaction sequence it was necessary to form 3-carbethoxypropyltriphenylphosphonium iodide (43) (plate IX). This compound was readily obtained from ethyl-4-bromobutyrate and triphenylphosphine. The proton magnetic resonance spectrum (n.m.r. NO-1) of the pure reaction product afforded evidence that the desired compound had been formed. A methyl triplet at δ = 1.3 (J = 7.5 Hz) and a methylene quartet at δ = 4.12 (J = 7.5 Hz) confirmed the presence of ethyl ester. The multiplet at δ = 2.0 was assigned to the methylene proton β to the phosphorous. A triplet at δ = 3.0 (J = 7.5 Hz) was due to the methylene proton α to the carbonyl group. The multiplet at δ = 3.8 - 4.0 was due to the methylene proton α to the phosphorous: a multiplet at δ = 7.78 confirmed the presence of the phenyl groups.

For our purpose we also prepared 4-pentenal (44) from allyl alcohol and n-butylvinylether using the procedure of Watanabe et al[20] The formation of this compound was supported by proton magnetic resonance spectrum (n.m.r. NO.-2). A multiplet at δ = 2.52 was due to four methylene protons. The multiplet at δ = 5.0 confirmed the presence of the terminal two protons of the double bond and the multiplet at

δ = 5.6 - 6.0 confirmed the third proton of the double bond. The aldehyde proton appeared as a multiplet at δ = 9.58.

The next step in the synthesis involved the use of Wittig reaction for the preparation of our starting compound ethyl-(Z)-4,8-nonadienoate (45). This compound was obtained by reacting the ylid produced from 3-carbethoxypropyltriphenylphosphonium iodide (43) and 4-pentenal (44). The proton magnetic resonance spectrum (n.m.r. NO-3) of the reaction product afforded evidence that the desired compound had been formed. A methyl triplet at δ =1.3 (J = 7.5 Hz) confirmed the presence of ethyl ester. A multiplet at δ = 2.1 - 2.5 was due to the eight protons of the methylene groups. The multiplet at δ = 4.9 - 5.2 showed the presence of the two terminal protons of the double bond, and a multiplet at δ = 5.5 was due to the two protons of the (4,5) double bond. The multiplet at δ = 5.8 - 6.0 confirmed the presence of the third proton of the terminal double bond. The infrared spectrum (IR NO-1) exhibited absorption bands at 1650 cm^{-1} (double bond), 1750 cm^{-1} (ester group) and 2940 cm^{-1} (C-H stretch). The mass spectrum of the compound gave a molecular ion peak at m/e 182.

We have observed that the stereochemistry of a Wittig reaction carried out in the presence of Crown ether is remarkably dependent on solvent. The cis-trans ratio of ethyl-4,8-nonadienoate varied with solvent as shown in Table - I. In methylene chloride the major product was cis

and in THF the major product was trans isomer. When we use sodium hydride as a base in DMF without Crown ether the major product was also cis isomer. The ratio of the cis-trans products was determined by gas chromatography using 10% SE-30 on gas chrom Q at 150°.

A comparison of the products from the Wittig reaction carried out in the different solvent is informative. The olefinic compounds obtained using DMF or methylene chloride are identical in the olefinic region of the n.m.r..Furthermore, the peak at δ =5.5 due to the two protons attached to the 4,5 double bond is identical in shape and chemical shift to the peak due to the double bond protons of (Z)-methyl-9-octadecenoate.

The nature of the carbon skeleton of the Wittig product was confirmed by hydrogenation. The hydrogenation product (45a) was identified as ethylnonate. The n.m.r. and IR spectra of the saturated ester (45a) were identical with authentic sample. Moreover, the saturated ester (45a) showed a single peak on gas chromatography whose retention time was identical with ethylnonate.

The next step in the synthesis required conversion of the doubly unsaturated ester (45) into the corresponding acid (46). This goal was readily achieved by treatment of (45) with aqueous potassium hydroxide under mild conditions to give the olefinic acid (46). The

proton magnetic spectrum (n.m.r. NO-4) had a multiplet at δ = 2.1 - 2.5 assigned to the eight methylene protons. The multiplet at δ = 4.9 - 5.2 confirmed the presence of the two teminal protons of the double bond. A multiplet at δ = 5.5 was due to the two protons of the 4,5 double bond. The multiplet at δ = 5.8 - 6 showed the presence of the third proton of the terminal double bond. The single proton of carboxylic acid function was shown downfield at δ = 9.89.

At this stage it was necessary to convert the unsaturated acid (46) into the acyl azide. Acylazides[24] are thermally unstable and rearrange to isocyanates on heating. They can therefore be synthesized only by those methods which operate at room temperature. For our purpose we used a solution of pyridine and hydrazoic acid in toluene. Pyridine and hydrazoic acid react in toluene to give an equillibrium mixture that contains a soluble source of azide anions. We first converted the acid (46) into acid chloride (47) by treating with thionyl chloride. The formation of the acid chloride was supported by IR spectroscopy. The infra-red spectrum (IR-NO-2) exhibited absorption bands at 1805 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-Cl$). The acid chloride (47) was then converted into the acyl azide (48) (plate X) by treating with a mixture of pyridine and hydrazoic acid in toluene at 0°. Pyridinium chloride percipitated instantly. The formation of the compound (48) was shown by IR spectroscopy (IR-NO-3) in which the carbonyl absorption of acid chloride was absent and two new bands appeared at 1720 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$) and 2130 cm^{-1} ($-N=\overset{+}{N}=\overset{-}{N}$). The proton magnetic resonance spectrum

(n.m.r. -NO-5) provided evidence that the desired compound had formed. The multiplet at δ = 2.18- 2.59 was due to the eight methylene protons. A triplet at about δ = 3.3 indicated that a small amount of isocyanate had already formed.The multiplet at δ = 4.9 - 5.2 showed the presence of two protons of the terminal double bond. The two protons of the 4,5 double bond appeared as a multiplet at δ = 5.5. The third proton of the terminal double bond appeared as a multiplet at δ = 5.8 - 6.0.

The next step in the synthesis involves the conversion of acyl azide (48) into isocyanate (49). This was carried out simply by heating the acyl azide (48) solution at 100^{o} for four hours. Visible evidence for this reaction was supplied by the evolution of gas from the reaction mixture. The formation of the isocyanate (49) was supported by IR and n.m.r. spectra. The strong absorption peak (IR - NO - 4) at 2260 cm^{-1} is characteristic of the isocyanate group.

The proton magnetic resonance spectrum (n.m.r. - NO-6) had a multiplet at δ = 2.18 - 2.59 which was assigned to the six methylene protons. The n.m.r. spectrum again indicated the presence of the protons associated with the double bonds and a triplet at δ = 3.36 (J = 7.5 Hz) assigned to the methylene protons adjacent to the nitrogen atom of the isocyanate group.

The next important step in the synthesis involves the hydrolysis of isocyanate (49) into amine (50). Organic isocyanates are

very often unstable being susceptible to thermolysis, photolysis or solvolysis. It should be pointed out that at this stage we need selective hydrolysis to keep the double bonds unaffected and acetic acid:water[26] (2:1) was the best reagent for hydrolysis of isocyanate (49) to unsaturated amine (50). When a solution of isocyanate (49) in toluene was heated with a mixture of acetic acid:water (2:1), it gave the desired amine (50). The proton magnetic resonance spectrum (n.m.r. - NO-7) afforded evidence that the desired compound had formed. A multiplet at δ = 2.18- 2.59 was due to six methylene protons. The triplet at δ = 2.7 confirmed the presence of the methylene group α to the nitrogen. This compound was contaminated with some residual isocyanate (triplet at δ = 3.30). The multiplet at δ = 4.9 - 5.2 confirmed the presence of the two terminal protons of the double bond. A multiplet at δ = 5.5 was due to the two protons of 4,5 double bond. The multiplet at δ = 5.8 - 6 showed the presence of the third proton of the terminal double bond. The formation of the amine (50) was further confirmed by hydrogenation of the amine (50) at atmospheric pressure using Adam's catalyst and comparing the n.m.r. and IR spectra which were identical with the authentic octylamine. The amine (50) was shown to be a mixture by tlc probably comprised of the E and Z isomers.

The amine (50) has a basic carbon skeleton for slaframine with appropriate functionality. At this point it was decided to protect the primary amino group. Two different groups were considered suitable

for this purpose.

The first method involved the conversion of amine (50) into trifluoroacetate derivative (51). The trifluoroacetyl group is an effective protecting group for amino groups. The ease with which this group can be introduced and more importantly the facility with which it can be removed makes it a highly attractive protecting group. The trifluoroacetate derivative (51) was obtained by treating an ether solution of the amine (50) with trifluoroacetic anhydride in alkali. The proton magnetic resonance spectrum (n.m.r. -NO-8) indicated that the desired compound had been produced. The multiplet at δ = 2 - 2.52 confirmed the presence of the six methylene protons. A quartet which appeared at δ = 3.48 (J = 7.0 Hz) was due to the two protons α to the nitrogen atom. The corresponding protons in the free amine were at δ = 2.7. The multiplet at δ = 4.9 - 5.0 confirmed the presence of two protons of the terminal double bond. A multiplet at δ = 5.5 indicated the presence of two protons of the 4,5 double bond. The third proton of the terminal double bond was indicated by a multiplet at δ = 5.8 - 6. The infrared spectrum (IR -NO-5) exhibited absorptions at 1560 cm^{-1} (double bond), 1740 cm^{-1} (- $\overset{O}{\overset{\|}{C}}$ -), 2985 cm^{-1} (C-H stretch) and 3460 cm^{-1} (N-H stretch).

The next step in the synthesis involved the conversion

of trifluoroacetate derivative (51) into an epoxide, but unfortunately this conversion was not successful.

A second approach involved the conversion of amine (50) into N-(benzyloxy carbonyl) derivative (52). This is also a suitable protective group since it can be easily removed, under mild conditions. The formation of N-benzyloxy carbonyl derivative (52) was achieved by treatment of an ethanolic solution of the amine (50) with benzyl chloroformate in alkali. Spectroscopic evidence supported the formation of the desired compound. In the proton magnetic spectrum (n.m.r. - NO-9) a multiplet at δ = 2 - 2.19 assigned the six allylic protons. The quartet at δ = 3.30 (J = 7 Hz) confirmed the presence of two protons α to the nitrogen. A multiplet at δ = 4.8 - 6 confirmed the presence of five olefinic protons. A sharp singlet at δ = 5.1 assigned the two protons of the methylene group adjacent to phenyl group. Another sharp peak which integrated less than two protons at δ = 4.66 possibily indicated the presence of the E-isomer of (52). The five protons of the phenyl group was indicated by absorption at δ = 7.36. The infrared spectrum (IR - NO-6) showed absorptions at 1545 cm^{-1} (double bond), 1750 cm^{-1} (- $\overset{O}{\overset{\|}{C}}$ -), 3080 cm^{-1} (C - H stretch for aromatic rings) and 3480 cm^{-1} (N - H stretch).

The next step in the synthesis involved the conversion of N-benzyloxycarbonyl derivative (52) into epoxide (53). This was achieved by treatment of (53) with m-chloroperoxybenzoic acid in methylene

chloride solution. The reaction product was purified by column chromatography on silica gel using benzene:ether (9:1) as eluent. The proton magnetic spectrum afforded evidence that the desired compound had been formed. In the proton magnetic spectrum (n.m.r - N0-10) a multiplet at δ = 1.5 showed the presence of six protons of the methylene group. The multiplet at δ = 2.3 confirmed the two protons of the terminal methylene group. A multiplet at δ = 3.0 indicated the presence of the three methine protons. A quartet at δ = 3.3 (J = 7.0 Hz) showed the presnece of two protons α to the nitrogen. A sharp singlet at δ = 5.1 confirmed the presence of the two methylene protons adjacent to the phenyl group. The five protons of the phenyl gruop was indicated by absorption at δ = 7.3.

This last reaction shows that our doubly unsaturated protected amine can be converted into the dioxirane by a per-acid oxidation. It should now be possible to selectively oxidize the terminal double bond to give the monooxirane as shown in the scheme* VII cyclization of this compound followed by a second epoxidation and cyclization should give a substance that could be converted into slaframine.

Another approach that we considered was the use of 1,4-cyclohexadiene as the starting material for the synthesis of an intermediate containing the Z-double bond. 1,4-Cyclohexadiene could

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* see Plate XIII

easily be converted to the mono-oxirane which when treated with sodium azide opened to the trans-azido alcohol.

Our plan was to oxidatively cleave the remaining double bond and reduce the azido group to give a compound with the desired stereochemistry and functionality (scheme - VI, plate XII). Unfortunately we could not achieve consistent and reproducible results in the oxidative cleavage of the double bond.

The starting compound necessary in this approach was 1,2-oxidocyclohex-4-ene (54) (plate - XI). This was obtained by the reaction of 1,4-cyclohexadiene with one equivalent of m-chloroperoxybenzoic acid. The nuclear magnetic resonance spectrum of the compound afforded evidence that the desired compound had formed. In the proton magnetic resonance spectrum (n.m.r. - NO-11) the methylene protons appeared as a multiplet at $\delta = 2.48$ and the methine protons appeared as a multiplet at $\delta = 3.24$. The two olefinic protons gave rise to a peak at $\delta = 5.6$ which exhibited only narrow coupling with adjacent protons.

The ^{13}C n.m.r. spectrum (^{13}C n.m.r. - NO-1) provided further evidence that the desired compound had formed. Carbon 3 and 6 were associated with peak at $\delta = 24.80$, carbon 1 and 2 were associated with peak at $\delta = 49.36$, and carbon 4 and 5 were associated with peak at $\delta = 120$.

The 1,2-oxidocyclohex-4-ene (54) was then converted into trans-2-azidocyclohex-4-enol (55) by treating with sodium azide and ammonium chloride in 80% ethanol for 16 hours under reflux conditions.

The formation of this compound was confirmed by n.m.r. and IR. In the proton magnetic resonance spectrum (n.m.r. -NO-12) the methylene group appeared as a multiplet at δ = 2.2. The hydroxyl proton appeared as a singlet at δ = 2.8. A multiplet at δ = 3.8 was due to the proton adjacent to the azido group and the proton adjacent to the hydroxyl group. A multiplet at δ = 5.6 confirmed the presence of the olefinic protons. The infrared spectrum (IR- NO-2) exhibited absorption bands at 2100 cm^{-1} (azido group) and at 3400 cm^{-1} (hydroxyl group). The formation of this compound was further confirmed by ^{13}C n.m.r. (^{13}C n.m.r. -NO-2). Carbon 3 and 6 were associated with peak at δ = 30.60 and δ = 33.62. Carbon 1 and 2 were associated with peak at δ = 70.62 and δ = 64.37. The carbon 4 and 5 were associated with peak at δ = 124.37 and δ = 125.

It was necessary to oxidatively cleave the carbon-carbon double bond in compound (55). For this purpose ozonolysis of (55) under various conditions was attempted but it was not possible to obtain the desired compound.

We then decided to protect the hydroxyl group by

forming the acetate derivative (56) of trans-2-azidocyclohex-4-enol in the usual manner. The formation of the compound (56) was confirmed by n.m.r. spectrum (n.m.r. - NO-13). A methyl singlet appeared at δ = 2.0 confirmed the presence of the acetate ester. The envelope at δ = 2 - 2.6 indicated the presence of the methylene protons. A multiplet at δ = 3.8 confirmed the presence of the protons α to the azido group.

A multiplet at δ = 4.81 - 5 confirmed the presence of the proton α to the acetate group. The olefinic protons were confirmed by multiplet at δ = 5.66. The ozonolysis on acetate derivative (56) was performed but again unsuccessful.

A second approach which was investigated involved the protection of the secondary alcohol group of (55) with 3,4-dihydropyran followed by ozonolysis. The tetrahydropyran group is stable under basic conditions, but can be readily removed under acidic conditions. A multiplet at δ = 1.5 - 2.8 in the proton magnetic resonance spectrum (n.m.r. -NO-14) of this product (56) was assigned to the ten methylene protons. The multiplet at δ = 3.8 confirmed the presence of four protons- two methylene protons, one methine proton α to the nitrogen and another methine proton α to the oxygen. The multiplet at δ = 4.9 was assigned to the single proton of the acetal. The two olefinic protons appeared as a multiplet at δ = 5.6 which exhibited only narrow coupling with adjacent protons. We again attempted ozonolysis of (57) under a variety

of conditions to obtain the desired intermediate for slaframine but, unfortunately, were not successful.

EXPERIMENTAL

ANALYTICAL AND PHYSICAL DATA

Melting points, determined with a Gallenkamp melting point apparatus and boiling points are uncorrected and reported in degrees centrigrade. Thin-layer chromatography was performed on glass plates coated with silica gel (Silica gel Woelm TLC, M. Woelm, Eschwege, Germany) to a thickness of 0.3 mm. The infrared spectra were taken by the author, using a Beckman spectrophotometer, Model IR-12.

The nuclear magnetic resonance spectra (n.m.r.) were recorded by Dr. T. Griffith and associates on a Bruker Model WP-80 n.m.r. spectrometer. All spectra were recorded in deuterated chloroform with tetramethylsilane as internal reference and are expressed in values as defined by the equation:

$$\delta = \frac{\text{observed shift (Hz)} \times 10^6}{\text{oscillator frequency (Hz)}}$$

The chemical shifts and coupling constants are only approximately obtained by first order analysis of splitting patterns for stated coupling constants and by measurements of approximate centres of multiplets for chemical shifts. The mass spectra were recorded by Mr. C. Mallard and Mr. K. Pringnitz on a Hitachi RMU-7 spectrometer. The elemental analyses were done with a Perkin-Elmer Elemental Analyser, Model 240.

The gas chromatography were done on a Perkin-Elmer Gas chromatograph Model 3920B using 10% SE-30 on gas chrom Q.

3-Carbethoxypropyltriphenylphosphonium iodide* [18] (43)

To a solution of sodium iodide (4.58 g) in ethylmethylketone (40 mL) was added dropwise a solution of ethyl-4-bromobutyrate (3.95 g) with stirring. The mixture was refluxed for 18 hours, after cooling the sodium bromide was filtered and the filtrate was concentrated to approximately 5 mL. Then benzene (20 mL) and triphenylphosphine (5.75 g) were added and the resulting mixture refluxed overnight. The benzene was removed on a rotary evaporator and the product was crystallised slowly by adding ether (70 mL) m.p. 152°, (yield, 4.50 g, 78%).

The proton magnetic resonance spectrum (n.m.r. NO-1)

δ = 1.3 (t, J = 7.5 Hz, 3 H) $-O-CH_2-C\underline{H}_3$

δ = 2.0 (m, 2H) $-CH_2-C\underline{H}_2-CH_2$

δ = 3.0 (t, J = 7.5 Hz, 3 H) $-C\underline{H}_2-\overset{\overset{O}{\|}}{C}-$

δ = 3.8 - 4.0 (m, 2 H) $-C\underline{H}_2-\overset{|}{\underset{|}{P}}-^{+}$

δ = 4.12 (q, J = 7.5 Hz, 2 H) $-O-C\underline{H}_2CH_3$

δ = 7.78 (m, 15 H) $(C_6\underline{H}_5)_3-$

*See plate IX

4-Pentenal [19] (44)

Allyl vinyl ether was prepared by the method of Watanabe and Conlon. [20] A mixture of allylalcohol (149 g) and n-butylvinyl ether (183 g) was heated with mercuric acetate (2.5 g) in a flask set up for distillation through a 22 inch glass-helices packed column. Heating was continued until crude allyl vinyl ether (157.5 g) had distilled at 66°-68° and no further distillation would occurr in this range. The crude material was redistilled to give allyl vinyl ether (122 g) b.p. 66-68°.

Pyrolysis of allyl vinyl ether was performed by the method of Hurd and Pollack [21] using pyrex helices packed pyrex tube at 300° with a nitrogen flow. In this manner 34 g of ether gave 25 g (70% yield) of distilled 4-Pentenal b.p. 103-104°.

The proton magnetic resonance spectrum (n.m.r. NO-2):

δ = 2.52 (m, 4 H) $-(C\underline{H}_2)_2-$

δ = 5.0 (m, 2 H) $C\underline{H}_2 = CH-$

δ = 5.6 - 6.0 (m, 1 H) $CH_2 = C\underline{H} - CH_2-$

δ = 9.58 (m, 1 H) $-C\underline{H}O$

Ethyl-(Z)-4,8-nonadienoate[18] (45)

Method A: To a solution of 3-carbethoxypropyltriphenylphosphonium iodide (43) (11.589 g; 0.023 mol) in dry DMF (30 mL) under nitrogen at 0° was added in portion sodium hydride (0.55 g, 0.023 mol). After the evolution of hydrogen had ceased, the dark orange solution was allowed to stand for one hour at room temperature with stirring. The solution was cooled to 10° and 4-pentenal (44) (1.83 g, 0.023 mol) added over a ten minute period with stirring. The resulting mixture was then stirred overnight at room temperature.

The reaction mixture was poured into 2N sulfuric acid (30 mL) and methylene chloride (30 mL) all at 0°. The organic layer was separated and the aqueous layer then extracted three times with 25mL portions of methylene chloride. The combined methylene chloride extracts were washed once with water and evaporated in vacuum. The residue was again extracted three times with 30 mL portion of ether, the combined ether extracts washed with water, dried over anhydrous magnesium sulfate and then evaporated to dryness. The residue was chromatographed on silica gel (60 g) using benzene as an eluent which gave the product (2.91 g, 80% yield) b.p. 98° (2.5 mm). On elemental analysis it gave C = 71.34%, H = 9.77%, required C = 72.52%, H = 9.89%.

The proton magnetic resonance spectrum (n.m.r. NO-3)

δ = 1.3 (t, J = 7.5 Hz, 3 H) $-O-CH_2-C\underline{H}_3$

δ = 2.1 - 2.5 (m, 8 H) $CH_2 = CH - C\underline{H}_2-$, $C\underline{H}_2 - \overset{O}{\overset{\|}{C}} -$

δ = 4.1 (q, J = 7.5 Hz, 2 H) $-O-C\underline{H}_2 - CH_3$

δ = 4.9 - 5.2 (m, 2 H) $C\underline{H}_2 = CH-$

δ = 5.5 (m, 2 H) $-C\underline{H} = C\underline{H}-$

δ = 5.8 - 6.0 (m, 1 H) $CH_2 = C\underline{H} - CH_2-$

It showed strong absorption in IR (IR - NO - 1) at 1650 cm^{-1} (double bond), 1750 cm^{-1} (ester group), and 2940 cm^{-1} (C - H stretch).

The mass spectrum of the compound gave molecular ion peak at m/e 182.

Method B [22] : To the 3-carbethoxypropyltriphenylphosphonium iodide (43) (1.092 g, 6 mmol), potassium carbonate (0.824 g, 6 mmol) in tetrahydrofuran (25 mL) was added 4-pentenal (44) (0.420 g, 5 mmol) and 18-Crown-6 (15 mg). The reaction mixture was refluxed for 18 hours. Then the solvent was evaporated on a rotary evaporator. The residue was extracted three times with 100 mL portions of petroleum

ether. The combined petroleum ether extracts was washed once with water, dried over anhydrous magnesium sulfate and then evaporated to dryness. The crude product was then chromatographed on silica gel using benzene as eluent which gave the product (0.655 g, yield = 60%).

Method C [22] : To a refluxing mixture of the 3-carbethoxypropyltriphenylphosphonium iodide (43) (1.092 g, 6.0 mmol), potassium carbonate (0.824 g, 6 mmol) and 18-Crown-6 (15 mg) in methylene chloride (25 mL) was added 4-pentenal (0.420 g,5 mmol).The reaction mixture was refluxed for 16 hours and then worked up as before which after chromatography gave the product,(0.70 g,yield = 65 %).

All products obtained by methods A, B and C were identified by n.m.r., infrared and mass spectra. The cis-trans ratio were determined by gas chromatography using 10% SE-30 on gas chrom Q at 150°C.

TABLE I:

Solvent	Cis-Trans ratio	Base	Retention time in minute		Yield
			cis	trans	
THF	1:3	K_2CO_3	2.28	1.38	60 %
CH_2Cl_2	5:1	K_2CO_3	2.28	1.38	65 %
DMF	2:1	NaH	2.27	1.38	80 %

Ethylnonate (45a)

The doubly unsaturated ester (45) (500 mg) was hydrogenated at atmospheric pressure in 100 mL of ethanol and 100 mg of Adams catalyst. After the uptake of hydrogen was complete the catalyst was filtered and the solvent evaporated to give 400 mg of the product (yield = 80%).

The compound was identical (n.m.r., IR and GC) with authentic ethylnonate.

4,8-Nonadienoic acid (46)

To a solution of ethyl-(Z)-4,8-nonadienoate (500 mg) in ethanol (10 mL), 10% potassium hydroxide solution (10 mL) was added and the solution allowed to stand overnight at room temperature. The ethanol was removed in vacuum on a rotary evaporator. The neutral material was removed by extracting with ether. The remaining aqueous solution was acidified with 10% sulfuric acid and then extracted three times with 100 mL portions of ether. The combined ether extract was washed with water, dried over anhydrous magnesium sulfate and then evaporated to dryness giving the product (300 mg, yield = 60%).

The proton magnetic resonance spectrum (n.m.r. - NO.4)

δ = 2.1 - 2.5 (m, 8 H) C = C - C$\underline{H}_2$, C$\underline{H}_2$ - C(=O) -

δ = 4.9 - 5.2 (m, 2 H) C$\underline{H}_2$ = C -

δ = 5.5 (m, 2 H) -C$\underline{H}$ = C$\underline{H}$ -

δ = 5.8 - 6.0 (m, 1 H) CH_2 = C$\underline{H}$ - CH_2

δ = 9.89 (1 H) - COO$\underline{H}$

Conversion of 4,8-Nonadienoic acid into Acyl azide [23] (48)

A solution of 4,8-nonadienoic acid (46) (308 mg, 2 mmol) in dry toluene (4 mL) and thionylchloride (4 mL) was stirred at room temperature for 4 hours. The reaction mixture was then evaporated in vacuum on a rotary evaporator leaving the acid chloride (47) as an oil.

IR (IR-NO-2) at 1805 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-Cl$). The acid chloride [24] (47) (344 mg, 2 mmol) was dissolved in dry toluene (4 mL) and cooled in an ice bath, the solution being carefully protected from moisture. From a dropping funnel a mixture of pyridine (158 mg, 2 mmol) and hydrazoic acid [25] (2 mL, 1.63 N) in toluene was slowly added, with stirring. After a few seconds pyridinium-chloride began to precipitate. When the addition was complete, the cooling bath was taken away and stirring continued at ambient temperature for 30 minutes. The precipitate was separated by suction filtration and the filtrate was subjected to aspirator vacuum on a rotary evaporator for a few minutes at ambient temperature to remove the excess hydrazoic acid giving the acyl azide* (48) (223 mg, yield = 65%).

The proton magnetic resonance spectrum NO-5:

δ = 2.18 - 2.59 (m, 8 H) $C=C-\underline{CH_2}$, $\underline{CH_2}-\overset{O}{\overset{\|}{C}}-$

δ =3.3 (due to isocyanate contamination)

δ = 4.9 - 5.2 (m, 2H) $\underline{CH_2}=C-$

δ = 5.5 (m, 2H) $-\underline{CH}=\underline{CH}-$

δ = 5.8 - 6 (m, 1H) $CH_2=\underline{CH}-CH_2-$

IR (IR-NO- 3) at 1720 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$) and 2130 cm^{-1} ($-N=\overset{+}{N}=\overset{-}{N}$).

*See plate X

Isocyanate[23] (49)

About 2 mL of the solution of acyl azide (48) in toluene was heated 4 hours to a temperature at which the evolution of nitrogen became brisk (about 100°). Finally the mixture was heated under reflux for 1 minute. The solvent was removed in vacuum on a rotary evaporator giving the isocyanate. Thin-layer chromatography of the product on silica gel using benzene as eluent gave a single spot.

The proton magnetic resonance spectrum (n.m.r. - NO-6)

δ = 2.18 - 2.59 (m, 6 H) C = C - $C\underline{H}_2$

δ = 3.36 (t, 2 H, J = 7.5 Hz) - N - $C\underline{H}_2$

δ = 4.9 - 5.2 (m, 2 H) $C\underline{H}_2$ = C -

δ = 5.5 (m, 2 H) - $C\underline{H}$ = $C\underline{H}$ -

δ = 5.8 - 6 (m, 1 H) CH_2 = $C\underline{H}$ - CH_2

IR (IR NO-4) at 2260 cm^{-1} (iso cyanate).

1-Amino-3,7-octadiene [26] (50)

To a solution of the isocyanate (49) (500 mg) in toluene (5 mL) was added acetic acid: water (2:1) (6 mL) and the resulting solution heated for 1 hour at 60°. Water (10 mL) was added and the resulting solution extracted with ether to remove the neutral compounds. The solution was then made alkaline with 10% sodium hydroxide solution. The basic solution was extracted with ether and the ether extract washed once with water, dried over anhydrous magnesium sulfate and then evaporated in vacuum on a rotary evaporator to dryness giving the product (200 mg, yield = 40%). Thin-layer chromatography of the product on silica gel using benzene:ether (9:1) as eluent showed two spots indicating that a mixture of products were present.

The proton magnetic resonance spectrum (n.m.r. - NO-7)

δ = 2.1 - 2.59 (m, 6 H) C = C - $C\underline{H}_2$ -

δ = 2.7 (t, 2H) $C\underline{H}_2$- N

δ = 3.30 (t, due to isocyanate contamination)

δ = 4.9 - 5.2 (m, 2 H) $C\underline{H}_2$ = C -

δ = 5.5 (m, 2 H) - $C\underline{H}$ = $C\underline{H}$ -

δ = 5.8 - 6 (m, 1 H) CH_2 = $C\underline{H}$ - CH_2 -

1-Trifluoroacetamido-3,7-octadiene [27] (51)

To a solution of the amine (50) (250 mg, 2 mmol) in dry ether (5 mL) was added sodium carbonate (318 mg, 3 mmol). The mixture was cooled in an ice bath and trifluoroacetic anhydride (1 mL) was added. The cooling bath was removed and the vigorous stirring continued for 20 minutes. Then the reaction mixture was poured into chloroform, the excess anhydride was destroyed with ice and the chloroform solution was washed with water, dried over anhydrous magnesium sulfate and then evaporated to dryness giving the product. The thin-layer chromatography on silica gel using benzene:ether (9:1) as eluent showed two spots indicating a mixture of products.

The proton magnetic spectrum (n.m.r. NO-8)

δ = 2 - 2.52 (m, 6 H) C = C - $\underline{CH_2}$

δ = 3.48 (q, J - 7.0 Hz, 2 H) - $\underline{CH_2}$ - NH

δ = 4.9 - 5.0 (m, 2 H) $\underline{CH_2}$ = C -

δ = 5.5 (m, 2 H) - $\underline{CH}$ = $\underline{CH}$ -

δ = 5.8 - 6 (m, 1 H) CH_2 = $\underline{CH}$ - CH_2 -

IR (IR - NO-5) at 1560 cm^{-1} (double bond), 1740 cm^{-1} (- $\overset{O}{\overset{\|}{C}}$ -), 2985 cm^{-1} (C - H stretch) and 3460 cm^{-1} (N - H stretch).

N-(Benzyloxycarbonyl)-3,7-octadiene [28] (52)

To a solution of the amine (50) (172 mg, 1.376 mmol) in ethanol (5 mL) cooled in an ice-water bath was added drop-wise benzylchloroformate (247.18 mg, 1.39 mmol). When approximately 1/2 of the acid chloride had been added, another solution of sodium carbonate (5 mL, 219.8 mg, 1.376 mmol) was added at such a rate that addition was complete slightly after the chloroformate. The reaction mixture was stirred for 5-10 minutes, then water (1mL) was added and stirring continued for 45 minutes. The ethanol was removed in vacuum on a rotary evaporator. To the residue water (25 mL) was added and the resulting aquous solution was extracted 4 times with 25 mL portions of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate. Evaporation of the solvent on a rotary evaporator gave a clear oil (250 mg). The thin-layer chromatography of the product on silica gel using benzene:ether (9:1) as eluent showed a mixture of products had formed. The crude product was purified by chromatography on a silica gel column using benzene:ether (9:1) as eluent. The product showed two spots on tlc.

The proton magnetic resonance spectrum (n.m.r. NO-9)

δ = 2 - 2.19 (m, 6 H) allylic protons

δ = 3.30 (q, J = 7 Hz, 2 H) - $\underline{CH_2}NH$ -

δ = 4.8 - 6 (m, 5 H) olefinic protons

δ = 5.1 (S, 2 H) - $\overset{O}{\overset{\|}{C}}$ - O - $\underline{CH_2}$ -

δ = 7.36 (5 H) $C_6\underline{H_5}$- aromatic

IR (IR NO-6) at 1545 cm^{-1} (double bond) 1750 cm^{-1} (- $\overset{O}{\overset{\|}{C}}$ -), 3080 cm^{-1} (C - H stretch for aromatic rings) and 3480 cm^{-1} (N - H stretch).

Epoxide [28] (53)

The solution of N-(benzyloxycarbonyl)-3,7-octadiene (52) (60 mg, 0.231 mmol) in methylene chloride (5 mL) was cooled in an ice-water bath. To this solution was added 91.67 mg (0.462 mmol) of m-chloroperoxybenzoic acid. The reaction mixture was stirred at 0° for 18 hours and allowed to warm to room temperature. After the addition of methylene chloride (50 mL), 20 mL of saturated sodium carbonate was added. This mixture was stirred for 20 minutes and the layers were separated. The organic layer was washed with an additional 2x20 mL of saturated sodium-bicarbonate solution. The combined aqueous solutions were back washed with methylene chloride (20 mL). The combined organic extracts were washed with water (25 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent on a rotary evaporator provided 50 mg of the product (53) as an oil. The thin-layer chromatography of the product on silica gel using benzene:ether (9:1) as eluent showed two spots, consistent with a mixture of cis-trans oxiranes.

The proton magnetic resonance spectrum (n.m.r. NO-10):

δ = 1.5 (m, 6 H) $-\underline{CH_2}-$

δ = 2.3 (m, 2 H) $\underline{CH_2}-\underline{CH}-$ (O)

δ = 3.0 (m, 3 H) $-\underline{CH}-\underline{CH}-$ (O)

δ = 3.3 (q, J - 7.0 Hz, 2 H) $-\underline{CH_2}-NH\overset{O}{\overset{\|}{C}}-$

δ = 5.1 (s, 2 H) $-\overset{O}{\overset{\|}{C}}-O-\underline{CH_2}-$

δ = 7.3 (5 H) $\underline{C_6H_5}$ - aromatic

1,2-Oxidocyclohex-4-ene* (54)

Cyclohexadiene (2.40 g, 0.03 mol) was dissolved in methylene chloride (200 mL) and m-chloroperoxybenzoic acid (6.10 g, 0.03 mol) added to the solution at 0°. After stirring for 3 hours at 0°, the solution was kept overnight in the cold. The solution was diluted with methylene chloride and then neutralized with sodium-bicarbonate solution, washed once with water, dried over anhydrous magnesium sulfate. The solvent was removed on a rotary evaporator giving the product (1.9 g, yield = 80%).

The proton magnetic resonance spectrum (n.m.r. - NO-11):

δ = 2.48 (m, 4 H) - CH_2 -

δ = 3.24 (m, 2 H) - CH(O) - CH(O) -

δ = 5.6 (m, 2 H) - CH = CH -

The ^{13}C n.m.r. spectrum (^{13}C n.m.r. NO-1):

δ = 24.80 (2C, $C_{(3)}$, $C_{(6)}$)

δ = 49.36 (2C, $C_{(1)}$, $C_{(2)}$)

δ = 120 (2C, $C_{(4)}$, $C_{(5)}$)

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*See plate XI

Trans-2-azidocyclohex-4-enol [29] (55)

1,2-Oxidocyclohex-4-ene (2.88g, 0.03 mol) dissolved in 80% ethanol (50 mL) was refluxed for 24 hours with sodium azide (2.60 g, 0.04 mol) and ammonium chloride (2.14 g, 0.04 mol). The reaction mixture was reduced to a small volume by evaporating the ethanol on a rotary evaporator. Then the residue was extracted three times with 100 mL portions of ether. The combined ether extracts were washed several times with water, dried over anhydrous sodium sulfate and the solvent was evaporated to dryness on a rotary evaporator giving the product (2.004 g, yield = 80%). The thin-layer chromatography of the product on silica gel with benzene:ether (9:1) as eluent showed a single spot which indicated the formation of the product.

The proton magnetic resonance spectrum (n.m.r. NO-12):

δ = 2.2 (m, 4 H), - $C\underline{H}_2$ -

δ = 2.8 (S, 1 H) - O$\underline{H}$

δ = 3.8 (m, 2 H) - $\underset{N_3}{\underset{|}{C\underline{H}}}$ -, - $\underset{|}{C\underline{H}}OH$

δ = 5.6 (m, 2 H) - $C\underline{H} = C\underline{H}$ -

The ^{13}C n.m.r. spectrum (^{13}C n.m.r. NO-2):

δ = 30.60 (1C, $C_{(3)}$), - $\underline{C}H_2$ - CHN_3

δ = 33.62 (1C, $C_{(6)}$), - $\underline{C}H_2$ - CHOH

δ = 64.37 (1C, $C_{(2)}$), - $\underline{C}H$ - N_3

δ = 70.62 (1C, $C_{(1)}$), - $\underline{C}H$ - OH

δ = 124.37, 125, (2C, $C_{(4)}$, $C_{(5)}$) - $\underline{C}H = \underline{C}H$ -

IR (IR - NO-7) at 2100 cm^{-1} (azido group), 2910 cm^{-1} (C - H stretch) and 3400 cm^{-1} (hydroxy group).

1-(Trans-2-azidocyclohex-4-ene)-acetate (56)

The acetylating agent 1 mL (pyridine:acetic anhydride, 1:1) was added to the trans-2-azidocyclohex-4-ene (55) (500 mg). The reaction mixture was kept overnight at room temperature. The solution was diluted with water and extracted 3 times with 100 mL portions of ether. The combined ether extracts were washed first with dilute hydrochloric acid, followed by dilute sodium-bicarbonate solution and finally with water. The ether extract was dried over anhydrous sodium sulfate, filtered and then the solvent was evaporated on rotary evaporator giving the product (250 mg, yield = 50 %).

The proton magnetic resonance spectrum (n.m.r. NO-13):

δ = 2.0 (s, 3H) $-COC\underline{H}_3$

δ = 2-2.6 (envelope, 4H) $-C\underline{H}_2-$

δ = 3.8 (m, 1H) $-C\underline{H}N_3-$

δ = 4.8-5.0 (m, 1H) $-C\underline{H}COCH_3$

δ = 5.66 (m, 2H) $-C\underline{H} = C\underline{H}-$

1-Tetrahydropyranloxyl-2-azidocyclohex-4-ene (57)

A solution of 2-azidocyclohex-4-enol (55) (1.39 g, .01 mol), 3,4-dihydropyran (0.92 g, 0.011 mol) and p-toluene sulphonic acid (0.20 g, 0.012 mol) in methylene chloride (200 mL) was stirred for 18 hours at room temperature. The reaction mixture was washed 3 times with dilute sodium bicarbonate solution, once with water, dried over anhydrous magnesium sulfate and evaporated on a rotary evaporator giving the product (1.112 g, yield = 80%).

The proton magnetic resonance spectrum (n.m.r. NO-14):

δ = 1.5 - 2.8 (m, 10 H) - $C\underline{H}_2$ -

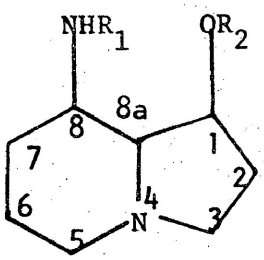
δ = 3.8 (m, 4 H) - $OC\underline{H}_2$, - $OC\underline{H}CHN_3$, - $C\underline{H}N_3$

δ = 4.9 (m, 1 H) - O - $\underline{C}H$ - O -

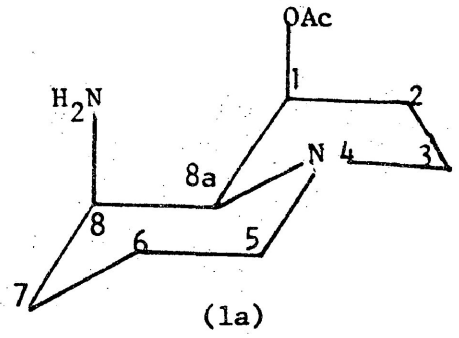
δ = 5.6 (m, 2 H) - $C\underline{H}$ = $C\underline{H}$ -

PLATES

PLATE 1



(1)

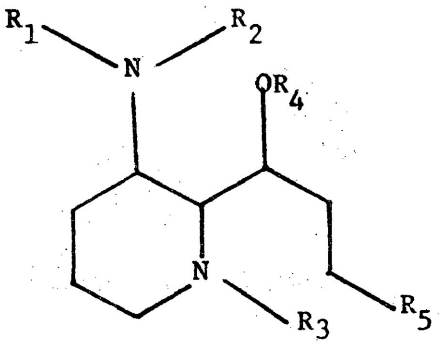


(1a)

1, R_1= H, R_2= Ac

2, R_1= R_2= H

3, R_1= R_2= Ac



(4)

4, R_1= R_4= Ac, R_2= H, R_3= CN, R_5= Br

5, R_1= C_2H_5, R_2= R_3= R_4= R_5= H

6, R_1= C_2H_5, R_2= R_3= CH_3, R_4= R_5= H

7, R_1= C_2H_5, R_2= R_3= R_4= Ac, R_5= H

PLATE 11

SCHEME 1

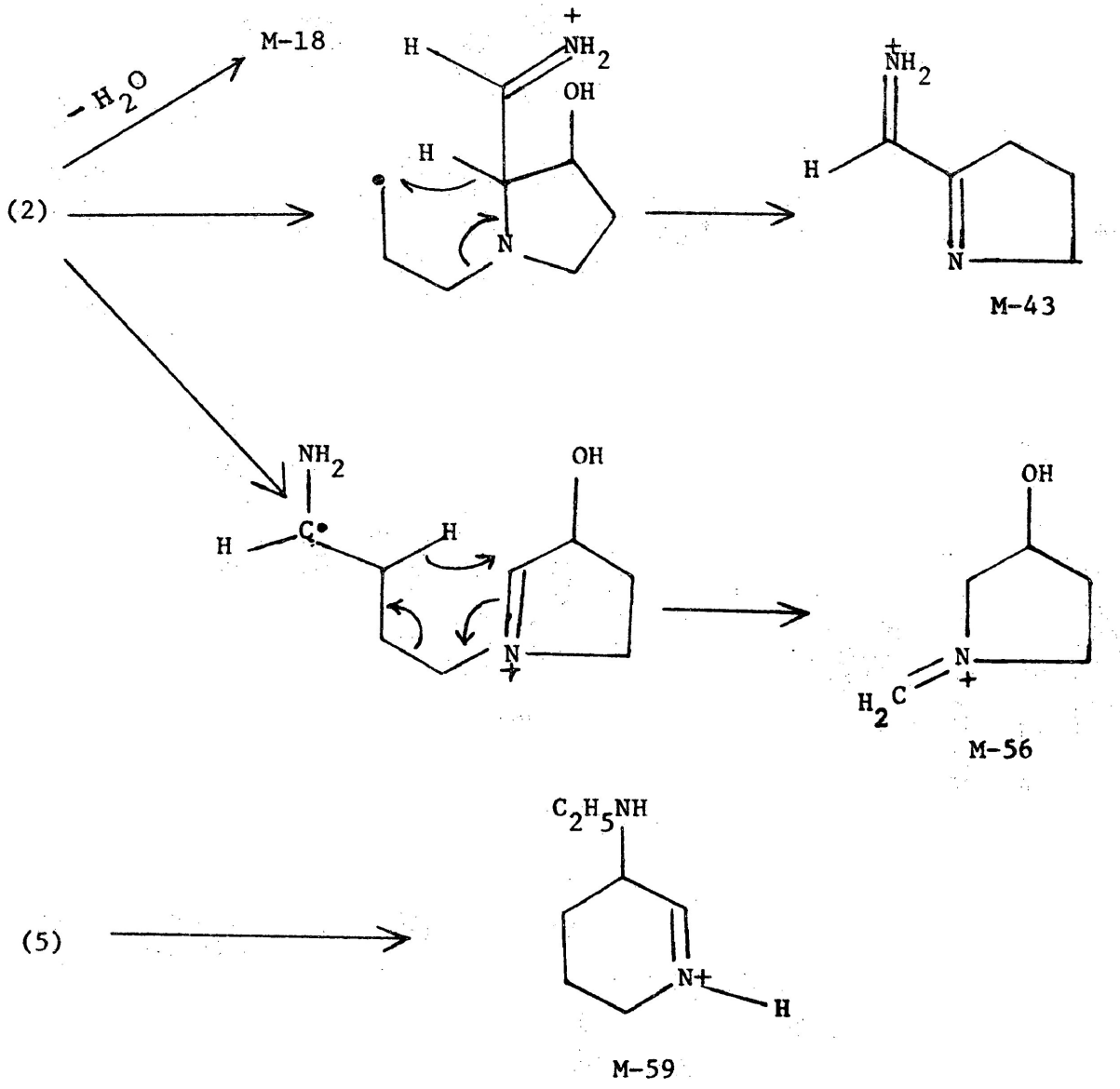
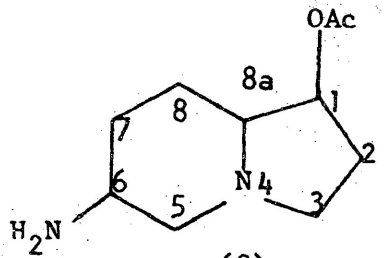


PLATE 111



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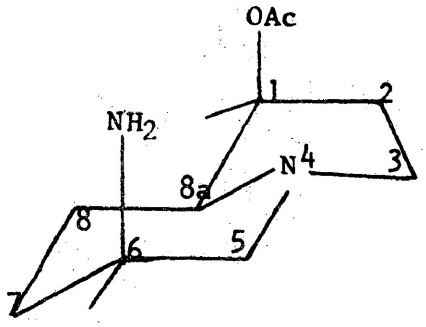

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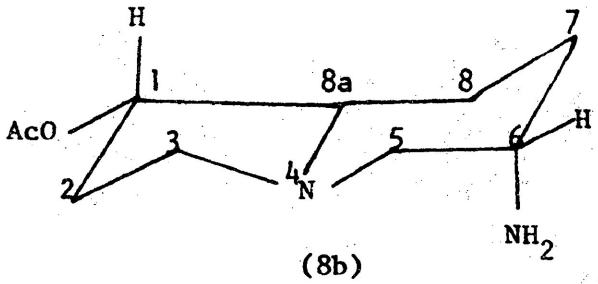
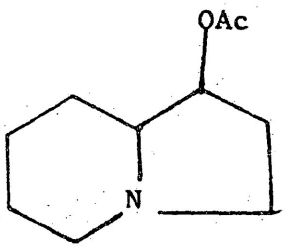


(8b)



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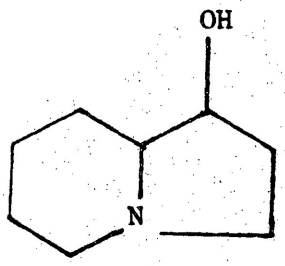
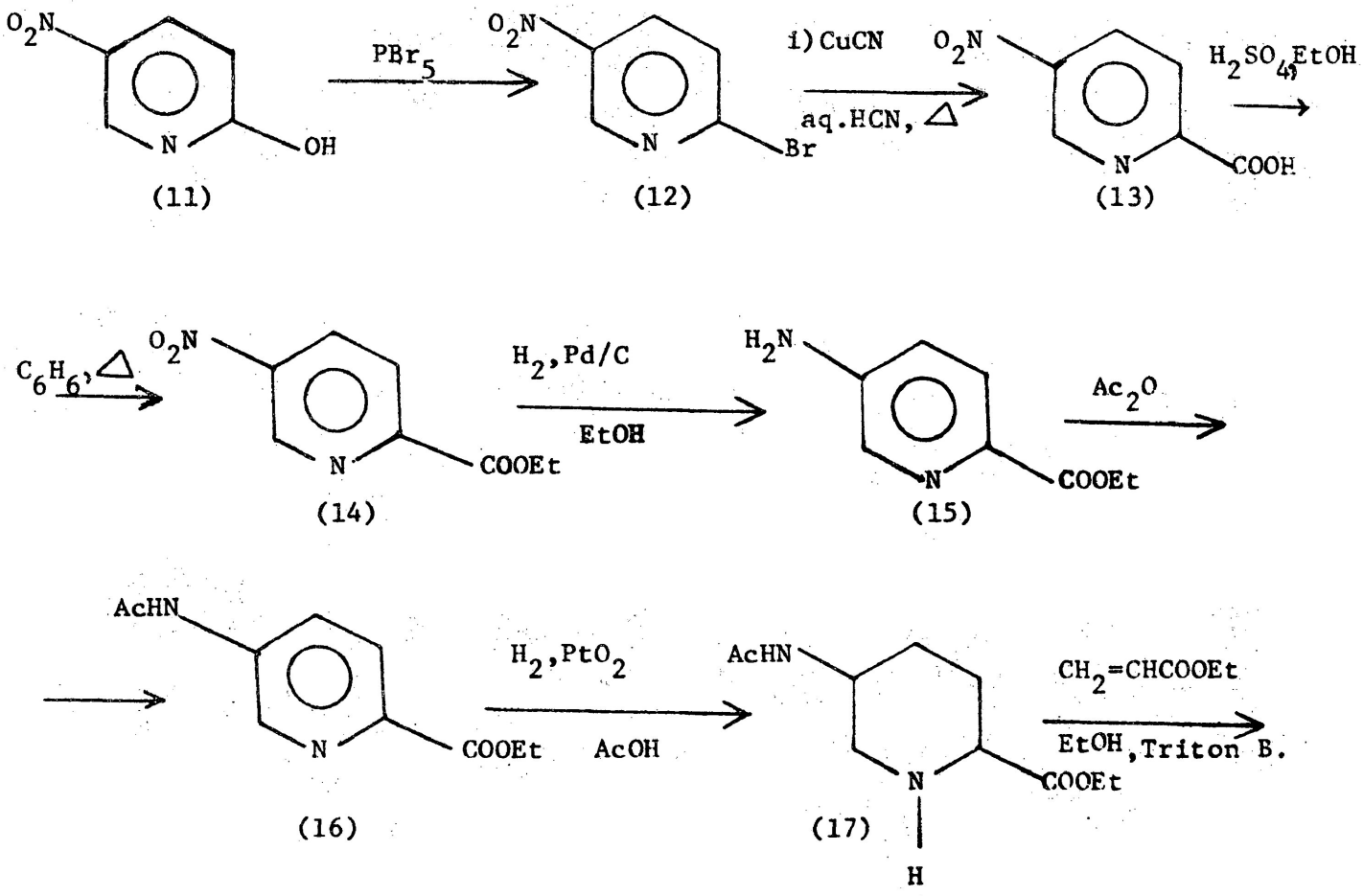


(10)

PLATE 1V

SCHEME 11



The Cartwright-Gardiner-Rinehart synthesis of slaframine.

PLATE V

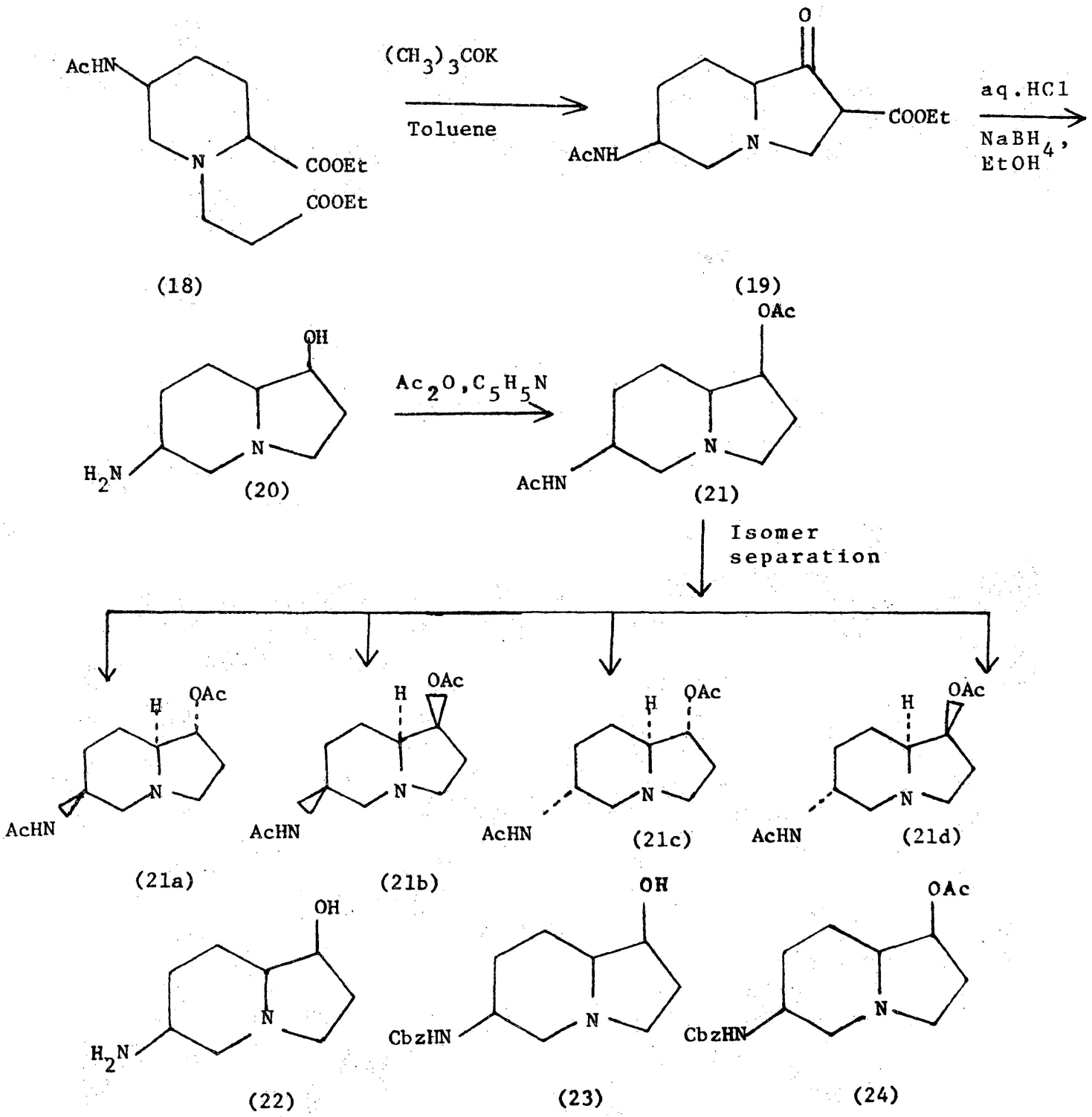
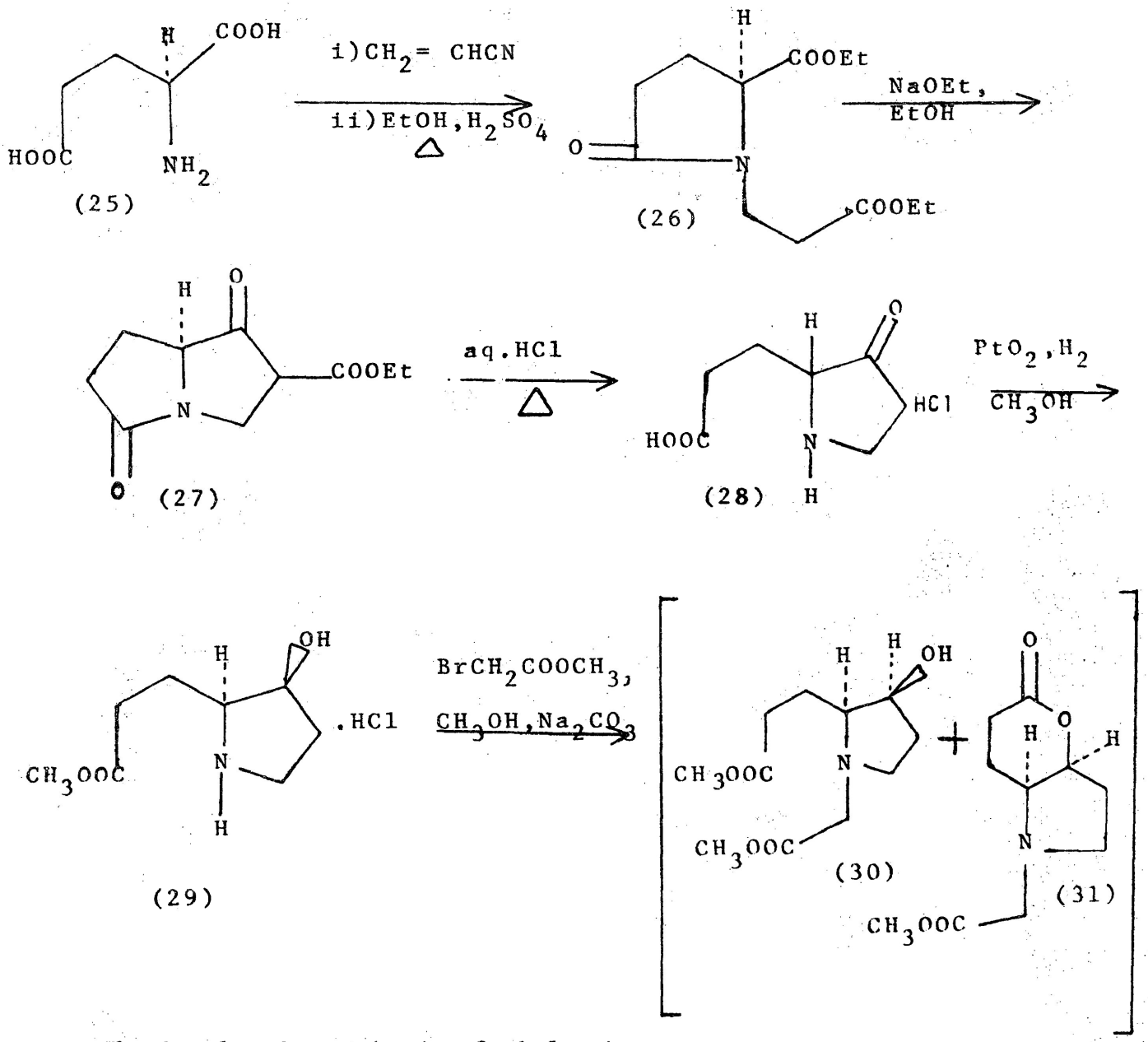

AcHN
COOEt
COOEt
(18)
$(CH_3)_3COK$
Toluene
O
AcNH
COOEt
(19)
aq.HCl
$NaBH_4$,
EtOH
OH
H_2N
(20)
Ac_2O, C_5H_5N
OAc
AcHN
(21)
Isomer
separation
H
OAc
AcHN
(21a)
(21b)
(21c)
(21d)
OH
H_2N
(22)
CbzHN
(23)
(24)

PLATE V1

SCHEME 111



The Gensler-Hu synthesis of slaframine.

PLATE V11

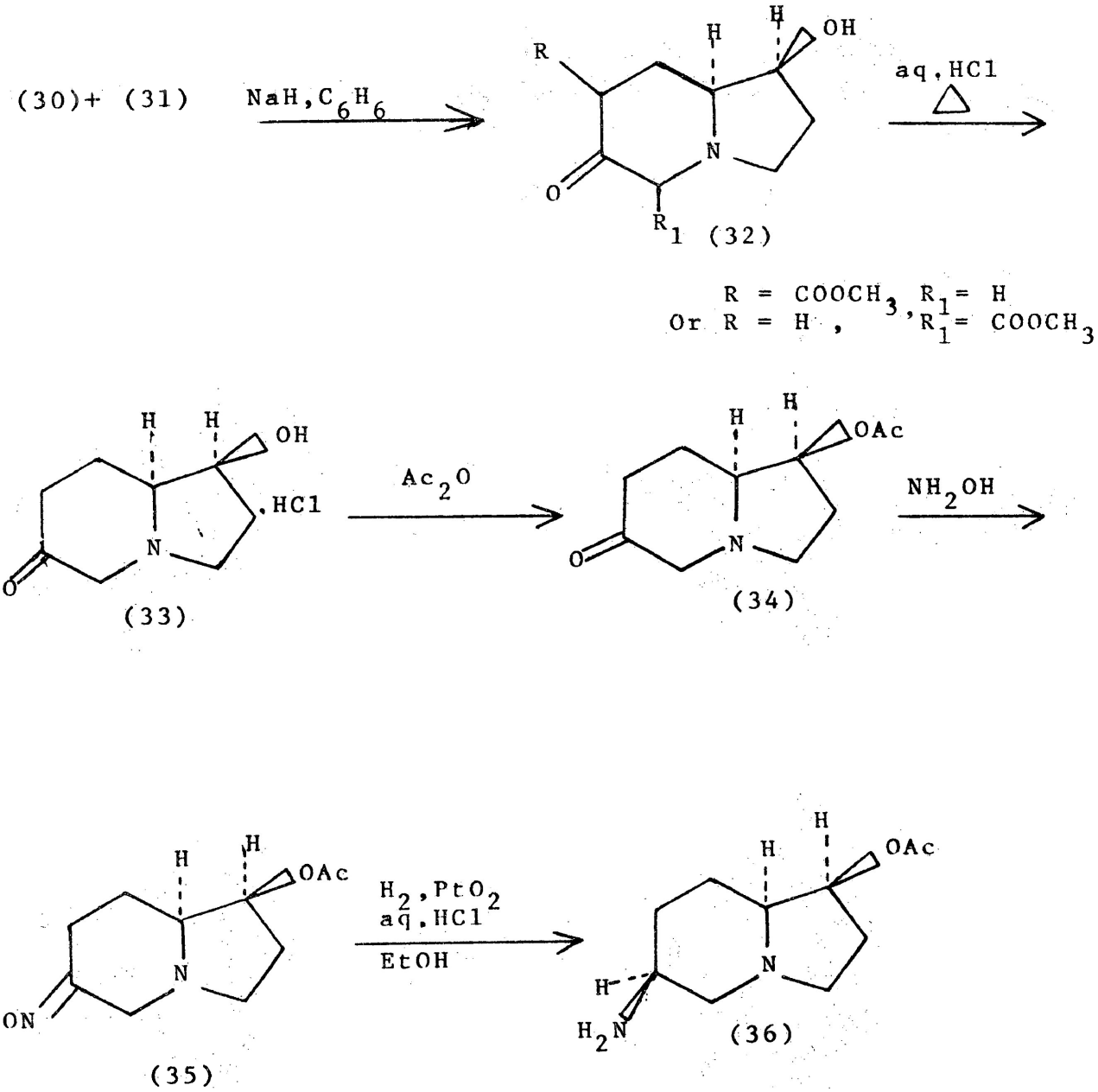
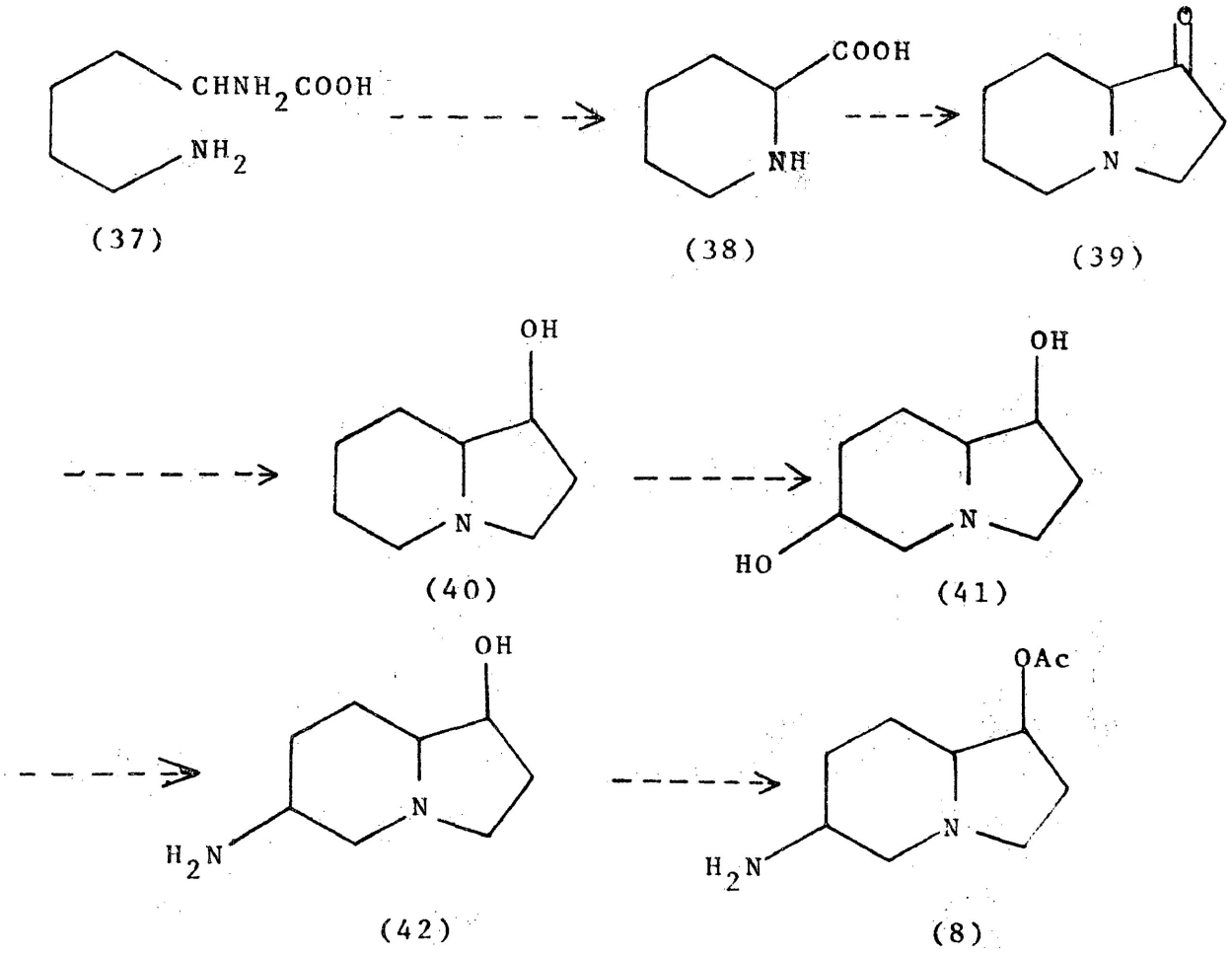

(30)+ (31)
NaH, C_6H_6
R
H
H
OH
N
O
R_1 (32)
aq, HCl
R = $COOCH_3$, R_1 = H
Or R = H , R_1 = $COOCH_3$
H
H
OH
.HCl
N
O
(33)
Ac_2O
H
H
OAc
N
O
(34)
NH_2OH
H
H
OAc
N
HON
(35)
H_2, PtO_2
aq. HCl
EtOH
H
H
OAc
N
H
H_2N
(36)

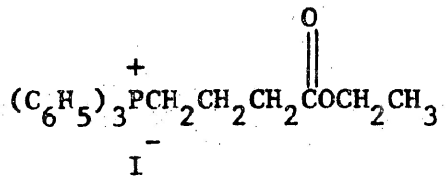
PLATE V111

SCHEME 1V

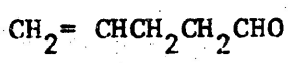


Proposed biosynthetic pathway to slaframine.

PLATE 1X



(43)



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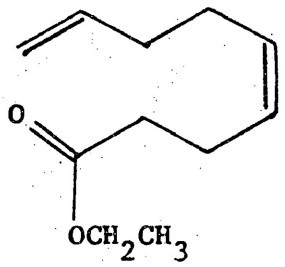

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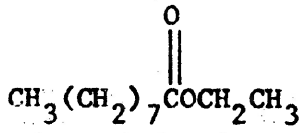
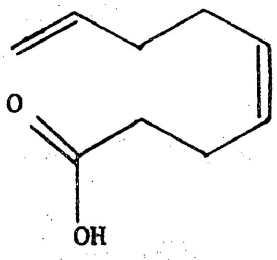


(45a)



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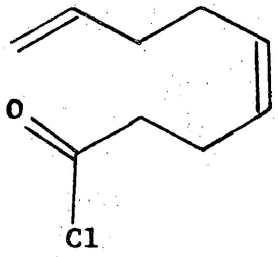
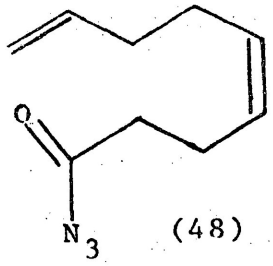


(47)

PLATE X



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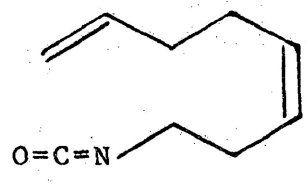

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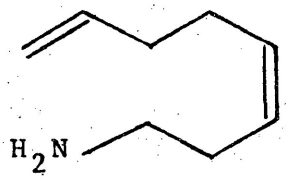

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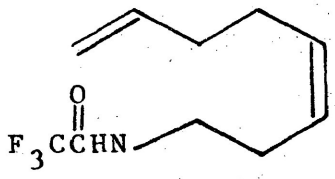

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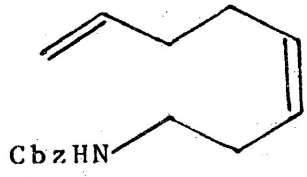

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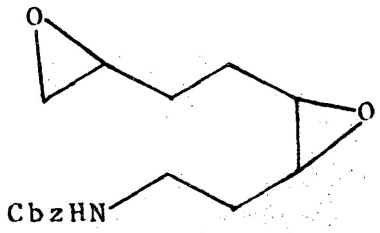

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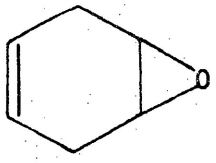
PLATE XI



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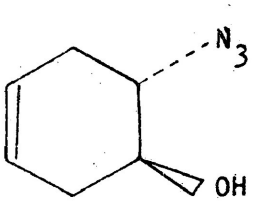

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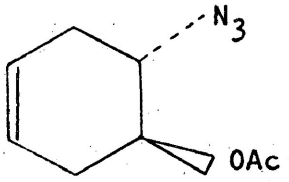

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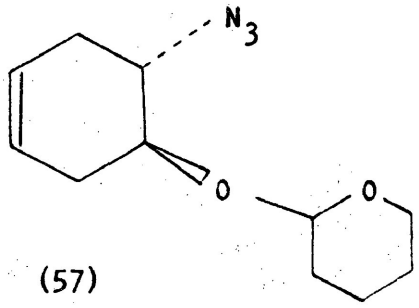

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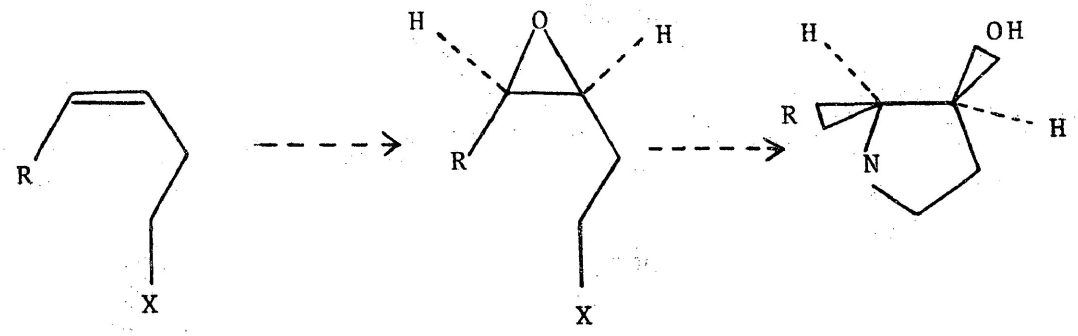
PLATE X11

SCHEME V



SCHEME V1

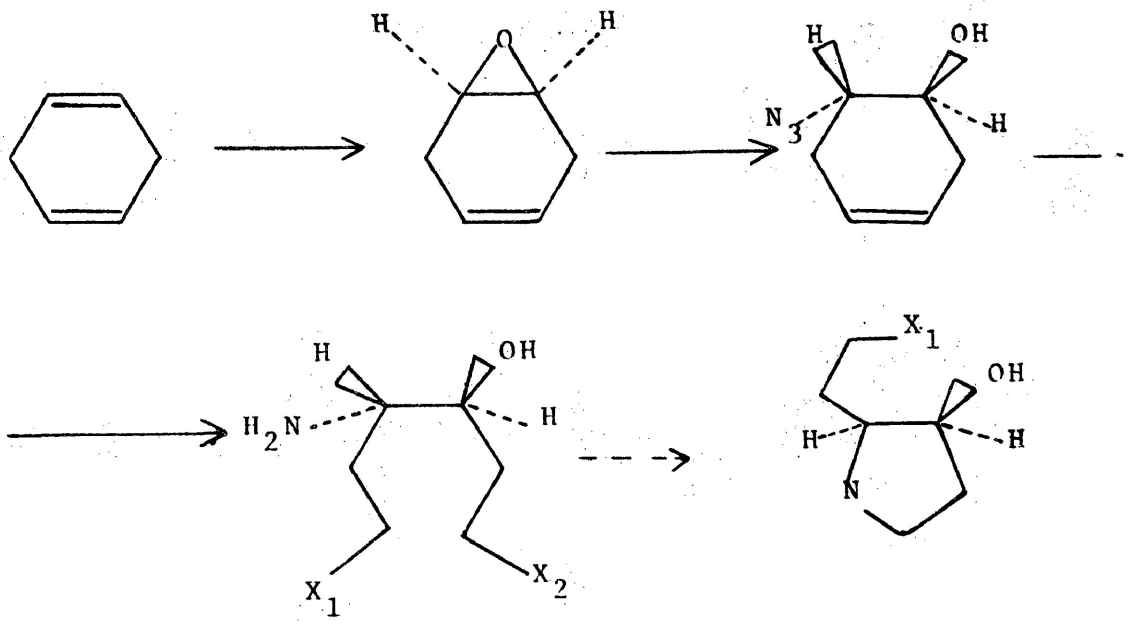
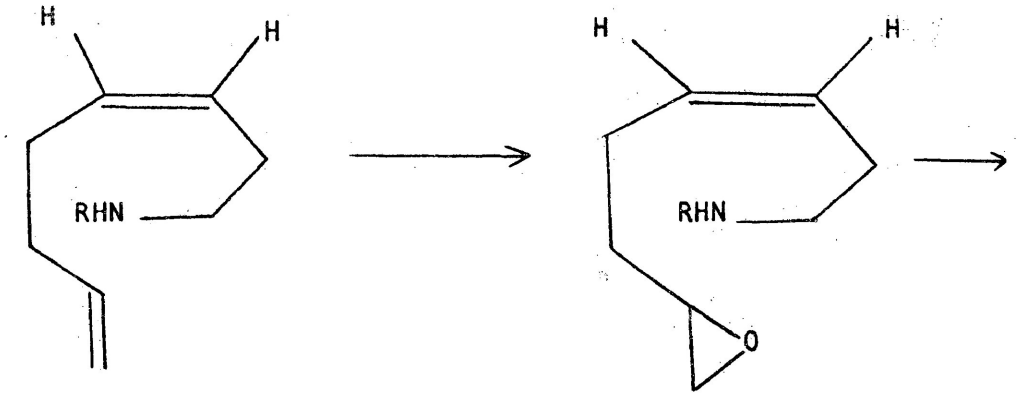
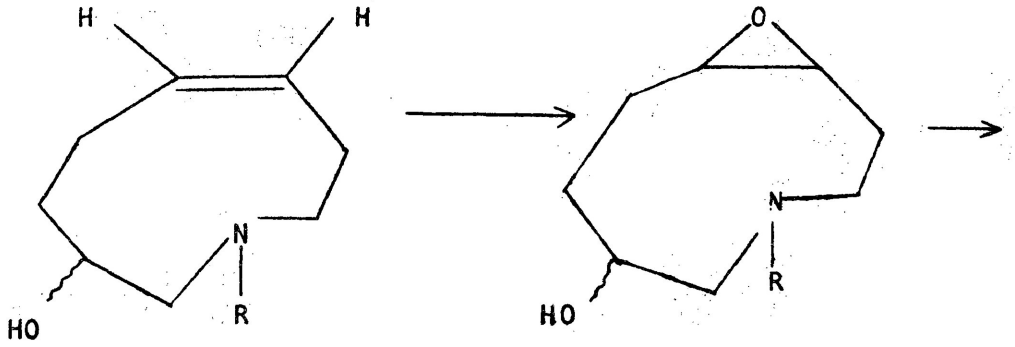


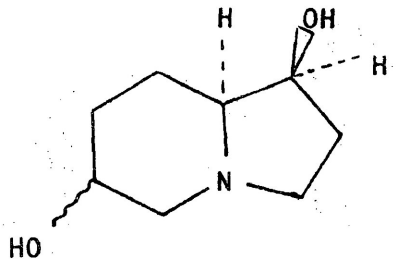
PLATE XIII

SCHEME VII

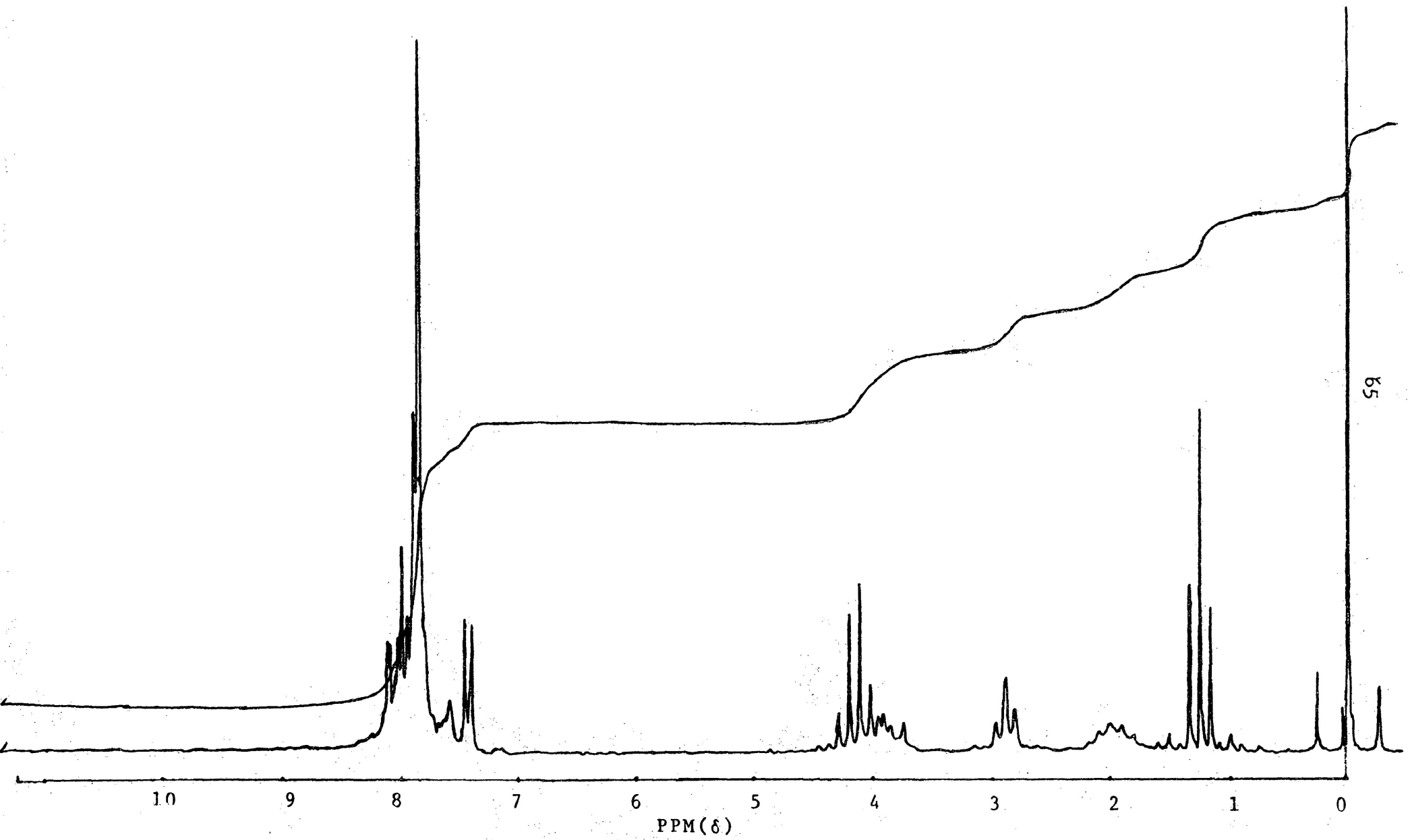


R = Cbz

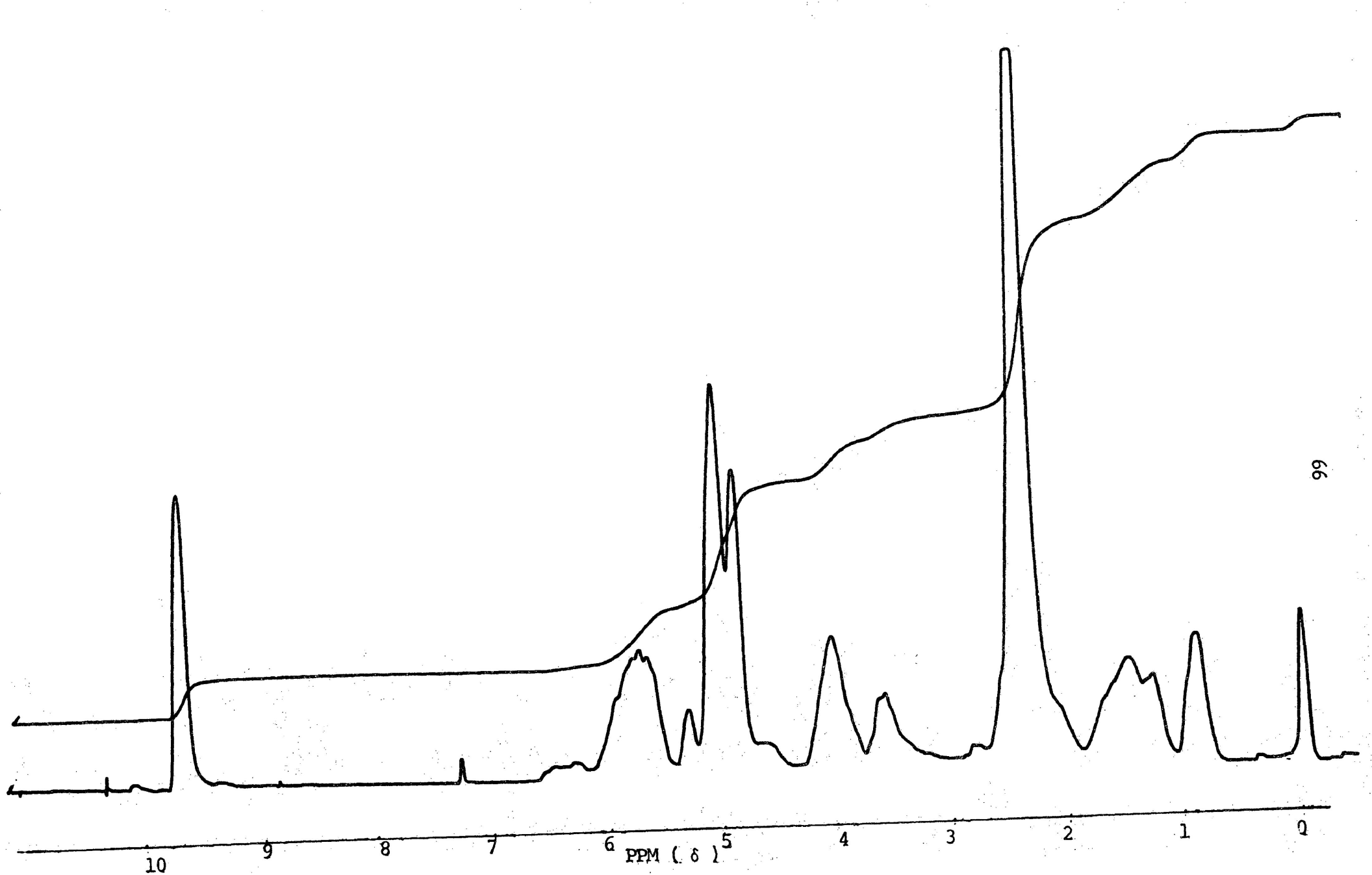




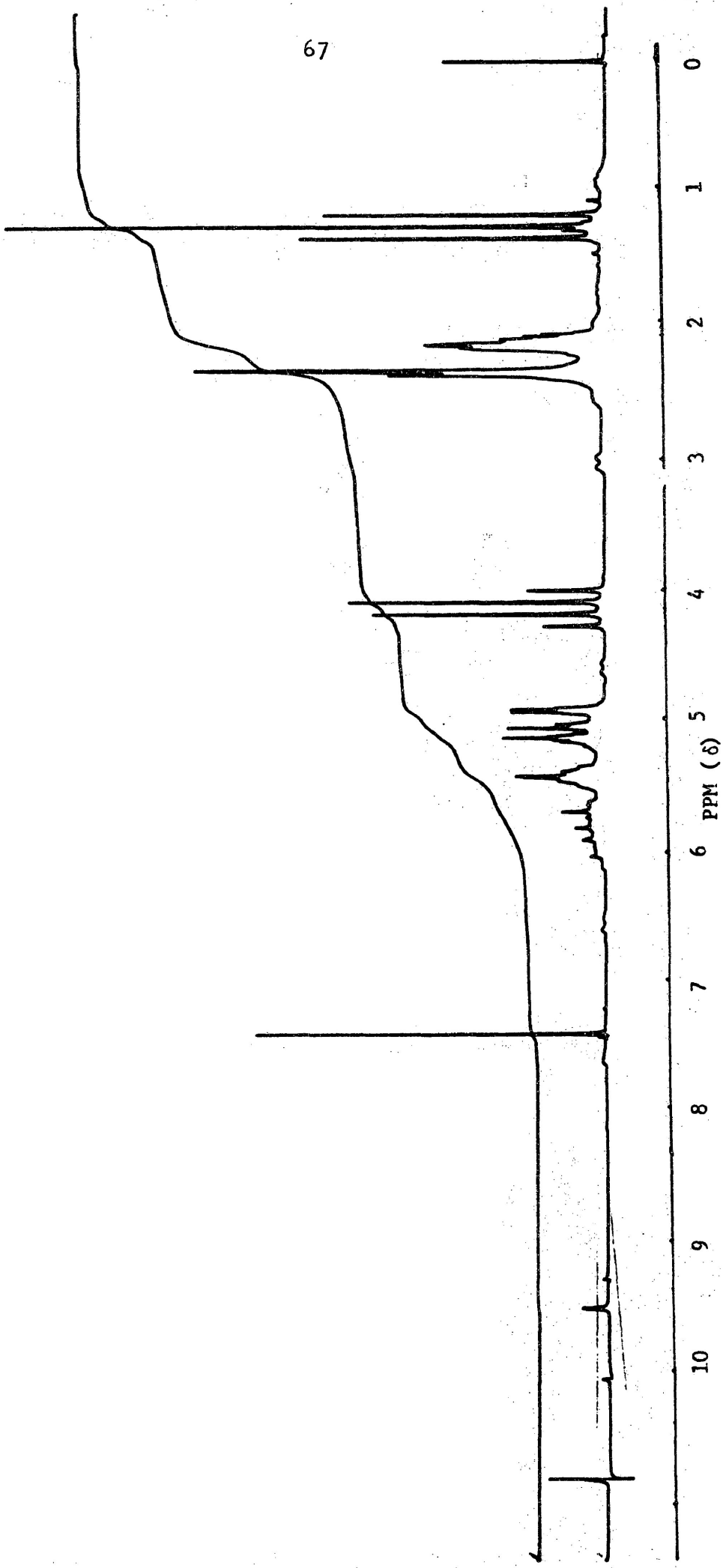
S P E C T R A



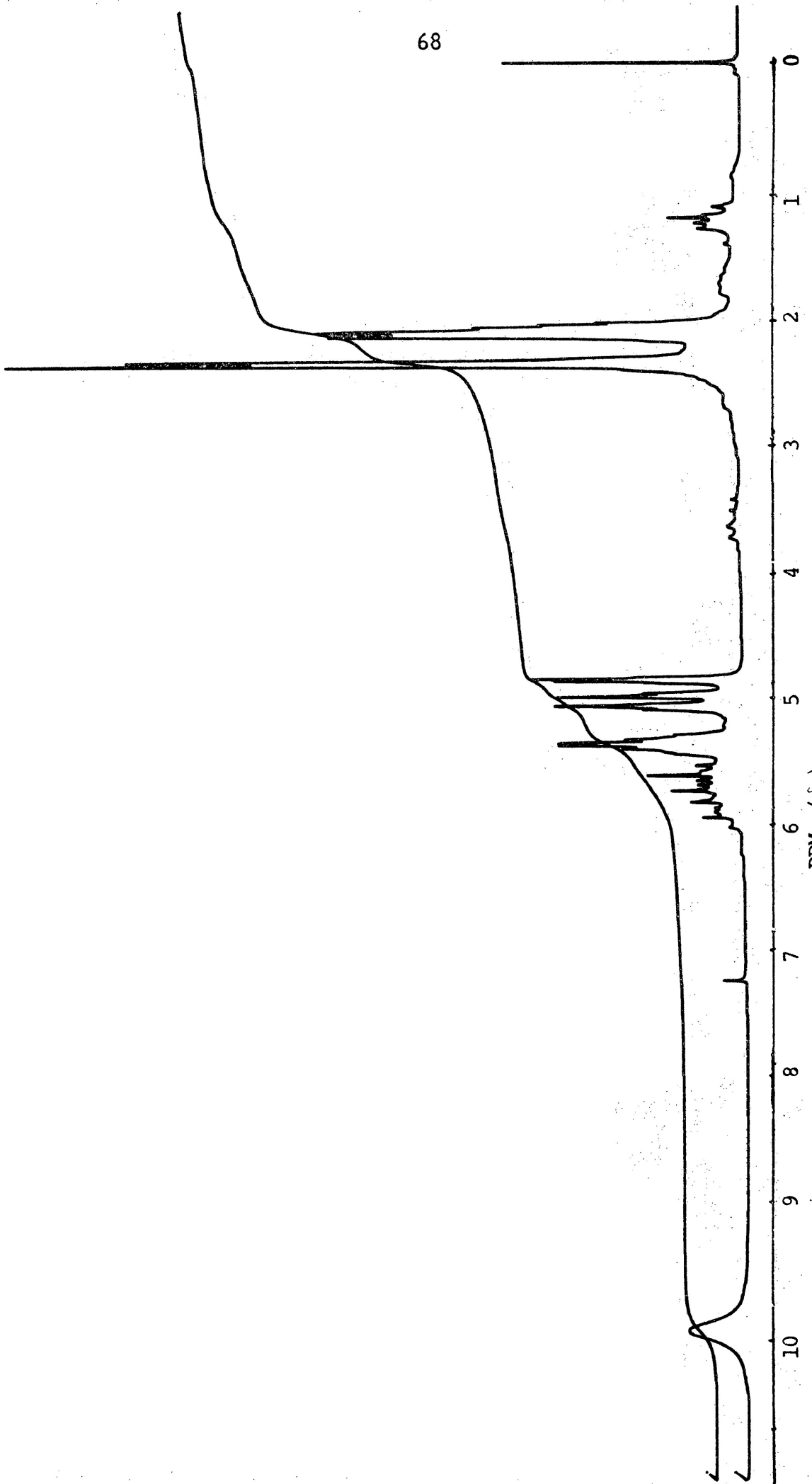
NMR NO.1 3-Carbethoxypropyltriphenylphosphoniumiodide



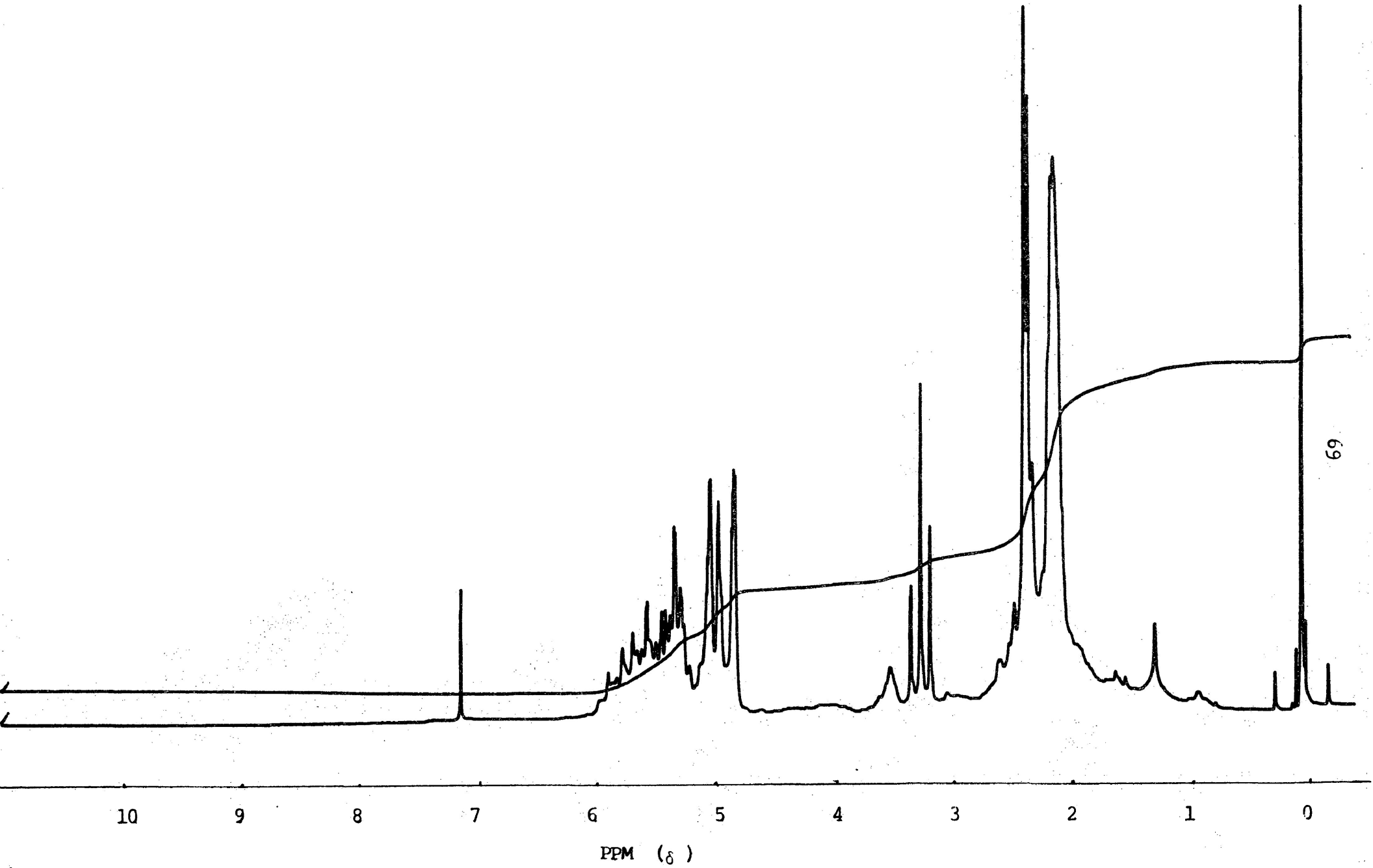
NMR NO.2 4-Pentenal



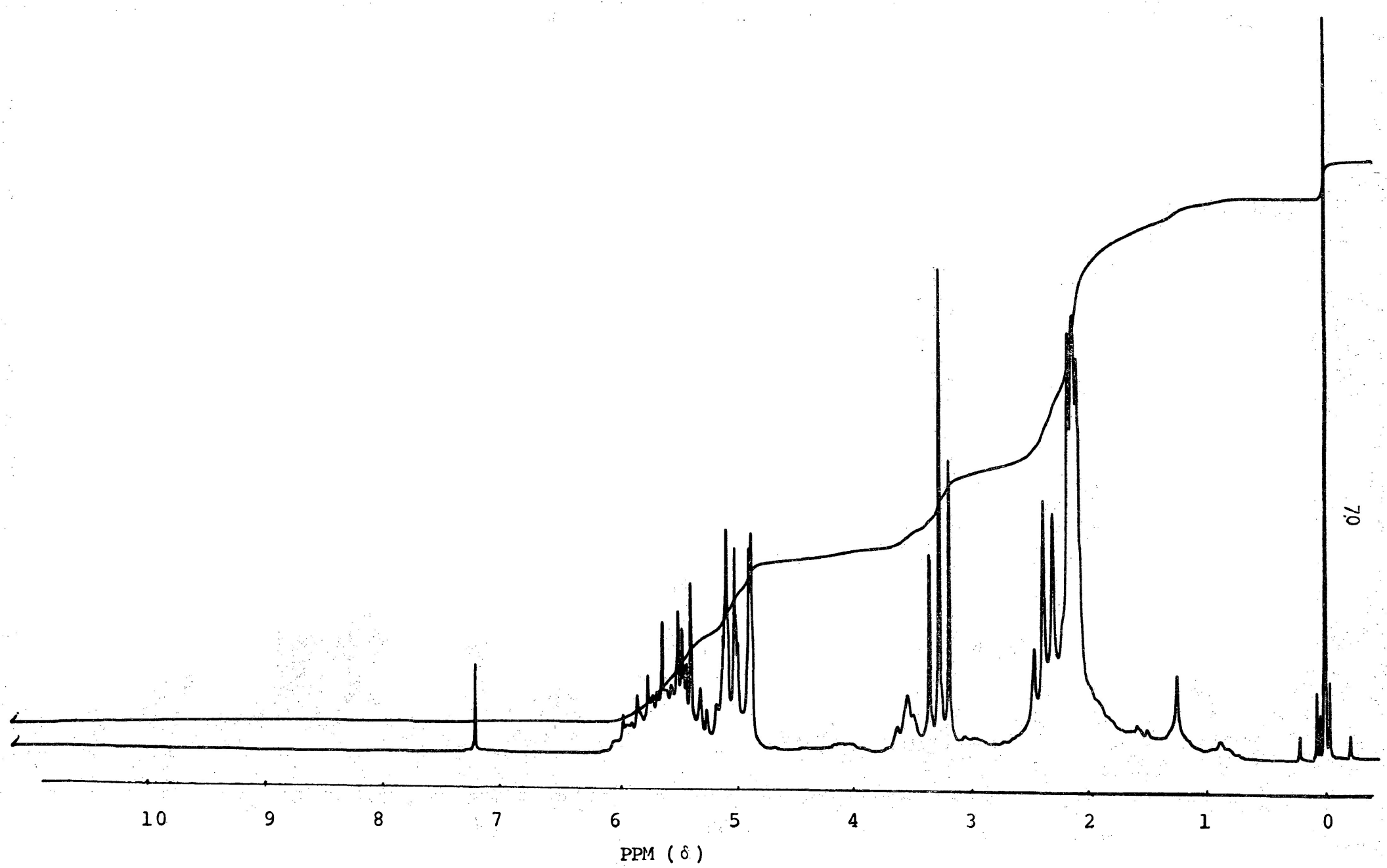
NMR NO.3 Ethyl-(Z)-4,8-nonadienoate



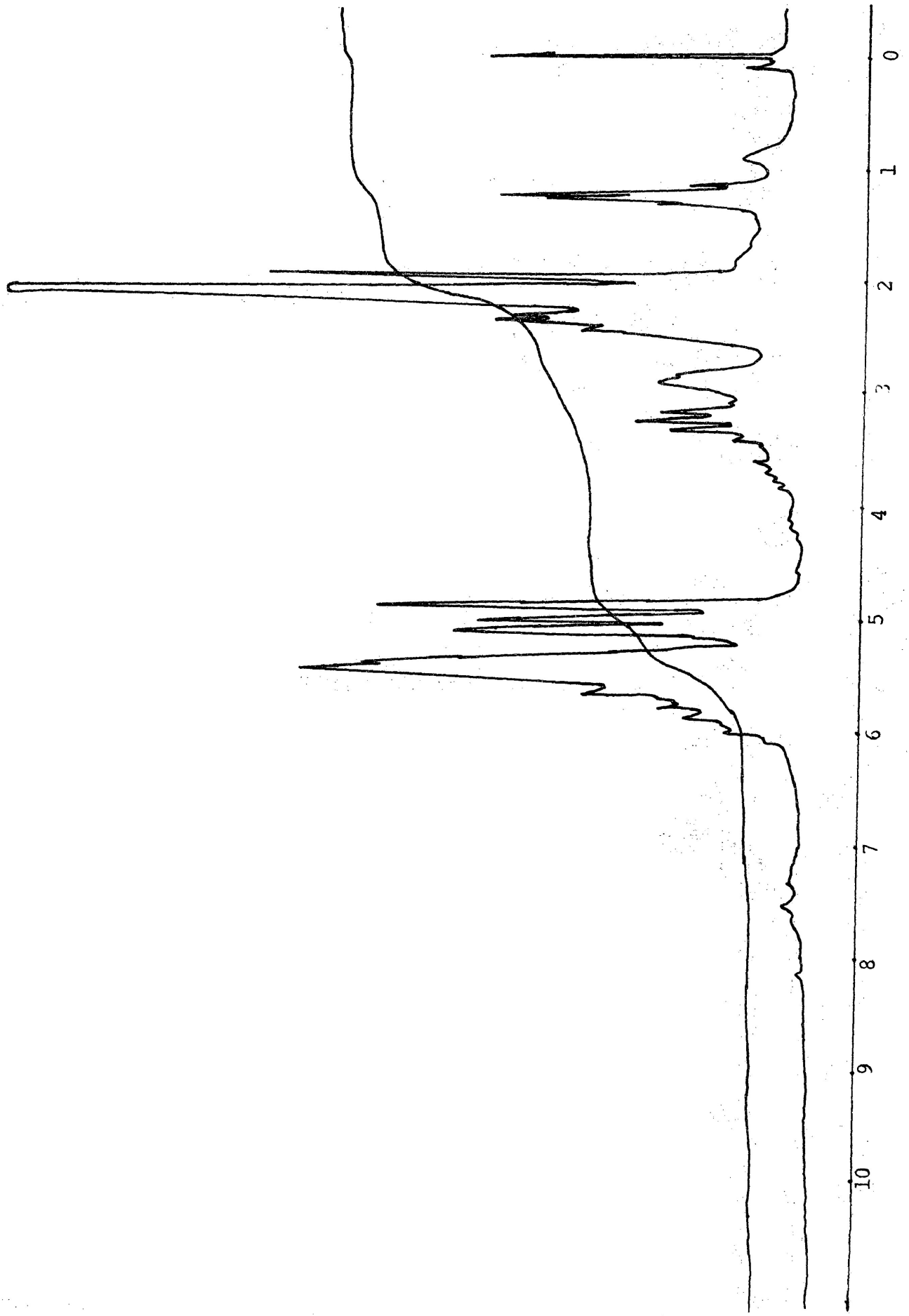
NMR NO.4 4,8-Nonadienoic acid.



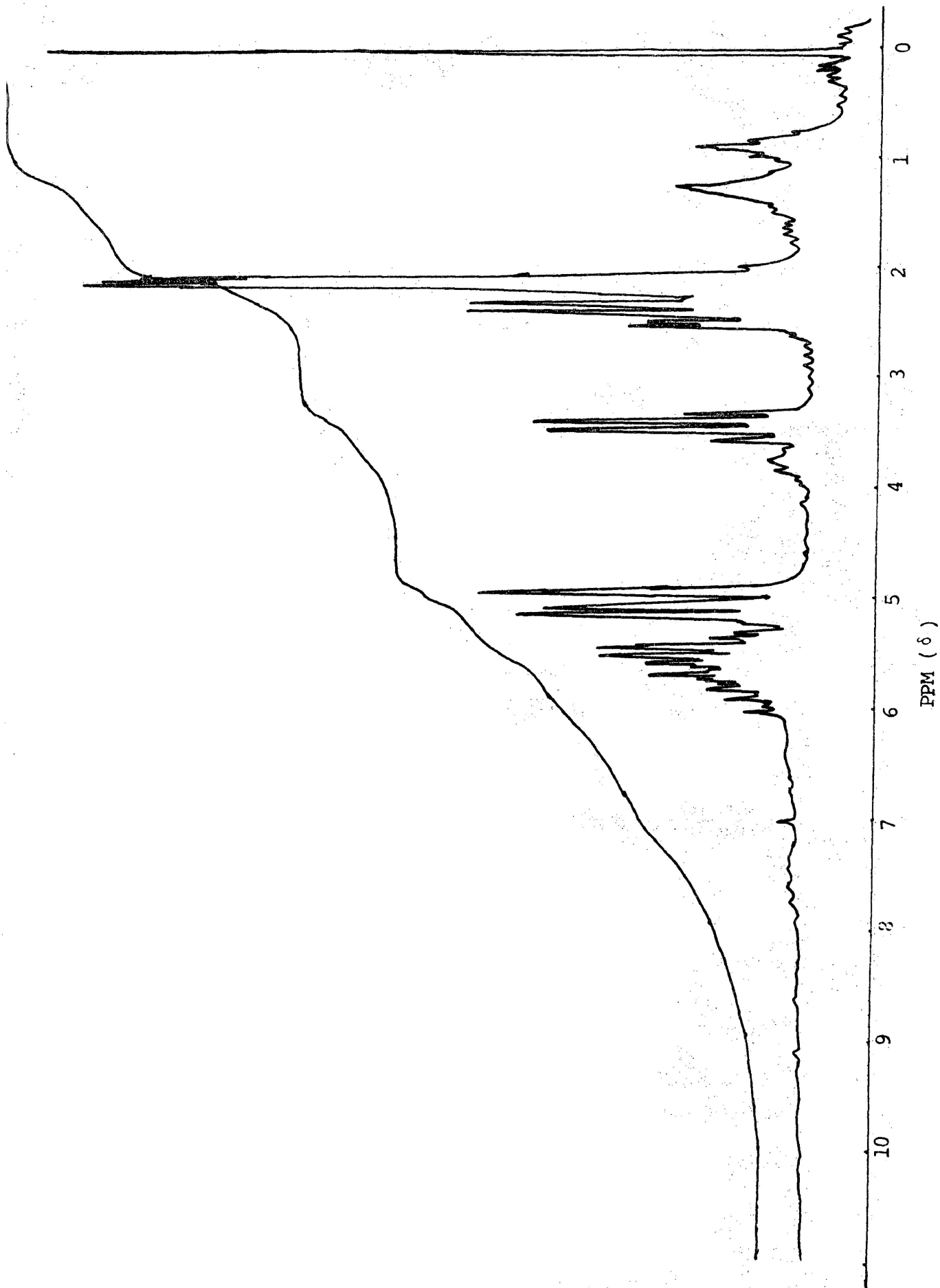
NMR NO.5 Acyl azide.



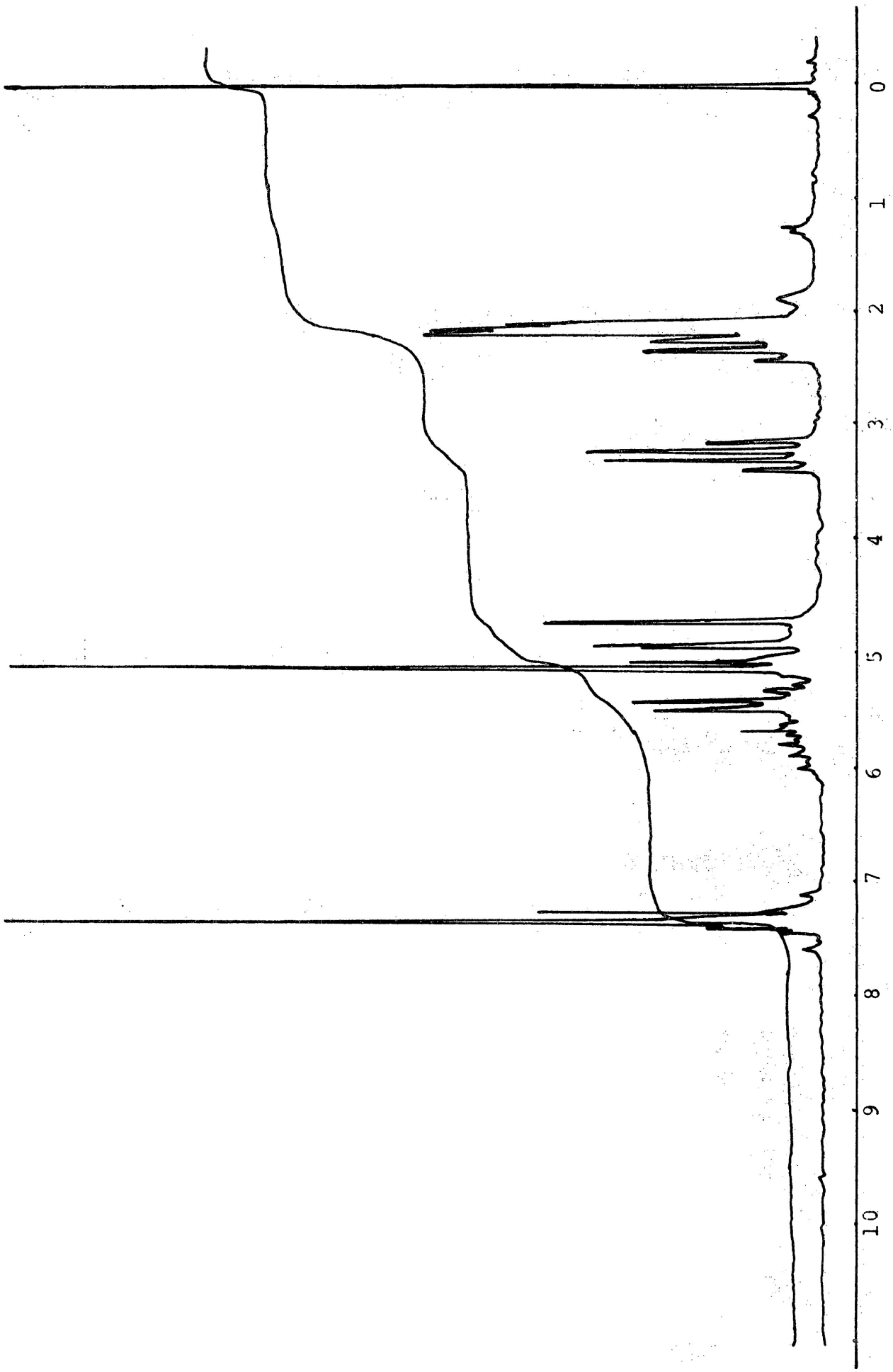
NMR NO.6 Isocyanate.



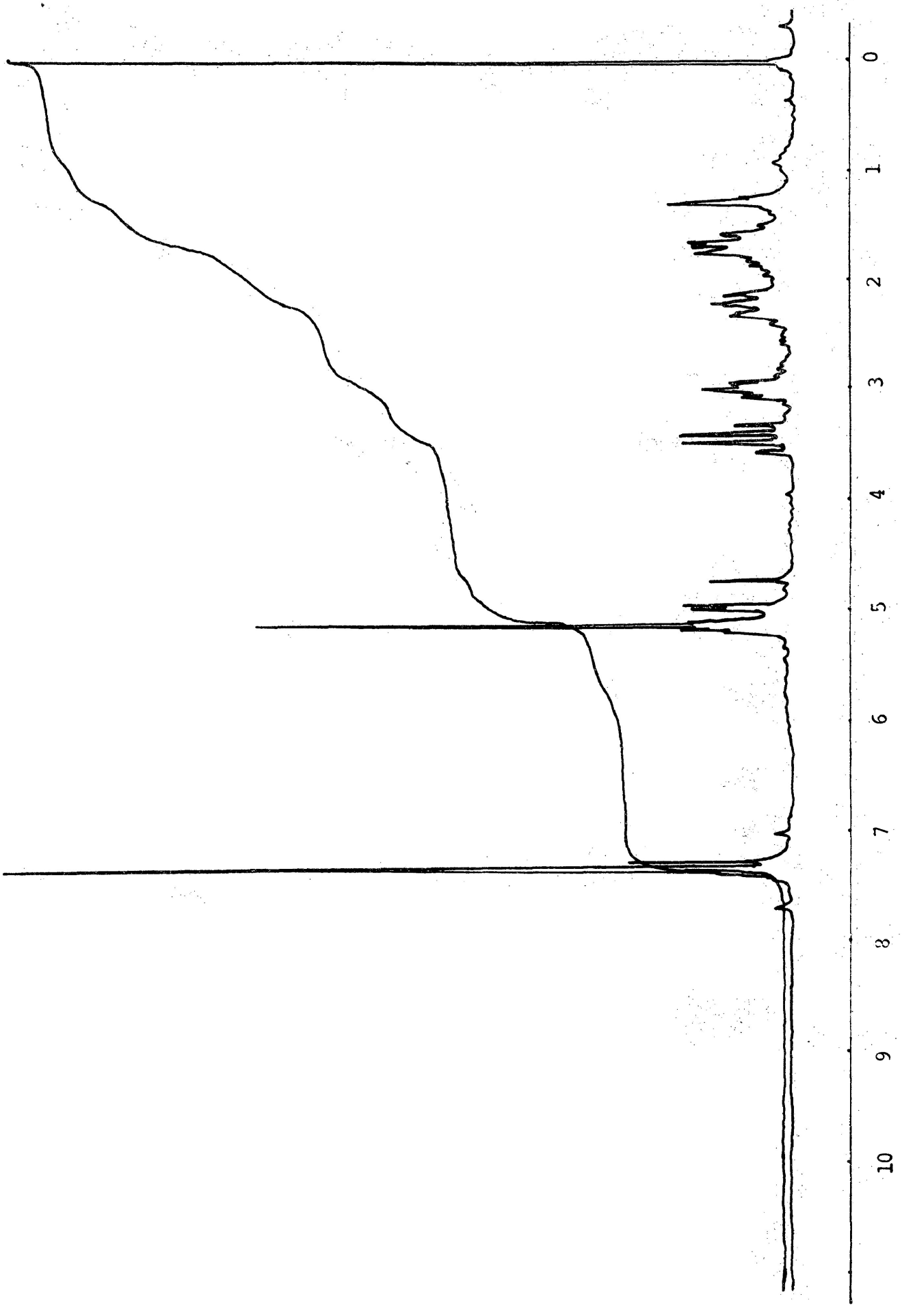
NMR NO.7 1-Amino-3,7-octadiene



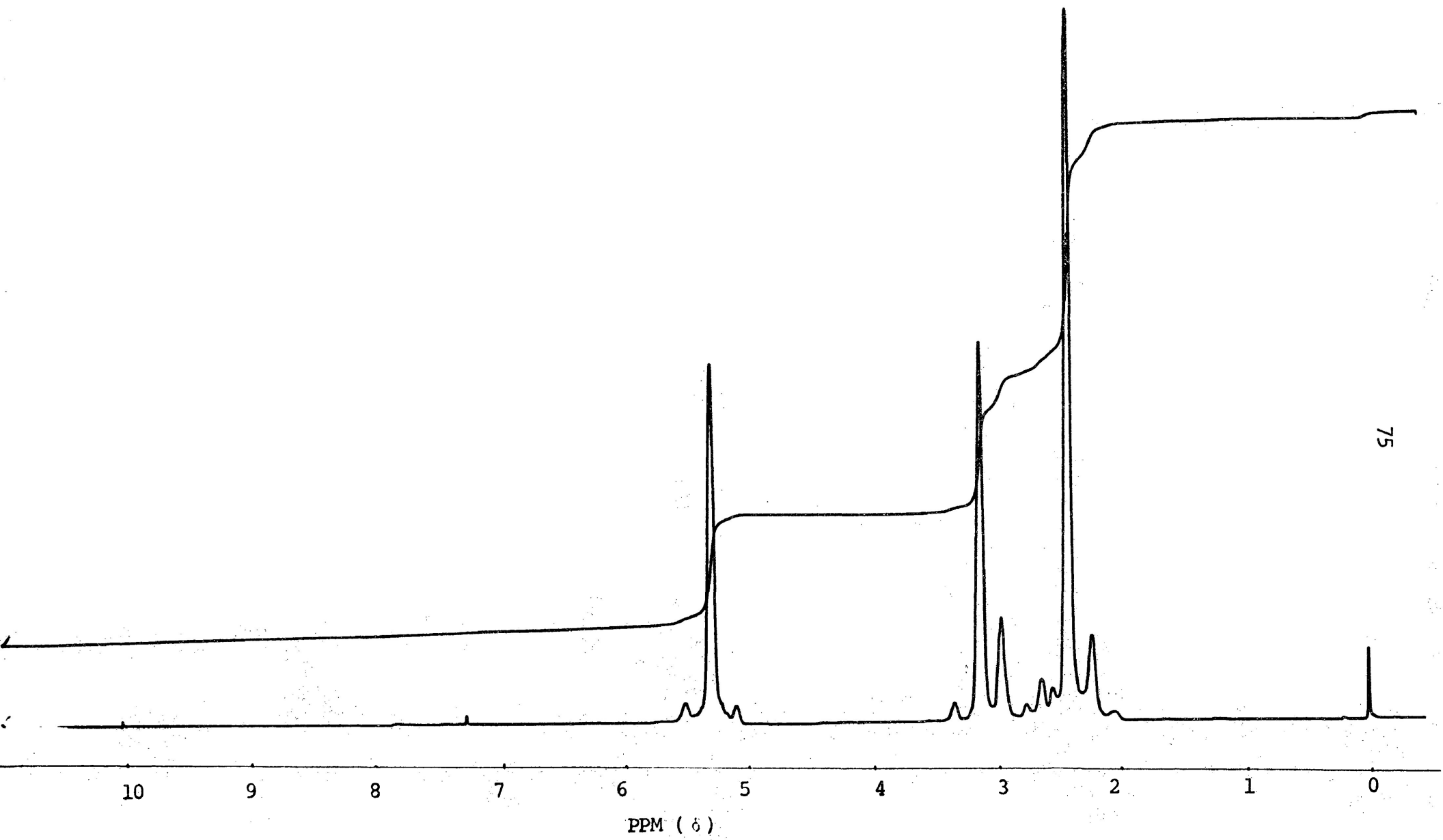
NMR NO.8 1-Trifluoroacetamido-3,7-octadiene



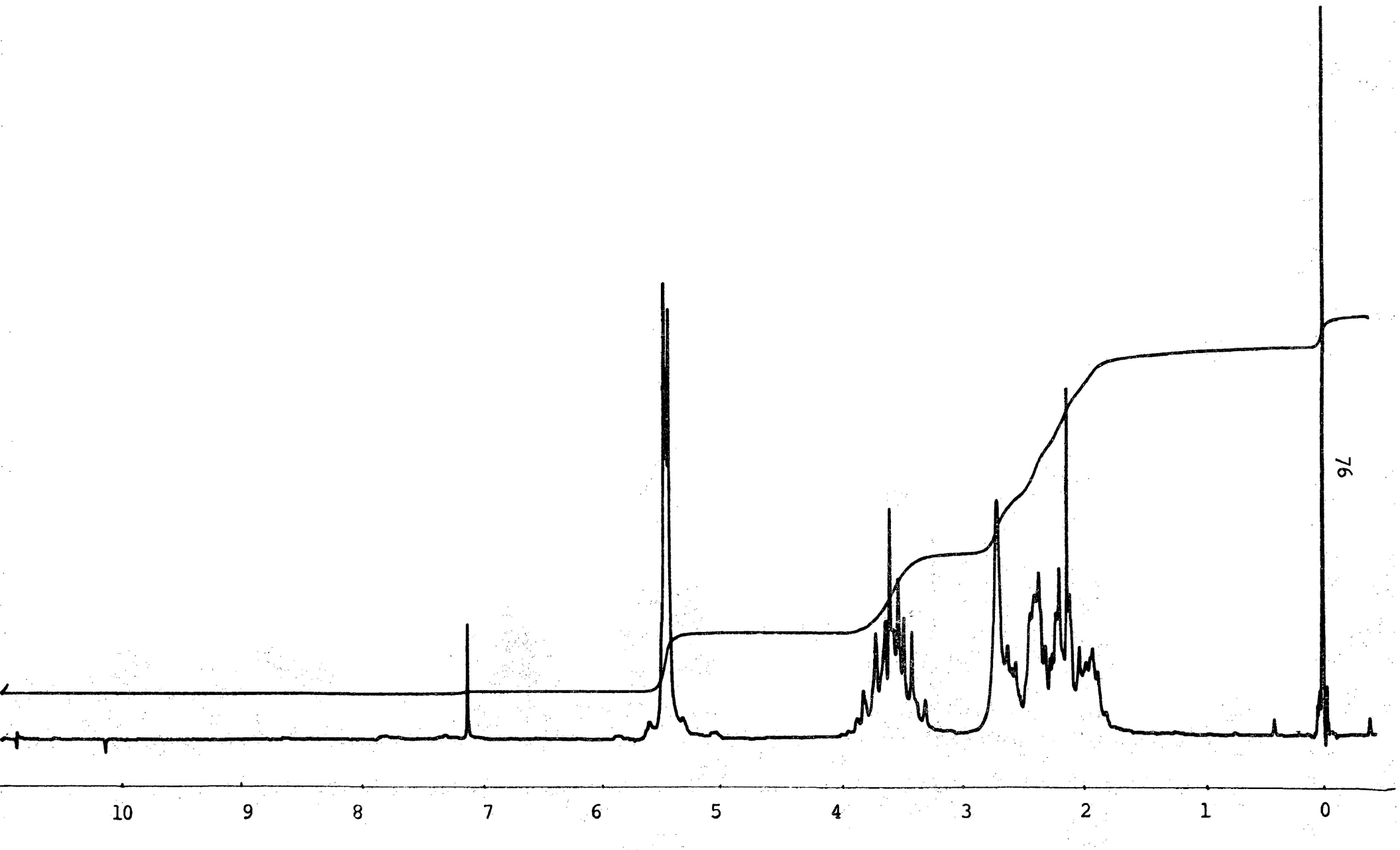
NMR NO.9 N- (Benzyloxycarbonyl)-3,7-octadiene



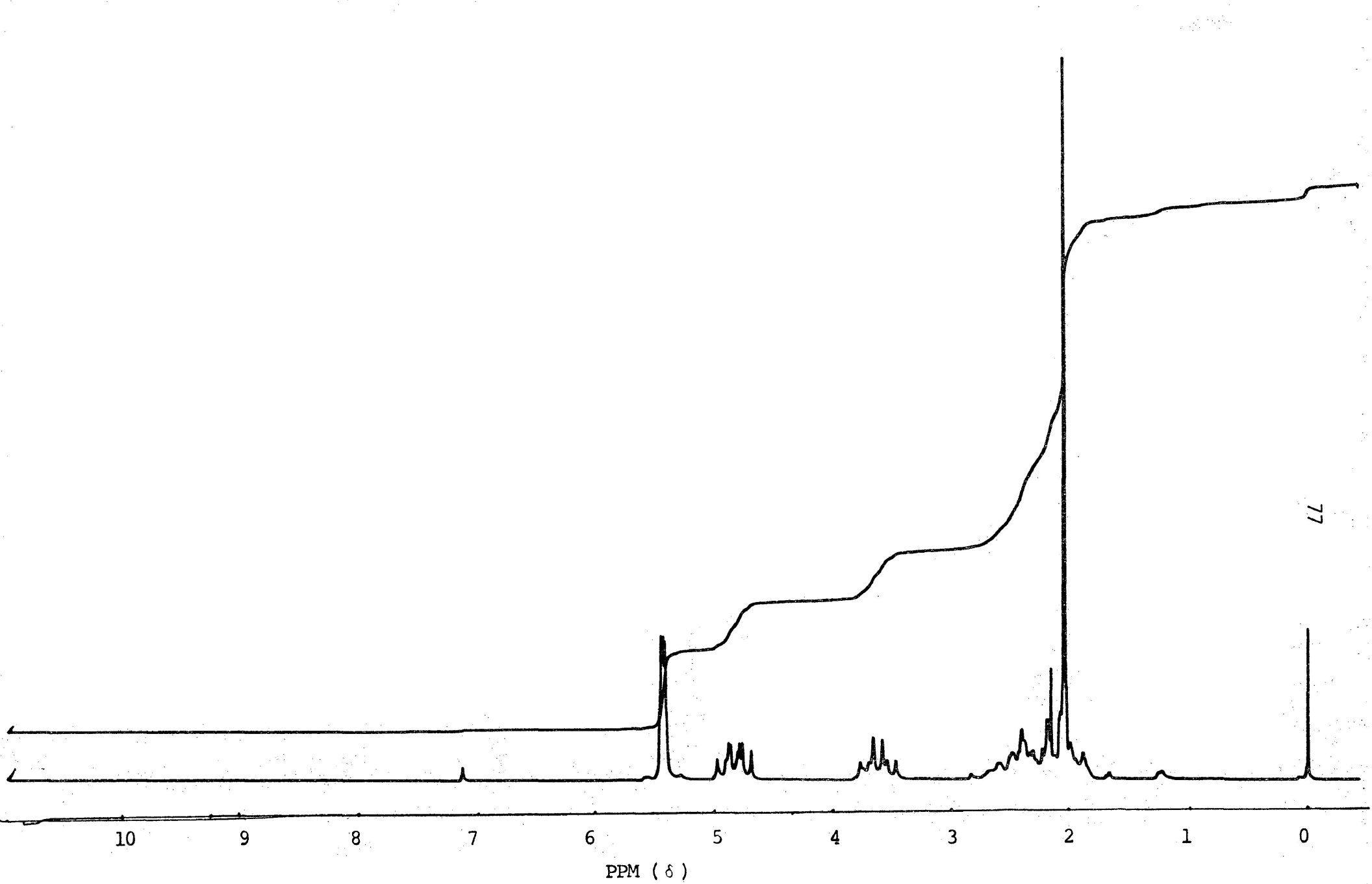
NMR NO.10 Epoxide.



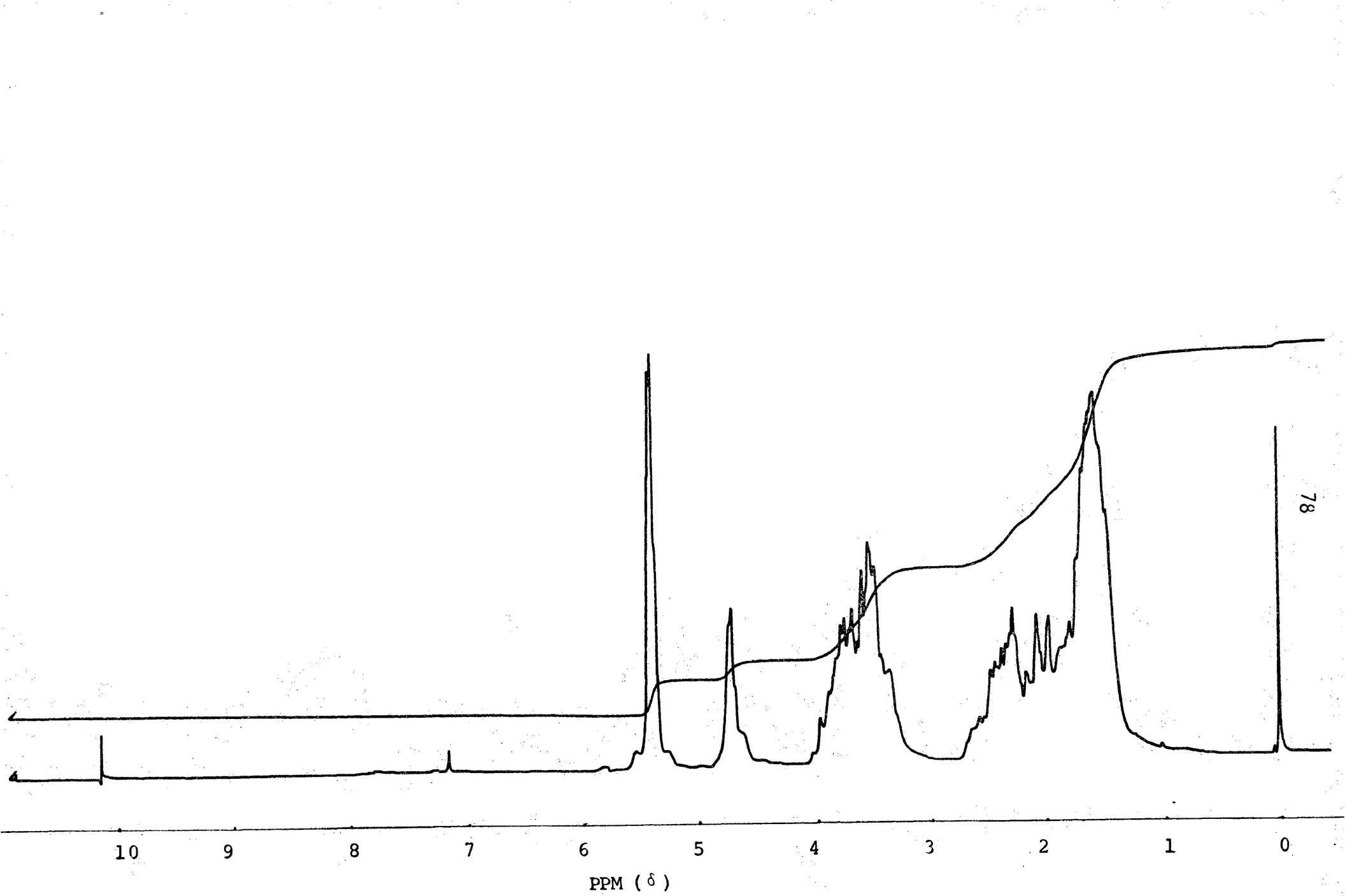
NMR NO.11 1,2-Oxidocyclohex-4-ene



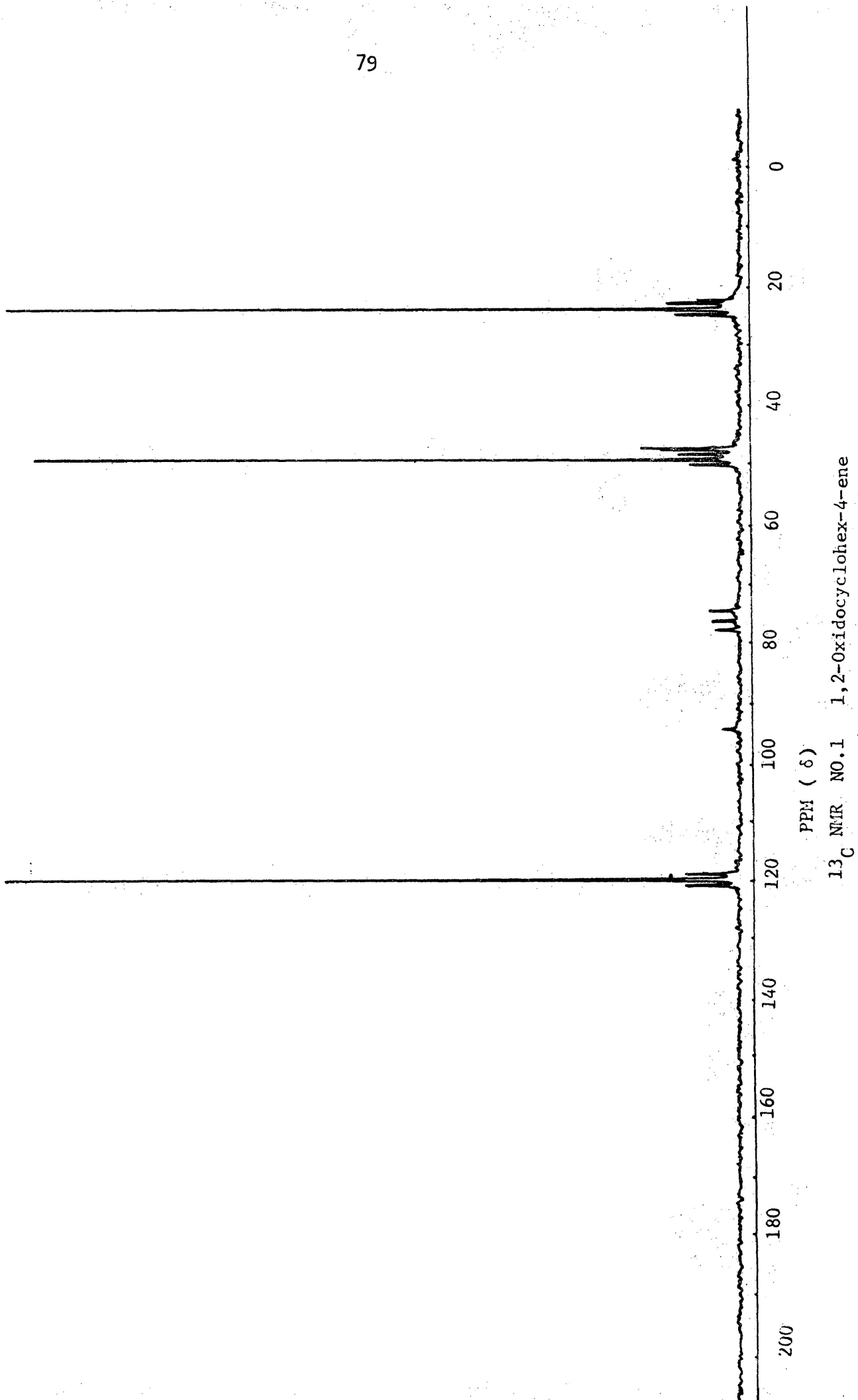
NMR NO.12 Trans-2-azidocyclohex-4-enol



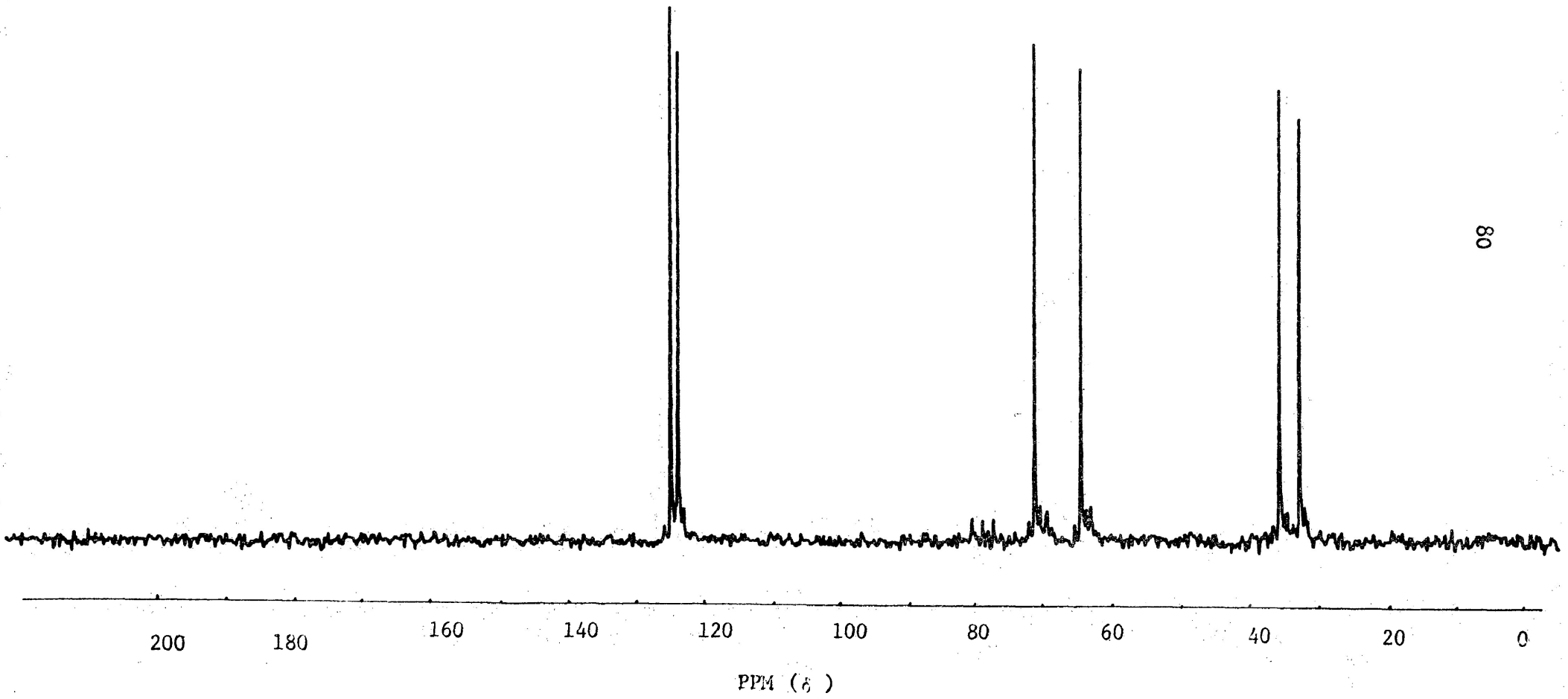
NMR NO.13 1-(Trans-2-azidocyclohex-4-ene)acetate



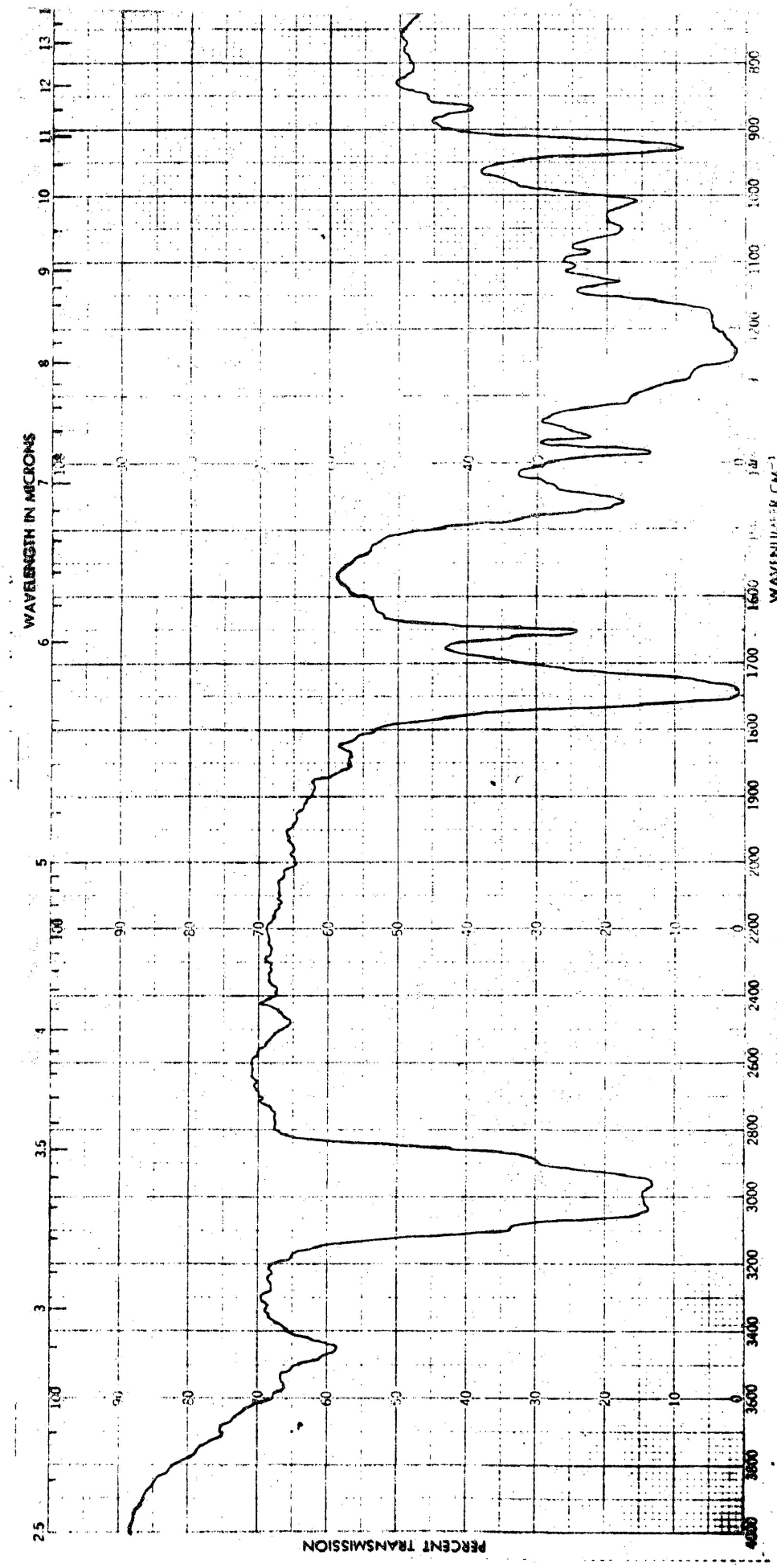
NMR NO.14 1-Tetrahydropyranloxyl-2-azidocyclohex-4-ene



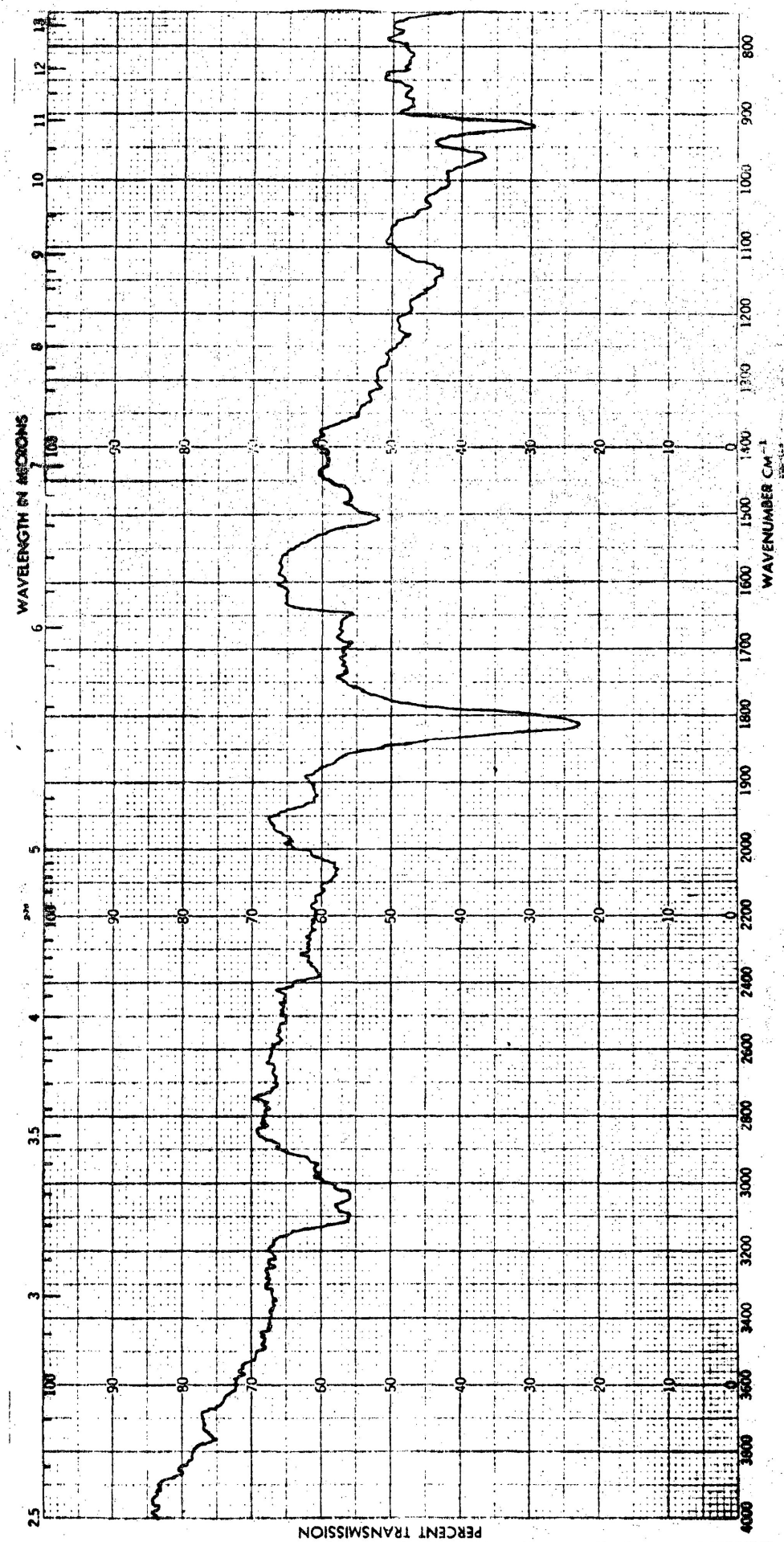
^{13}C NMR NO.1 1,2-Oxidocyclohex-4-ene



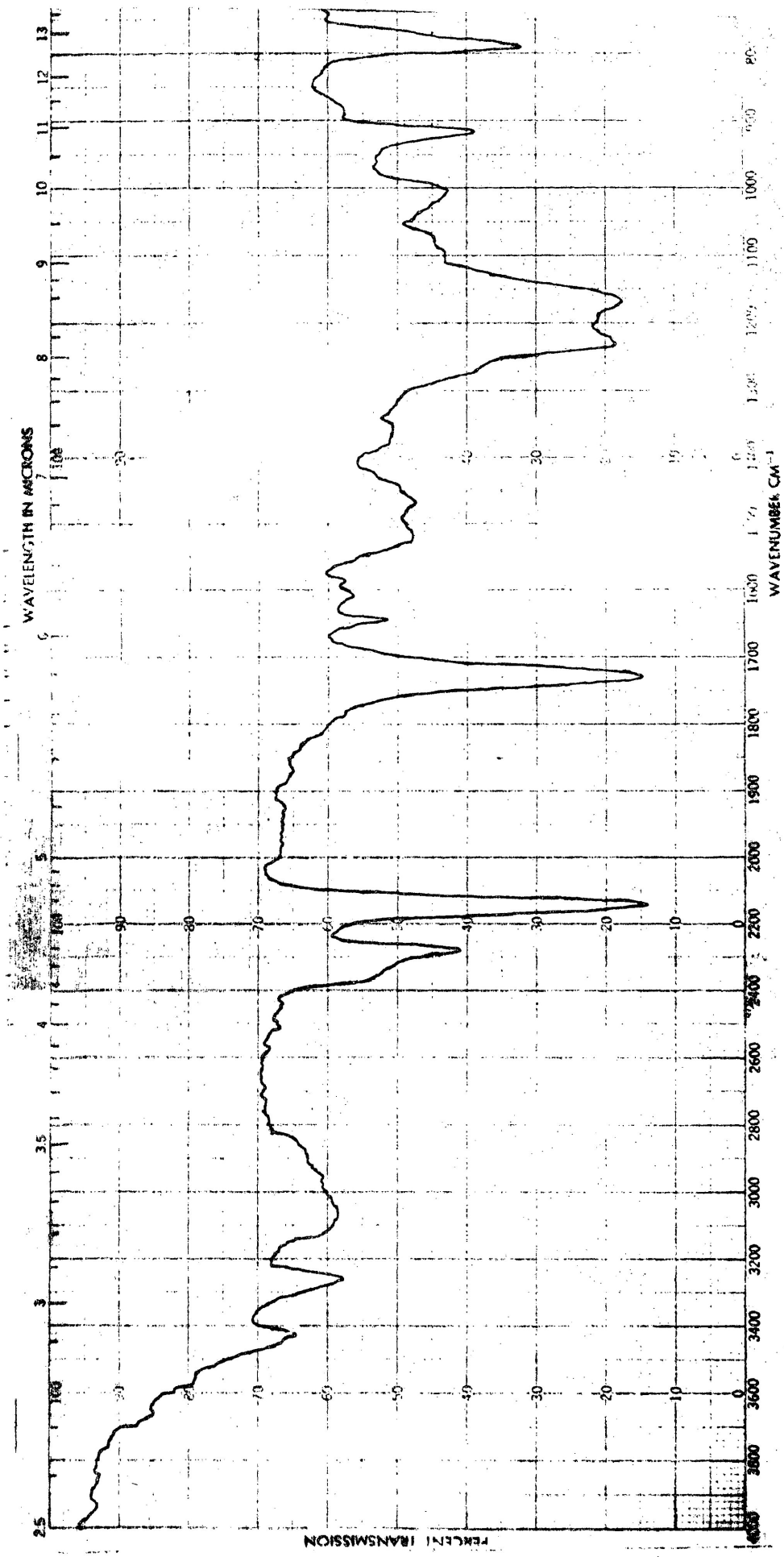
^{13}C NMR NO.2 Trans-2-azidocyclohex-4-enol



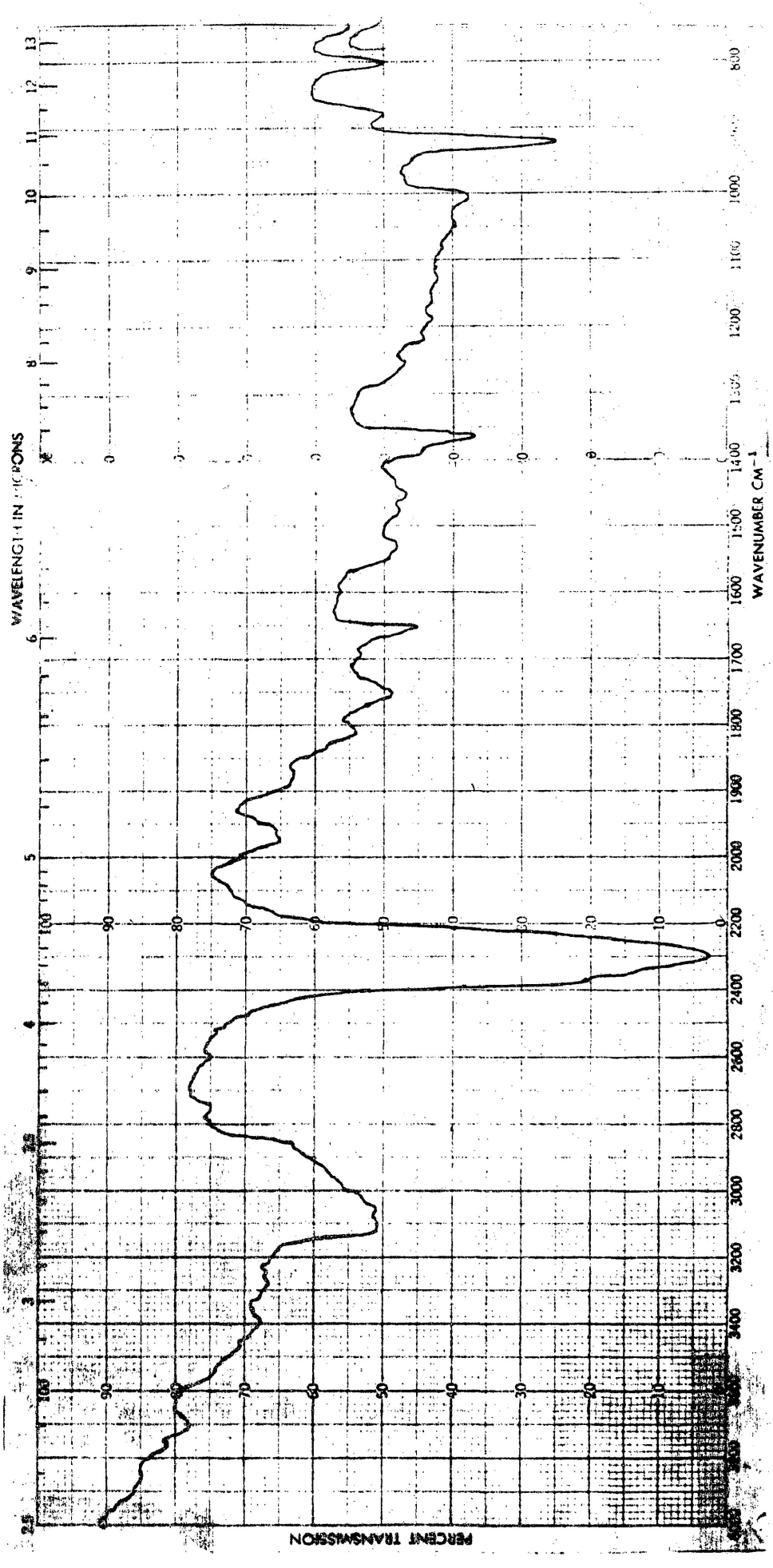
IR NO.1 Ethyl-(Z)-4,8-nonadienoate



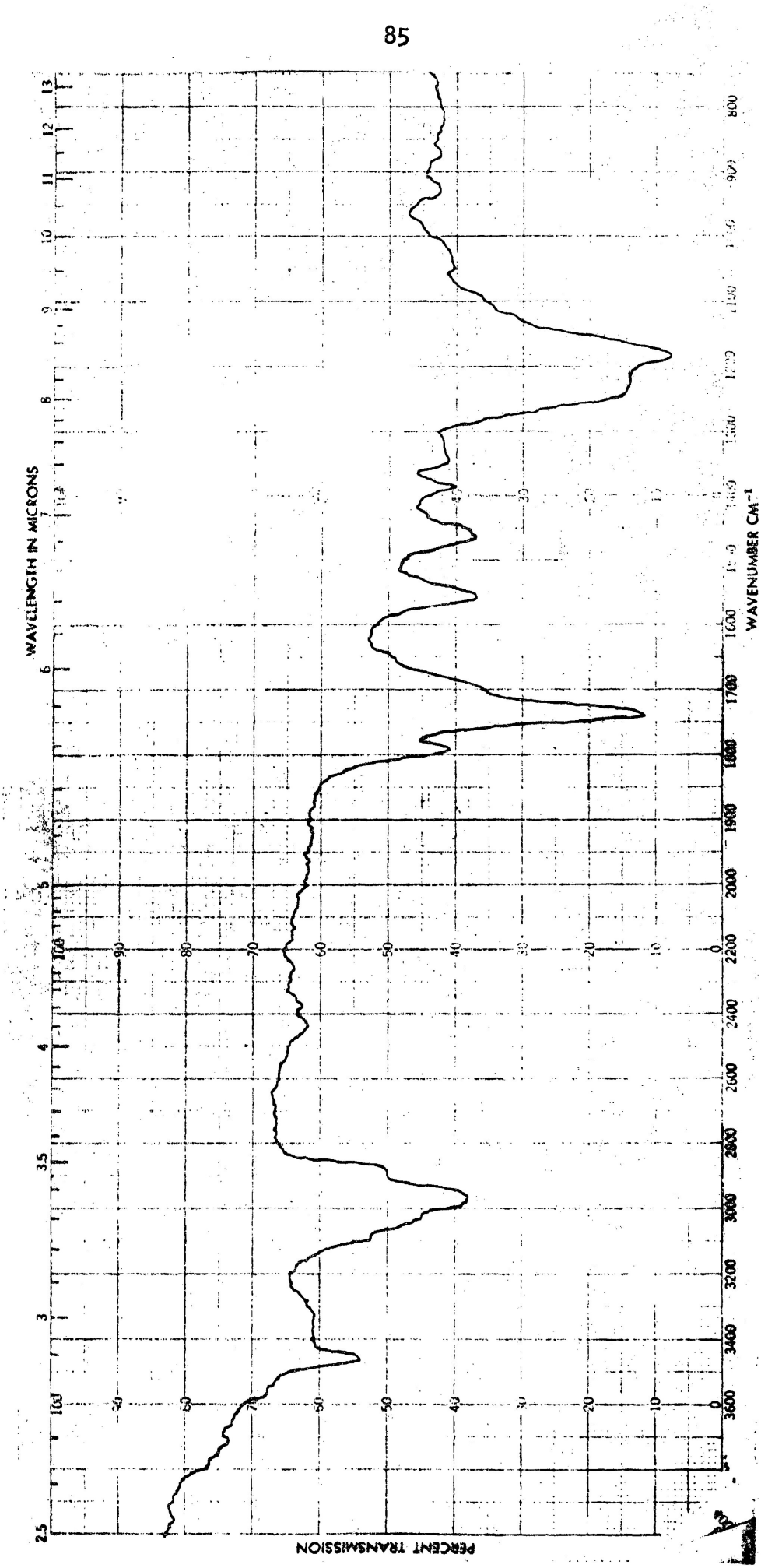
IR NO.2 Acid Chloride



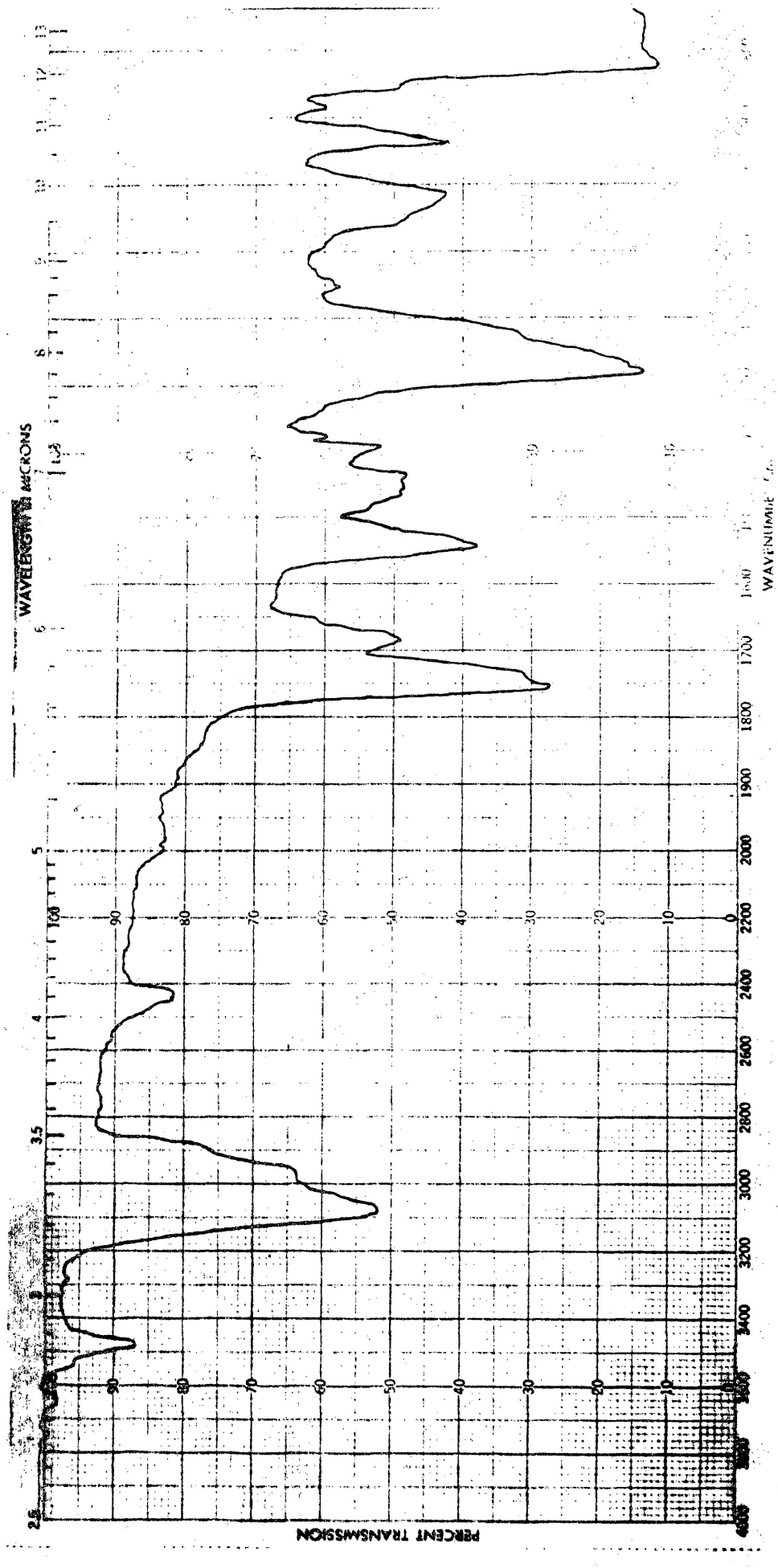
IR NO.3 Acyl Azide



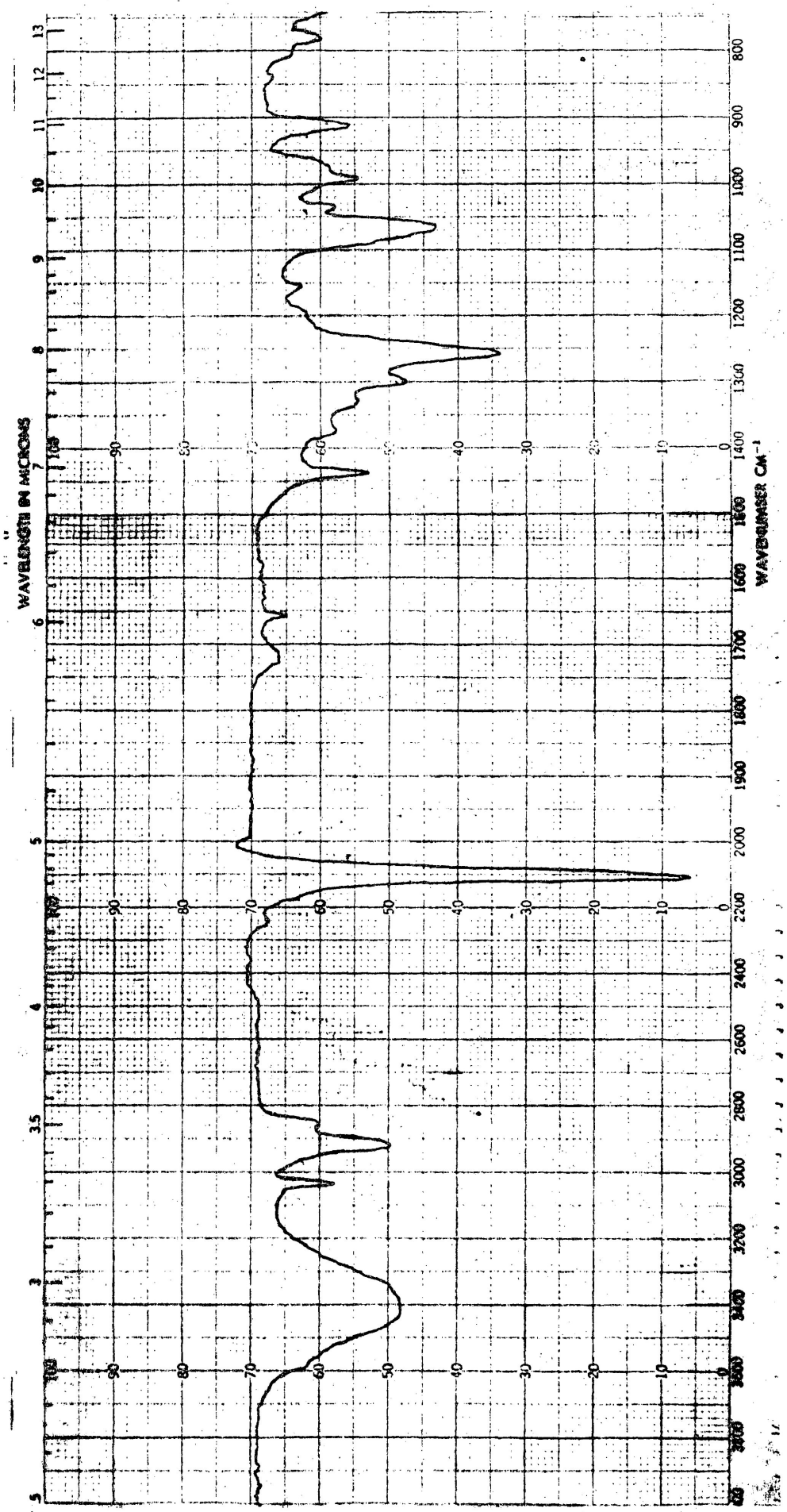
IR NO.4 Isocyanate



IR NO.5 1-Trifluoroacetamido-3,7-octadiene



IR NO.6 N-(Benzyloxycarbonyl)-3,7-octadiene



IR NO.7 Trans-2-azidocyclohex-4-enol

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