THE SYNTHESIS AND CHARACTERIZATION OF N-(5-HYDROXY-1-HEXYL) NICOTINAMIDE AS A PRECURSOR TO AN NICOTINAMIDE ADENINE DINUCLEOTIDE ANALOGUE

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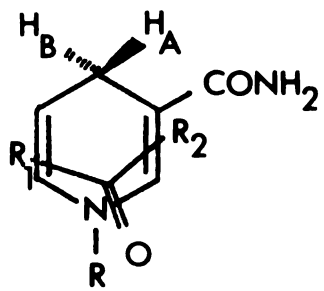
ABSTRACT

THE SYNTHESIS AND CHARACTERIZATION OF N-(5-HYDROXY-1-HEXYL)-NICOTINAMIDE AS A PRECURSOR TO AN NICOTINAMIDE-ADENINE DINUCLEOTIDE ANALOGUE

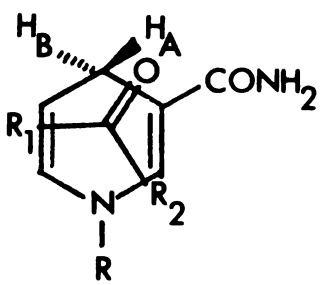
By

Michael Kenneth May

The stereospecificity of the nicotinamide adenine dinucleotide (NAD^+ - NADH) coenzyme oxidation-reduction of carbonyl compounds with the "dehydrogenase" enzymes has been a topic of controversy for many years. One of the questions that must be answered is the relationship of the substrate to the enzyme-coenzyme during the oxidation-reduction process (I,II).



I

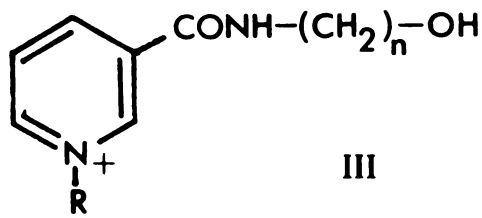


II

R = ribose—diphosphate—ribose—adenine

This question might be answered if the substrate were covalently bonded to the enzyme in such a manner as to limit the possible alignments by taking away from the substrate its freedom to rotate within its plane. By synthesizing NAD^+ analogues with aminoalcohols and studying the stereospecificity of their oxidation and reduction the above question might be answered.

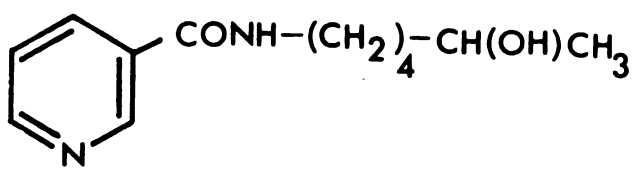
Primary aminoalcohols are currently being used to synthesize the analogues of the coenzyme (III) in order to determine optimum chain length and reaction conditions.



$n = 3,4,5$

III

This thesis describes the synthesis and characterization of IV, the N-(5-hydroxy-1-hexyl)nicotinamide, which is the precursor of a coenzyme analogue with a secondary hydroxyl group.



IV

THE SYNTHESIS AND CHARACTERIZATION OF N-(5-HYDROXY-1-HEXYL)NICOTINAMIDE AS A PRECURSOR TO AN NICOTINAMIDE ADENINE DINUCLEOTIDE ANALOGUE

By

Michael Kenneth May

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To My Grandmother

Ethel J. Day

"Columbus elaborated a deep plan to find a new route to an old country. Circumstance revised his plan for him, and he found a new world. And he gets the credit of it to this day.
He hadn't anything to do with it"

— Mark Twain

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TABLE OF CONTENTS

LIST OF TABLES

Introduction

The enzymatic reduction of aldehydes and ketones to the corresponding alcohols has been known for many years (1). These reactions are both reversible and highly stereospecific with respect to both the coenzyme and substrate (2). The stereospecificity of nucleotides - especially the pyridine coenzymes - has received considerable attention in recent years and has been the subject of several recent reviews (3,4).

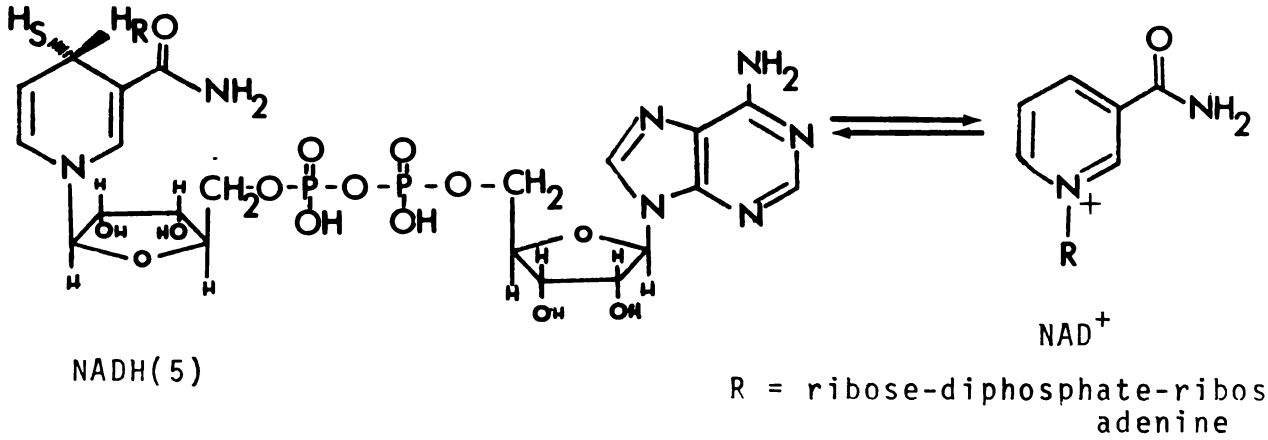


Figure 1

The steroespecificity of the enzymes - the dehyrdogenases - with respect to the diasterotopic protons at carbon 4 of Nicotinamide Adenine Dinucleotide (NADH) has been well established (6,7,8). The dehydrogenases can be classified as "A-type" or "B-type" enzymes depending upon which proton is transfered during the reduction of various aldehydes and ketones.

To elucidate the substrate orientation during the reduction process, Prelog studied several enzymatic reductions and determined the absolute configuration of the alcohols produced. However, only "B-type" enzymes were used in the study (9). On the basis of his results, he postulated a model in which the aldehyde or ketone was aligned such that the larger group was always away from the $-CONH_2$ group in the pyridine ring, with the carbonyl pointed down toward the nitrogen (Figure 2)

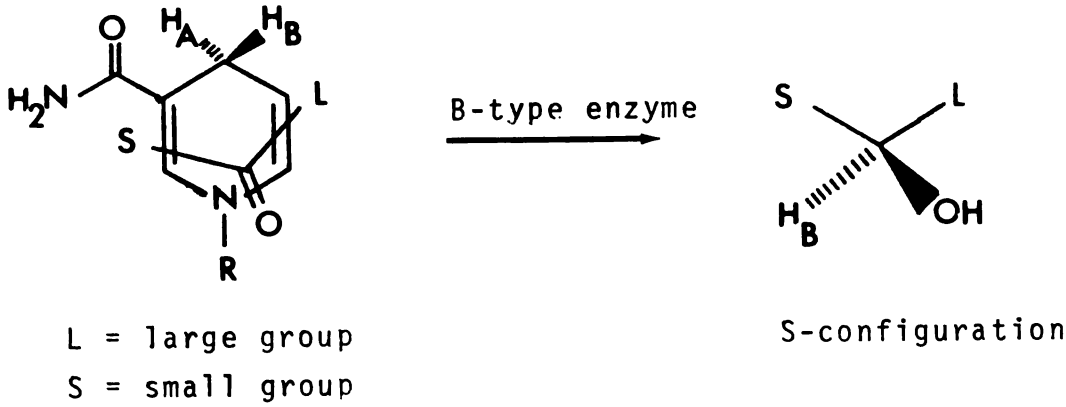


Figure 2

Prelog's justification of this model was based on the following:
a) steric (coenzyme-substrate) interactions which are smaller when "S" is over the $-CONH_2$ and "L" is over the hydrogen, than vice-versa,
b) hydrophilic and hydrophpbic interactions. Similar suggestions involving hydrophilic and hydrophobic interactions were made earlier by Kosower, Vennesland, and Westheimer (2,10,11). On the basis of the first assumption of steric interactions of the large group with the amido group, Prelog predicted that alcohols with R-configuration would

result from reductions of aldehydes and ketones with A-type enzymes (Figure 3)

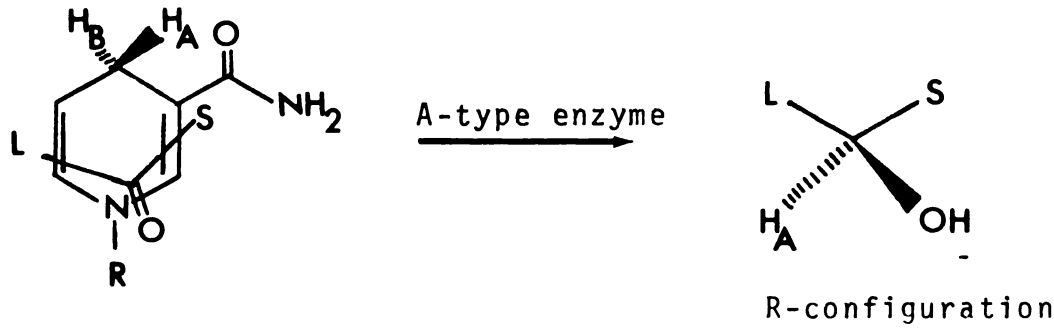


Figure 3

The enzymatic reduction of acetaldehyde-1-d with NADH and yeast alcohol dehydrogenase (YADH), and A-type enzyme, gave S(-)ethanol-1-d (11). Later work by Lemieux (12) established the absolute configuration of ethanol-1-d to be the R(+) configuration. This contradictory information was pointed out by Karabatsos (13), who suggested that the $-CONH_2$ group of the coenzyme played no role in the determination of the absolute configuration of the product alcohols. The spacial arrangement of substrate-coenzyme was suggested to be as in Figure 4, rather than Figure 3.

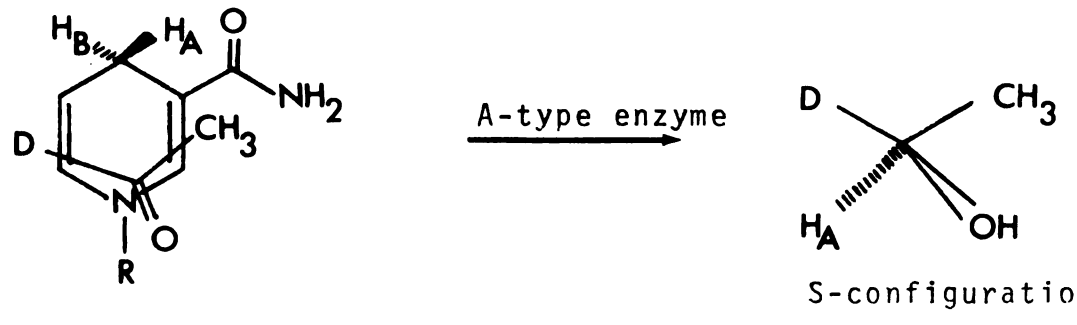


Figure 4

As further support for his suggestion, Karabatsos cited the observation that in the oxidation of (R,S)-2-octanol with yeast alcohol dehydrogenase and NAD^+ only the (S)-2-octanol reacted to give the corresponding ketone (Figure 5) (14).

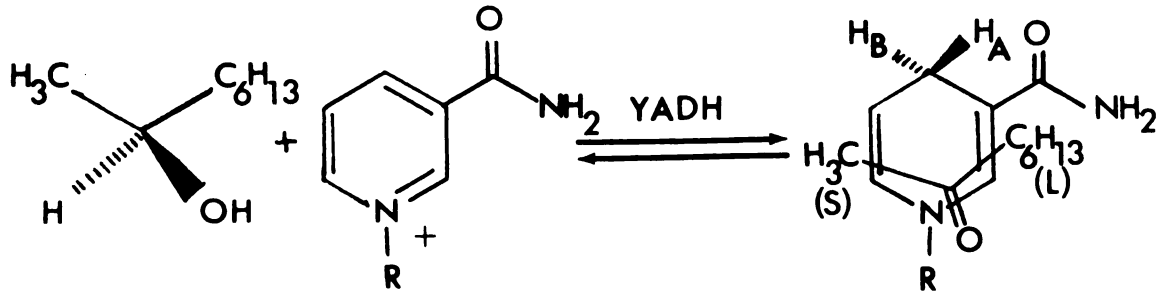


Figure 5

Also, fermenting yeast reduction of various 1-d-aldehydes and several ketones gave alcohols whose absolute configuration was "S"-, i.e.: the one model suggested by Karabatsos (15-17).

The enzymatic reduction of either D- or L- lactaldehyde with NADD and liver alcohol dehydrogenase (LADH) yielded propanediol-1-d whose absolute configuration at C-1 was "R" (18). This result indicated that both D- and L- lactaldehyde, as well as acetaldehyde, have the same substrate-coenzyme relationship (Figure 6).

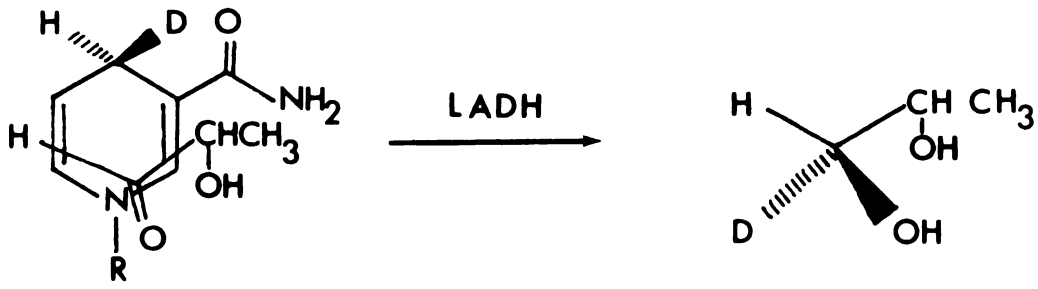
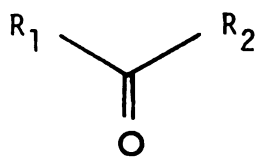


Figure 6

This finding, however, brought into question the importance of hydrophilic-hydrophobic interactions vis-a-vis steric interactions involving substrate and enzyme. To test the relative importance of hydrophobic-hydrophilic interactions and steric interactions, enzymatic reductions were run on substrates with groups of comparable size but different hydrophilicities (Table 1).



R_1	R_2
CH_3-	$HOCH_2$-
CH_3-	CH_3CH_2-
CH_3-	$ClCH_2$-

Table 1

Reduction of hydroxyacetone with glycerol dehydrogenase - an A-type enzyme - gave 2-(R) propanediol (8). The corresponding spacial relationship is shown in Figure 7 and has been interpreted on the basis of hydrophobic-hydrophilic interactions being more important than steric interactions

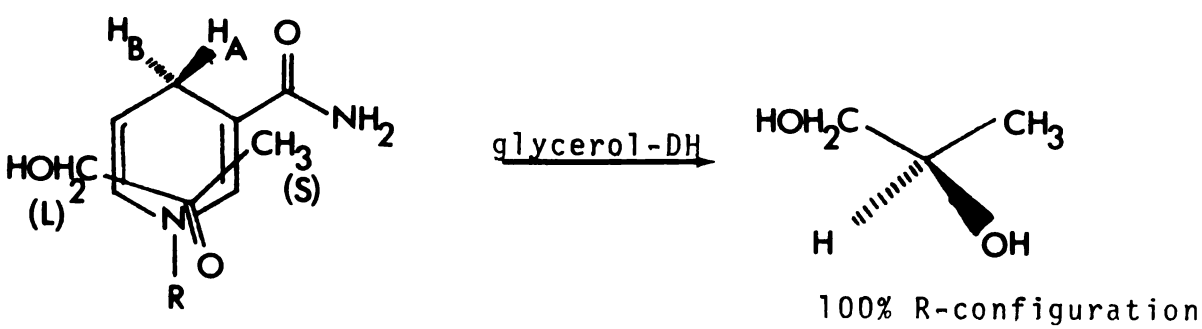


Figure 7

In contrast, the fermenting yeast reduction of methyl ethyl ketone yielded 64-67% S- and 33-36% R-2-butanol (17); a similar result was obtained by purified liver alcohol dehydrogenase (19). The results, which fit Figure 5, have been interpreted in terms of steric interactions being more important than hydrophobic-hydrophilic interactions. Previous studies by several independent investigators using both A- and B-type enzymes have given widely varying results (20-22).

The models presented so far, and the rationale for their validity of not, are based on the idea of having the carbonyl group pointed toward the nitrogen of the pyridine ring. Kosowar (10) observed a λ_{max} pf 340 mμ and 325 mμ for the NADH and the NADH·ADH· carbonyl complex respectively. He suggested that this shift in the spectrum could be attributed to the effect of a positively charged nitrogen, present only in the complex, on the chromophore shown in Figure 8.

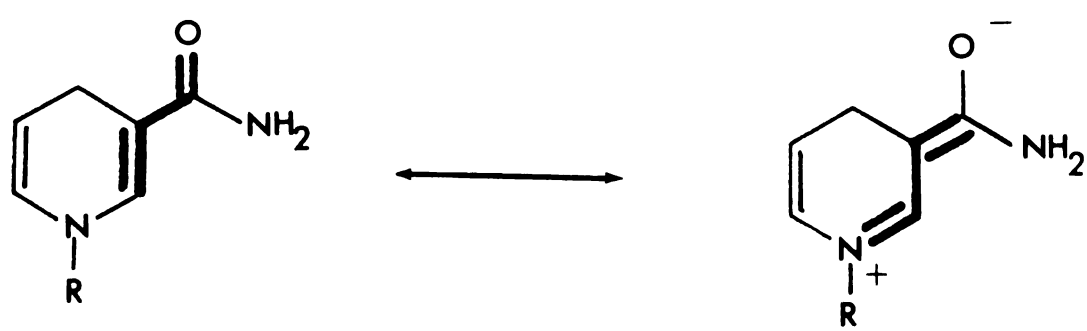


Figure 8

The calculated increase in the transition energy of the $\pi \rightarrow \pi^*$ transition in the presence of an alkylammonium ion, hydrogen bonded to two groups as shown in Figure 9, corresponds closely to the observed energy change. It is evident, therefore, that the model having the carbonyl group pointed towards the nitrogen of the ring is not based on the best evidence.

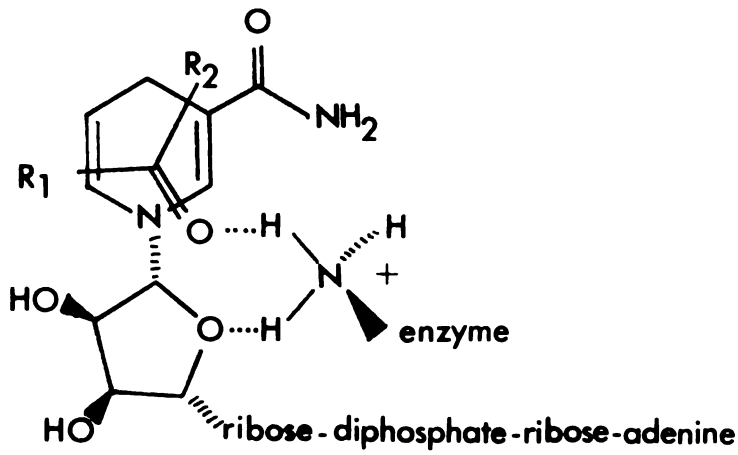


Figure 9

It now seems obvious that any model proposed must account for the possibility of having the carbonyl group either "up" or "down" on face A or face B of the pyridine ring. The available data from product stereospecificities agree with either orientation of the carbonyl group (Figure 10).

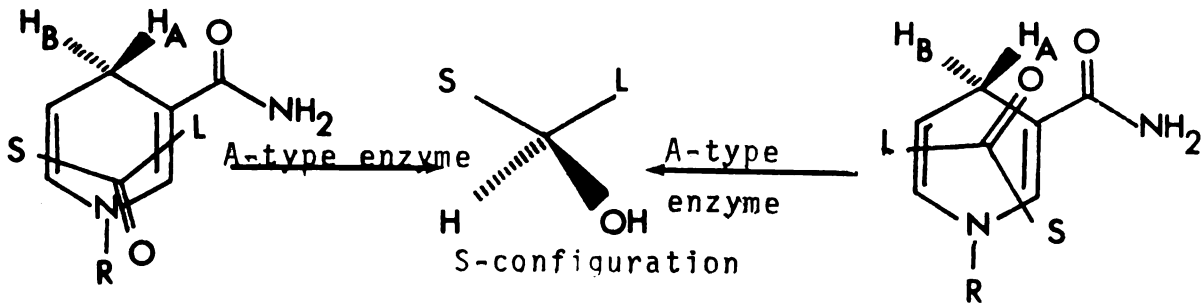


Figure 10

To prove whether the carbonyl is "up" or "down" in these reductions, the substrates should be covalently bonded on the coenzyme in such a manner as to distinguish between the two possibilities by taking away from the substrate its freedom to rotate within its plane. Absolute configuration studied as summarized in Figure 11 would answer the question of whether the carbonyl is "up" or "down".

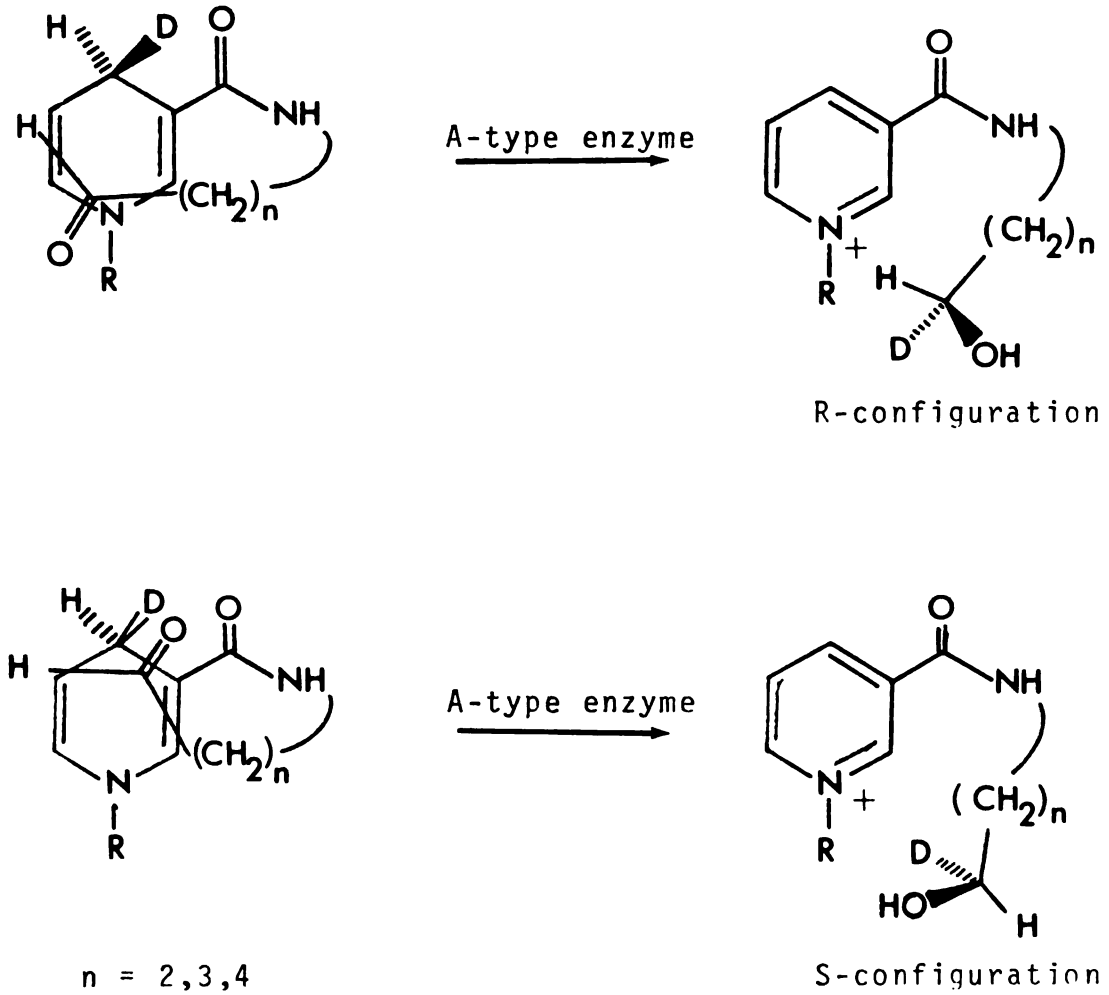
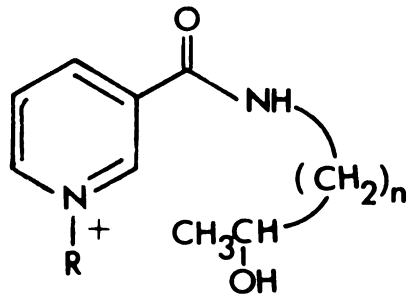


Figure 11

Since many NAD^+ analogues have been synthesized, most notedly in studies by Kaplan (23), we have attempted to prepare the analogues presented in Figure 11 by using alcohols of varying chain length and reaction conditions. Primary aminoalcohols commercially available have been used to prepare coenzyme analogues. At the same time we have approached this problem by using secondary aminoalcohols, as shown in Figure 12.



(R,S-configuration)

n = 3,4,5

Figure 12

The major advantage to using the secondary alcohol is the ease of determination of the stereochemistry of the reaction as opposed to that of using secondary alcohols involved the difficulties in the synthesis of these compounds. There is no reliable synthesis of secondary aminoalcohols in the literature. The only reported synthesis of 5-hydroxy--1-hexyl-amine - the major object of this study - goes with very low yield (24).

The purpose of this study was the synthesis and characterization of 5-hydroxy-1-hexylamine and its nicotinamide derivative, N-(5-hydroxy-1-hexylamine)nicotinamide as a precursor to the NAD^+ analogue. Several methods for improved synthesis of the five and seven carbon secondary alcohols have also been investigated.

Experimental

A. Reagents and Compounds

Benzyltrimethylammonium hydroxide, acrylonitrile, 2-methyltetrahydropyran, acetonitrile, tetrahydrofuran, 4-acetylbutylric acid, α-acetyl-γ-buryrolacetone, cyclopropyl methyl ketone, ethyl acetoacetate, thionyl chloride, and 1,1,1,3,3,3-hexamethyldisilizane were obtained from Aldrich Chemical Company, Inc.; acetone, boron trifluoride etherate complex, boron tribromide etherate complex, ammonia, sodium borohydride, Vitride-T(sodium bis(2-methoxyethoxy)aluminum hydride) from Matheson Coleman and Bell (Division of the Matheson Company, Inc.); ethylene glycol, sodium cyanide, potassium cyanide from Mallinckrodt AR Chemical Company; p-toluenesulfonic acid, celite 545 from Fisher Scientific, Inc.; nicotinic acid from Sigma Chemical Company; Raney nickel powder from W. R. Grace Chemicals (Division of Grace Inc.); Bio-sil A from BioRad Laboratories; and lithium aluminum hydride from Alfa Inorganics (Alfa Ventron Inc.)

B. Solvents

Diethyl ether, dimethylsulfoxide, benzezen triethylamine, carbon tetrachloride, and methylene chloride were dried and purified from commercially available material by standard methods (25-27). Absolute ethanol, 95% ethanol, methanol, pyridine, acetone, and ethyl acetate were also obtained from commercial sources and used with further drying or purification. Chloroform-d_1/1%TMS (v/v), Chloroform-d_1, pyridine-d_5, and acetonitrile-d_3 were purchased from Norell Chemical Company, Inc.

C. Preparation of Compounds

1. 5-Oxocapronitrile

The compound was prepared by a procedure reported by Albertson (28). The following is a description of that procedure.

To a solution of 3.27g of sodium in 400 ml of absolute ethanol was added 600 ml (4.73 moles) of ethyl acetoacetate. With stirring, 246 (3.59 moles) of acrylonitrile was added slowly over a period of ten hours such that the temperature did not exceed 45°. The alcohol was distilled off and the residue washed with 100 ml of water to which 10 ml of acetic acid had been added. The residue was distilled through a 6-in. vacuum-jacketed Vigreaux column to give 281.2g (42.7% yield) of ethyl(2-cyanoethyl)-acetoacetate, b.p. 132.0°-134.0°/0.2 mm. The compound was a pale yellow liquid.

The 5-oxocapronitrile was prepared by adding 200g (1.09 moles) of ethyl(2-cyanoethyl)-acetoacetate to a solution of 200g of sodium carbonate in 1800 ml of water. The two layer mixture was refluxed for four hours during which time it became homogeneous. The product was salted out with potassium carbonate and the aqueous layer extracted with 5-100 ml portions of ether. Distillation of the residue after removal of the ether yielded 93.66 (77% yield) of 5-oxocapronitrile, b.p. 66.0°-68.5° /1.0 mm

IR: CO 1710 cm^{-1}(s)

CN 2240 cm^{-1}(m)

Previous attempts at preparing this compound proved unsuccessful or proceeded with very low yield. Since a large portion of this work was spent in an attempt to prepare 5-oxocapronitrile, several unsuccessful methods will be briefly outlined.

The initial attempt at the preparation was a simple Michael addition of acetone and acrylonitrile. The synthesis was taken from a procedure by Baumgarten (29). A mixture of 1470 ml (1160g,20 moles) of acetone and 10 ml of benzyltrimethylammonium hydroxide (40% in methanol) was heated to reflux. To this hot solution was added 132.2 ml (106g, 2 moles) of acrylonitrile dropwise over a period of three hours. The solution was heated for one hour after the addition was completed. The catalyst was neutralized to Congo Red with 3 N hydrochloric acid and the excess solvent removed. Distillation of the residue with a 15 in. electrically heated column with a ground glass spiral insert yielded 9.62 (0.130 mole) of a dark yellow liquid, b.p. 108°-110°/14mm. Gas chromatrographic analysis of the liquid showed it to be a mixture of several compounds. Separation of the major components on the gas chromatograph indicated a mixture of the product, the mono- and dicyanoethylated adducts as well as polymers of acrylonitrile. Several distillations of the impure mixture failed to yield pure product. Attempted reduction of the mixture with lithium aluminum hydride and distillation of the reduction mixture also failed to yield the desired compound.

A second attempt to obtain the 5-oxocapronitrile via the chloroketone proved also to be unseccessful. The preparation of 5-chloro-2-pentanone from α-acetyl -γ-butyrolacetone was reported by Cannon (30). The synthesis of the chloroketone went in 80% yield. Attempts at substitution of the chlorine with sodium cyanide and potassium cyanice were unsuccessful. Several methods of cyanation (31-34) from previous literature prepatations on the reduced chloroketone (using sodium borohydride) as well as the ethylene glycol ketal yielded predominantly

starting material and tar. After several attempts, this method, too, was abandoned.

Attempts were also made to prepare the 5-oxocapronitrile from cyclopropyl methyl ketone after work reported by Rodig and Johnston (34) and Djerassi (33) by using both potassium cyanide and sodium cyanide to affect ring opening with nucleophilic substitution. Work up of the resulting black solutions showed again a predominance of starting material and small amounts of impurity which were not characterized.

2. 5-Hydroxycapronitrile

To a solution of 77 ml (0.653 mole) of the 5-oxocapronitrile in 1-liter of benzene was added slowly with stirring a solution of 101 ml (0.360 mole) of Vitride-T (70% benzene) in 500 ml of benzene. The solution changed from colorless to deep yellow near the end of the addition. The yellow mixture was heated under reflux for 12-18 hours. The mixture was colled and quenched with alternate additions of 73 ml of water, 73 ml of 15% sodium hydroxide, and 219 ml of water. The solution was filtered and the residue distilled through a 6 in vacuum-jacketed Vigreaux column to yield a colorless liquid, b.p. 55.0°-56.6° /0.1 mm. The reduction proceeded with nearly quantitative yield.

IR: OH 3600 cm^{-1}(m) 3476 cm^{-1}(broad)

CN 2244 cm^{-1}(m)

An alternate method for the reduction was also attempted.

To a solution of 25 ml (0.215 mole) of the 5-oxocapronitrile in 400 ml of ether was added slowly a mixture of 25g (0.662 mole) of sodium borohydride at ice-salt bath temperature. After the addition was

completed the mixture was allowed to stir overnight while slowly warming to room temperature. The mixture was neutralized with dilute sulfuric acid, diluted to twice the volume, and the organic layer salted out with sodium chloride. The aqueous layer was separated and extracted with 10-100 ml portions of ether. The ether layers were combined and the ether removed. Distillation of the organic residue gave quantitative yields by this procedure (35).

Attempts to reverse the addition, i.e.; add the solution of 5-oxocapronitrile to the reducing reagent led to complexation of the nitrile with subsequent formation of several undesired side products. Addition of the 5-oxocapronitrile solution to the Vitride solution solidified upon heating. Selective reduction of the carbonyl with lithium aluminum hydride was also unsuccessful. The major product from the lithium aluminum hydride reduction appears to be piperidines or piperidones (28). Similar results are obtained from direct reduction of 5-oxocapronitrile with Raney nickel and hydrogen (36).

3. 5-Hydroxy-1-hexylamine

A solution of 24.11g (0.212 mole) of the 5-hydroxycapronitrile, in 400 ml of 10N methanolic ammonia (47) and freshly prepared W-2 Raney nickel catalyst (48) were placed under 50 psi of hydrogen in a Parr apparatus at room temperature. After shaking 36 hours the pressure drop was within 2 psi of the theoretical amount. The catalyst was filtered from the solution by using Celite 545 and the solvent was removed. Distillation gave a nearly quantitative yield of a colorless liquid, b.p. 62.0°-62.5° /0.1 mm.

IR: NH_2 3362 cm^{-1}(m) 3656 cm^{-1}(sh)

OH 3600 cm^{-1}(m) 3188 cm^{-1}(broad)

Synthesis of the desired aminoalcohol was attempted directly from the reduction of the amide of 4-acetylbutyric acid. A mixture of 15g (0.115 mole) of the acid, 12.85 ml (14.28g, 0.230 mole) of ethylene glycol, 0.050g p-toluenesulfonic acid, and 250 ml of benzene was heated to reflux in a flask equiped with a Dean-Stark trap for 36 hours (or until the calculated amount of water had been collected.) The solution was cooled, washed with saturated bicarbonate solution, and the benzene removed. Distillation of the residue and characterization of the product by nmr indicated the major product to be the ketal of the hydroxy ester. The ester was heated in a 10% potassium hydroxide solution until the layers become homogeneous. The solution was neutralized with dilute hydrochloric acid and extracted with ehter. Distillation yielded only a small amount (less than 2 grams) of a brown residue presumed to be the ketal of the acid. The procedure was abandoned due to the lack of good yield and expense of starting materials.

A study of Ritter-type ring openings of furans and 2-methylpyrans 5-hydroxyalkylacetamides was undertaken to determine whether this method would provide a suitable pathway to the direct synthesis of the aminoalcohol. Solutions of tetrahydrofuran and 2-methyltetrahydropyran in dimethylsulfoxide, hexamethylphosphoramide, chlorobenzene, and xylene were treated with boron trifluoride, boron tribromide, and stannic chloride. These solutions were heated to reflux and solutions of acetronitrile in each of the reaction solvents were added to the hot solutions. Work-up of the resulting mixture failed to yield any

of the desired acetamides. Nmr showed the major products to be the expected halogenated ring-opened structures with considerable amounts of starting material recovered along with traces of uncharacterized side products.

4. Nicotinyl Chloride

To a suspension of 400 ml of carbon tetrachloride and 64.8g (0.400 mole) of potassium nicotinate was added slowly, with stirring, 36 ml (0.500 mole) of thionyl chloride under a continuous stream of nitrogen. After the addition was completed the solution was allowed to reflux for 12-14 hours. The nitrogen was stopped after the evolution of SO_2 gas became slow. The solution was filtered and the solvent removed under vacuum. The residue was again filtered and distilled to yield a colorless liquid, b.p. 30.0-31.5°/0.08 mm. Yields were not determined since the product hydrolyzed readily on contact with air.

5. 5-Hydroxy-1-hexylamine Trimethylsilyl Ether

To 14.74g (0.126 mole) of the aminoalcohol was added 26.30 ml (0.126 mole) of 1,1,1,3,3,3-hexamethyldisilizane and 0.1g dried ammonium chloride under nitrogen atmosphere. The two-phase mixture was allowed to heat slowly to 125 and heated at this temperature for one hour. The reaction went with an initial vigorous evolution of ammonia and the two phases became one. Distillation of the solution yielded 12.0g (81.4% yield) of the completely silated alcohol, b.p. 41.5-44.5°/0.2 mm.

6. N-(5-Hydroxy-1-hexyl)nicotinamide

A solution of 10.03g (0.053 mole) of the aminoalcohol silyl ether, 65 ml of triethylamine, and 65 ml of pyridine was heated to 100-115 with moderate stirring under nitrogen. To the hot solution, 5.78 ml (0.053 mole) of nicotinyl chloride was injected slowly by syringe through a rubber septum. Heating and stirring were continued for 2 hours after the addition. After cooling, the solution was filtered and the triethylamine, 10 ml of water was added and the solution was refluxed for 2 hours. The pyridine-water mixture was removed under vacuum until only a dark, viscous residue remained. Caution must be taken to insure removal of all solvent since trace amounts inhibit recrystallization of the amide. A small amount of the residue was dissolved in chloroform and thin layer chromatography analysis of it by using 9:1 chloroform-methanol showed the residue to be a mixture of trace amounts of the ester, nicotinic acid, and product. The 3-, 4-, 5-, and 6-carbon primary hydroxyamides have R_f values which closely correspond to the secondary hydroxyamide. The chloroform solution of the mixture was separated on a 7-foot Bio-Sil A (100-200 mesh) silica gel column attached to a UV spectrophotometer. The product was eluted with the 9:1 chloroform-methanol mixture. After evaporation of the solvent, the resulting oil was crystallized from acetone and recrystallized to constant melting point. The amide is a tan powder, m.p. 44.5°-45.2°

IR: CO 1664 cm^{-1}(s) 1651 cm^{-1}(sh) 1589 cm^{-1}(s)

NH 3636 cm^{-1}(m)

OH 3607 cm^{-1}(m) 3430 cm^{-1}(m) 3318 cm^{-1}(broad)

D. Instrumentation

1. N.m.r. Spectra

A Varian A56/60D analytical spectrometer operated at at ambient temperature was used to obtain all n.m.r. spectra. The undergassed samples were run in concentrations based on weight percent. Tetramethylsilane(TMS) was used as the internal reference standard (δ=0.00). Magnetic field sweep widths were calibrated by the audio-frequency side band technique. All spectra were recorded at a radio-frequency field strength well below the value necessary to observe the onset of saturation.

2. Infrared Spectra

All samples were run on a Perkin-Elmer 237B Grating Infrared Spectrophotometer using 0.0922 mm path-length KBr liquid cells. Samples were all 5% (w/w) in methylene chloride. Wavelength calibrations were made using polystyrene film.

3. Mass Spectra

Samples were analyzed on a LKB 900 Gas Chromotograph - Mass Spectrometer (LKB Instruments, Inc.). Bar graphs were recorded by PDP8/I (Digital Equipment Corp.) and displays printed by Tektronix Display and Hardcopy unit.

Results and Discussion

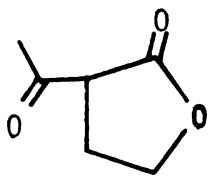
A. Reactions for the preparation of the compounds

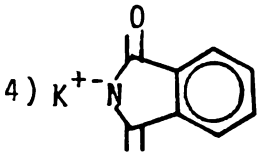
Generally aminoalcohols containing primary amino and primary hydroxy groups are easily prepared and can be obtained commercially. Aminoalcohols containing primary amino and secondary hydroxy groups are not easily prepared in high yields. This study outlines a reliable method of preparing 5-hydroxy-1-hexylamine and provides a basis for good methods of preparation of similar animoalcohols of varying chain length (equations 1, 2 and 3).

a) $CH_3COCH_2CO_2C_2H_5 \xrightarrow[3)\ Na_2CO_3,\ \text{reflux}]{1)\ C_2H_5ONa/C_2H_5OH \quad 2)\ CH_2CHCN,\ 25°\text{-}45°} CH_3CO(CH_2)_3CN$ (1)

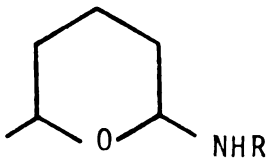
b) $CH_3CO(CH_2)_3CN \xrightarrow[\text{or}\quad Na(OCH_3OC_2H_5)AlH/\ C_6H_6,\ \text{reflux}]{NaBH_4/(C_2H_5)_2O,\ C_2H_5OH,0°} CH_3CH(OH)(CH_2)_3CN$

c) $CH_3CH(OH)(CH_2)_3CN \xrightarrow[50\ \text{psi, R.T.}]{\text{Raney-nickel}(H_2)/\ CH_3OH,NH_3} CH_3CH(OH)(CH_2)_4NH_2$



1) HCl, heat
2) $NaBH_4/(C_2H_5)_2O, 0°$
3) HMDS, 125°, N_2
4) 
5) OH

$\longrightarrow CH_3CH(OH)(CH_2)_4NH_2$ (2)



1) BF_3, heat
2) H_2O
3) reduce

$\longrightarrow CH_3CH(OH)(CH_2)_5NH_2$ (3)

The previously reported synthesis of 5-hydroxy-1-hexylamine by Baumgarten (29) and Okahara (24) results in very low yields of pure product.

The initial compound in the synthesis of 5-hydroxy-1-hexylamine is 5-oxocapronitrile. This compound contains functional groups which are readily converted to the desired amino alcohol. The first attempt to prepare 5-oxocapronitrile was a simple "Michael addition" of acetone and acrylonitrile (equation 4).

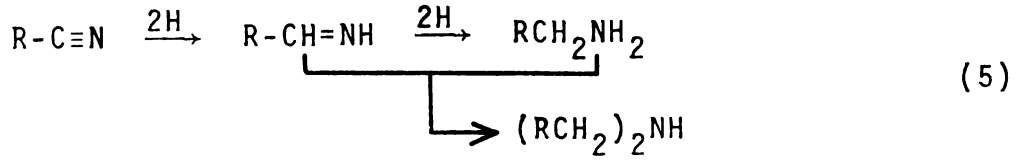
$$(CH_3)_2CO + CH_2CHCN \xrightarrow{\text{base}} CH_3COCH_2CH_2CH_2CN \qquad (4)$$

Apparently, acrylonitrile is so powerful a cyanoethylating agent in the presence of simple ketones that monocyanoethylation is only observed in 8% yield of less, even when a 10:1 molar excess of the

ketone is used (28). The major product appears to be the polycyanoethyl adduct (possibly with replacement of all of the protons in the case of acetone). Since β-ketoesters give better yields of monocyanoethyl derivatives, a better yield of the desired product was realized by using ethyl acetoacetate and acryonitrile (28). Aqueous carbonate gave the pure ketonitrile in high yield (equation 1a). Reduction of both functional groups of the 5-oxocapronitrile was expected to give the desired aminoalcohol.

Work done by Albertson indicated that direct reduction of 5-oxocapronitrile with either lithium aluminum hydride or Raney-nickel leads to piperidines, piperidones, or both (28). Okahara on the other hand has reported preparation of the 5-hydroxy-1-hexylamine by alternate reductions with lithium aluminum hydride followed by Raney-nickel; but includes no experimental details (24). Several attempts to duplicate this procedure were unsuccessful. The selective reduction of the carbonyl was accomplished by using milder reducing conditions, i.e.: sodium borohydride or sodium bis(2-methoxyethoxy)aluminum hydride which do not reduce nitriles (equation 1b). Reduction of the carbonyl eliminate the possibilities of cyclization thus allowing the nitrile reduction to proceed smoothly.

Reductions of nitriles to primary amines generally go in low yields with formation of the appropriate secondary amines as the major side products via aldimine intermediates (37) (equation 5).

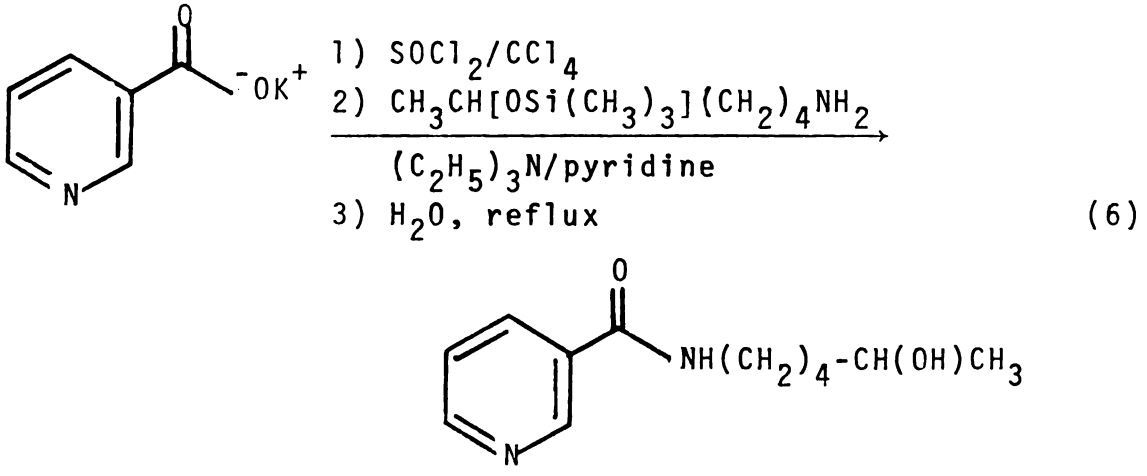


(5)

Reduction of nitriles in 10 N methanolic ammonia (a saturated solution in ammonia) results in quantitative yield of the primary amine when Raney-nickel catalyst W-2 is used (38). This method was found to give the 5-hydroxy-1-hexylamine in greater than 90% yield with no secondary amine formation (equation 1c).

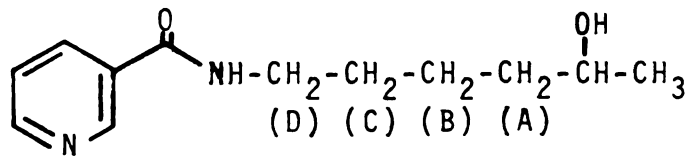
As previously stated, this study has resulted in finding general methods for the preparation of the 5- and 7-carbon analogues of this type of aminoalcohol (equations 2 and 3). Work is currently being done using the chloroketone synthesis from α-acetyl γ-butyrolacetone (equation 2) to give the 4-hydroxy-1-pentylamine via the "Gabriel" synthesis. The problems of cyclization have been eliminated by prior reduction of the carbonyl with sodium borohydride and protection of the hydroxy group with 1,1,1,3,3,3-hexamethyldisilizane. A suggested pathway for the 7-carbon analogue - resulting from studies with pyrans and substituted pyrans (see experimental) - from the aminopyrans has also been attempted (equation 3).

Methods for the synthesis of the N-(hydroxyalkyl)-nicotinamides were previously well established (39) (equation 6).



(6)

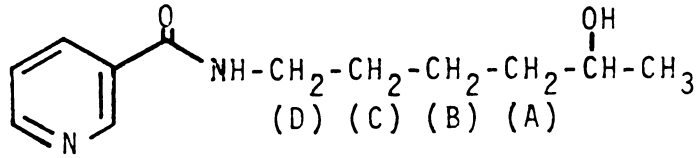
Table 2 - Chemical Shifts (δ values)[a] of N-(5-hydroxy-1-hexyl)-nicotinamide in $CDCl_3$ at Various Concentrations



Concentration(%)[b]	$-CH_3$ ν[c]	$-CH_2$-[d](A,B,C) ν	CH-[e] $-CH_2$-(D) ν	-OH[f] -NH- ν
50	1.15 -68.8	1.48 -89.0	3.61 -216.5	4.55 -272.8
40	1.13 -68.0	1.47 -88.1	3.61 -216.3	4.17 -250.0
30	1.13 -68.0	1.48 -88.5	3.40 -204.0	3.84 -230.5
20	1.15 -58.8	1.48 -89.0	3.34 -200.3	3.53 -211.5
10	1.15 -69.3	1.49 -89.5	----g -----	3.10 -185.8
5	1.18 -71.0	1.53 -91.5	---- -----	2.48 -148.8

[a]Internal standard tetramethysilane (δ = 0.00); [b]Conc w/w; [c]Chemical shift taken at 250 sweep width (Hz); [d]Center of Multiplet; [e]Appear as multiplet; [f]Appear as single absorption; [g]Signal lost in baseline "noise".

Table 3 - Chemical Shifts (δ values)[a] of N-(5-hydroxy-1-hexyl)-nicotinamide in Pyridine -d_5 at Various Concentrations



Concentration	$-CH_3-$ν[c]	$-CH_2-$[d](A,B,C)ν	CH-[e] $-CH_2-$(D)ν	-OH[f] -NH-ν
50	1.26 -75.8	1.63 -98.0	3.80 -227.8	5.39 -323.6
40	1.26 -75.8	1.64 -98.3	3.75 -225.0	5.38 -322.6
30	1.27 -76.3	1.65 -98.8	----g ----	5.32 -319.5
20	1.27 -76.3	1.65 -99.0	---- ----	5.26 -315.9
10	1.28 -77.0	1.66 -99.8	---- ----	5.20 -311.8
5	1.28 -77.0	1.67 -100.0	---- ----	5.06 -303.6

[a]Internal standard tetramethylsilane (δ = 0.00); [b]Conc w/w; [c]Chemical shift taken at 250 sweep width (Hz); [d]Center of Multiplet; [e]Appear as multiplet; [f]Appear as single absorption; [g]Signal lost in baseline "noise".

Table 4 - Chemical Shifts (δ values)[a] of N-(5-hydroxy-1-hexyl)-nicotinamide in CD_3CN at Various Concentrations

Concentration	$-CH_3\nu$[c]	$-CH_2-$[d] (A,B,C)ν	CH-[e] $-CH_2-$(D)ν	-OH/-NH-ν[f]
50	1.11 -66.8	1.48 -88.5	3.55 -213.3	3.65/4.18 -219.0/-250.8
40	1.10	1.44 -86.5	3.34 - 200.3	3.28 -197.0
30	1.09 -65.3	1.43 -85.8	3.34 -200.5	3.10 -186.0
20	1.08 -65.0	1.45 -87.3	3.39 -203.3	2.83 -169.6
10	1.09 -65.5	1.45 -86.8	2.91 -174.8	2.90/3.58 -174.0/214.5
5	1.09 -65.5	1.44 -86.3	----g ----	---- ----

[a]Internal standard tetramethylsilane (δ = 0.00); [b]Conc w/w; [c]Chemical shift taken at 250 sweep width (Hz); [d]Center of Multiplet; [e]Appear as Multiplet; [f]Appears as two absorptions which merge and move apart again.

chloroform show a single sharp absorption for the two -NH- and -OH protons at all concentrations. The position of the peak is concentration dependent, moving up-field as the concentration decreases. The chemical shift of the methyl doublet remains relatively constant with variations in concentration. The methylene and methine absorptions move only slightly up-field with decreasing concentration in all solvents (Tables 2 and 3).

The -NH- and -OH absorption in deuterated acetonitrile gave surprisingly different results from those obtained in the other solvents. At the 50% concentration the spectra showed two broad resonances resulting from slow exchange of the two labile protons. On dilution, the two resonances merge to give a single sharp absorption (Figure 13). Further dilution results in their reappearance. This phenomenon may be interpreted as slow exchange between the -NH- and -OH protons due to substrate-substrate intermolecular hydrogen bonding of either the -OH or -NH- porton with the nitrogen of the ring (Figure 14).

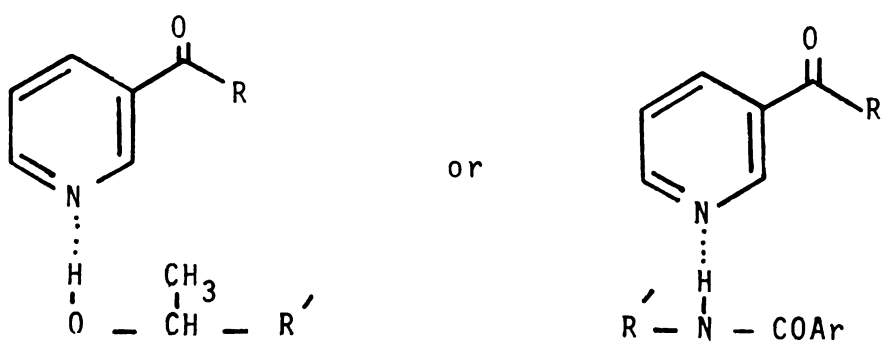


Figure 14

As the concentration is decreased, this hydrogen bond is also decreased. Fast exchange between the -OH and -NH- protons results in the appearance of a sharp single absorption. Further decrease in concentration may lead to intramolecular hydrogen bonding becoming important (Figure 15) which again slows the exchange rate between the -NH- and -OH protons (Figure 16 and Table 4).

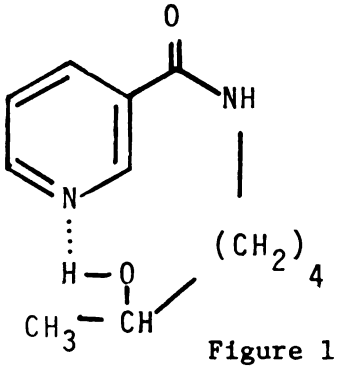


Figure 15

Infrared absorptions in the -OH and -NH- stretching regions indicated that both inter- and intramolecular hydrogen bonding is occurring. In addition to the non-hydrogen bonded -OH absorption at 3607 cm^{-1}, a broad absorption at 3318 cm^{-1} indicated intermolecular hydrogen bonding of the -OH. A moderate absorption at 3430 cm^{-1} shows the presence of some intramolecular hydrogen bonding.

Since the final objective of these studies is to elucidate the spacial relationship between coenzyme and substrate in the alcohol dehydrogenase oxidation-reduction reactions, this apparent "folding" of the alkyl chain deserves further investi ation. More definite conculsions must await results to be obtained from the longer chain N-hydroxyalkylnicotinamides in other solvents.

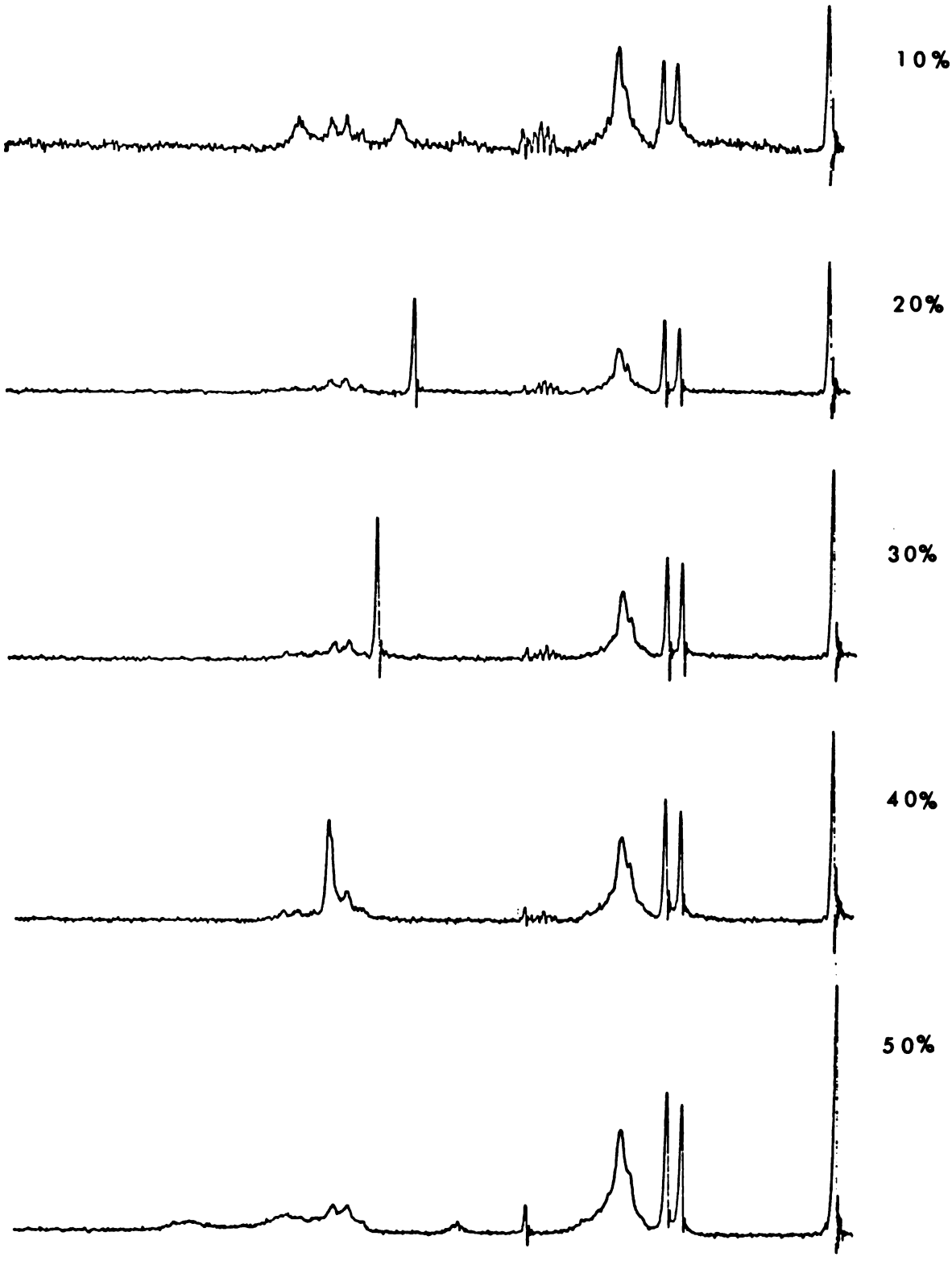


Figure 16. N.m.r. spectra of N-(5-hydroxy-1-hexyl)nicotinamide at various concentrations

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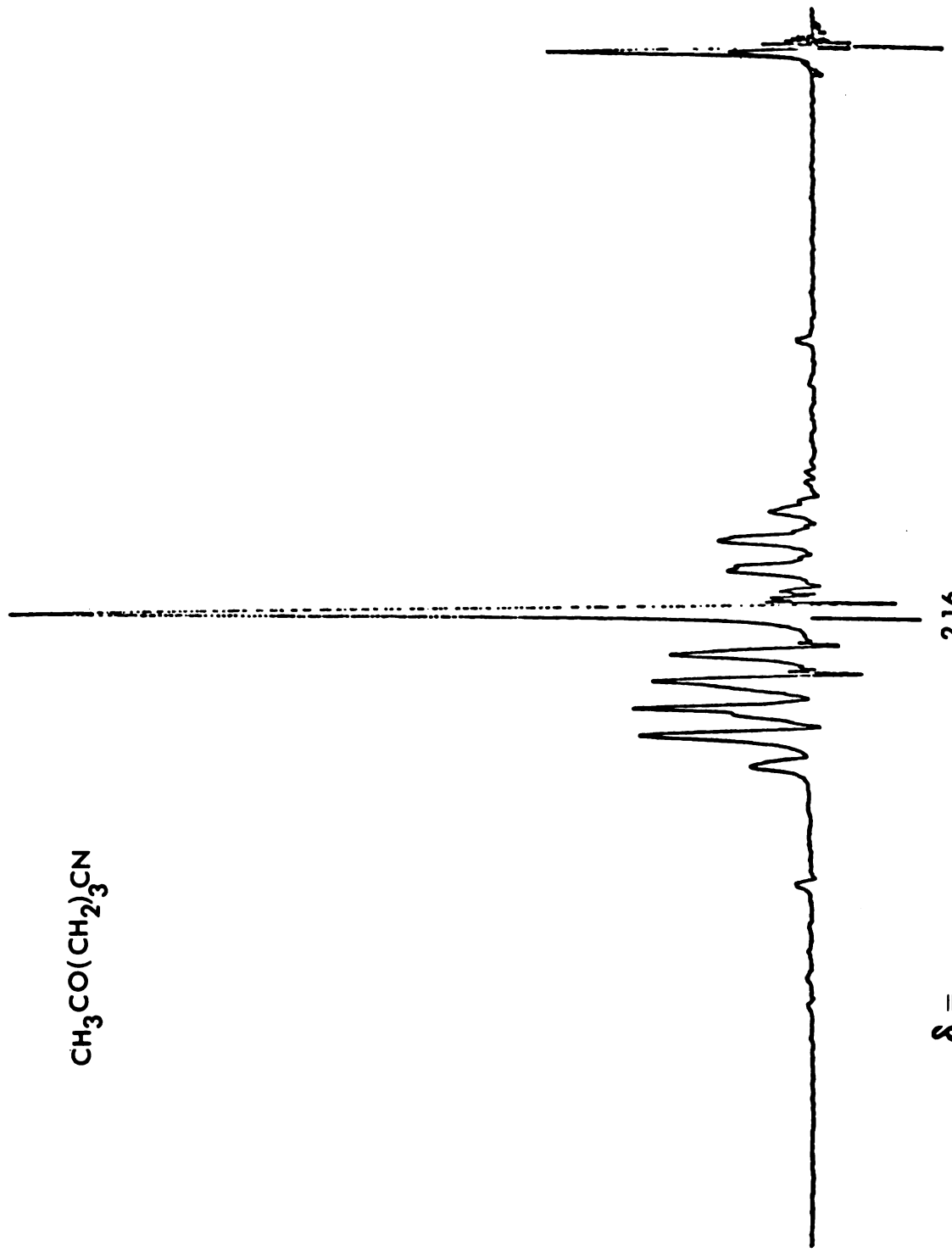
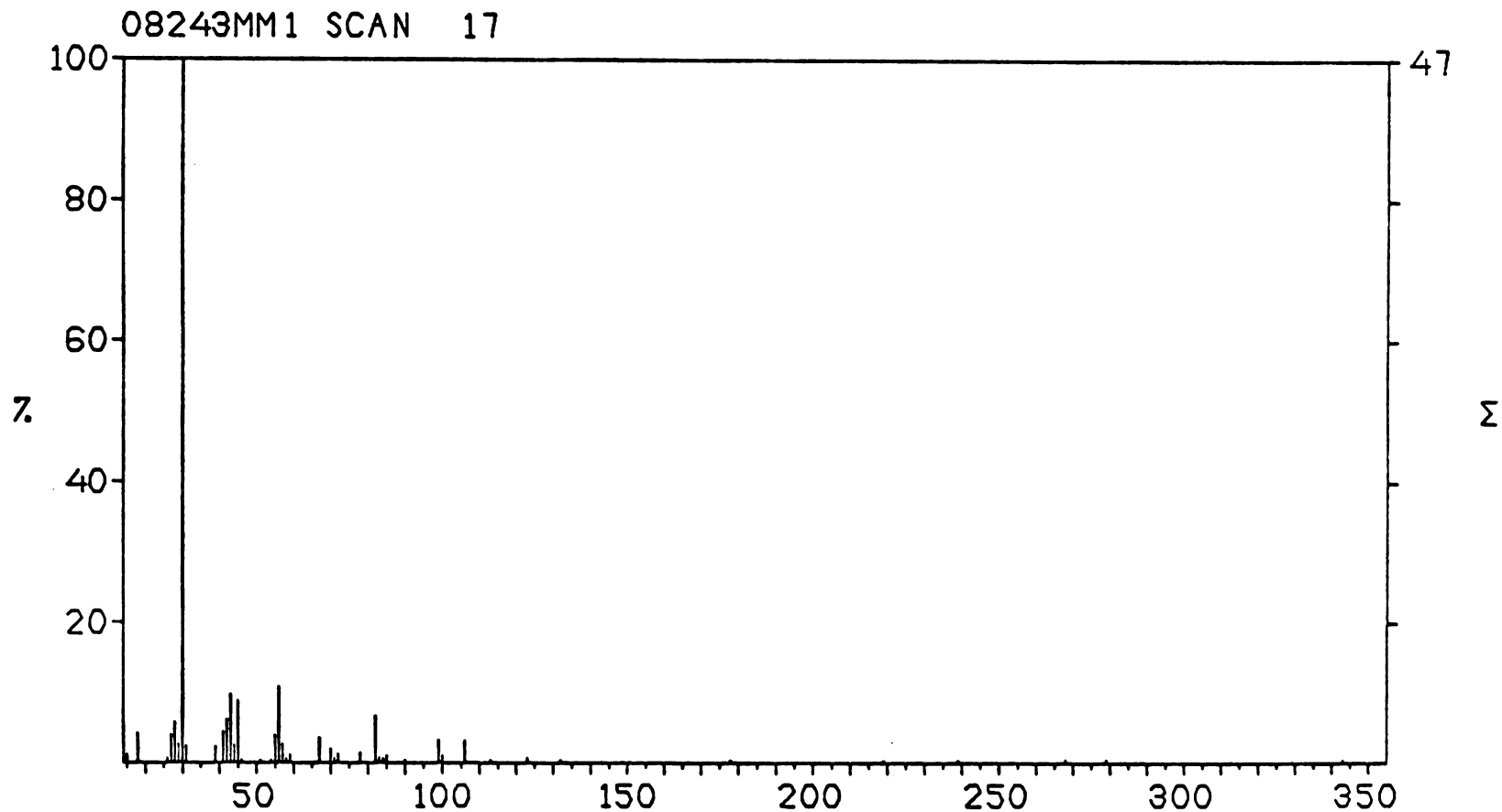
APPENDIX

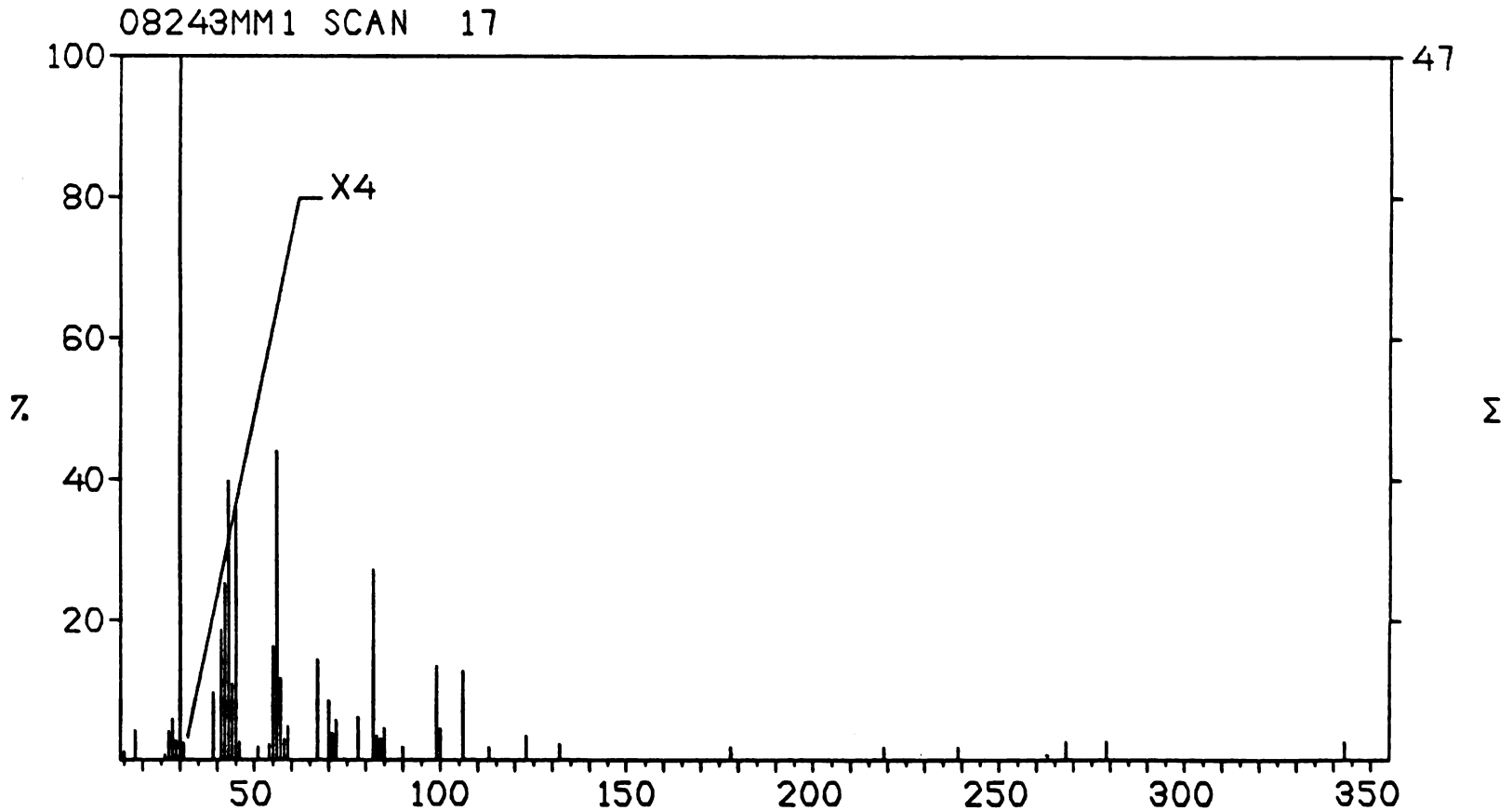


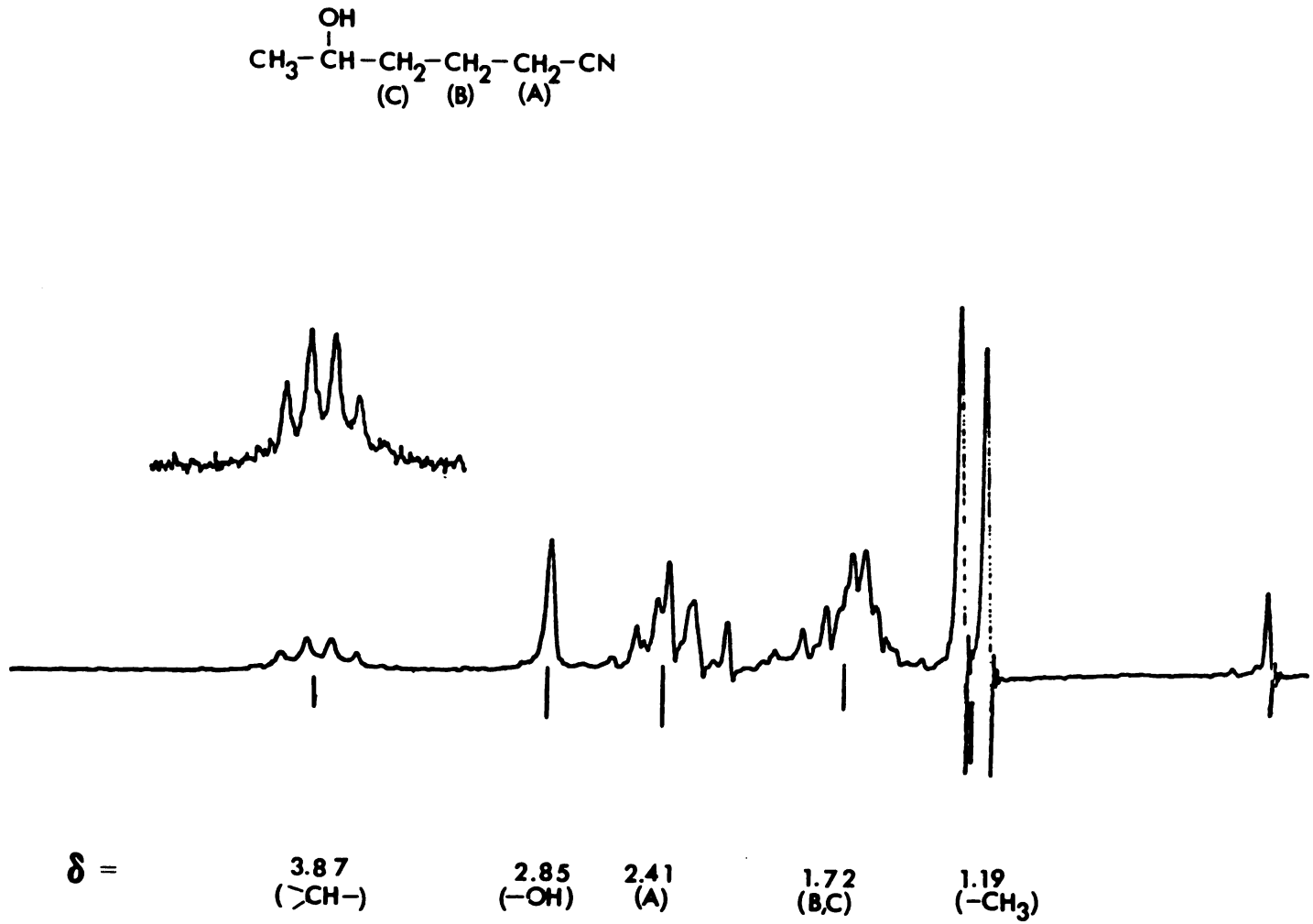
A. N.m.r. spectrum of 5-oxocapronitrile (conc. 20%) in $CDCl_3$/1%(v/v)TMS

B. Mass spectrum of 5-Oxocapronitrile



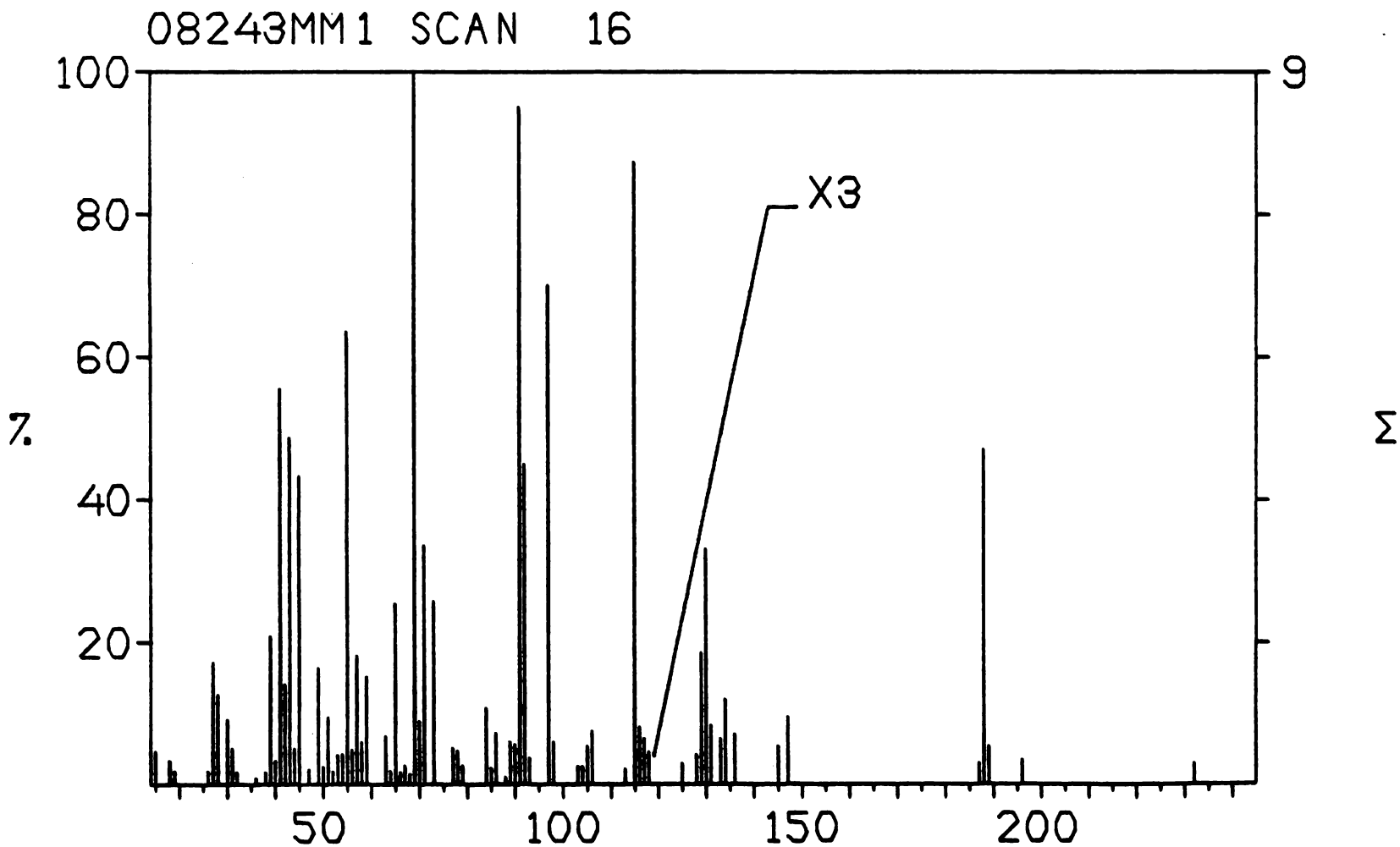
C. Mass spectrum of 5-Oxocapronitrile



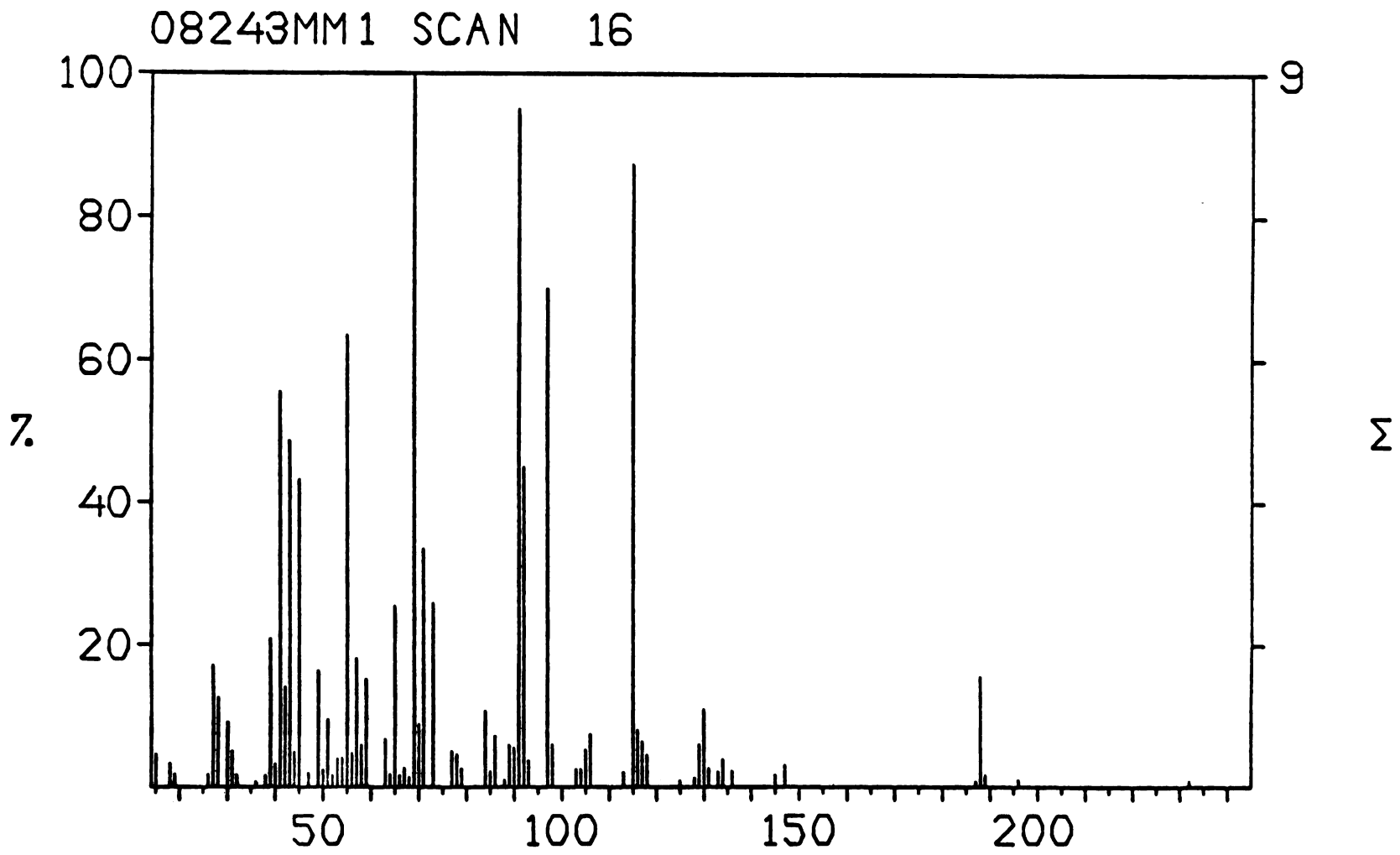


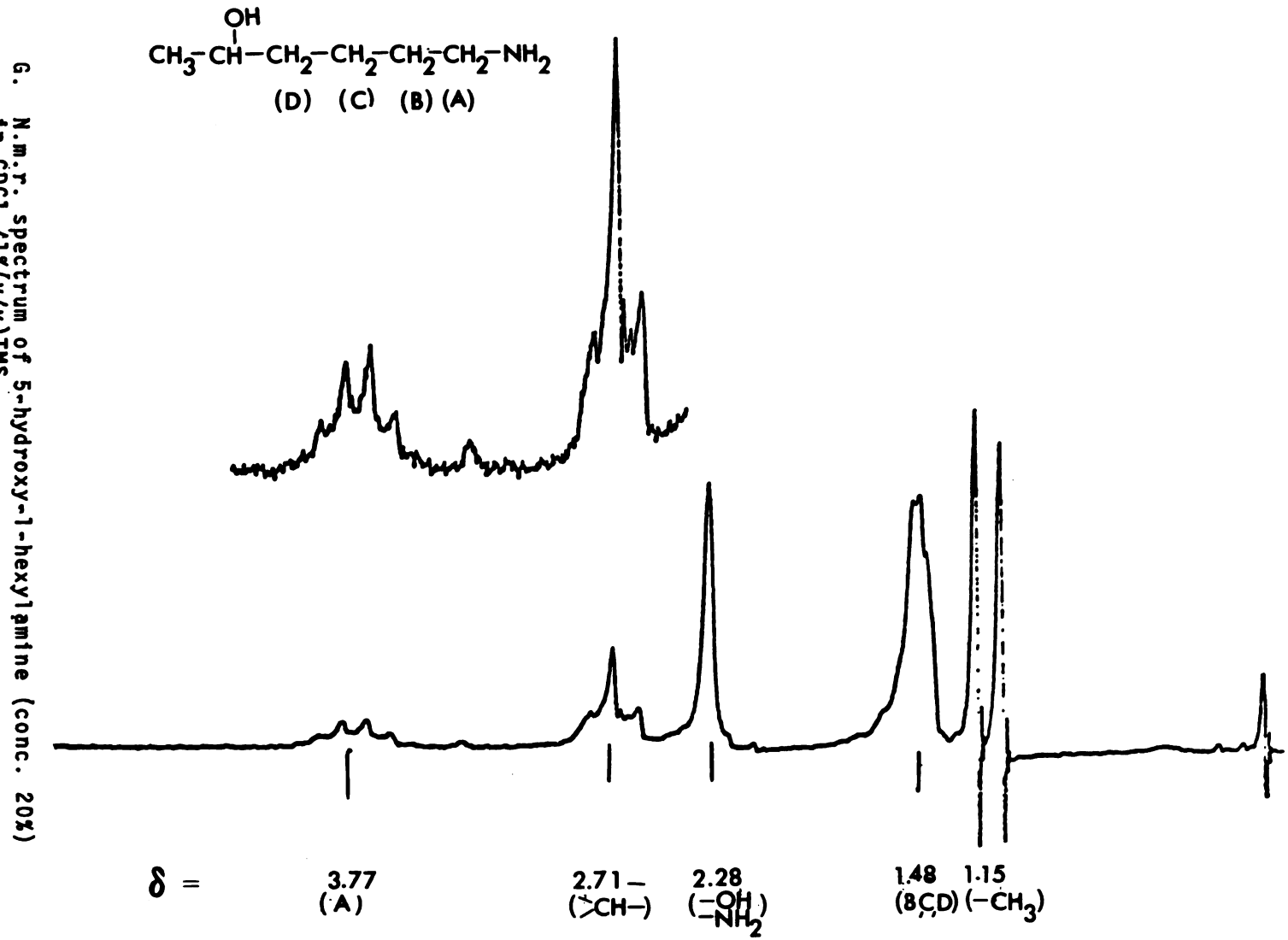
D. N.m.r. spectrum of 5-hydroxycapronitrile (conc. 20%) in $CDCl_3$/1%(v/v)TMS

E. Mass spectrum of 5-Hydroxycapronitrile

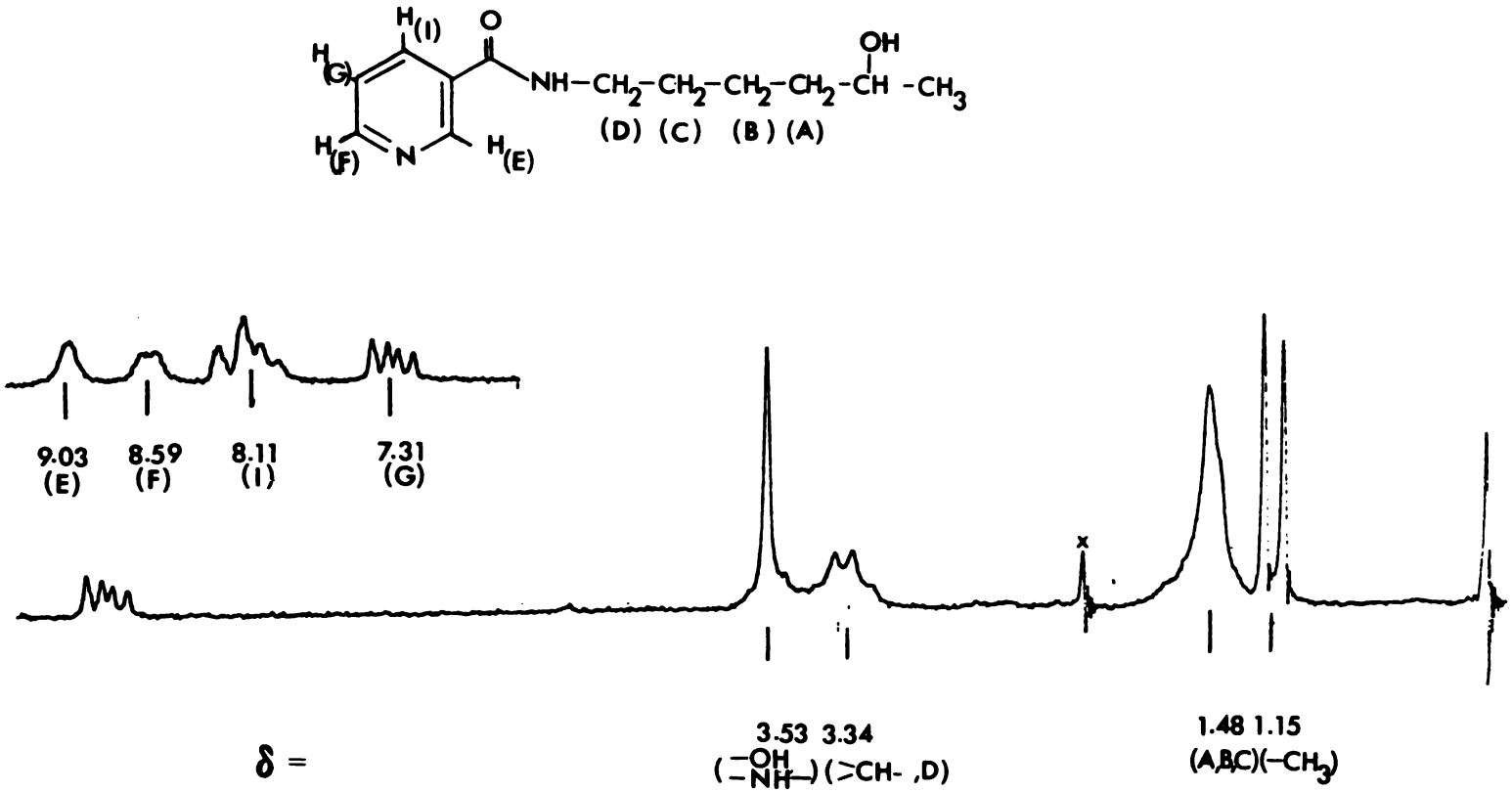


F. Mass spectrum of 5-Hydroxycapronitrile



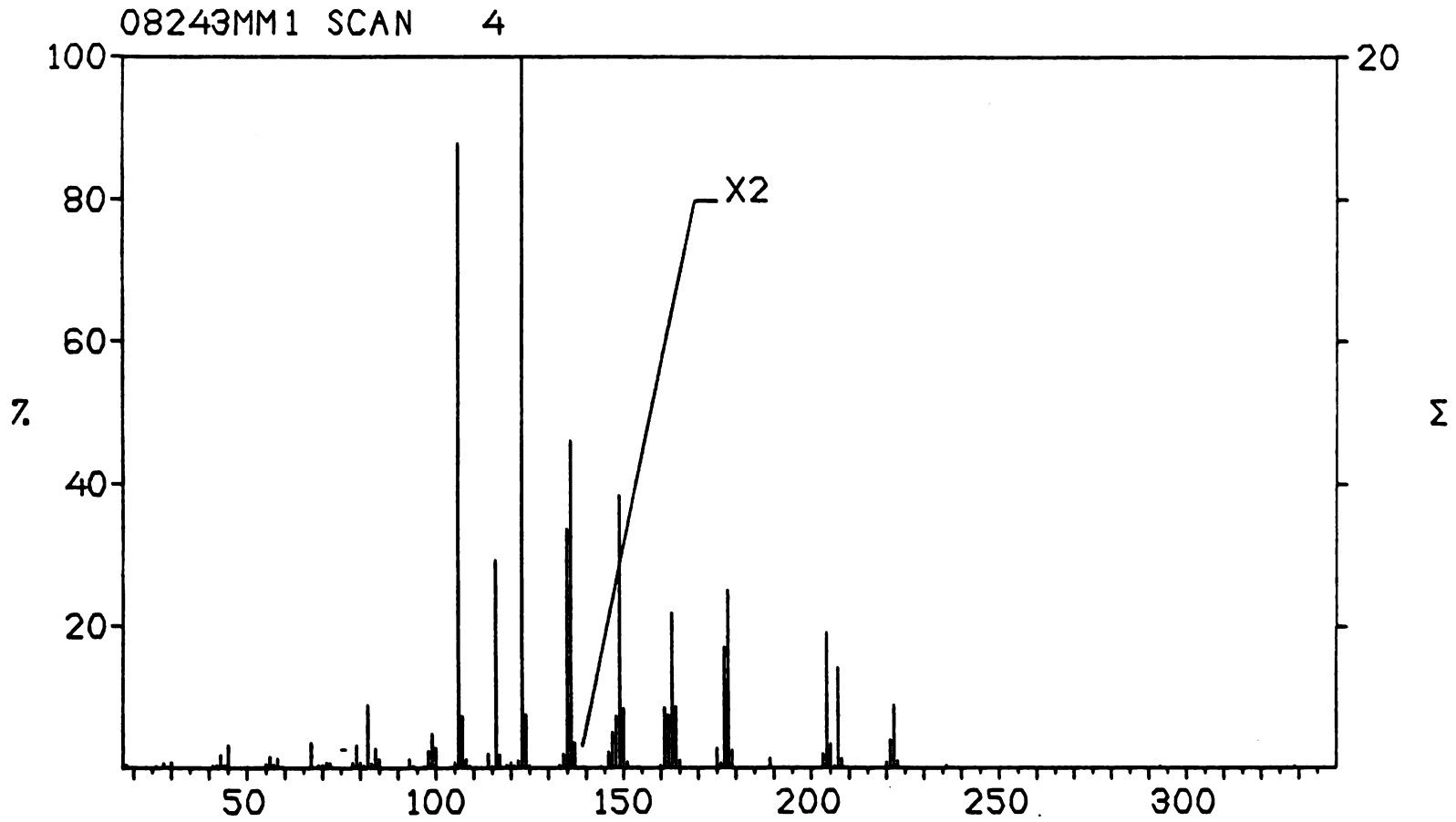


G. N.m.r. spectrum of 5-hydroxy-1-hexylamine (conc. 20%) in $CDCl_3$/1%(v/v)TMS



H. N.m.r. spectrum of N-(5-hydroxy-1-hexyl)nicotinamide (conc. 20%) in $CDCl_3$/1%(v/v)TMS

I. Mass spectrum of N-(5-hydroxy-1-hexyl)nicotinamide



J. Mass spectrum of N-(5-hydroxy-1-hexyl)nicotinamide