STEREOCHEMICAL STUDIES OF NITROSAMINES: THE INDUCED CIRCULAR DICHROISM OF ACHIRAL NITROSAMINES

DISSERTATION

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By

Howard M. Fribush, B.S.

Denton, Texas

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The induced circular dichroism (ICD) of several chiral nitrosamines and various chiral reagents has been investigated. The interaction is attributed to a 1:1 hydrogen bonded complex between the NO group of the nitrosamine and the hydroxyl groups of alcohols and polyols, or the amino group of amines. Only those chiral reagents possessing large differences in size of the groups about the hydrogen bonding site contributed to CD anomalies. The acyclic 2-octanols did not give observable Cotton effects, presumably due to the similarity in size of the methyl and methylene groups and rotational freedom of the acyclic system. The signs of the Cotton effects could be correlated with the absolute configuration of the sterically hindered alcohols and amines.

Large ICD effects using carbohydrates in aqueous media suggested that the complex is strong enough to overcome competition of hydrogen bonding with the solvent. Relative to the parent nitrosamines, smaller ICD curves were observed with hydroxynitrosamines, suggesting that the hydrogen

bonding of the carbohydrates with the nitroso function competes with that of the hydroxy groups. The increase of nitrosamine solubility and mutagenicity in the presence of polyols lends support to complex formation.

Only the alpha, axial hydrogens of conformationally biased, heterocyclic nitrosamines were found to undergo selective hydrogen-deuterium exchange, suggesting that this feature is critical for nitrosamine carcinogenicity.

CHAPTER I

INTRODUCTION

In the past thirty years the nitrosamines have emerged as one of the most potent class of carcinogens that is ubiquitous in the environment. This is ironic as nitrosamines have been routinely used since the nineetenth century in qualitative tests for secondary amines. Dimethylnitrosamine, the first nitrosamine studied for its carcinogenicity, is highly toxic and is a very potent carcinogen in a variety of animals.[1] It produces carcinomas of the liver, and in relatively large doses in humans causes liver damage ultimately leading to death.[2]

Nitrosamines have been found in tobacco smoke, cosmetics, skin lotions, shampoos, cutting fluids, water supplies, nitrite-preserved meats, smoked fish, certain alcoholic beverages, herbicides and other agricultural chemicals, human feces, and body fluids. Recently, certain non-prescription drugs have been shown to be precursors of carcinogenic nitrosamines.[3] Yet, in spite of the considerable amount of knowledge acquired about the carcinogenicity and general toxicity of these substances in animals, it is still not certain whether they pose a threat to man as carcinogens.

The most interesting biological attributes of carcinogenic nitrosamines are their organ specificity, relative potency and broad action. Nitrosamines have induced malignancies in every animal thus far tested. Not all nitrosamines are carcinogenic, and their relative effectiveness, or potency, varies greatly among even structurally related compounds. The nitrosamines tend also to elicit a varied response from different animals. Some nitrosamines cause tumors in different organs depending on the animal species.

It is generally accepted that the process of nitrosamine carcinogenesis begins with the oxidation of the carcinogen by a mixed-function oxidase (MFO). This oxidation is similar to the process of detoxification of substances in the liver.[4] The product of this (MFO) defense system with nitrosamines is a highly reactive electrophilic species that alkylates DNA, thus causing the production of abnormal cells.[5] The chemical mechanism is shown in Fig. 1. A mixed-function oxidase-nitrosamine complex has never been isolated; however, the oxidative enzyme for nitrosamines has been isolated.[6] Furthermore, the mutagenicity tests, such as the Ames test, generally give poor results with nitrosamine substrates even using the S-9 microsomal fraction. Thus the discovery of a MFO-nitrosamine complex would be of significance in determining the metabolic fate of nitrosamines.

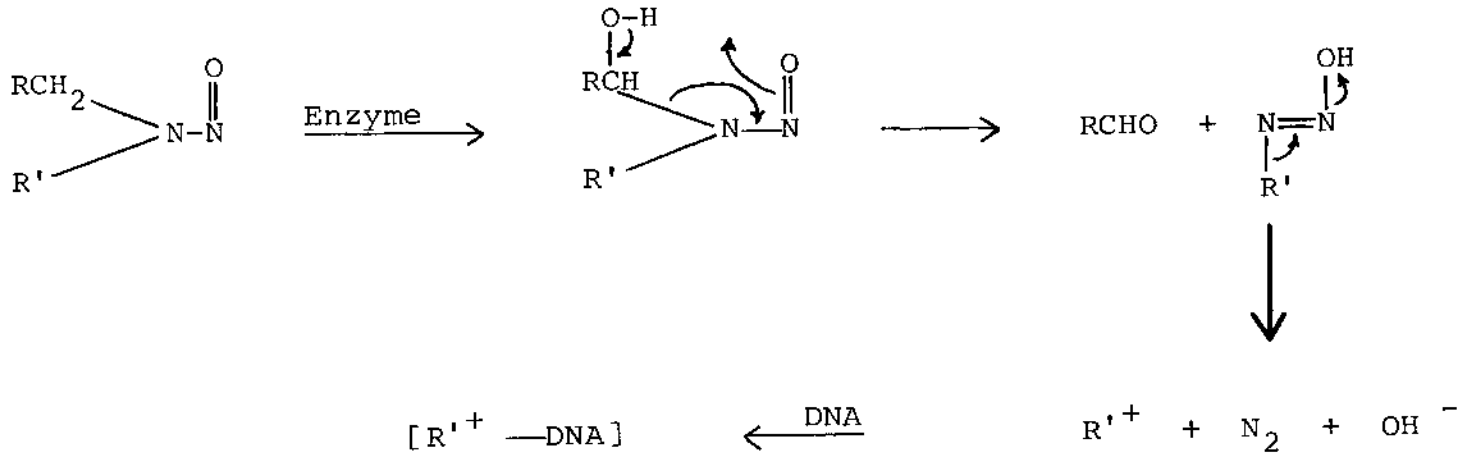

FIG. 1 - The Accepted Chemical Mechanism of Nitrosamine Carcinogenesis.

A method for studying the complex of the MFO with nitrosamines was investigated based on circular dichroism measurements. This technique utilized the phenomenon of induced circular dichroism. An achiral nitrosamine which absorbs light between 330 and 380 nm would show no Cotton effect in the CD spectrum. A chiral compound which has no absorption of light in the range 250-400 nm would also show no Cotton effect in the CD spectrum. However, if an interaction occurs between the achiral nitrosamine and chiral, UV transparent enzyme model, an anomalous curve in the CD will occur at the wavelength of the absorption maximum of the nitrosamine. Model systems for the complex formed between a nitrosamine and an MFO involved chiral alcohols, amines, and polyhydroxylated compounds as the UV-transparent, hydrogen-bonding donors. The formation of such a complex was supported by the collection of solubility data of the nitrosamines in a solution of the enzyme model.

A consideration of the stereochemical correlation of the reactivity of nitrosamines to alkylation conditions and relative carcinogenicity suggested that these processes might be related. This provided another reason for studying the mechanism of the MFO activation. The anion of cyclic, conformationally-biased nitrosamines undergoes reaction with electrophiles, with alkylation occurring stereospecifically as well as regiospecifically.[7,8] This

stereoelectronic control may be important in an enzymatic reaction as well. To lend support to a stereoselective pathway of nitrosamine metabolism, hydrogen-deuterium exchange studies were carried out on several conformationally biased nitrosamines.

This study, therefore, was initiated to investigate the existence of a strong interaction between a nitrosamine and a model of the oxidative enzyme (a chiral alcohol) by studying the stereochemical effect of chiral reagents on the nitrosamine functionality.

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CHAPTER II

HISTORICAL

This dissertation concerns the induced circular dichroism of nitrosamines, the chemistry of which has been the object of much recent research. For this reason, a review of the nitrosamines as well as induced circular dichroism will be included.

Nitrosamines are relatively stable, polar molecules. The electronic structure of the function leads to an absorption band in the 350 nm region which causes them to be quite yellow in color. This electronic structure also provides a charge distribution producing several unusual properties in the nitrosamine.[1]

That the nitrosamine group is polar has been demonstrated in several ways. A large dipole moment of 3.98 D for dimethylnitrosamine has been calculated by Tanaka.[2] A quantum mechanical calculation on the energy levels and orbital coefficients for nitrosamines reveals that the polar resonance form can contribute up to 49% of the ground state and about the same extent to the excited state. Nitrosamines have also been shown to be good Lewis bases. A pK_B of -3 has been calculated for several nitrosamines in hydrocarbon solvent.[3] Cationic or electrophilic species are reported[4-6] to react

at oxygen to give salts, while the site of protonation and hydrogen bonding is not clear.[7-10]

The delocalization of the "amino nitrogen" free pair by resonance with the π bond of the NO is required for the highly polar structure. This delocalization of electrons increases the double bond character of the nitrogen-nitrogen bond producing an energy barrier to rotation (Fig. 2). This energy barrier to rotation about the N-N bond has been determined in various ways to be about 25 kcal/mole.[11-15]

The N-nitroso group is angular, so unsymmetrical nitrosamines give rise to Z and E isomerism.[16] This isomerism can be detected by nuclear magnetic resonance which shows that the hydrogens on the alpha carbons are not equivalent.[17-19] The anisotropic effect is caused by shielding cones which extend perpendicular to the C_{α}-N^{+}=N-O^{-} plane and which are curved toward the <u>syn</u> side (Fig. 3). This effect results in a greater shielding zone on the <u>syn</u> side than on the <u>anti</u> side. The deshielding zone lies in the C_{α}-N^{+}=N-O^{-} plane, and studies on conformationally biased nitrosopiperidines revealed a stronger deshielding region on the <u>syn</u> side.[20,21] The occurrence of geometrical isomers of unsymmetrical nitrosamines has also been attributed to a barrier to nitrogen inversion.[22,23] In some cases the nitrosamine geometrical stereoisomers have been separated.[24,25]

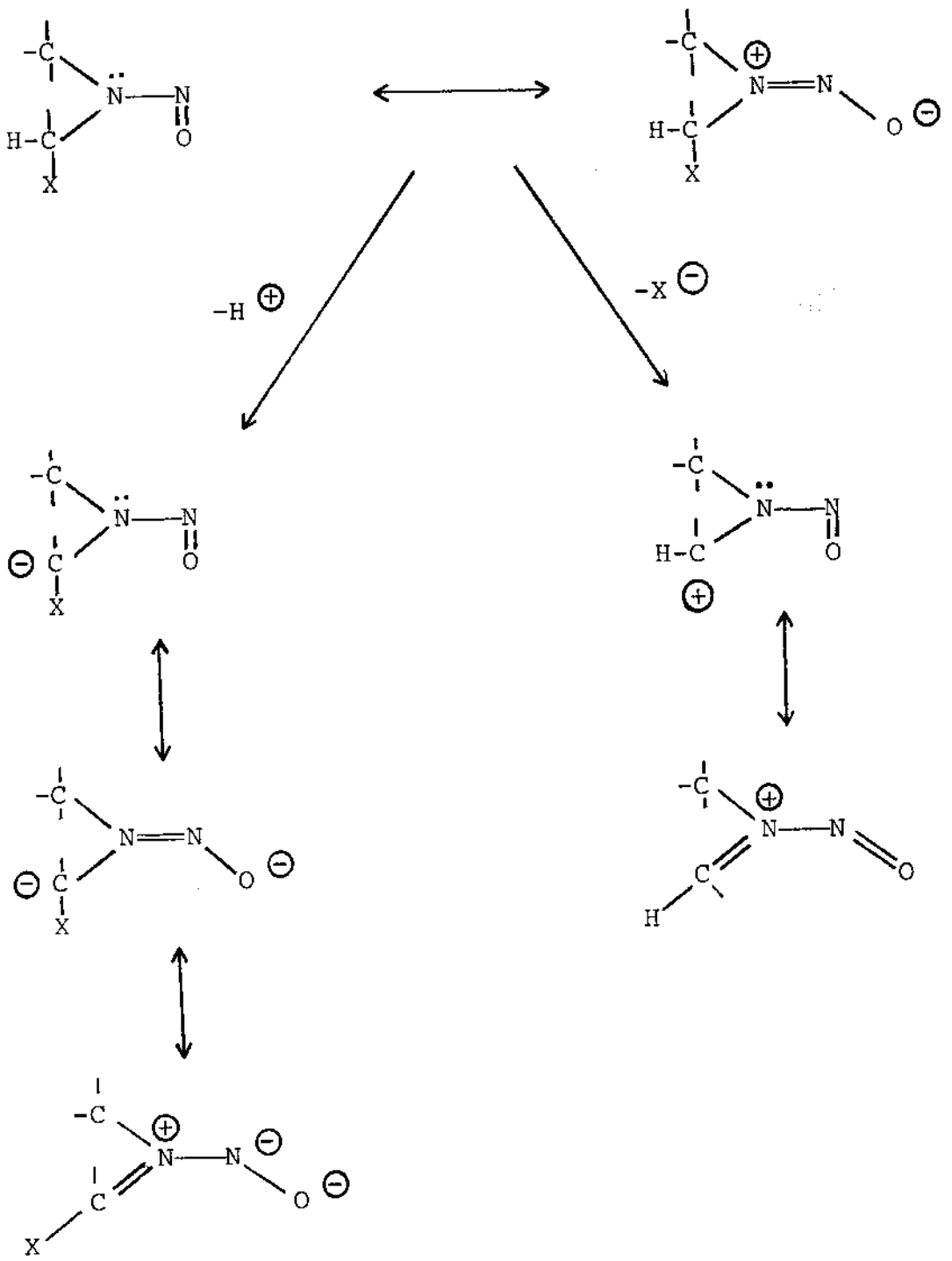


Fig. 2--Resonance forms of nitrosamines and their α-carbanions and α-carbocations showing the delocalization of charge.

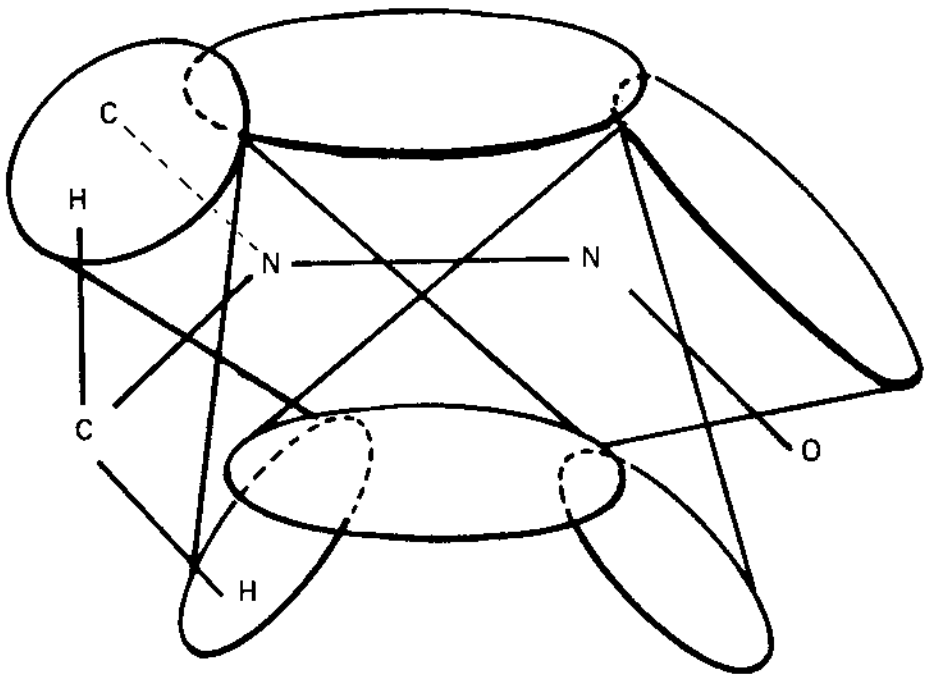


Fig. 3--The anisotropic effect of the nitrosamine group. The shielding cones lie above and below the plane of the nitroso group. The deshielding region lies in the plane of the nitrosamine function.[68]

The steric size of the NO group resulting from restricted rotation has been studied in cyclic systems. Fraser has shown that the conformational free energy of syn alpha methyl group is about 3.9 kcal/mole favoring the axial form.[26]

The polarization of the nitrosamine function would be expected to have an effect on the acidity of the alpha hydrogens. This acidity of the alpha protons of nitrosamines was first reported by Keefer[27] by a study of deuterium exchange of 1-nitrosopiperazine. This deuterium exchange reaction was extended to prepare labeled piperidines by Portoghese.[28] Later, the anion of conformationally biased nitrosamines was found to undergo stereospecific and regiospecific alkylation.[29] These observations were explained on the basis of the resonance model outlined earlier. In this case, the anion generated would be derived from loss of the axial proton. In this way the σ-orbital of the developing negative charge could overlap with the π-system of the nitrosamine function (Fig. 4). Alkylation would follow an axial route to avoid a boat-like transition state.

The stabilization of a negative charge at the alpha position of nitrosamines was described by Seebach and Enders[30] as "umpolung", a term coined to indicate that the nitroso group attached to the amine nitrogen reverses the normal polarity of the alpha carbon (Fig. 5).

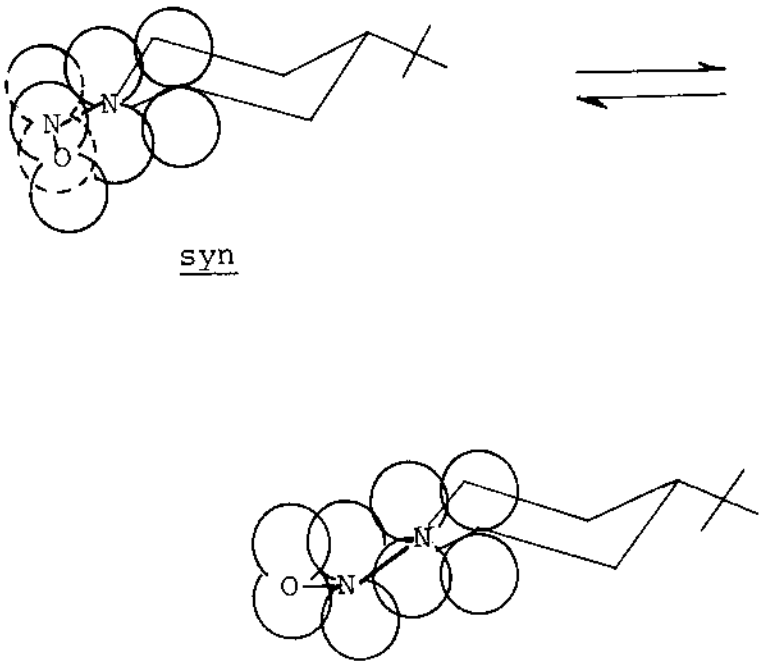


Fig. 4--Orbital stabilization of a developing charge at the α-carbon of a conformationally biased nitrosamine.

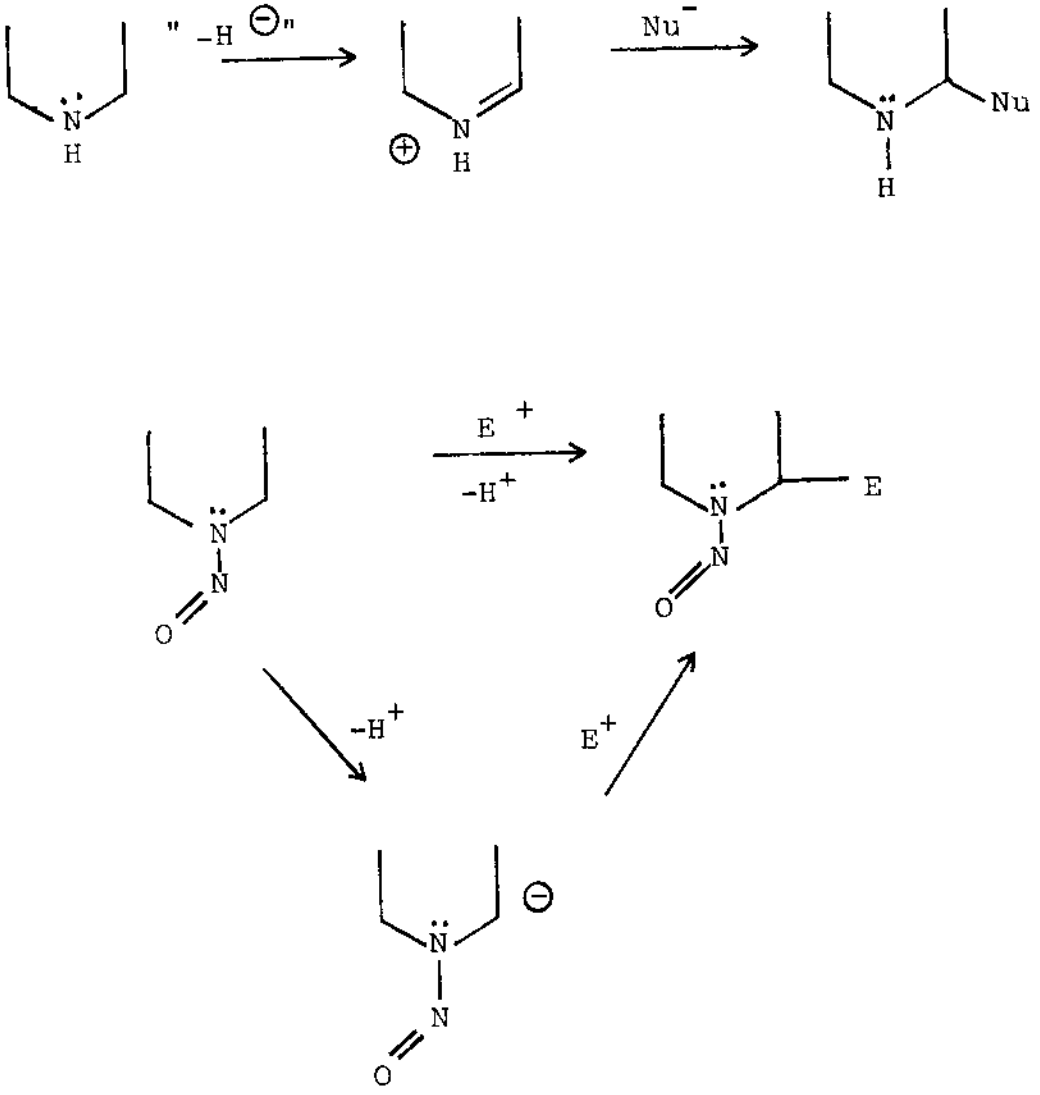


Fig. 5--"Umpolung" of the nitrosamine function.[30]

Wiessler[31] reported that nitrosamines were also able to stabilize a positive charge at the alpha carbon. This finding was confirmed by Baldwin.[32]

Induced Circular Dichroism (ICD)

The induction of optical activity into achiral molecules was first observed by Blout and Stryer[33] from solutions containing dyes and polypeptides. Since that time induced chirality has been shown to be a general phenomenon, as asymmetry has been induced in ketones[34], aromatic compounds[35], pyrroles[36], organometallic complexes[37], inorganic compounds[38], polymers[39], nitroalkanes[37], azo compounds[37], and thioketones.[40] In addition, optical activity has been induced in micelle[41] and liquid crystal[42] systems. This phenomenon has been used for determining optical purities[43a] and absolute configurations.[43b] These experiments have, in turn, given rise to interest in the intermolecular interactions involved.

The characteristic large rotatory dispersion curves ($[\alpha]$ = +150 deg/g-cm) of dye molecules in the presence of polyglutamic acid observed by Blout and Stryer[44] were suggestive of complexes resulting from "configurationally-induced" or "conformationally-induced" optical rotatory power. Configurationally-induced optical rotatory power was thought to arise from the interaction of the symmetric dye chromophore with the local asymmetric environment about an alpha carbon atom of the polypeptide. Conformationally-

induced optical rotatory power would arise from the interaction of the coiled chiral polypeptide with a dissymmetric arrangement of several dye molecules.

Similar findings in analogous systems have been confirmed by others. Model studies using cryptates have revealed that molecular receptors may use intermolecular interactions for the selective binding of substrates.[45] The ICD curve of acridine orange in the presence of RNA was attributed to intercalation, in which each dye molecule is sandwiched between two adjacent base pairs.[46] The type of bond attaching the dye to the chiral framework was not defined but may be hydrogen bonding, charge transfer, or π-complex.

The ICD resulting from the intercalation of methylene blue, proflavine and ethidium bromide into DNA revealed that all of these dyes compete for the same binding sites.[48] The ICD of acridine orange and RNA and DNA suggested that the dye binds similarly to the two RNA's, but differently to DNA.[47] These results were attributed to a transmittance of effects to adjacent bound molecules either electronically or by asymmetric perturbation. Furthermore, as the number of bound dyes in a helical sequence decreased the molecular ellipticity per bound dye decreased.[49]

The difference in ICD sign from complexes between 4-nitroaniline and DNA or RNA was thought to be due to the difference in orientation of the chromophore with respect to

the helix axis.[50]

Recent work has focused on dextrin capture leading to ICD. Aqueous solutions of 4-benzoylbenzoic acid and β-cyclodextrin exhibited large concentration and pH-dependent curves.[51] A plot of the Benesi-Hildebrand relation generated an equilibrium constant from which the pK_a of the acid was estimated in reasonable agreement with literature values.

The magnitudes of the induced circular dichroic effects were attributed to steric favorability of the benzoic acids towards the asymmetric cavity of the dextrin, which is composed of glucose units. Thus 4-benzoylbenzoic acid exhibited stronger ICD bands than did the 2- or 3-isomers. Model systems of the isomeric benzoylbenzoic acids in the presence of D-(+)-glucose also produced Cotton effects having analogous magnitudes; thus, the authors concluded that the benzoylbenzoic acids were included inside the dextrin ring with the carbonyl group exposed to the asymmetric environment of the cavity.

These results are in direct contrast with those of Ito et al.[52] who reported that the carbonyl group of racemic 3-methylcyclohexanone in the presence of β-cyclodextrin was outside the ring. Although these authors failed to provide solid evidence for this model, it is possible that the difference in orientation of the ketone in this case could be governed by the chirality of each enantiomer. Hence one

enantiomer would orient itself in a preferred fashion, while its antipode would face the opposite direction. The ICD from complexes between β-cyclodextrin and aromatic compounds[53,54,55] or 2-hydroxytropone[56] has been attributed to axial inclusion.

Ion-pair dissociation has also been cited as a source of the ICD in the benzoylbenzoic acid-amphetamine system.[57] IR studies ruled out the possibility that the ring-chain tautomer ruled out the possibility that the ring-chain tautomer of benzoylbenzoic acid was responsible for the ICD, and NMR, IR and GC studies ruled out the possibility that the ICD was due to an amide of a keto-acid. Conductivity experiments indicated that the ionic concentration was very low even in methanol; thus, the possibility that the carboxylate ion was acting as a hydrogen bond acceptor was also discarded.

Previous studies[58,59], which showed that organometallic salts gave contact- and solvent-separated ion-pairs in ethereal solvents, were used as an analogy to explain a solvent effect in the present system. In this case the reduction in the magnitude of the ICD was probably caused by the solvation of the organic cation by the ethers, leading to ion-pair dissociation.

The magnitude of asymmetric induction is proportional to the average distance between a chiral center and the chromophore[60]; thus, a contact ion-pair would be expected to show

a larger ICD than a solvent-separated ion pair. This in turn would be expected to show a larger ICD than free ions (Fig. 6). At low temperature, the increase of the ICD in this system suggested a decrease in intramolecular rotation and conformational homogeneity.

This and related systems were also used to predict the absolute configurations of amines, based on the steric effects of the system[61] (Fig. 7). In this empirical model the benzoylbenzoate anion approaches the ammonium cation from the less hindered side, while the phenyl moiety of the benzoyl group also orients in such a way as to minimize the steric effect. As a result of the sign of the ICD is always positive when R_1 is larger than R_2 and negative when R_1 is smaller than R_2.

Micellular solutions containing achiral substrates have also given rise to ICD effects. The origin of the ICD appears to depend on the structure of the micelle-achiral reagent complex.[41] In the presence of R-(-)-amphetamine or R-(+)-cetyl-α-phenethylamine, 4-alkyl-2-benzoylbenzoic acids (alkyl>6 carbon atoms) in salt solutions of concentration less than 10^{-3}M produced large ([θ] +100-1800 deg-cm^2/dmol ICD effects. Since the magnitude of the ICD increased markedly with increased chain length, a micelle model was assumed. The non-polar head group of the amine would be in the core of the micelle, and the achiral molecule would lie

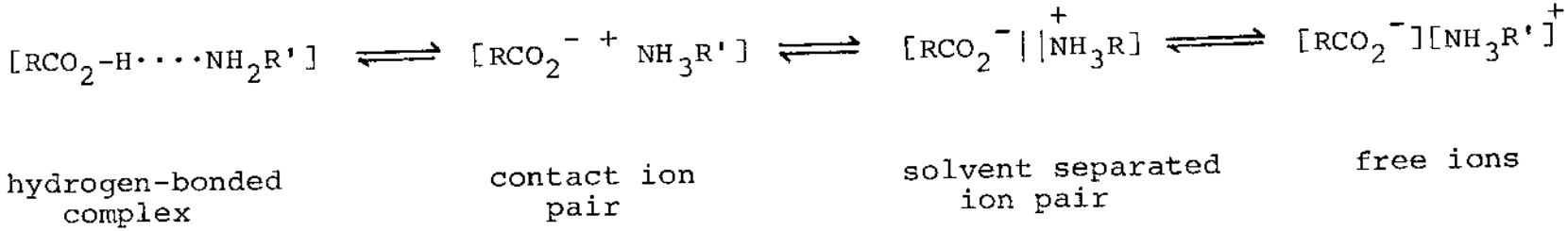
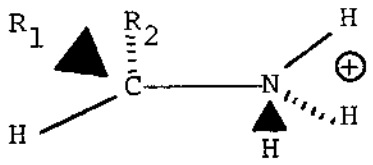
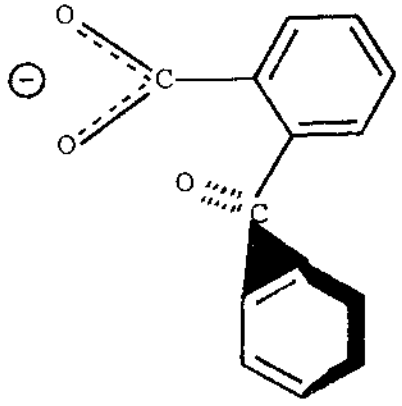


Fig. 6--The possible origin of the ICD for the benzoylbenzoic acid-amphetamine system.





Relative Sizes of R_1 and R_2	Sign of ICD for $n \rightarrow \pi^*$
$R_1 > R_2$	+
$R_1 < R_2$	−

Fig. 7--Prediction of the absolute configuration of amines.

inside the benzene environment.[41a]

Achiral ketones dissolved in aqueous sodium desoxycholate solution produced ICD bands that were dependent on the type of ketone. Small ketones produced no ICD, whereas larger ketones exhibited ICD. The sign of the ICD as well as the magnitude could also be correlated with ketone structure. The formation of the micelle was necessary for inducing optical activity, for in a solvent in which desoxycholic acid does not form micelles (methanol) no ICD was observed.[41b]

Optically inactive molecules also become chiral when they are dissolved in cholesteric liquid crystals.[42] The sign of the ICD bands in the N-(p-methoxybenzilidine)-p-butylaniline--cholesteryl chloride system was dependent upon the sense of the cholesteric helix, being positive for a left hand helix, and negative for a right-hand helix. As with the macromolecules, the origin of the ICD in this system was dependent on a macroscopic helical structure, and not just a chiral solvent.

In simple systems hydrogen bonding has been cited by various authors as the cause of the ICD effect. Benzil and benzophenone exhibit ICD in the $n \rightarrow \pi^*$ region in (+)-L-butane-2,3-diol as solvent. This phenomenon was described by Bosnich[62] who first applied the term "induced circular dichroism". This effect was only assumed to be caused by hydrogen bonding; however, there existed the remote possibil-

ities that either the optically active solvent or the hydrogen bonding itself could cause the molecules to assume a preferred dissymmetric conformation.

Bolard[63,64] observed ICD curves resulting from complexes of cyclohexanone-menthol and acetone-menthol systems in CCl_4. From the shift of the carbonyl stretching vibration in the IR and the $n \rightarrow \pi^*$ transition in the UV spectra after the addition of menthol, hydrogen bonding was attributed to the association. The magnitude of the ICD, furthermore, was found to be dependent on the concentration of menthol. Assuming that the ICD occurred in a 1:1 ketone-alcohol complex, Bolard[63,64] calculated the induced rotational strength of the acetone-menthol solution from the probable geometry of the complex, but was unable to predict the sign of the ICD-band.

In several systems where hydrogen bonding was a possibility, no ICD effects were observed. ICD from complexes of various chiral tetrahydrofuranols and several achiral and racemic ketones in acetonitrile was reported by Hayward.[65,66] When the chiral reagents were replaced by D-(+)-glucose and its derivatives, (+)-2-octanol, or (-)-bornyl acetate in acetonitrile, no ICD curves were observed. The authors hypothesized that in the absence of hydrogen bonding the origin of ICD was in the perturbation of the chromophore by the dissymmetric field of the chiral medium. This ex-

planation could be extended to imply that in enzymatic reactions and chiroptical studies of biopolymer structure, binding of small molecules at specific sites in the chiral macromolecule may not be a prerequisite for induction of optical activity. The phenomenon, furthermore, exhibited temperature dependence, with increasing ellipticity as the temperature decreased.

Induced circular dichroism, therefore, has been shown to result from several general interactions depending on the nature of the complex. This paper describes the first observation of the induction of optical activity into achiral nitrosamines, detected by induced circular dichroism. As will be discussed in subsequent chapters of this dissertation, the origin of the ICD in these systems appears to be simple hydrogen bonding.

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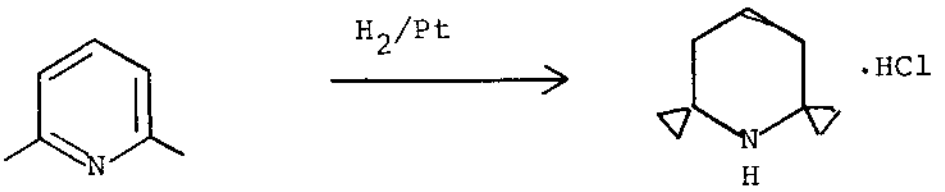
CHAPTER III

EXPERIMENTAL

General

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Hitachi-Perkin Elmer R24B and are presented in units of parts-per-million (ppm) downfield from tetramethylsilane used as an internal standard. IR spectra were recorded on a Beckman IR-33 and are presented in units of reciprocal centimeters (cm^{-1}). UV spectra were recorded on a Beckman 25 spectrophotometer and are presented in nanometers (nm) followed by the molar absorptivity (ε). ^{13}C NMR spectra were recorded on a JEOL Jnn PFT-100. CD spectra were recorded on a Cary 61 spectropolarimeter under the direction of Dr. Don Gray of the University of Texas at Dallas and on a Jasco J40A spectropolarimeter, and are presented in units of deg-cm^{2}/dmol. Optical rotations were measured on a Rudolph Model 80 polarimeter and are presented in units of deg-ml/g-dm. The term "brine" is used to represent a saturated aqueous solution of sodium chloride.

Preparation of cis-2,6-Dimethylpiperidine Hydrochloride (1).

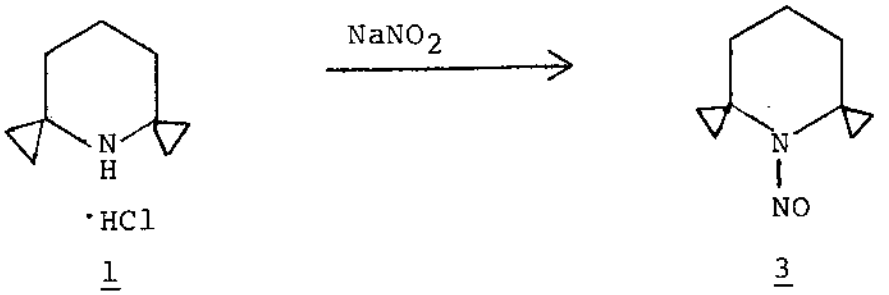


2 1

A solution of 10 g (90 mmol) of 2,6-dimethylpyridine (2) (Aldrich #L390-0) in 200 ml of 95% ethanol was acidified with concentrated HCl. The solution was hydrogenated in a Parr shaker over 200 mg of PtO_2 at 50 psi of hydrogen. Shaking was continued until there was no further uptake of hydrogen, and the final pressure of hydrogen was 23 psi. The platinum was removed by filtration, and the solvent was evaporated under reduced pressure. Recrystallization of the residue from methanol gave 4 g (30%) of cis-2,6-dimethylpiperidine hydrochloride (1) as white needles, mp 288-290°; lit.[1] mp 289-291°.

^{1}H NMR (D_2O): 1.5(d); 2.3(m); 3.5(m).

Preparation of 1-Nitroso-cis-2,6-dimethylpiperidine (3).



1 3

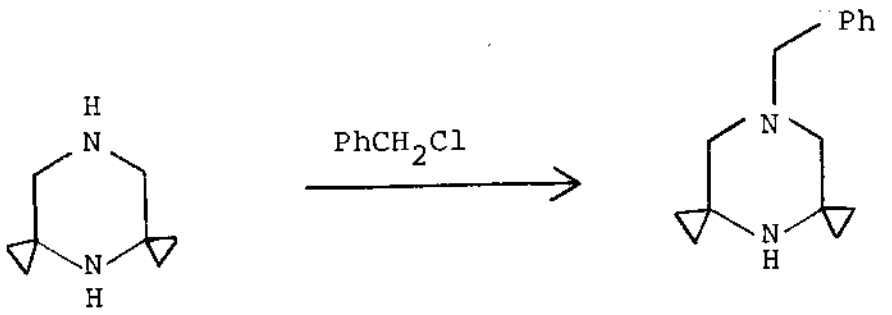
A solution of 5 g (33.5 mmol) of cis-2,6-dimethylpiperidine·HCl (pH 3) in 15 ml of distilled water was treated with a saturated aqueous solution of $NaNO_2$. The yellow oil which separated was removed by extraction with ether, and the aqueous phase was extracted three times with 20 ml portions of ether. The combined ether extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water, and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$ and the ether was removed by evaporation to leave a yellow oil, which crystallized from petroleum ether at -78° to give 2.5 g (53%) of 1-nitroso-cis-2,6-dimethylpiperidine (3) as a yellow solid, mp 30-31°; lit.[2] 30-31°.

^1H NMR ($CDCl_3$): δ1.15(d); 1.40(d); 1.55(m); 1.80(m); 4.83(m).

IR (Film): 2975(m); 2940(s); 2870(m); 1465(m); 1435(m).

UV: 365(76); 377(60).

Preparation of 4-Benzyl-cis-2,6-dimethylpiperazine (4).



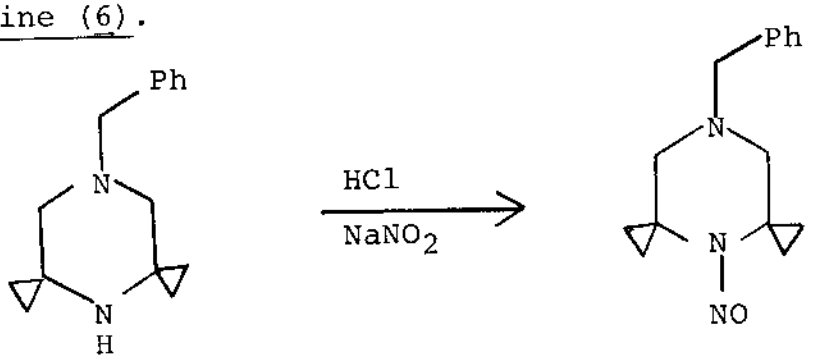
5 4

To 3 g (23.8 mmol) of cis-2,6-dimethylpiperazine (5) (Aldrich D17,980-9) in 50 ml of dry acetone in a 250 ml, 3-necked, round-bottom flask equipped with a reflux condenser and dropping funnel was added 3 g (23.8 mmol) of benzyl chloride dropwise over a 5 min period. The resulting mixture was heated under reflux for 3 hrs. The solid which precipitated was removed by filtration and dissolved in 50 ml of distilled water. The resulting solution was saturated with anhydrous K_2CO_3. The oil which separated was taken up in $CHCl_3$, and the aqueous phase was extracted three times with 20 ml of $CHCl_3$. The organic phase was washed with 20 ml of brine and filtered through a layer of $MgSO_4$. The solvent was removed under reduced pressure to give 2 g (38.5%) of a clear oil which was distilled under reduced pressure to give 4-benzyl-cis-2,6-dimethylpiperazine (4), bp 75-80° at 0.32 mm; lit.[3] 100-104° at 1.6 mm.

^{1}H NMR ($CDCl_3$): δ 0.9(d); 1.2(s); 1.5(t); 2.6(broad d); 2.8(m); 3.35(s); 7.1(s).

IR (Film): 3250(b); 3000(s); 2800(s); 1375(m); 690(b); 700(s); 1310(s).

Preparation of 1-Nitroso-4-benzyl-cis-2,6-dimethylpiperazine (6).



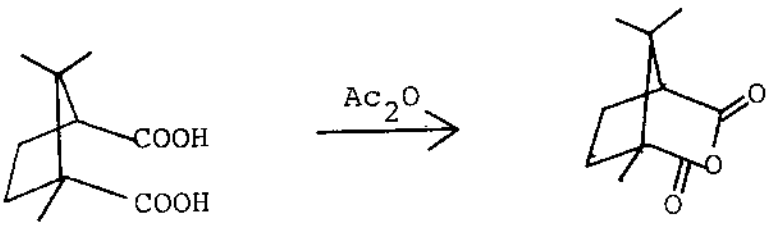
4 6

A saturated aqueous solution of $NaNO_2$ was added to 0.5 g (2.45 mmol) of 4-benzyl-cis-2,6-dimethylpiperazine (4) in 10 ml concentrated HCl at 0°. A yellow oil separated, and was taken up in $CHCl_3$. The aqueous phase was extracted three times with 20 ml of $CHCl_3$. The combined organic layers were washed with 20 ml of 5% HCl, 20 ml of distilled water, 20 ml of brine and filtered through a layer of $MgSO_4$. The solvent was removed under reduced pressure to leave 412 mg (82.4%) of 6 as a yellow oil, mp (picrate) 152-154°.

Anal for $C_{13}H_{19}N_3O$: Calc: C, 49.35; H, 4.76; N, 18.18. Found: C, 49.54; H, 4.90; N, 17.92.

^{1}H NMR (DMSO-d_6): δ 1.2(d); 1.46(d); 2.06(q); 2.43(q); 2.73(s); 2.9(d); 3.05(s); 3.7(s); 4.98(m); 7.5(s).

Preparation of (+)-Camphoric Acid Anhydride (8).



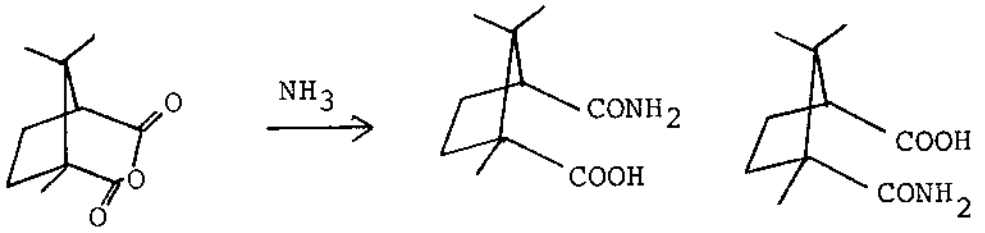
7 8

Following a procedure of ApSimon and Cooney[4], a solution of 150 g (0.75 m) of (+)-camphoric acid (7) (Aldrich #C40-9) in 200 ml (2 mol) of acetic anhydride in a 1 ℓ round bottom flask was heated under reflux for 2 hrs. On cooling, transparent needles separated. The crystals were isolated by filtration and air dried. The filtrate was treated with distilled water to precipitate any remaining solid. The solid, 136 g (100%) of (+)-camphoric acid anhydride (8), melted at 221-222°; lit.[4] 222-223°.

^1H NMR ($CDCl_3$): δ 1.0(s); 1.08(s); 1.2(s); 2.0(m); 2.75(d).

IR (KBr): 3000(s); 2930(m); 1830(s); 1730(s); 1220(s); 1245(s); 1280(s); 1042(s); 945(s).

Preparation of Camphorimide (9).



8

9

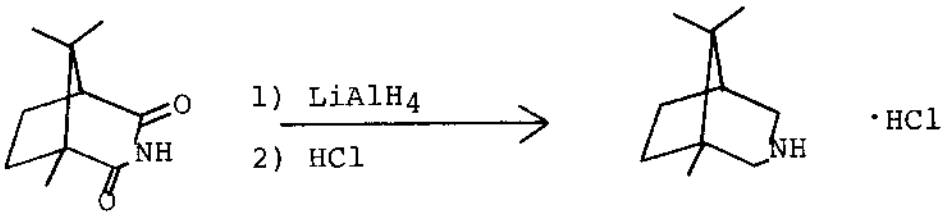
A solution of 138 g (0.76 mol) of (+)-camphoric acid anhydride (8) (vide supra) in 1 ℓ dry THF was treated with ammonia to cause the precipitation of a water-soluble white solid. The solid was removed by filtration and any THF remaining was removed by evaporation under reduced pressure. The solid (10 g) was sublimed under an aspirator pressure at 150° to 180°. The white solid which sublimed was collected, and the residue was dissolved in $CHCl_3$, filtered, and resublimed. The purified white solid was dissolved in $CHCl_3$, washed with 100 ml of 5% $NaHCO_3$, 100 ml of distilled water, 100 ml of 5% HCl, 100 ml of distilled water and 100 ml of brine. The organic phase was filtered through a layer of $MgSO_4$, and the solvent was removed under reduced pressure to give 7.4 g

(74.4%) of camphorimide (9) as a white powder, mp 244-246°; lit.[4] 246-248°.

^{1}H NMR ($CDCl_3$): δ 1.0(d); 1.2(s); 2.0(s); 2.75(m); 6.6(broad s).

IR (KBr): 3220(s); 3100(m); 2980(s); 1700(b); 1355(m); 1310(m); 1235(m); 1093(s).

Preparation of Camphidine Hydrochloride (10).



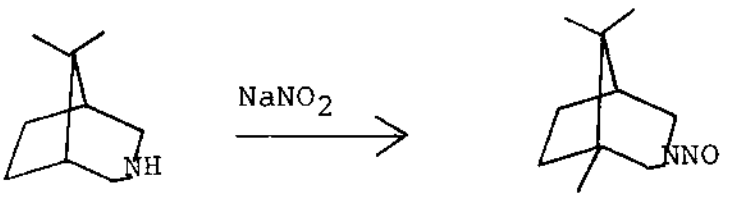
9 10

In a 1 ℓ, 3-necked round bottom flask equipped with a dropping funnel and reflux condenser was placed 11.4 g (0.3 mol) of lithium aluminum hydride and 200 ml of dry THF. The mixture was cooled to 0° and 46 g (0.25 m) of camphorimide (9) in 200 ml dry THF was added over a 1.5 hr period. The mixture was heated under reflux for 18 hrs and, after cooling, 81 g (0.25 m) of $Na_2SO_4 \cdot 10H_2O$ was added. When the evolution of hydrogen ceased the mixture was filtered and the filter cake was washed with 200 ml ether. The organic phase was filtered through Florosil and the solvent was removed under reduced pressure to give a yellow oil which crystallized on standing to give camphidine (11), mp 183-

186°; lit.[5] mp 185-187°. The amine was dissolved in 100 ml of dry ether and dry HCl was passed into the solution, producing 10.2 g (30%) of camphidine hydrochloride[5], mp 287-289°; lit.[5] mp 288-290°.

^{1}H NMR (D_2O): δ 0.8(d); 1.0(s); 1.6(m); 2.2(d); 2.5(d); 3.0(g); 4.18(s).

Preparation of N-Nitrosocamphidine (12).



·HCl

10 12

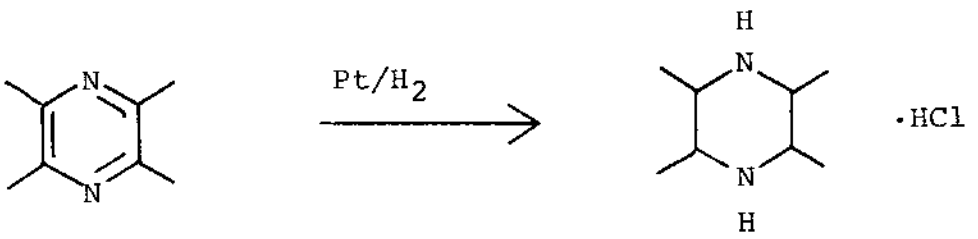
A stirred solution (pH 3) of 10 g (66.7 mmol) of camphidine hydrochloride (10) in 20 ml of distilled water in a 250 ml Erlenmeyer flask at 0° was treated dropwise with a saturated aqueous solution of $NaNO_2$ over an 0.5 hr period. The yellow oil which separated crystallized on standing. The solid was removed by filtration, air dried and recrystallized from 95% ethanol to give 5.5 g (30.7%) of N-nitrosocamphidine[5] (12), mp 165-166°; lit.[5] mp 166.5-167°.

^{1}H NMR (DMSO-d_6): δ 0.95(s); 1.02(s); 1.15(s); 1.8(m); 4.2(m).

CD (95% EtOH, c=0.1): $[\theta]_{351}$ +573.

General Procedure for Deuterium Exchange Studies. A solution of the nitrosamine in 0.5 ml of DMSO-d_6 was treated with 4 drops of 6N NaOD in D_2O in an NMR tube. The NMR spectra were determined at specific time intervals after being heated at 100° in a water bath.

Preparation of 2,3,5,6-Tetramethylpiperazine Hydrochloride (13).



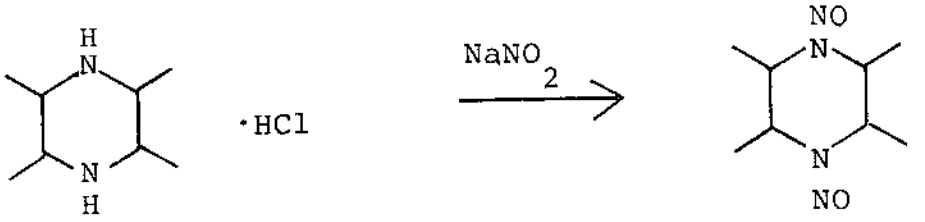
14 13

A solution of 5 g (36.8 mmol) of tetramethylpyrazine (14) (Aldrich #18,393-8) in 200 ml of 95% ethanol was acidified with concentrated HCl and was treated with hydrogen at 1 atm over 50 mg of PtO_2 as catalyst. Shaking was continued until there was no further uptake of hydrogen and 65 psi was absorbed. The catalyst was removed by filtration and the solvent was removed by evaporation under reduced pressure. Recrystallization of the residual solid from methanol gave 4.1 g (63.5%) of 2,3,5,6-tetramethylpiperazine hydrochloride (13) as yellow crystals, mp 295-300°(d); lit.[6] mp 300°(d).

^1H NMR (D_2O): δ 1.05(d); 3.15(broad d).

^{13}C NMR (D_2O): δ 36.014; 72.999.

Preparation of 1,4-Dinitroso-2,4,5,6-tetramethylpiperazine (15).



13 15

A saturated aqueous solution of $NaNO_2$ was added dropwise over a 1 hr period to a stirred solution of 5 g (28.5 mmol) of 2,3,5,6-tetramethylpiperazine hydrochloride (13) in 25 ml distilled water in a 250 ml Erlenmeyer flask. A white fluffy solid precipitated immediately and was isolated by filtration, air dried, and recrystallized from 95% ethanol to give 3.8 g (66.7%) of 1,4-dinitroso-2,3,5,6-tetramethylpiperazine (15) as yellowish needles[6], mp 170-172°; lit.[7] 174°.

^1H NMR ($CDCl_3$): δ 1.1(d); 1.65(d); 4.5(broad d).

^{13}C NMR ($CDCl_3$): δ 11.600; 15.094; 54.166; 55.137.

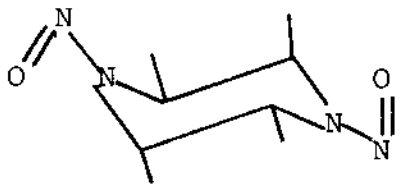
Anal for $C_8H_{16}N_4O_2$: Calc: C, 47.99; H, 8.05; N, 27.98. Found: C, 48.08; H, 8.19; N, 27.92.

IR ($CDCl_3$): 31.60(w); 2960(s); 1465(m); 1430(w).

UV (95% EtOH): 357(111).

Conformational Analysis of 1,4-Dinitroso-2,3,5,6-tetramethylpiperazine (15). A solution of 1,4-dinitroso-2,3,5,6-tetramethylpiperazine (15) in $CDCl_3$ was treated with 25 mg of

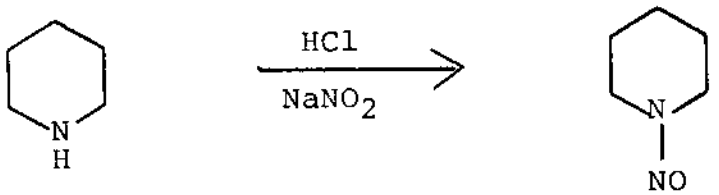
Euroshift F [$(Eu(FOD)_3$; Pierce Chemical Co.)]. The NMR of the solution showed a noticeable shift of the methyl peaks, as well as a slight separation of the methine resonances. The shift reagent was added in 5 mg increments until the methine region was completely separated into two sets of octets, indicating the following stereochemistry:



15

Thus the parent amine 13 must possess the same stereochemistry as the dinitrosamine 15.

Preparation of 1-Nitrosopiperidine (18).



16 18

A solution of 5 g (52.6 mmol) of piperidine (16) (Aldrich #10,409-4) in 5 ml of distilled water was prepared by adding concentrated HCl until a solution was complete. The solution was cooled to 0° and a saturated aqueous solution of $NaNO_2$ was added dropwise over a 1 hr period until the pH reached 3. A yellow oil separated and was taken up in ether. The aqueous phase was extracted three times with 50 ml of

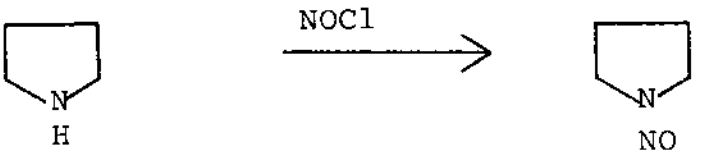
ether, and the combined ether extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$ and the ether was removed under reduced pressure to give 3.2 g (53.4% of 1-nitrosopiperidine (17) as a light yellow oil[8], bp 60-63° at 1 mm, lit.[8] 100° at 14 mm.

^1H NMR ($CDCl_3$): δ 1.7(m); 3.7(t); 4.1(t).

IR (film): 2940(s); 2860(s); 1460(s); 1445(m); 1425(m).

UV (Hexane): 377(65); 364(70).

Preparation of 1-Nitrosopyrrolidine (19).



18 19

A stirred solution of 5 g (70.4 mmol) of pyrrolidine (18) (Aldrich #P7,380-3) in 150 ml of anhydrous ether and 5.6 g (70.4 mmol) of dry pyridine in a 250 ml, 3-necked, round-bottom flask equipped with a fritted gas inlet tube and a dry ice-acetone cold finger was treated with an excess of gaseous nitrosyl chloride. Pyridine hydrochloride separated and the reaction was stirred at room temperature for an additional hour. The solid which formed was removed by filtration and washed with 100 ml ether. The ether was evaporated under reduced pressure giving 4.5 g (64%) of 1-

nitrosopyrrolidine[8] (as a yellow oil), bp 98° at 12 nm; lit.[9] 69-70° at 1 mm.

^{1}H NMR ($CDCl_3$): δ 2,05(m); 3.45(m); 4.2(m).

IR (Film): 2980(w); 2880(w); 1458(w); 1423(s).

UV (Isooctane): 355(950); 368(1700); 380(950).

Preparation of Dibenzylnitrosamine (21).

$$\underset{\underline{22}}{PhCH_2NHCH_2Ph\cdot HCl} \xrightarrow{NaNO_2} \underset{\underline{21}}{PhCH_2NCH_2Ph}\,(NO)$$

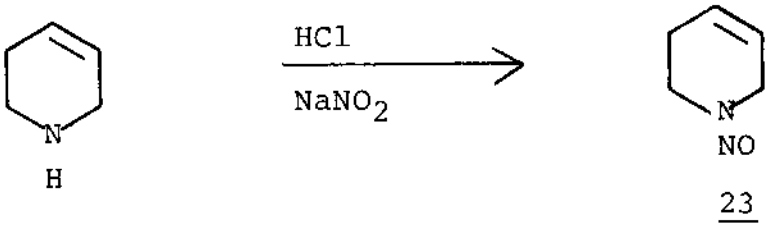
A saturated aqueous solution of $NaNO_2$ was added dropwise over a 2 hr period to a hot, stirred solution of 15 g (64.2 mmol) of dibenzylamine hydrochloride, mp 255-256° (recrystallized from 95% ethanol) in 100 ml distilled water in a 250 ml Erlenmeyer flask. The reaction was exothermic and NO_2 was evolved. The addition of $NaNO_2$ is accompanied by the precipitation of the nitrosamine. The mixture was cooled to room temperature, and after 24 hrs, crystallization was complete. The solid was separated by filtration and was recrystallized from hexane to give 13.7 g (93.6%) of dibenzylnitrosamine (21) as a yellow solid[10], mp 59-60°; lit.[10] mp 59-60°.

^{1}H NMR ($CDCl_3$): δ 4.5(s); 5.0(s); 7.05(m).

IR ($CDCl_3$): 3160(s); 3040(s); 2940(s); 1450(m).

UV (95% Ethanol): 361(79).

Preparation of 1-Nitroso-1,2,3,6-tetrahydropyridine (23).



23

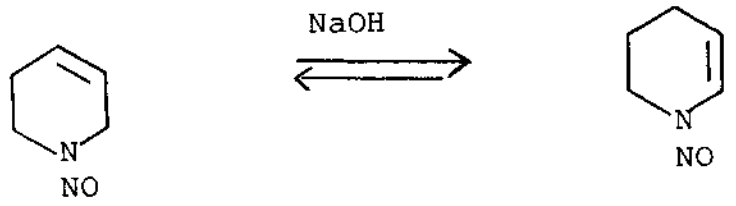
A stirred solution of 5 g (44.6 mmol) of 1,2,3,6-tetrahydropyridine (Pfaltz and Bauer #T05440) and 20 ml of concentrated HCl in a 250 ml Erlenmeyer flask at 0° was treated dropwise with a saturated aqueous solution of $NaNO_2$ over a 1 hr period. The pH was 3. The reaction mixture was stirred for another hour and a dark oil separated from the yellow solution. The oil was taken up in ether, and the aqueous phase was extracted with three portions of 50 ml of ether. The combined ether extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water, and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$, and the ether was removed under reduced pressure giving 4.3 g (63.8%) of 1-nitroso-1,2,3,6-tetrahydropyridine (23) as a light yellow oil (bp 60-65° at 0.25 mm); lit.[11] bp 44-45° at 0.2 mm).

^1H NMR ($CDCl_3$): δ 2.48(m); 3.7(t); 3.9(m); 4.21(t); 5.65(m)

IR (Film): 3045(s); 2985(w); 2930(w); 2850(w); 1430(b).

UV (95% Ethanol): 350(96).

Preparation of 1-Nitroso-1,2,3,4-tetrahydropyridine (24).



23 24

A solution of 5 g (44.6 mmol) of 1-nitroso-1,2,3,6-tetrahydropyridine and 1.8 g (44.6 mmol) of NaOH pellets in 100 ml of dry methanol was placed in a 250 ml round-bottom flask equipped with a reflux condenser. The resulting solution was heated under reflux overnight and the solvent was evaporated under reduced pressure. The residue was taken up in 100 ml distilled water, and the aqueous solution was extracted with three portions of 50 ml of ether. The combined ether extracts were washed with 20 ml of brine and filtered through a layer of $MgSO_4$. The solvent was evaporated under reduced pressure giving 4.7 g (94.1%) of 1-nitroso-1,2,3,4-tetrahydropyridine (24) as a light yellow oil, bp 64-66° at 0.16 mm; lit.[11] bp 42° at 0.04 mm.

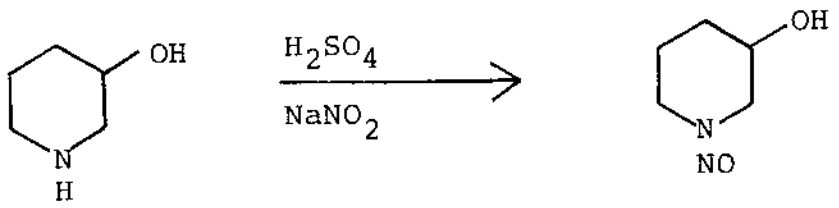
^1H NMR ($CDCl_3$): δ 2.0(m); 3.6(t); 4.22(t); 5.21(m); 7.35(broad d).

IR (Film): 3090(w); 2930(m); 2885(w); 2845(w); 1440(s).

UV (Hexane): 360(200); 375(260); 390(240); 276(8800); 208(4400).

(95% Ethanol): 277(7200); 208(4000).

Preparation of 1-Nitroso-3-hydroxypiperidine (26).



26

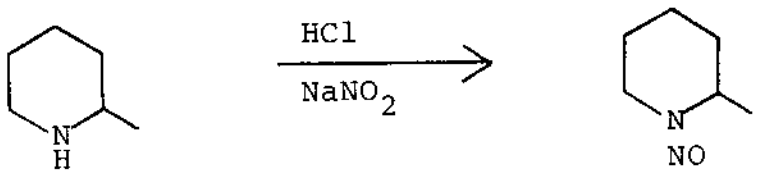
A solution of 10 g (100 mmol) of 3-hydroxypiperidine (Fluka AG, Buchs SG #56210) in 30 ml of 10% H_2SO_4 in a 250 Erlenmeyer flask at 0° was treated dropwise with a saturated aqueous solution of $NaNO_2$ over a 1 hr period. The pH was 3. The orange solution was filtered and extracted three times with 50 ml portions of $CHCl_3$, and the combined organic extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water, and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$ and the solvent was removed under reduced pressure to give 7.2 g (56%) of 1-nitroso-3-hydroxypiperidine (26) as a yellow oil (bp 110-113° at 0.07 mm) which crystallized after distillation to a light yellow solid[12], mp 45°; lit.[12] 45°.

^1H NMR ($CDCl_3$): δ 1.3-1.8(m); 3.4-4.5(m); 3.6(s).

IR (Film): 3345(b); 2930(s); 3860(m); 1415(b); 1080(s).

UV (95% Ethanol): 349(61).

Preparation of 1-Nitroso-2-methylpiperidine (28).



27 28

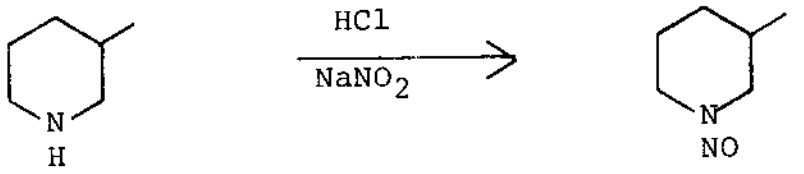
A stirred solution of 5 g (51.5 mmol) of 2-methylpiperidine (27) (Aldrich #M7,280-3) in 20 ml of HCl at 0° was treated with a saturated aqueous solution of $NaNO_2$ dropwise over a 1 hr period. The pH was 3. The yellow oil which separated was taken up in ether, and the aqueous phase was extracted with three portions of 50 ml of ether. The combined ether extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water, and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$, and the ether was removed under reduced pressure to give 3.6 g (55.5%) of 1-nitroso-2-methylpiperidine (28) as a light yellow oil[13], bp 69-74° at 0.25 mm; lit.[13] bp 82-84° at 8 mm.

^1H NMR (CCl_4): δ 1.07(d); 1.5(d); 1.7(m); 3.6(t); 4.4(m); 5.0(m).

IR (Film): 2970(w); 2940(m); 2860(w); 1470(w); 1455(w); 1430(m).

UV (Hexane): 377(67); 364(71).

Preparation of 1-Nitroso-3-methylpiperidine (30).



29 30

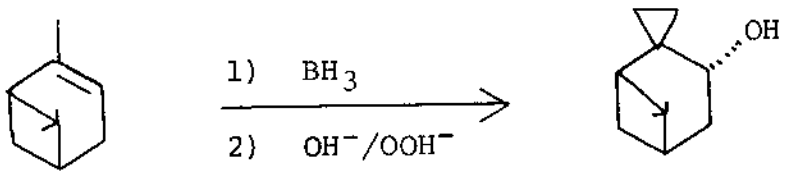
A stirred solution of 5 g (50.5 mmol) of 3-methylpiperidine (29) (Aldrich #M7,300-1) in 20 ml of concentrated HCl at 0° was treated dropwise with a saturated aqueous solution of $NaNO_2$ to a pH of 3. A yellow oil separated. The oil was taken up in ether, and the aqueous phase was extracted with three portions of 50 ml of ether. The combined ether extracts were washed with 20 ml of 5% HCl, 20 ml of distilled water, and 20 ml of brine. The organic phase was filtered through a layer of $MgSO_4$, and the solvent was removed under reduced pressure to give 4.2 g (65%) of 1-nitroso-3-methylpiperidine (30) as a light yellow oil[14], bp 65-67° at 0.5 mm; lit.[14] 88-89° at 10 mm.

^1H NMR ($CDCl_3$): δ 1.05(d); 1.7(m); 2.0-4.0(m); 4.55(t).

IR (Film): 2950(b); 2870(m); 1467(m); 1458(m); 1430(m).

UV (Hexane): 364(110); 377(84).

Preparation of (+)-3-Pinanol (32).



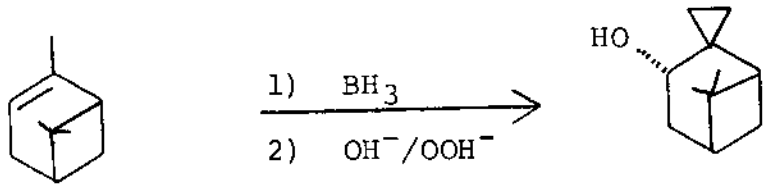
31 32

A solution of 1.55 g (40 mmol) of sodium borohydride and 13.6 g (10 mmol) of (-)-α-pinene (31) (The Glidden Co.; $[\alpha]_D$ (neat) -45.0) in 50 ml dry diglyme (distilled from $LiAlH_4$) was treated with 7 ml (55 mmol) of boron trifluoride etherate. The reaction was stirred at room temperature for 2 hrs at which time 20 ml of 3M NaOH was added in one portion. The flask was immersed in an ice water bath and 11 ml of 30% H_2O_2 was added very slowly. The solution was stirred for an additional 30 min at room temperature. The reaction mixture was extracted with three portions of 20 ml of ether, and the combined ether extracts were washed with five portions of 50 ml of ice water to remove any diglyme. The organic phase was filtered through a layer of $MgSO_4$, and the solvent was removed under reduced pressure giving a clear oil which crystallized on standing. The solid was recrystallized from petroleum ether to give 1.2 g (78.4%) of (+)-3-pinanol as white needles, mp 54-55°; $[\alpha]_D$ (c=10, Benzene) +31.7; lit.[15] mp 54-55°; $[\alpha]_D$ (c=10, Benzene) +32.8.

^1H NMR ($CDCl_3$): δ 0.9(s); 1.05(s); 1.15(s); 1.2(s); 1.85(broad t); 3.95(m).

IR (KBr): 3420(b); 2900(s); 1420(s); 1380(s); 1115(b).

Preparation of (-)-3-Pinanol (34).



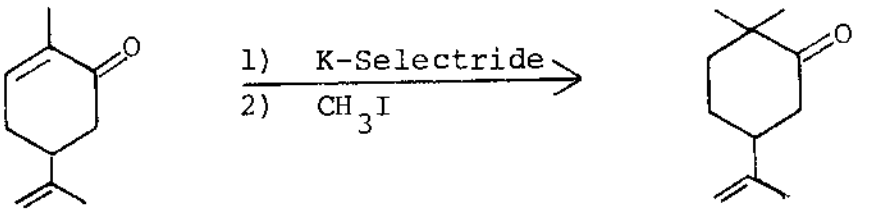
33 34

This reaction followed the procedure for the preparation of (+)-3-pinanol (32) (vide supra). (+)-α-Pinene (33) (Aldrich #P4,568-0) $[\alpha]_D$ (neat) +46.5 gave (50%) (-)-3-pinanol[15], mp 54-55°; $[\alpha]_D$ -31.5° (c=10, Benzene); lit.[15] $[\alpha]_D$ -32.8° (c=10, Benzene).

^1H NMR ($CDCl_3$): δ 0.9(s); 1.05(s); 1.15(s); 1.2(s); 1.85(broad t); 3.95(m).

IR (KBr): 3420(b); 2900(s); 1420(s); 1380(s); 1115(b).

Preparation of (-)-2-Methyldihydrocarvone (36).



35 36

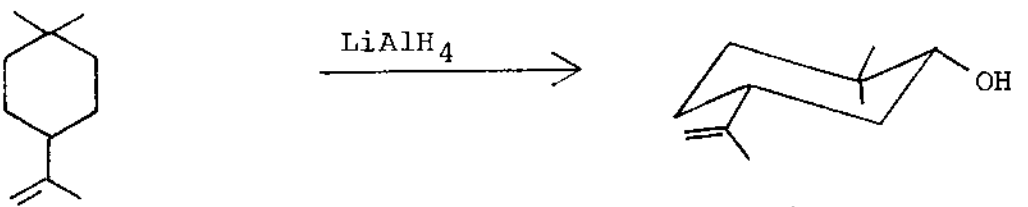
A solution of 5 g (33.3 mmol) of (+)-carvone (35) (Aldrich #11,169-0) ($[\alpha]_D$ (neat) +58) and 20 ml of dry THF at -78° was placed in a dry 250 ml, 3-necked, round-bottom flask

under N_2. To this solution was added rapidly 74 ml (37 mmol) of a 0.5M solution of K-Selectride (potassium trisecbutylborohydride).[16] The solution turned yellow and was stirred at -78° for 1 hr, at which time 6.15 g (43.3 mmol) of cold methyl iodide was added rapidly. After 5 min at -78°, the cooling bath was removed, and the reaction was stirred for an added 2 hrs. At this time a white precipitate had formed, and 30 ml of a 2.8M solution of NaOH and 30 ml of 30% H_2O_2 was added at 0°. After the reaction mixture had become colorless and homogeneous, it was stirred overnight at room temperature. The aqueous phase was extracted with three portions of 50 ml of hexane. The combined organic extracts were washed with 20 ml of distilled water, 20 ml of 10% $NaHSO_3$, 20 ml brine and filtered through a layer of $MgSO_4$. The solvent was removed under reduced pressure to give 5.3 g (96.4%) of (36), a colorless oil, bp 42-47° at 0.1 mm; $[\alpha]_D$ -57 (c=4.9, MeOH); lit.[16] bp 50-53° at 0.25 mm.

^1H NMR (CCl_4): δ 1.0(s); 1.1(s); 1.7(s); 2.3(broad s); 4.7(s).

IR (Film): 2800(b); 1710(s); 1645(s).

Preparation of (-)-2-Methyldihydrocarveol (37).



36 37

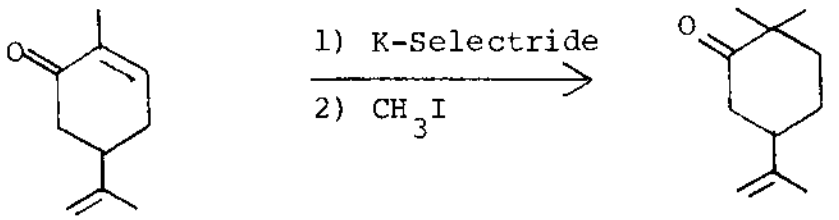
To a slurry of 2 g (53 mmol) of lithium aluminum hydride in 100 ml dry ether in a 250 ml, 3-necked round-bottom flask was added 5 g (30 mmol) of (-)-2-methyldihydrocarvone (36) (vide supra); $[\alpha]_D$ -57 deg-ml/g-dm (c=4.9, MeOH) dissolved in 20 ml dry ether. The reaction was stirred overnight at room temperature and 17 g (53 mmol) $Na_2SO_4 \cdot 10H_2O$ was added to decompose the excess hydride. The mixture was filtered and the filter cake washed with 100 ml of ether. The solvent was removed under reduced pressure to give 4.6 g (91.3%) of (-)-2-methyldihydrocarveol (37) as a colorless oil, bp 62-67° at 0.05 mm; $[\alpha]_D$ -6.20 deg-ml/g-dm; (c=5.09, MeOH).

^1H NMR (CCl_4): δ 0.85(s); 0.98(s); 1.37(broad s); 1.7(s); 2.95(broad s); 3.25(q); 4.6(s).

IR (Film): 3350(b); 3070(s); 2900(b); 1640(s).

Anal for $C_{11}H_{20}O$: Calc: 78.51; H, 9.51; O, 11.98. Found: C, 78.47; H, 9.62; O, 12.07.

Preparation of (+)-2-Methyldihydrocarvone (39).



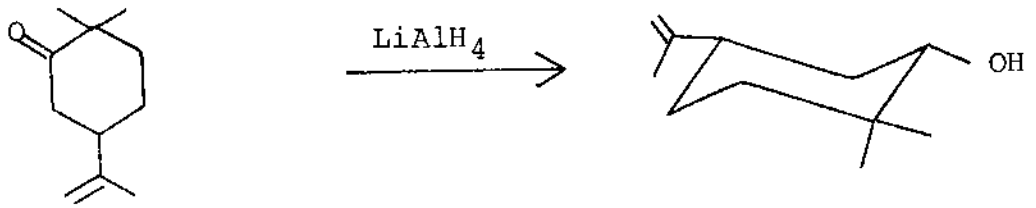
38 39

This reaction followed the procedure for the preparation of (-)-2-methyldihydrocarvone (vide supra). The reaction of 5 g (33.3 mmol) of (-)-carvone (38), (Aldrich, #12,493-1); $[\alpha]_D$ -58 (neat) gave 5 g (95%) of (+)-2-methyldihydrocarvone[16] (39), $[\alpha]_D$ +56 (c=4.9, MeOH); bp 42-48° at 0.1 mm; lit.[16] bp 54-57° at 0.25 mm.

^1H NMR (CCl_4): δ 1.0(s); 1.1(s); 1.7(s); 2.3(broad s); 4.7(s).

IR (Film): 2800(b); 1710(s); 1645(s).

Preparation of (+)-Methyldihydrocarveol (40).



39 40

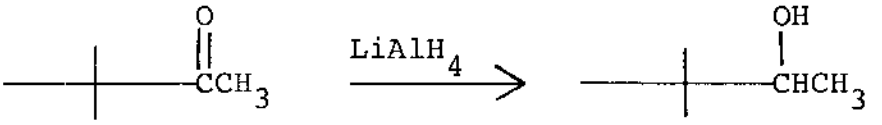
This preparation followed the procedure for the preparation of (-)-methyldihydrocarveol (37) (vide supra). This conversion of 5 g (30 mmol) of (+)-2-methyldihydrocarvone (39) $[\alpha]_D$ +56 (c=4.9, MeOH), gave 4.5 g (91%) of (+)-2-methyldihydrocarveol (40), bp 62-67° at 0.05 mm; $[\alpha]_D$ +5.9 (c=5.0, MeOH).

^1H NMR (CCl_4): δ 0.85(s); 0.98(s); 1.37(broad s); 1.7(s); 2.95(broad s); 3.25(q); 4.6(s).

IR (Film): 3350(b); 3070(s); 2900(b); 1640(s).

Anal for $C_{11}H_{20}O$: Calc: C, 78.51; H, 9.51; O, 11.98. Found: C, 78.64; H, 9.42; O, 11.91.

Preparation of (±)-3,3-Dimethyl-2-butanol (42).

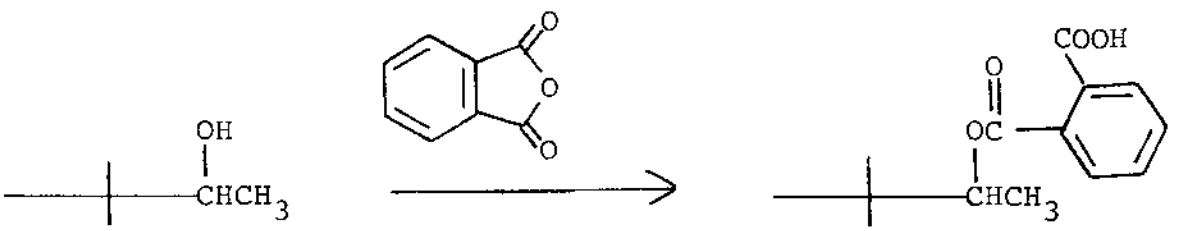


42

A solution of 20 g (0.2 mol) of pinacolone (Aldrich #P4,560-5) in 50 ml of dry ether was added to a stirred slurry of 12 g (0.31 m) of lithium aluminum hydride in 100 ml dry ether in a standard 500 ml, 3-necked set-up. The reaction was stirred overnight under N_2 and 100 g (0.32 m) of $Na_2SO_4 \cdot 10H_2O$ was added. The mixture was filtered after the evolution of hydrogen had ceased, and the filter cake was washed with 100 ml of ether. The solvent was removed under reduced pressure to give 18.4 g (90%) of (±)-3,3-dimethyl-2-butanol (42) as a volatile, clear oil[17] bp 119-120°; lit.[17] bp 119-120°.

IR (Film): 3350(b); 2860(s); 1080(s).

Preparation of (±)-3,3-Dimethyl-2-butyl Phthalate (43).

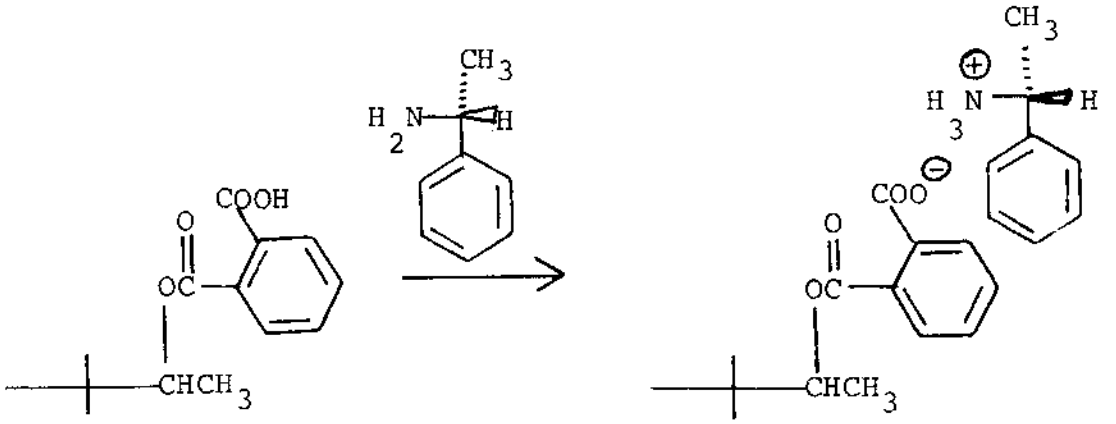


42

A mixture of 54 g (0.53 mol) of (±)-3,3-dimethyl-2-butanol (42), 80 g (0.54 mol) of phthalic anhydride and 300 ml dry pyridine was heated until the solids all dissolved. The reaction was allowed to stand for 12 hr at room temperature and then was heated on a steam bath for 15 min. The solution was acidified with 5% HCl and extracted with three portions of 100 ml of ether. The combined ether layers were concentrated by distillation under reduced pressure and the residue was dissolved in 10% $NaHCO_3$. The solution was washed with two portions of 100 ml of ether and was acidified with 50 ml of cold, concentrated HCl. The white precipitate which separated was removed by filtration and dried under reduced pressure for two days. The solid was recrystallized from petroleum ether, affording 75 g (56.6%) of (±)-3,3-dimethyl-2-butyl phthalate (43) as a white powder[18], mp 85-87°; lit.[18] 85-86°.

^1H NMR ($CDCl_3$): δ 0.92(s); 1.25(d); 4.8(q); 7.45(m); 7.88(broad s).

Preparation and Fractional Crystallization of (+)-α-Methylbenzylammonium-(±)-3-Dimethyl-2-butyl phthalate (44).



43 44

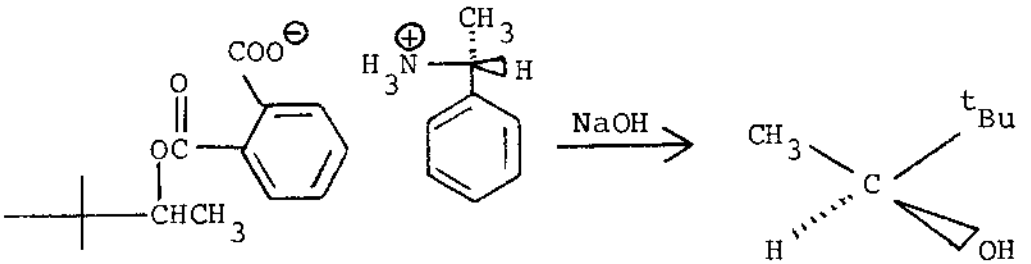
The salt was prepared by mixing a concentrated solution of 73.4 g (0.29 mol) of (±)-3,3-dimethyl-2-butyl phthalate (43) in 125 ml anhydrous methanol with 31.6 g (0.29 m) of neat (+)-α-methylbenzylamine (45). The solution was stirred at 0° until crystallization was complete. The solvent was removed under reduced pressure (water bath temperature 40°), leaving 105 g (100%) of a white, water-soluble solid, mp 98-100°, $[\alpha]_D$ (MeOH) +7. A small amount was recrystallized three times from hexane, giving colorless needles, $[\alpha]_D$ +23.6. The remainder was divided in half, and each portion was recrystallized four times from hexane to give (+)-α-methylbenzylammonium-(±)-3,3-dimethyl-2-butyl phthalate (44); mp 100-102°, $[\alpha]_D$ +17, (c=4.7 in MeOH).

Anal for $C_{22}H_{29}NO_4$: Calc: C, 70.13; H, 7.87; N, 3.77. Found: C, 70.19; H, 8.04; N, 3.60.

^{1}H NMR (D_2O): δ 0.9(s); 1.25(d); 2.6(d); 4.0-5.0(m); 7.32(s).

IR (KBr): 2950(b); 1710(s); 1620(m); 1375(s); 1260(s); 1135(m); 1065(s); 755(m); 690(m).

Hydrolysis of (+)-α-Methylbenzylammonium-3,3-dimethyl-2-butyl phthalate (42).



44 42

In a 500 ml Erlenmeyer flask was placed a solution of 5 g (13.5 mmol) of (+)-α-methylbenzylammonium-3,3-dimethyl-2-butyl phthalate (44) in 200 ml of hot, distilled water, and a large excess of NaOH pellets was added slowly. When the solution cooled to room temperature, it was extracted with three portions of 25 ml of ether. The aqueous phase was transferred to a 500 ml, 3-necked round-bottom flask equipped with a reflux condenser and thermometer, and warmed to 50-60° for 6 hrs. During this period oil droplets separated. The mixture was extracted with three portions of 50 ml ether. The combined ether extracts were washed with 20 ml of brine and filtered through a layer of $MgSO_4$. The solvent was carefully evaporated under reduced

pressure to give 1.13 g (82%) of (42) as a clear oil[17], bp 119-120°; $[\alpha]_D$ (c=0.1, MeOH) +1.55 (19.1% optically pure); lit.[17] bp 119-120°; $[\alpha]_D$ 7.71.

General Procedure for Induced CD Spectra. The chiral reagent was weighed into a tapered 2 ml volumetric flask and diluted to the desired volume with the appropriate solvent. This solution, which varied from 1.0-2.0 M, was used to determine the baseline. The solution was added to a separate 2 ml volumetric flask containing the nitrosamine and 1-2 drops of solvent. After thorough mixing, this solution was transferred to a 1 mm cell and the spectrum was scanned from 400 nm to 285 nm. The concentration of the nitrosamines was approximately 0.13 M. The exact data are given in Tables I and II and Appendix C.

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CHAPTER IV

RESULTS AND DISCUSSION

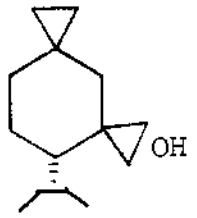
Induced Circular Dichroism

The strong interaction between hydroxy compounds and nitrosamines has been confirmed by induced circular dichroism studies as well as by solubility properties. In the case of the induced circular dichroism of nitrosamine-alcohol complexes, only sterically hindered alcohols induced chirality into the nitrosamines. The use of the enantiomeric 2-octanols produced no CD curve. These results probably reflect either the population of rotamers in the acyclic, free-rotating alcohols or the similarity of magnetic effects of CH_2 and CH_3, since the sterically hindered (-)-3,3-dimethyl-2-butanol--1-nitrosopyrrolidine complex produced a large induced circular dichroism.

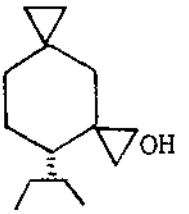
A series of cyclic alcohols (Table I) containing groups of substantially different sizes about the carbinol carbon also produced similar induced circular dichroism curves where 3, 17, 19, and 21 were used as the nitrosamines. The sign of the induced CD effect could be related to the configuration of the carbinol carbon of the alcohols.

TABLE I

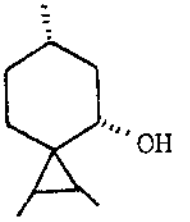
CHIRAL COMPOUNDS USED TO INDUCE CIRCULAR DICHROISM



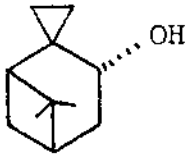
R-(-)-Menthol



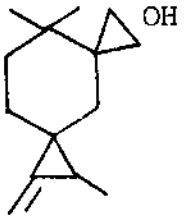
R-(-)-Isopulegol



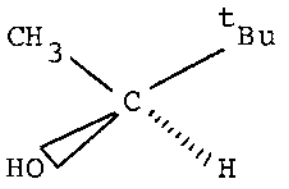
S-(+)-Isomenthol



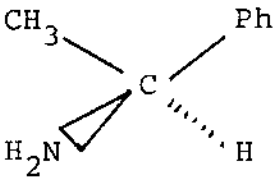
R-(-)-3-Pinanol



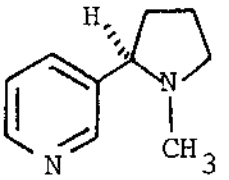
S-(+)-2-Methyl-Dihydrocarveol



S-(+)-3,3-Dimethyl-2-Butanol



S-(-)-α-Methyl-Benzylamine



S-(-)-Nicotine

NOTE: Stereochemistry denoted for the asymmetric carbon bearing the hetero atom.

TABLE II

THE ICD CURVES WITH ACHIRAL NITROSAMINES

AND CHIRAL ALCOHOLS IN ISOOCTANE[11]

Nitrosamine / Chiral Alcohol	R-Menthol		S-Menthol		R-Pino-campheol		S-Pino-campheol		R-Isopulegol		S-Isomenthol	
	Sign	λ(nm)	Sign	λ(nm)	Sign	λ(nm)	Sign	λ(nm)	Sign	λ(nm)	Sign	λ(nm)
1-Nitroso-2,6-dimethyl-piperidine (II)	+	358	-	356	+	360	-	358	+	355 (365) (380)	-	360 (370)
1-Nitroso-pyrrolidine (XIII)	+	355 (365)	-	350								
Dibenzyl-nitrosamine (VIII)	+	355 (365)										

The ICD curves of R-(-)-menthol, R-(-)-isopulegol, R-(-)-3-pinanol and R-(-)-2-methyldihydrocarveol were measured with the nitrosamines in Table II and in each case the Cotton effect observed in the ICD was positive. Conversely, when S-(+)-menthol, S-(+)-isomenthol, S-(+)-pinanol and S-(+)-2-methyldihydrocarveol were used, the ICD gave the expected opposite, negative Cotton effects.

The results of this study were used to formulate an empirical relationship (Table III), which could then be used to predict the absolute configuration of sterically hindered alcohols. The rule predicts a positive Cotton effect if the relative positions of the large, medium and small groups about the carbinol carbon lie in a clockwise manner with the hydroxyl group oriented above the plane of the paper. A negative Cotton effect would be observed if the large, medium and small groups arrangement is counterclockwise.

To test this hypothesis a partially resolved sample of 3,3-dimethyl-2-butanol was prepared by asymmetric reduction of pinacolone. The reaction should produce the S configuration and the negative Cotton effect observed with the mixture of the 3,3-dimethyl-2-butanol and N-nitrosopyrrolidine confirmed this configuration.

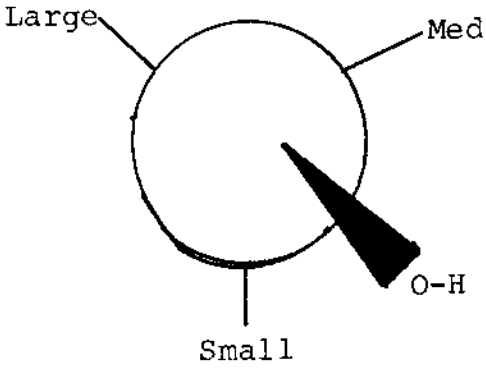
This relationship also applies to amines such as the optically active α-methylbenzylamines and S-(-)-nicotine. A solution of S-(-)-α-methylbenzylamine and nitrosamines 3 or

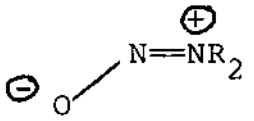
TABLE III

PREDICTION OF THE ABSOLUTE CONFIGURATION OF ALCOHOLS

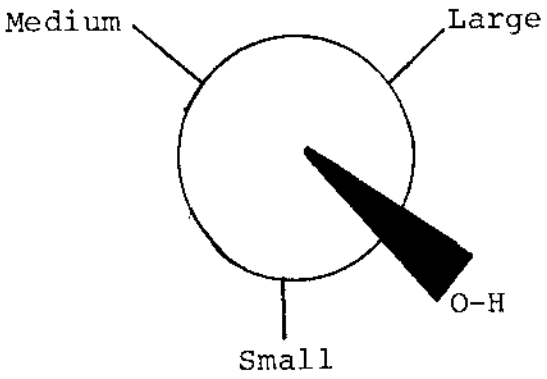
USING ICD OF NITROSAMINE-ALCOHOL COMPLEXES

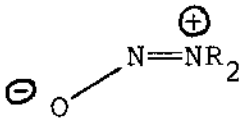
(+)-Cotton Effect





(-)-Cotton Effect





19 in isooctane gave negative Cotton effects in the ICD curve. On the other hand, the use of the enantiomeric R-(+)-α-methylbenzylamine with 3 or 19 produced the mirror image, positive Cotton effects. The fine structure in these ICD curves suggests that a mechanism other than hydrogen bonding may be operating here.

Nitrosamine 3 exists as a racemic mixture and thus could be the source of the Cotton effect when exposed to UV light. However, induced CD curves of similar magnitudes are obtained with the totally achiral nitrosamine 19; thus, the potential chirality of the racemic mixture does not contribute to the CD curve. These results also rule out the possibility of hydrogen bonding at the amino nitrogen of the nitrosamine, since the two axial methyl groups of 3 should exhibit some effect on the hydrogen bonding at that site.

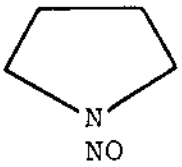
The magnitude of the Cotton effect observed with the 1-nitroso-cis-2,6-dimethylpiperidine (3)-menthol system was found to depend on the concentration of both the nitrosamine and the menthol. The increase in concentration of the nitrosamine holding the menthol concentration constant, and the increase of menthol concentration keeping the nitrosamine concentration constant, gave linear increases in the intensity of the ellipticity in the CD maximum. These results suggest that the induced CD is derived from a 1:1 complex between the nitrosamine and menthol.

Polyhydroxy compounds, such as carbohydrates and ascorbic acid, also induced chirality into achiral nitrosamines. These results are summarized in Table IV. Sucrose, mannose and ascorbic acid produce positive CD curves using 1-nitrosopyrrolidine (19). Lactose, soluble starch and agar produce negative CD curves. Glucose produces no induced CD effect. Positive induced CD curves were also obtained with 1-nitrosopiperidine (18), 1-nitroso-1,2,3,4-tetrahydropyridine (24), 1-nitroso-3-hydroxypiperidine (26), bis-(2-hydroxypropyl)-nitrosamine (48), 1-nitroso-4-hydroxyhexahydroazepine (49), and bis-(2-hydroxyethyl) nitrosamine (50) using a 1M solution of ascorbic acid to effect the ICD curve (Table V). The magnitude of the ICD of the hydroxynitrosamines 26, 48, and 50 was greatly reduced as compared with the standard nitrosamines 18 and 19. This suggested that inter- or intramolecular hydrogen bonding of the hydroxynitrosamines competed with the hydrogen bonding of the chiral alcohol with the nitrosamine.

The experiments utilizing the monohydric, chiral alcohols described previously employed a solvent which did not compete with the alcohol in hydrogen bonding with the nitrosamine. In aqueous solution the solvent would be expected to compete with the hydrogen bonding of carbohydrates. Regardless, large induced CD curves were observed, suggesting that the interaction between the highly polar chromophore of

TABLE IV

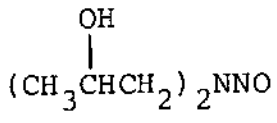
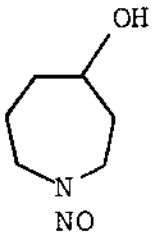
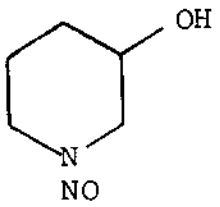
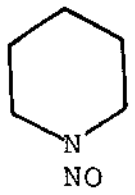
ICE IN AQUEOUS SOLUTION



Polyol	[θ]
Glucose	0
Sucrose	+21.0
Lactose	- 4.4
Mannose	+13.0
Agar	-----
Starch (sol)	-----
Ascorbic Acid	+41.5

TABLE V

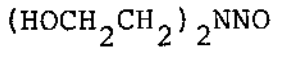
ICD OF HYDROXYNITROSAMINES--ASCORBIC ACID (1.0M)

Hydroxynitrosamines	[θ]
	+0.36
	+15.0
$(HOCH_2CH_2)_2NNO$	+0.51
	+1.28
	+3.37

the nitrosamines and the polyhydric alcohols is strong.

48

49



50

There are several factors which suggest that the ICD from compounds containing hydroxyl groups and N-nitroso compounds is due to formation of a complex. The magnitude of the Cotton effect is directly related to the concentration of both the nitrosamine and the polyol. The Cotton effect, furthermore, does not appear to be a result of a chemical reaction between the nitrosamine and polyol, since increases in polyol concentration well past the stoichiometric amount caused increases in the ICD curve. The reproducibility of the magnitude of the ICD curves on rescanning the spectrum also eliminated the possibility of a photochemical reaction as an explanation for the ICD curves, for a photochemical reaction between nitrosamines and ascorbic acid has been reported.[1]

Solubility Studies

Nitrosamines 15, 17 and 24 were shown to be qualitatively more soluble in 1M ascorbic acid solution than in

water (Tables VI-IX). Nitrosamine 17 was shown to be only slightly more soluble in 1M sucrose solution than in water (Table IX). In order for the solubility of nitrosamines to be increased on the addition of carbohydrate or ascorbic acid solution as compared with water, a water soluble complex must be formed. These results support the idea of a strong interaction resulting in complex formation.

Further support of complex formation was provided by the results of Ames test for mutagenicity.[2] The Ames assay with test strain TA100 using an S-9 fraction for metabolism gave about twice the number of revertants if sucrose was present during the incubation. Substituting ascorbic acid for the sucrose under the same conditions increased the mutagenicity of 24 by almost three-fold. This effect was attributed to either (1) the stabilization of the α-hydroxylated metabolite by the polyol near the active site of the mixed function oxidase; (2) the stabilization of the ultimate carcinogen, producing a more effective transport mechanism, or (3) competition between the agar and the polyol for binding to the nitrosamine.[2]

Hydrogen-Deuterium Exchange Studies

Within a series of nitrosamines, the carcinogenic potency seems to be dependent on the ease of metabolic conversion to an α-hydroxy derivative. The carcinogenicity also seems to be related to the presence of an acidic hydrogen on

TABLE VI

EFFECT OF ASCORBIC ACID ON NITROSOPIPERIDINE SOLUBILITY IN AQUEOUS SOLUTION

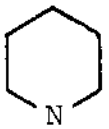
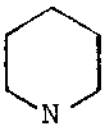
System	Stirred Time (min)	Absorbance (338 nm)
 (500 mg) + 1 ml H_2O	5	0.785
	10	0.797
	15	0.762
 (500 mg) + 1 ml H_2O L-Ascorbic Acid (500 mg)	25	1.126

TABLE VII

EFFECT OF ASCORBIC ACID ON 2,3,5,6-TETRAMETHYLDINITROSOPIPERAZINE IN AQUEOUS SOLUTION

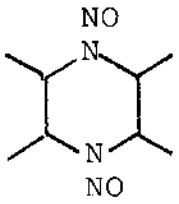
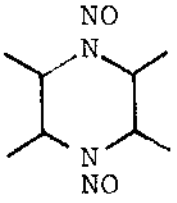
System	Stirred Time (min)	Absorbance (350 nm)
 (500 mg) + 1 ml H_2O	10	0.163
	20	0.167
 (500 mg) + 1 ml H_2O L-Ascorbic Acid (500 mg)	300	0.394

TABLE VIII

EFFECT OF ASCORBIC ACID ON 1-NITROSO-1,2,3,4-TETRAHYDROPYRIDINE IN AQUEOUS SOLUTION

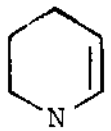
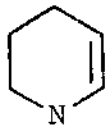
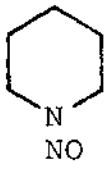
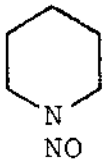
System	Stirred Time (min)	Absorbance (369 nm)
 (500 mg) + 1 ml H_2O	10	0.169
	20	0.168
 (500 mg) + 1 ml H_2O L-Ascorbic Acid (500 mg)	30	0.170
	40	0.167
	300	0.179

TABLE IX

EFFECT OF SUCROSE ON NITROSOPIPERIDINE

SOLUBILITY IN AQUEOUS SOLUTION

System	Stirred Time (min)	Absorbance (358 nm)
 (500 mg) + 1 ml H_2O	10	0.293
	20	0.289
 (500 mg) + 1 ml H_2O (+)-Sucrose (500 mg)	30	0.331
	110	0.320

the α-position of the nitrosamine. Thus any stereoselectivity of H-D exchange might be correlated with a structure-activity relationship of the carcinogenicity of nitrosamines. To determine the stereoselectivity of the acidity of the hydrogens α to the nitrosamine function, the hydrogen-deuterium exchange of several conformationally-biased, nitrogen heterocycles was measured. It was observed that stereoelectronic control of deuterium exchange favored reaction of an alpha axial hydrogen in six membered heterocyclic nitrosamines. The steric interaction of an N-nitroso group with an adjacent equatorial alkyl group has been shown to be sufficient to cause 1-nitroso-cis-2,6-dimethylpiperidine (3) and 1-nitroso-4-benzyl-cis-2,6-dimethylpiperazine (6) to exist predominantly in a distorted chair conformation with the methyl groups axial.[3] The tertiary butyl group of 4-t-butyl-1-nitroso-piperidine (45) and the diequatorial methyl groups of 4-benzyl-1-nitroso-cis-3,5-dimethylpiperazine (46) act as holding groups to stabilize the conformation. The deuterium exchange of 45 has been reported.[4] The amide function of 4-benzoyl-1-nitroso-3,5-dimethylpiperazine (47) acts similarly to give a conformational equilibrium favoring the conformation with the methyl groups axial.[5] The conformation of the bicyclic compound N-nitrosocamphidine (12) has been reported[6] to be a chair form. (For structures see Fig. 8, p 76).

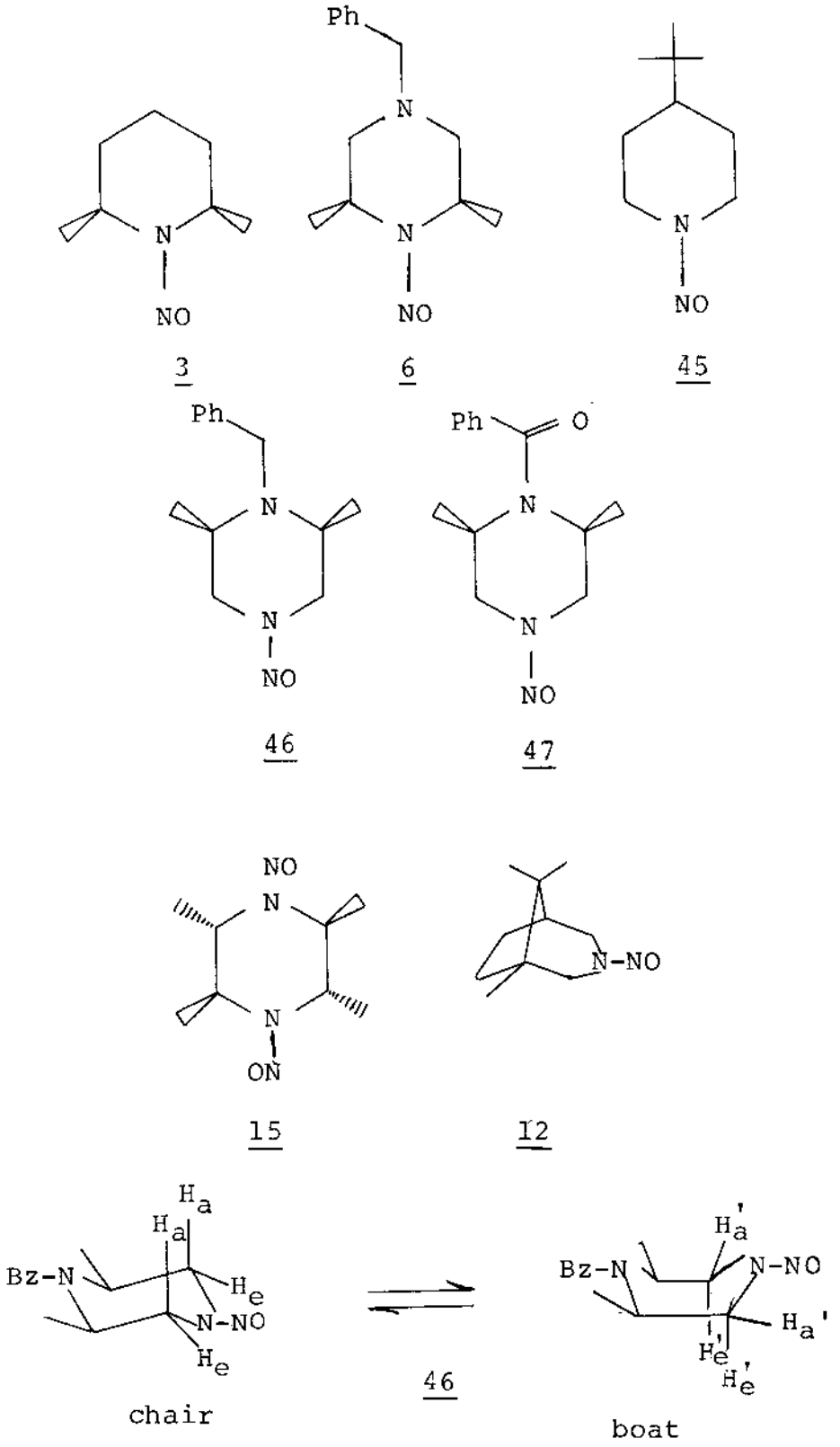


Fig. 8--Nitrosamines used in hydrogen-deuterium exchange studies.

The stereochemistry and conformation of an isomer of 1,4-dinitroso-2,3,5,6-tetramethylpiperazine has been determined by spectral methods (see Experimental). The ^{1}H NMR spectra of the parent amine showed a single doublet for the methyl groups and two singlets in the ^{13}C spectra. This observation limits the structure to a conformationally mobile chair form containing two axial methyl and two equatorial methyl groups (Fig. 9). Structure 15a can be eliminated since it would form only the mononitrosamine (m/e 170). The ^{1}H NMR of the dinitroso derivative (m/e 200) shows two methyl doublets, while the ^{13}C NMR gave the expected four signals. The first order ^{1}H NMR spectrum of the dinitrosoamine is consistent with the structure of the highly symmetrical nitrosamine 15b (Ci symmetry), rather than the asymmetric 15c which would show only two signals in the ^{13}C NMR. This hypothesis was confirmed by the separation of the methine region of the ^{1}H NMR spectrum into two distinct octets using the achiral lanthanide shift reagent $(EuFOD)_3$. The methine region of the NMR of the dinitroso derivative of 15c would be more complex. The melting point, furthermore, corresponded with that of the γ-isomer reported by Kipping[7], and the structure assigned by Harris and Spragg[8], based on the ^{1}H NMR.

Thus, if the relative acidity of an alpha hydrogen of a nitrosamine is subject to stereoelectronic control, compounds

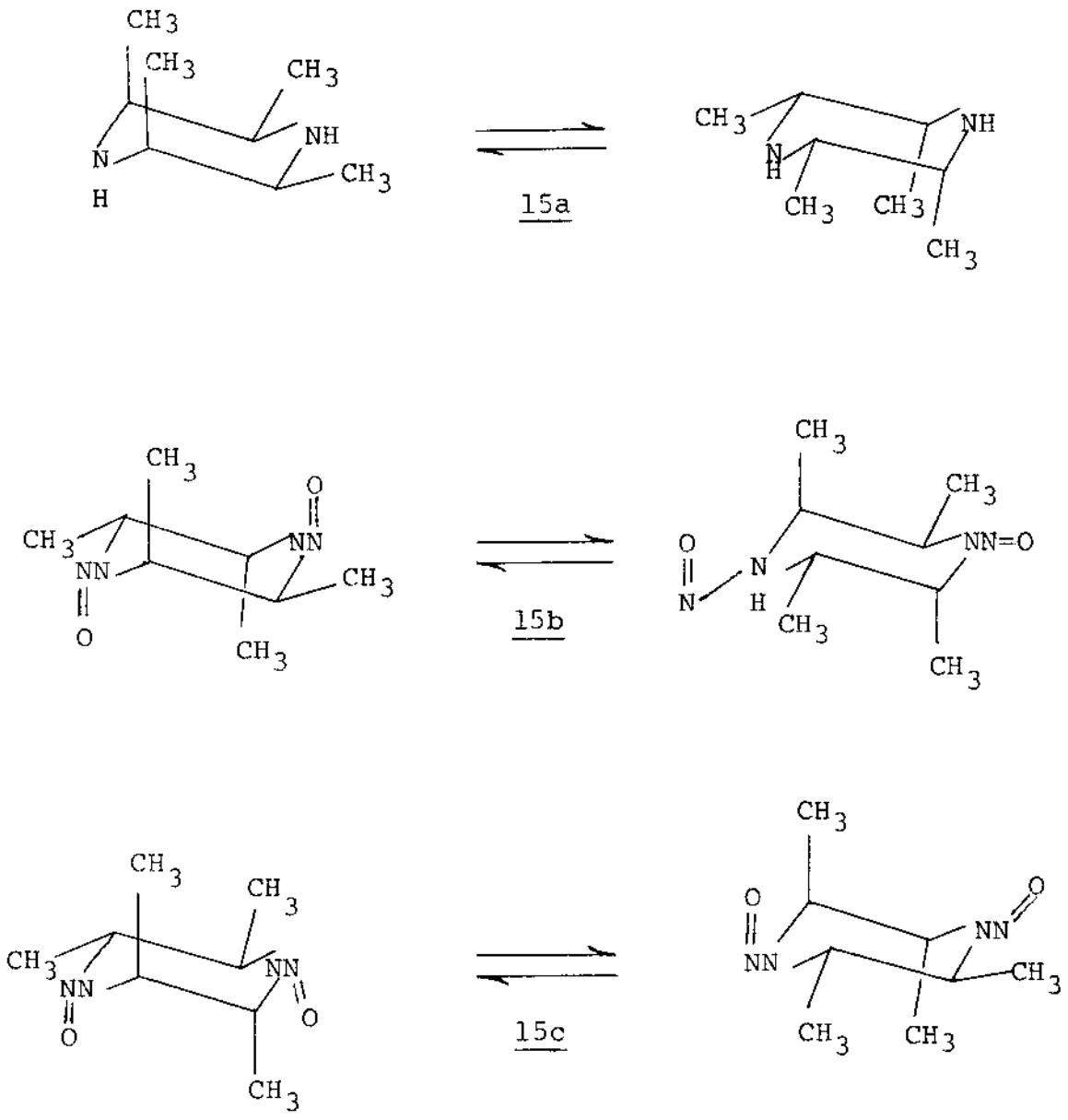


Fig. 9--Possible structures of 2,3,5,6-tetramethylpiperazine and its dinitroso derivative.

3 and 6 should show no deuterium exchange, while 12, 15, 45, 46, and 47 should undergo selective deuterium exchange.

The attempted deuterium exchange of the protons attached to the 2- and 6-positions of compounds 3 and 6 in sodium deuteroxide and deuterium oxide failed to show any change after 171 hr at 100°. Since the methyl substituents at the 2- and 6-positions of 3 and 6 are known to be axial, this lack of exchange provides inconclusive, negative evidence that the hydrogens alpha to a nitrosamine function in a cyclic system are acidic only if they are axially oriented.

Compound 47 exists in a single chair conformation with the methyl groups axial, leading to two doublets at δ1.1 and δ1.3 ppm in the NMR spectrum. A multiplet at δ3.5 ppm results from the diequatorial methine hydrogens at the 3- and 5-positions. The AB quartets at δ3.15 and δ4.3 ppm were assigned to the syn-axial and the anti-axial protons, respectively. The anti-equatorial hydrogen alpha to the nitrosamine function exhibits a doublet centered at δ4.45 ppm while the syn-equatorial hydrogen gives the same absorption pattern at δ4.8 ppm. Nitrosamine 47 was dissolved in deuterated dimethylsulfoxide containing deuterium oxide and sodium deuteroxide, and the resulting mixture was heated to 100°C. Complete exchange of the two alpha-axial hydrogens took place within 15 min. The NMR signal for the alpha-equatorial protons collapsed into one broad absorption band.

No further change in the spectrum was observed after heating the reaction mixture for 13 hr.[10]

Nitrosamine 46 exists primarily in the chair conformation with the two methyl groups in the equatorial orientation. The NMR spectrum of 46 shows a doublet at δ4.83 ppm for the syn- and anti-equatorial protons. Signals at δ2.8 and δ3.8 ppm correspond to the syn- and anti-axial protons alpha to the nitrosamine function. Exchange of the two axial protons in the favored chair conformation took place within 20 min. The doublet at δ4.8 ppm became a singlet and gradually disappeared on continued heating of the reaction mixture at 100°. The exchange of these protons, equatorial in the preferred conformation, probably occurred because ring inversion to a boat-like conformation is possible with only a slight increase in non-bonded repulsion. In this boat form the alpha hydrogen originally equatorial becomes nearly axially oriented. The deuterium exchange of the compound 45 has been reported.[9]

The 100 MHz ^{1}H NMR spectrum of 12 in DMSO-d_6 exhibits a multiplet at δ4.2 for the syn-axial and anti-equatorial protons. The syn- and anti-axial alpha-hydrogens appear at δ2.7 and δ2.9 ppm, respectively, as broad doublets. The exchange experiment with the nitrosamine 12 was carried out in DMSO-d_6 containing sodium deuteroxide in deuterium oxide solution at 100°. The exchange was followed by the disappearance of the resonance signal for the alpha-axial protons

at δ2.7 and δ2.9 ppm, and the collapse of the multiplet at δ4.2 ppm for the alpha-equatorial protons to a pair of broad doublets. Complete exchange of the four alpha protons occurred after 45 hr. The temperature was sufficient to allow a conformational inversion of the ring causing the alpha-equatorial protons to become axial.

The ^{1}H NMR spectrum of 15 gives the two methyl doublets at δ1.1 and δ1.65, and a multiplet at δ4.5 ppm for the alpha protons. Here, the exchange was followed by the collapse of the methyl doublets to singlets. As a result of the decreased coupling of the deuterium as compared to protons, exchange of the methine protons occurred after 24 hr at 80°C or 1.5 hr at 100°C. These results are summarized in Table VIII.

It is reasonably clear from the above experiments that the H-D exchange is subject to stereoelectronic control. The hydrogens alpha to a nitrosamine function in a six-membered cyclic system are acidic only if they are axially oriented. This observation supports the hypothesis that the anion formed by removal of the alpha hydrogen is stabilized by the overlap of the unshared electrons with the pi-system of the nitrosamine function, and it is these hydrogens which are most likely to be reactive in an enzymatic reaction as well. These data do not allow a distinction to be made between the syn- and anti-axial hydrogens; however, the results of Fraser[8] suggest that the syn-hydrogen is more reactive than

TABLE X

H-D EXCHANGE STUDIES OF VARIOUS NITROSAMINES

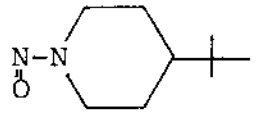
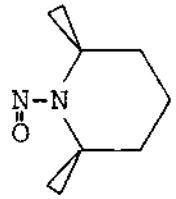
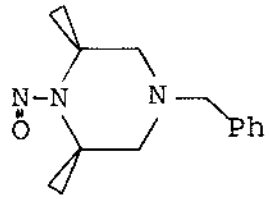
			Time (hr)	Exchange	Temp.	Stereochemistry
1)		(45)	0.5	2 H's	100°	Axial
2)		(3)	171	None	100°	-----
3)		(6)	171	None	100°	-----

Table X . (continued)

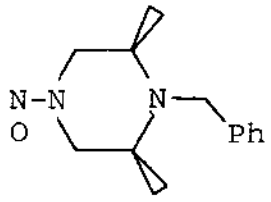
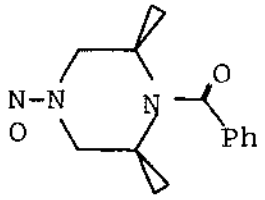
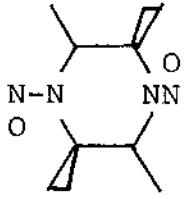
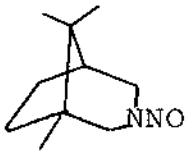
			Time (hr)	Exchange	Temp.	Stereochemistry
4)		(46)	0.3	4 H's	100°	Axial
5)		(47)	0.25	2 H's	100°	Axial
6)		(15)	1.5	4 H's	100°	Axial
			24	4 H's	80°	Axial

Table X. (continued)

			Time (hr)	Exchange	Temp.	Stereochemistry
7)		(12)	45	4 H's	100°	Pseudo-axial

the anti-hydrogen.

The results of this study have shown that (1) the highly polar chromophore of nitrosamines can complex to molecules via hydrogen bonding or other mechanisms; (2) this effect is easily observed by ICD, which can be used to relate the absolute configuration of alcohols and amines to the sign of the ICD; (3) the solubility properties of nitrosamines are altered by complexation with polyols; and (4) these results confirm that a proton in the axial position alpha to the nitrosamine function is acidic and is probably an important biochemical feature of the metabolism of nitrosamines which are carcinogenic.[12]

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APPENDIX A

NUCLEAR MAGNETIC RESONANCE SPECTRA

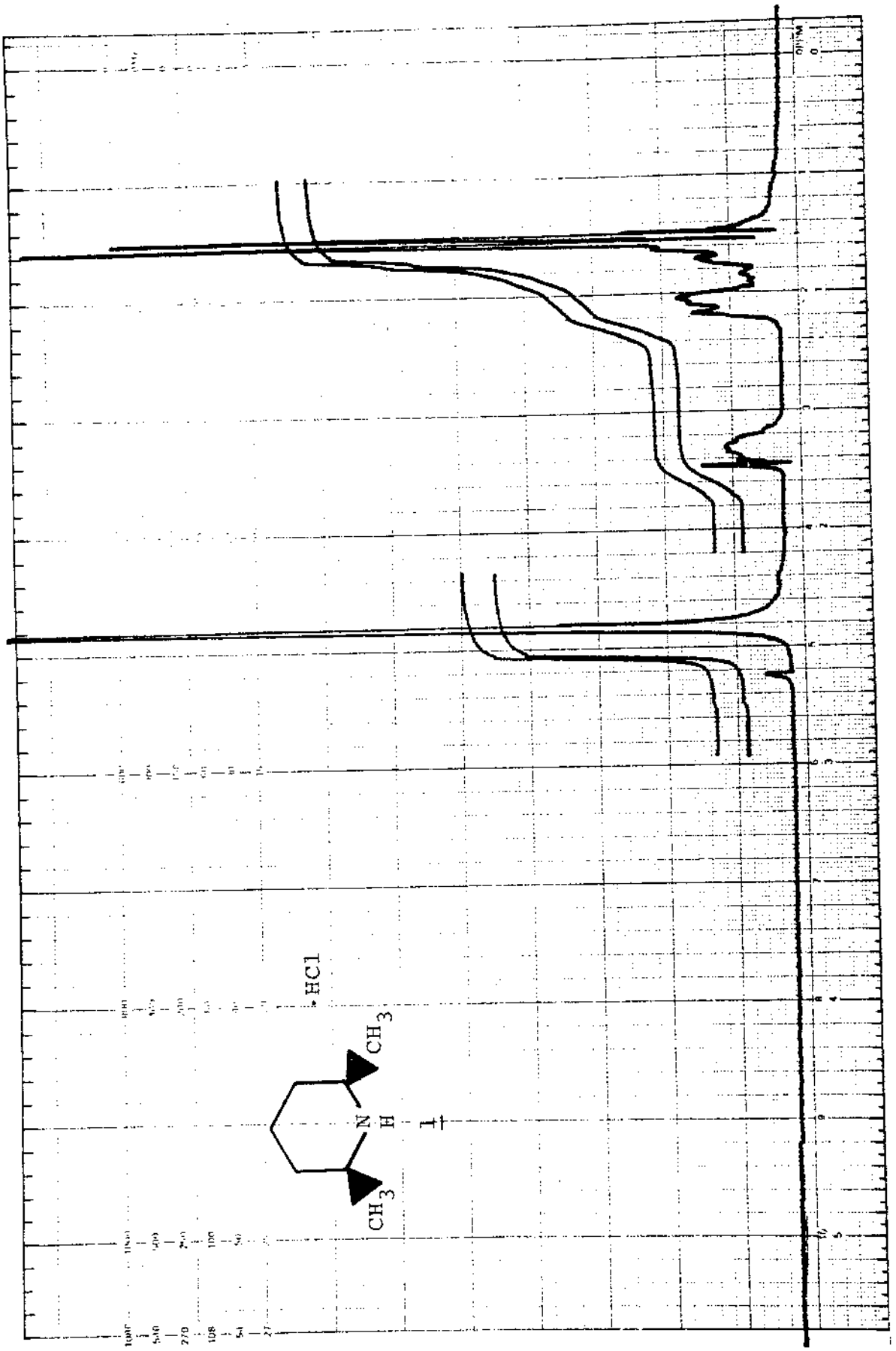


Fig. 1--The NMR spectrum of compound 1.

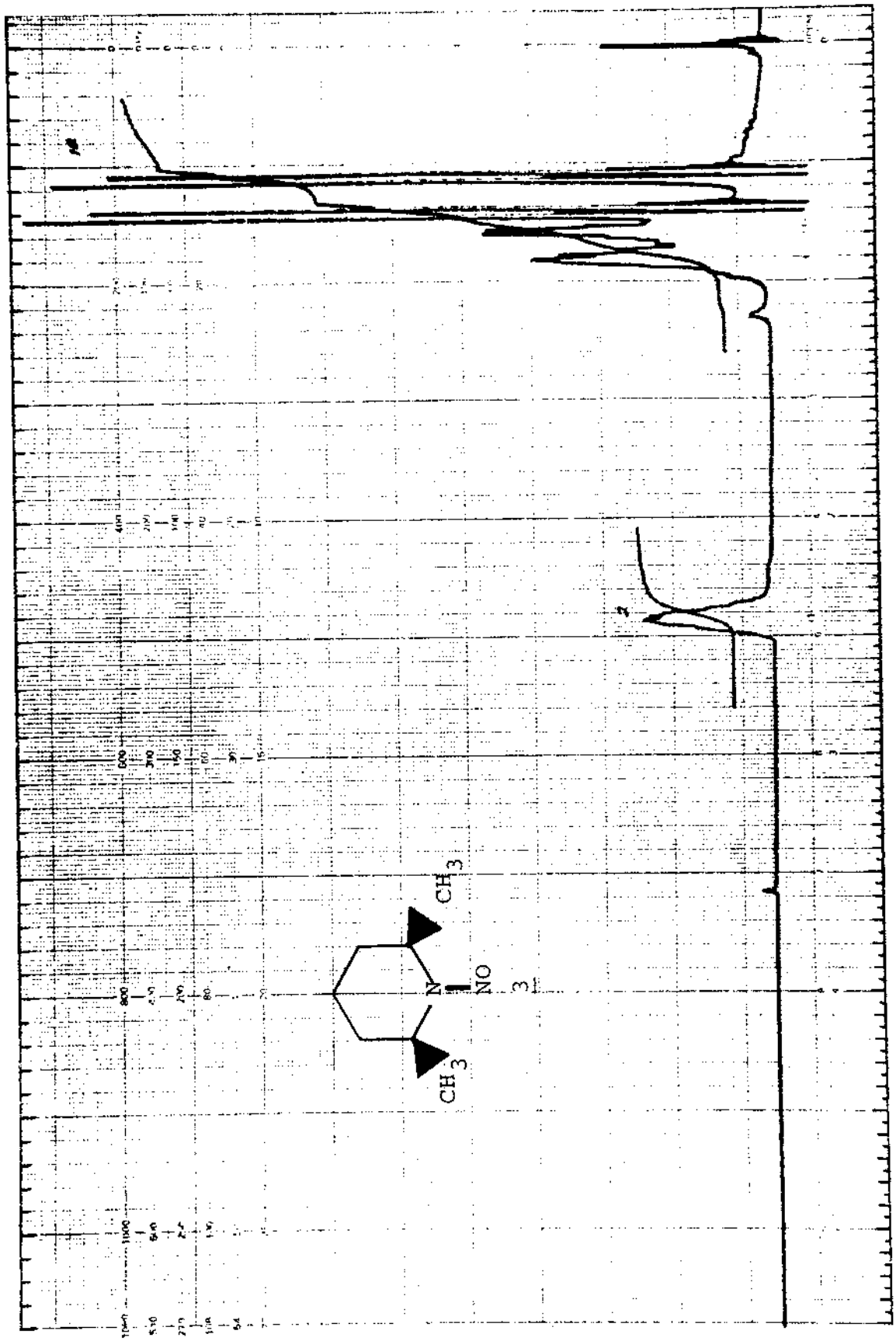
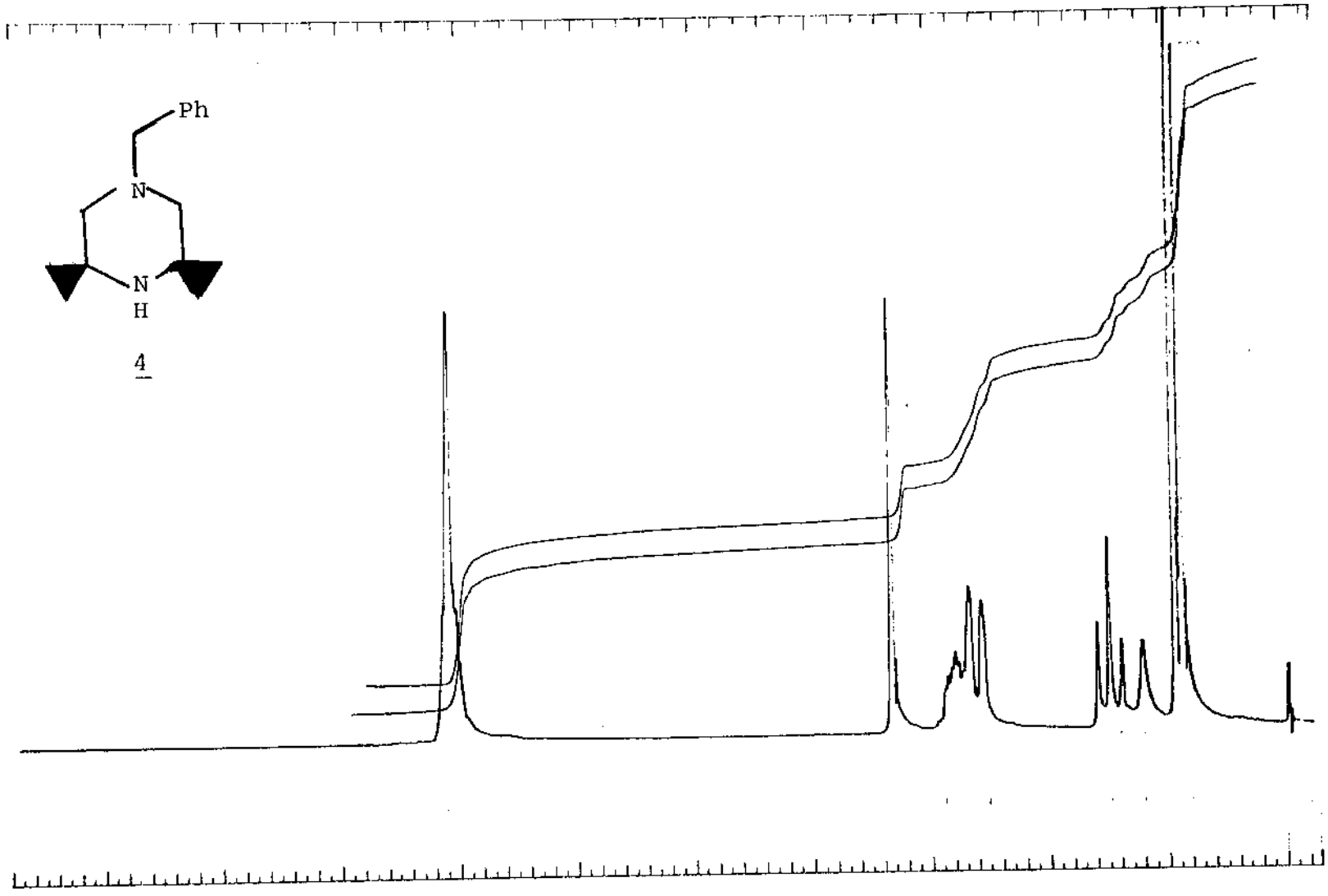


Fig. 2--The NMR spectrum of compound 3.



4

Fig. 3--The NMR spectrum of compound 4.

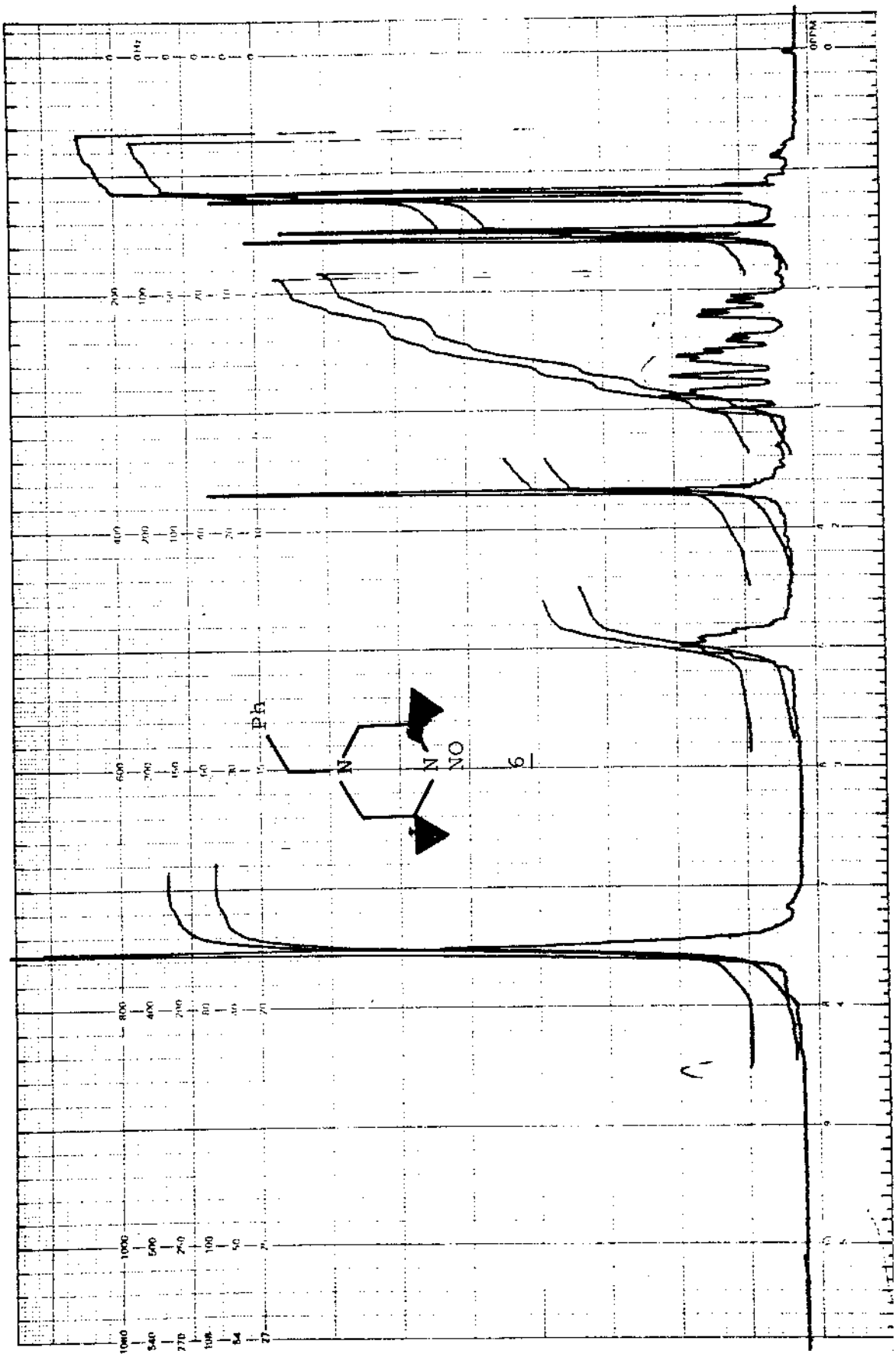


Fig. 4--The NMR spectrum of compound 6.

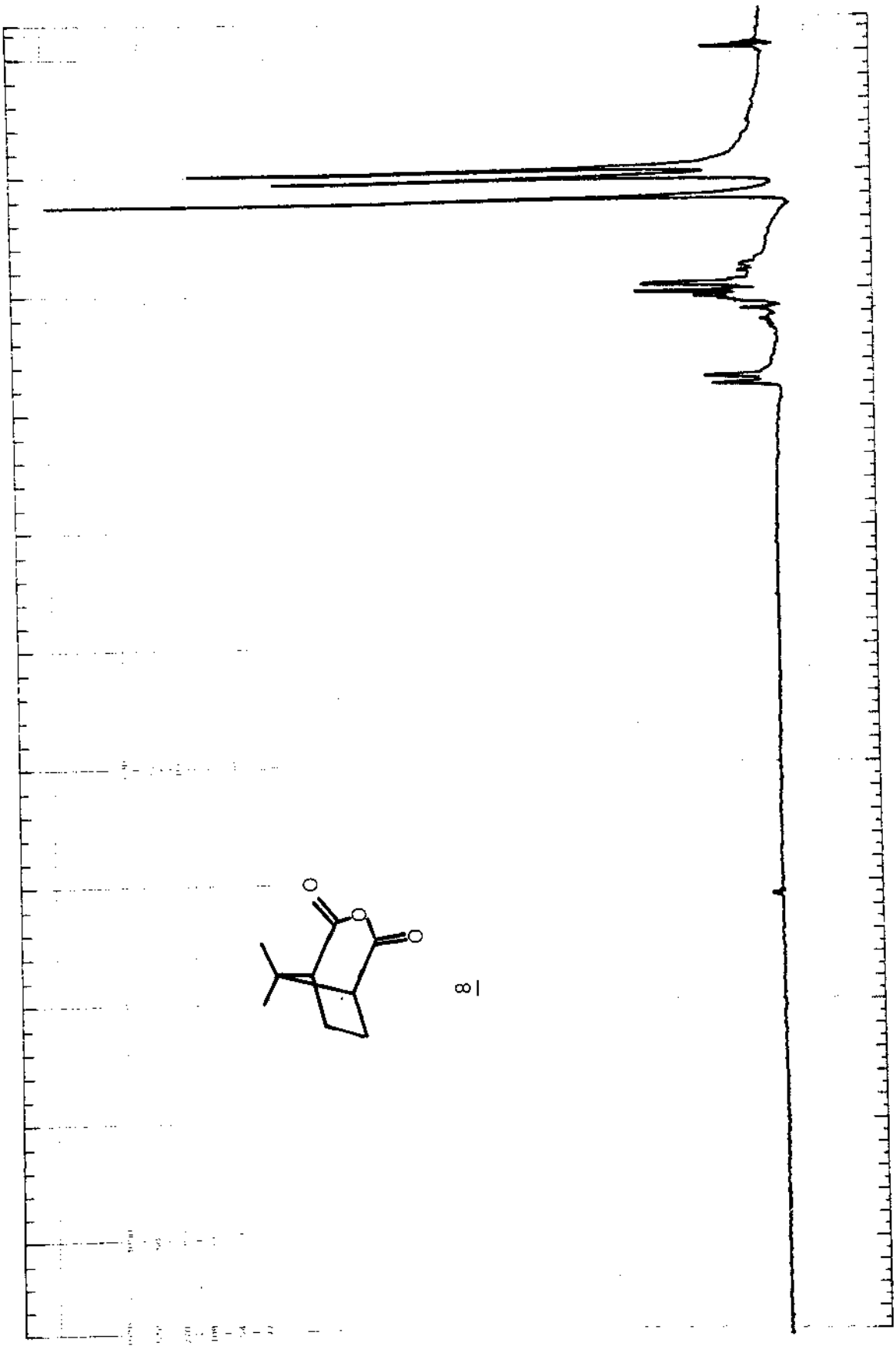


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Fig. 5--The NMR spectrum of compound 8.

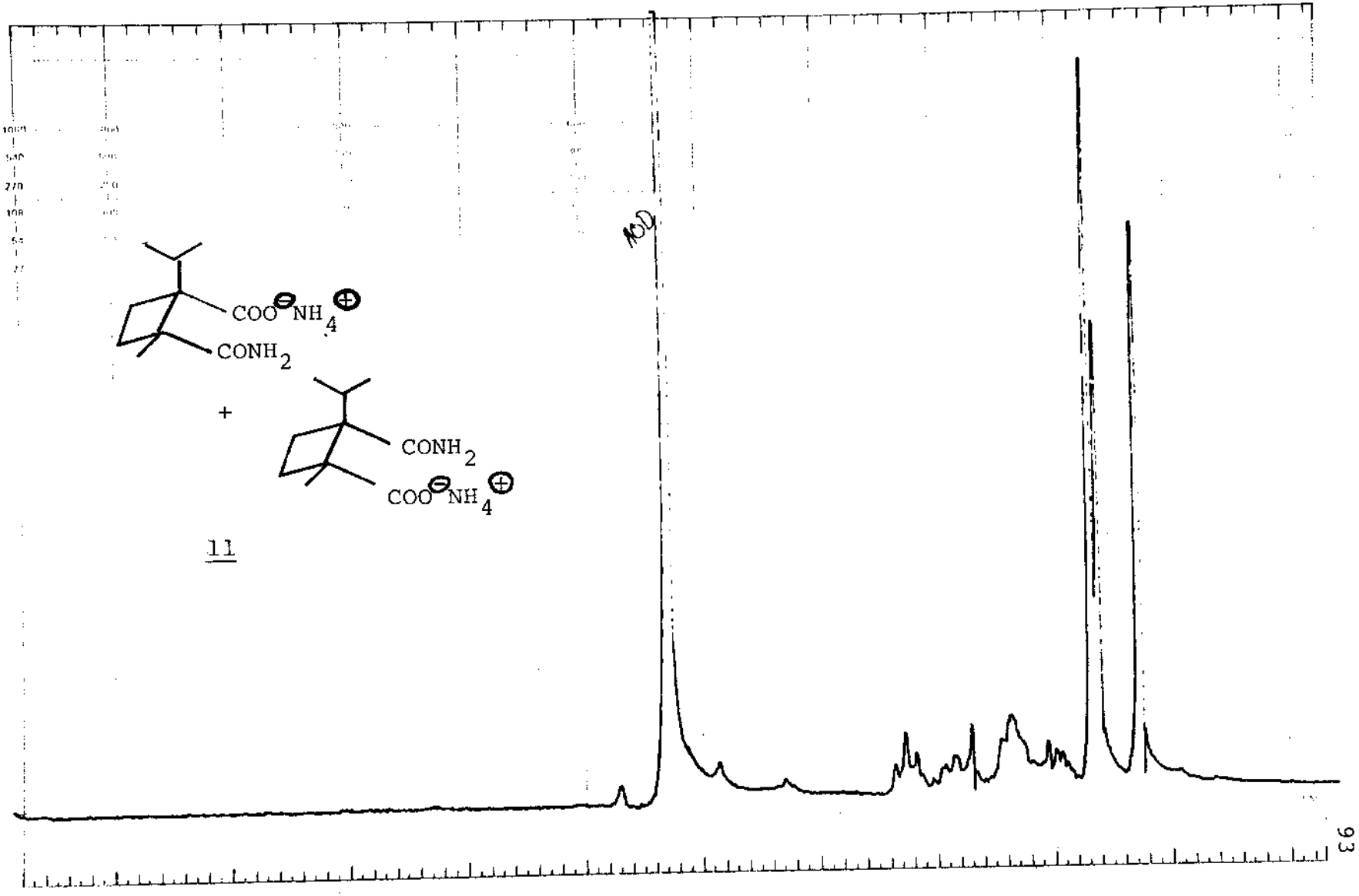


Fig. 6--The NMR spectrum of compound 11.

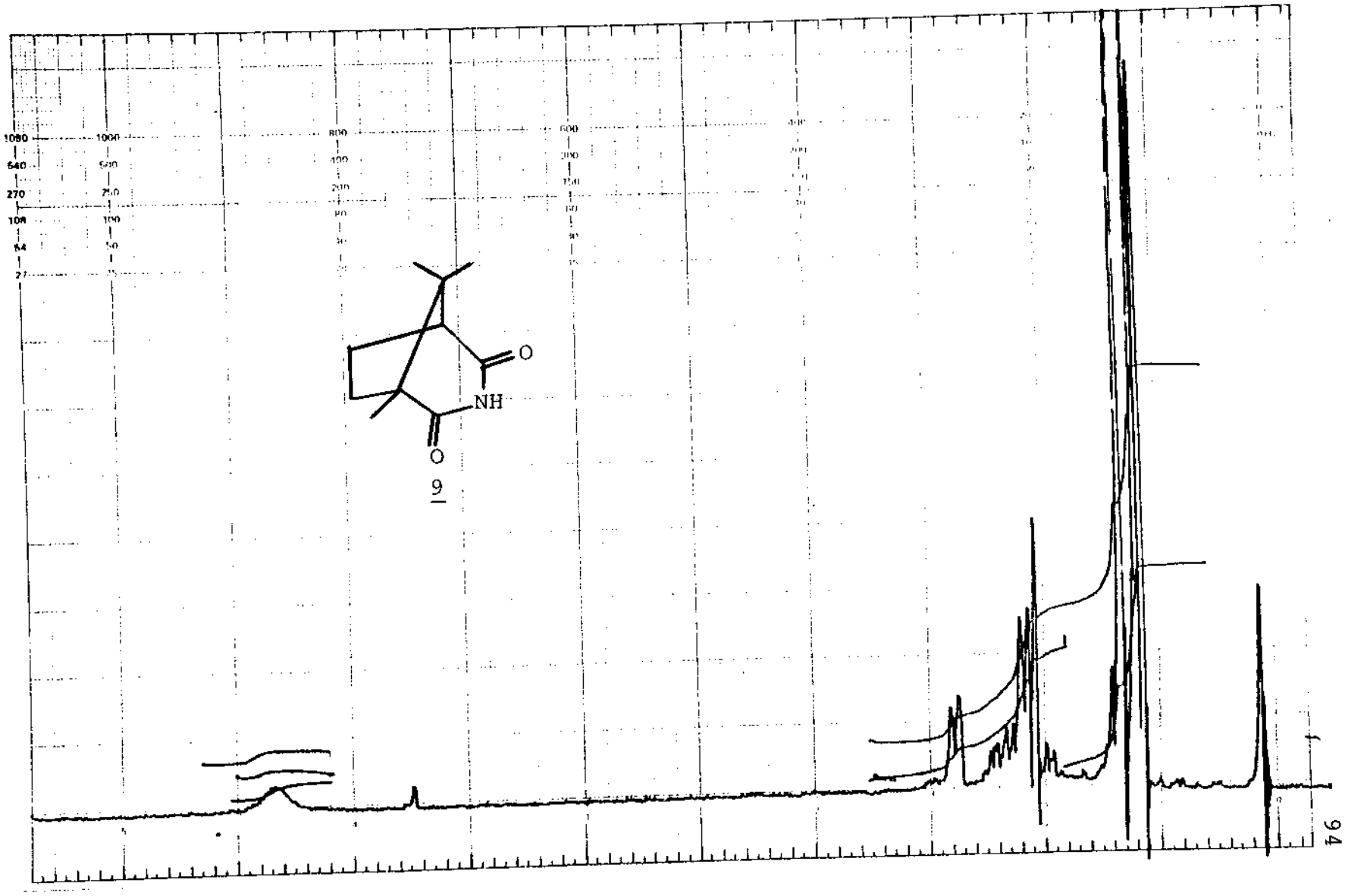
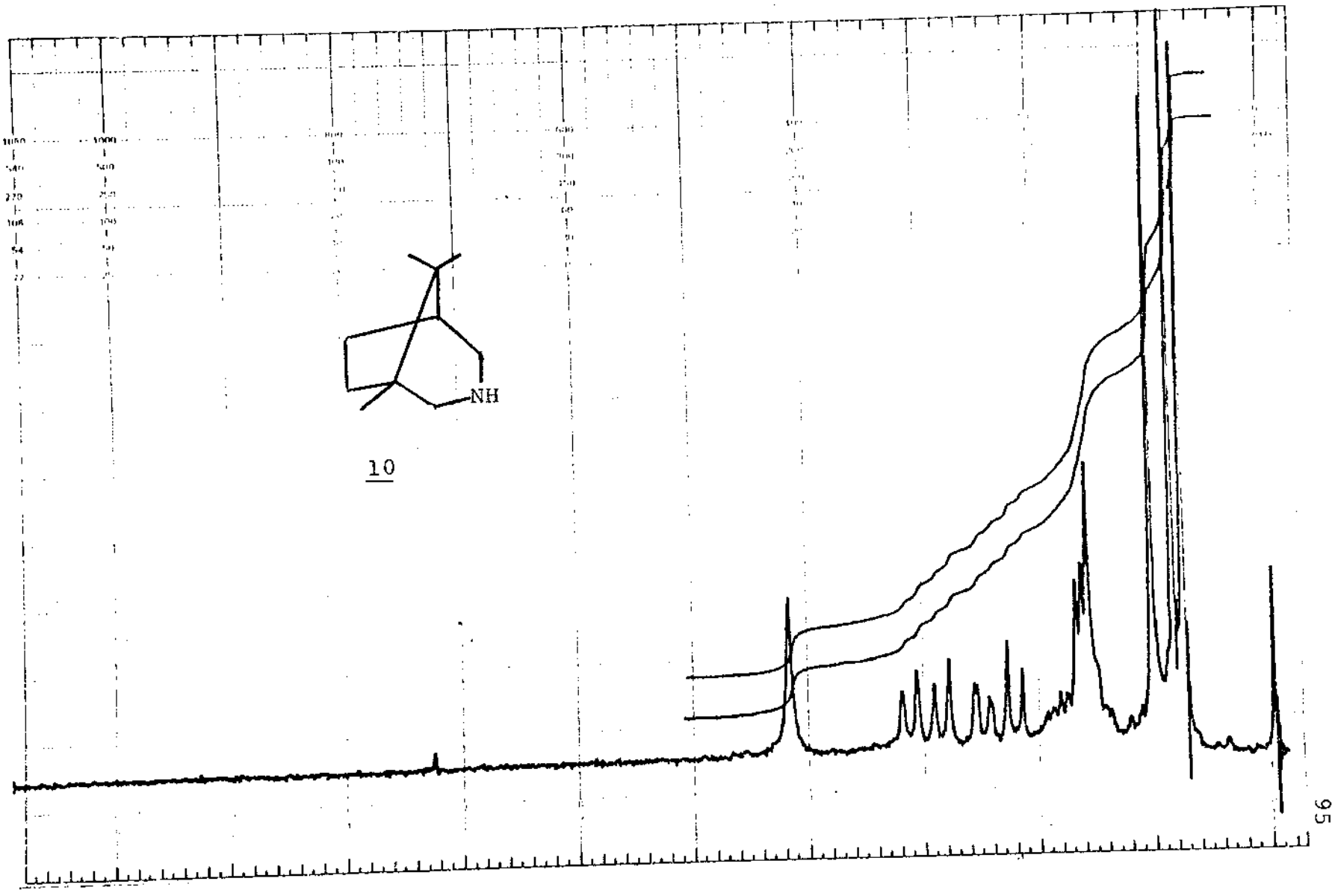


Fig. 7--The NMR spectrum of compound 9.



10

Fig. 8--The NMR spectrum of compound 10.

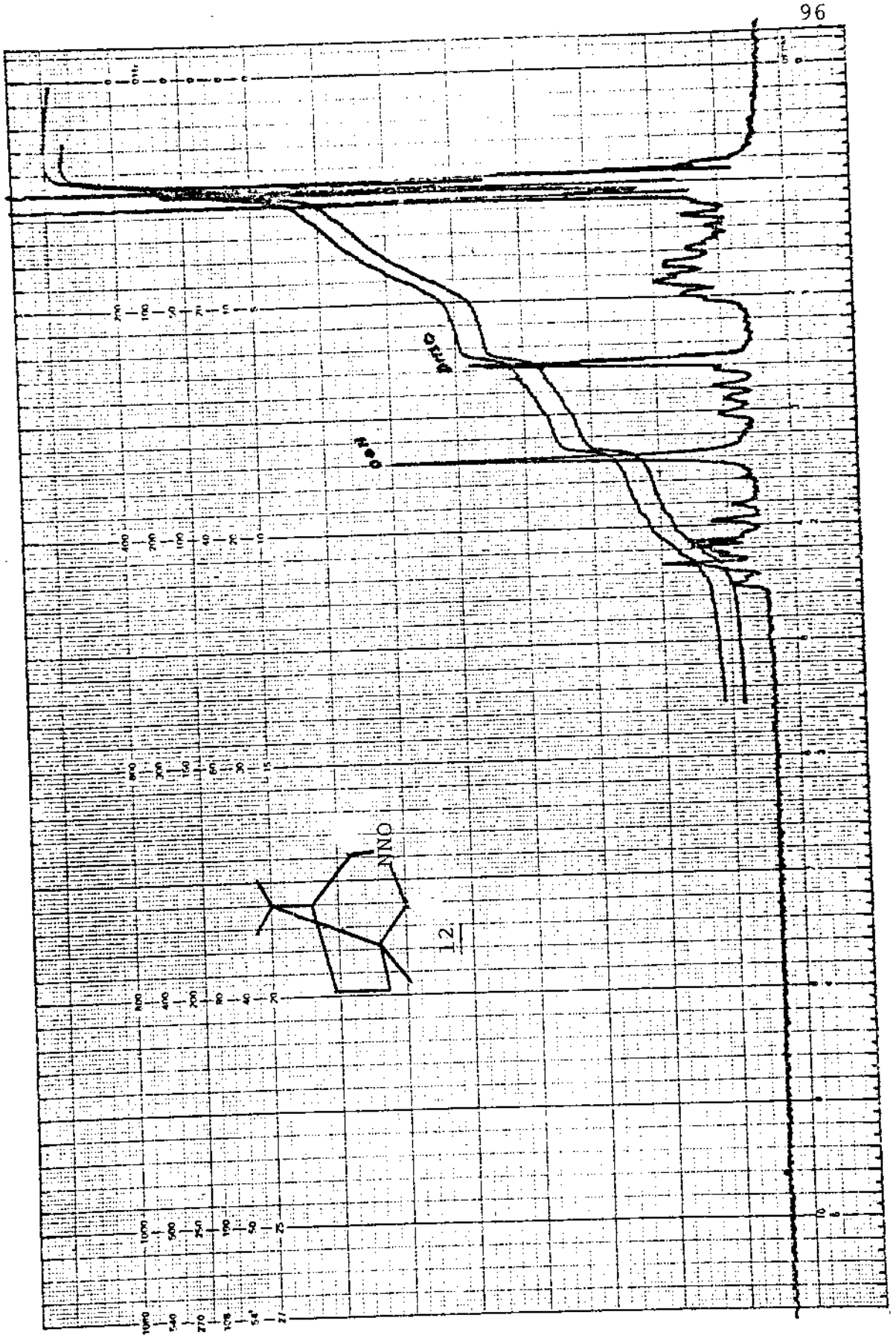


Fig. 9--The NMR spectrum of compound 12.

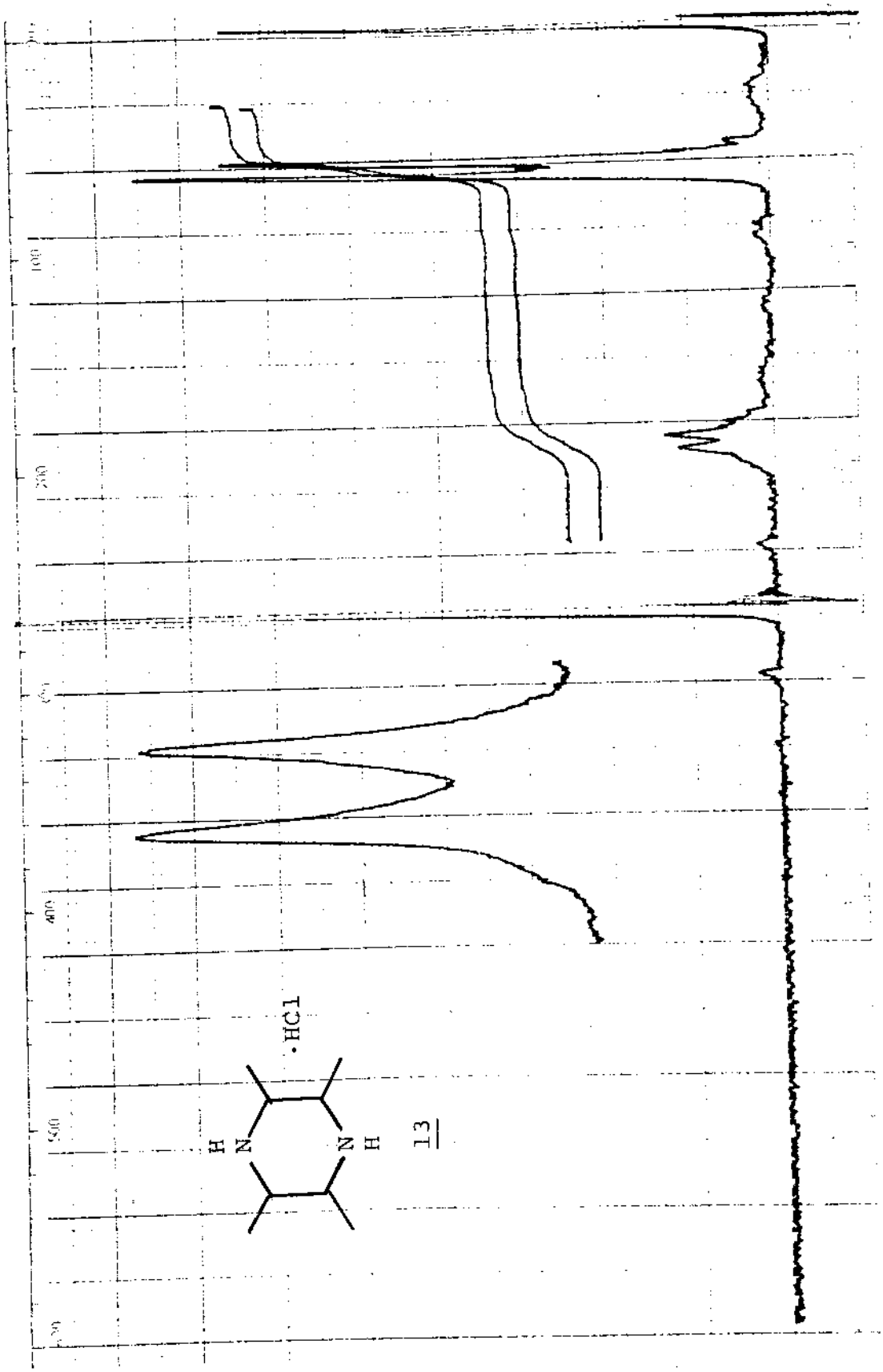


Fig. 10--The NMR spectrum of compound 13.

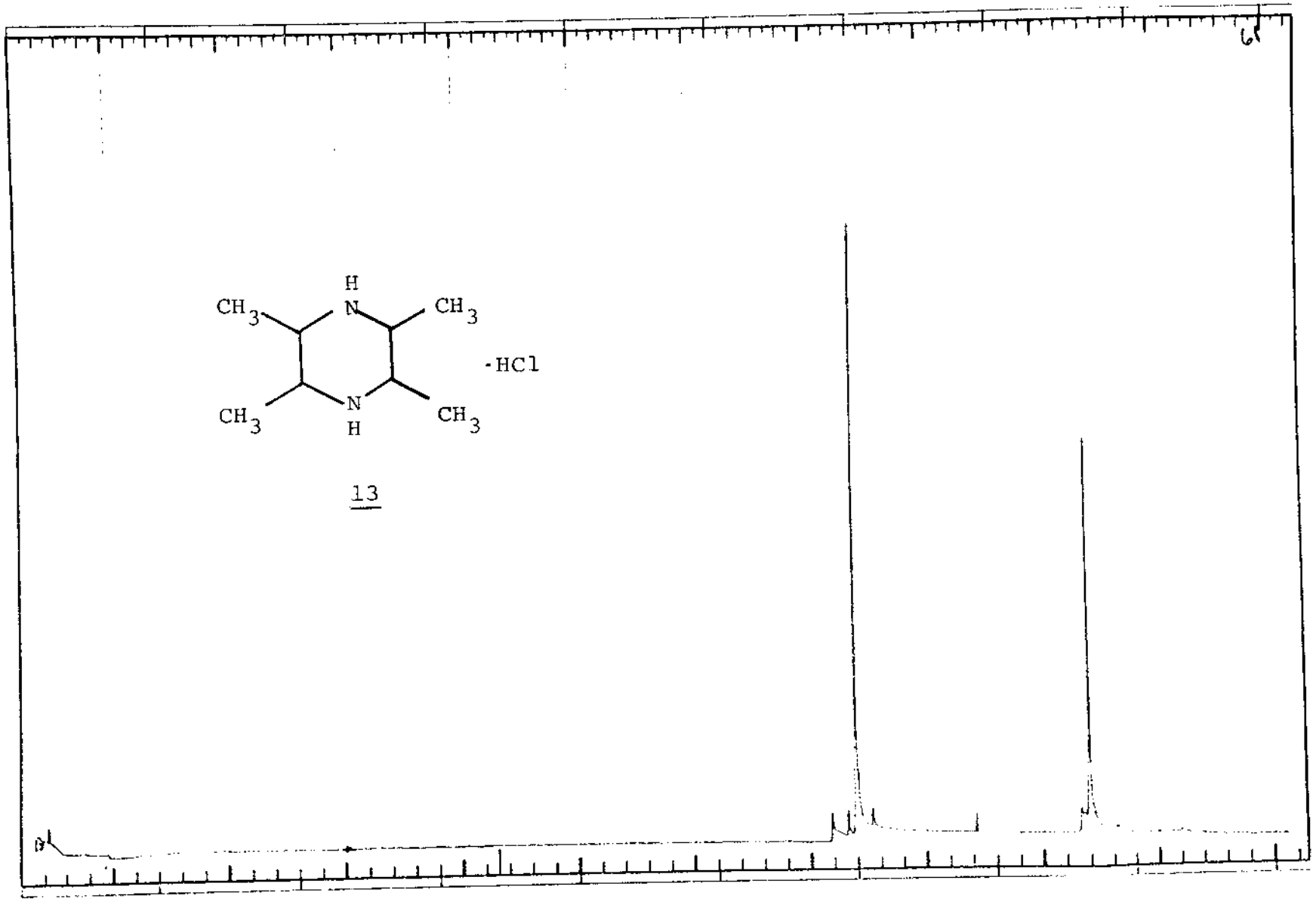


Fig. 11--The ^{13}C NMR spectrum of compound 13.

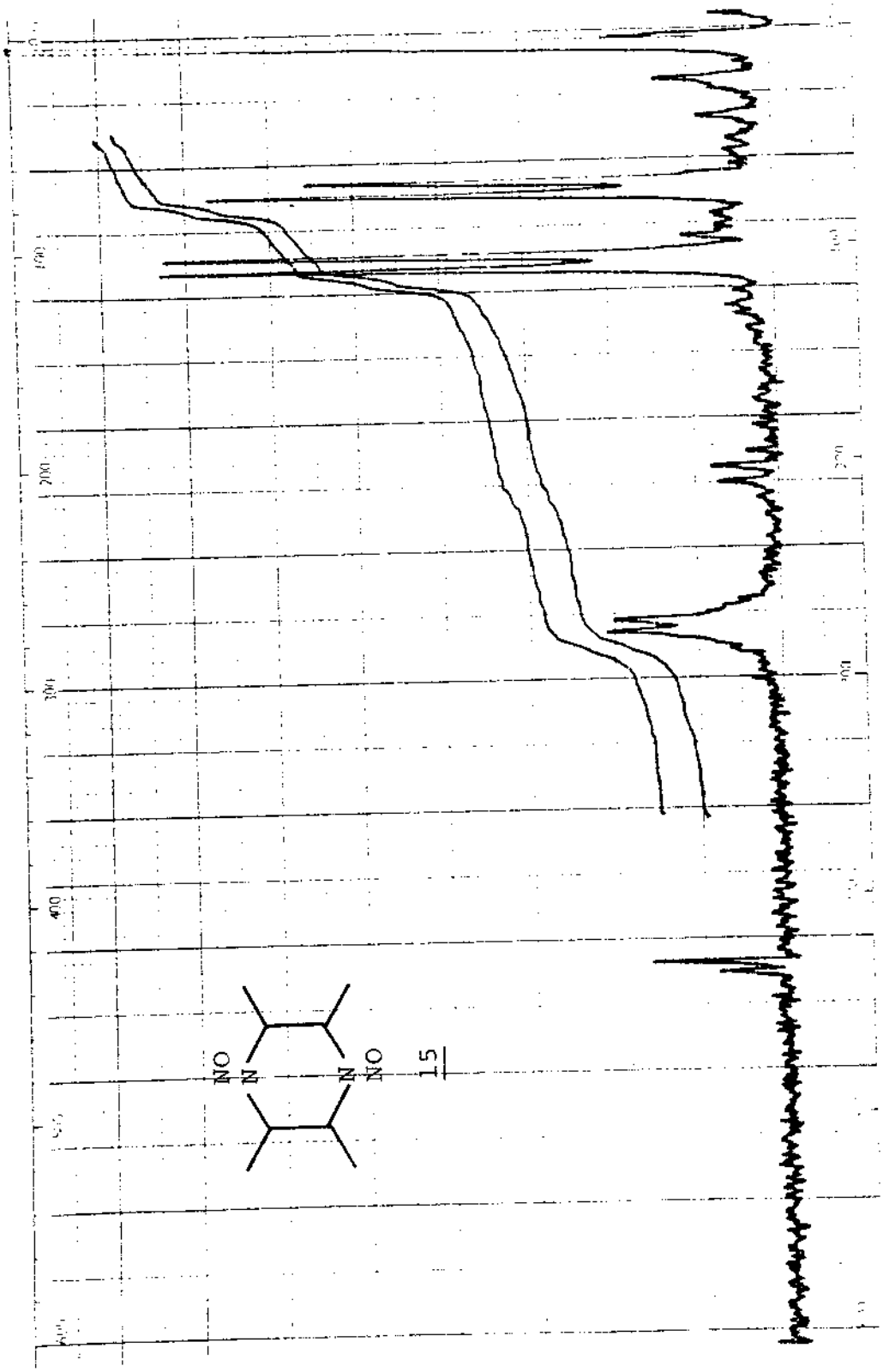
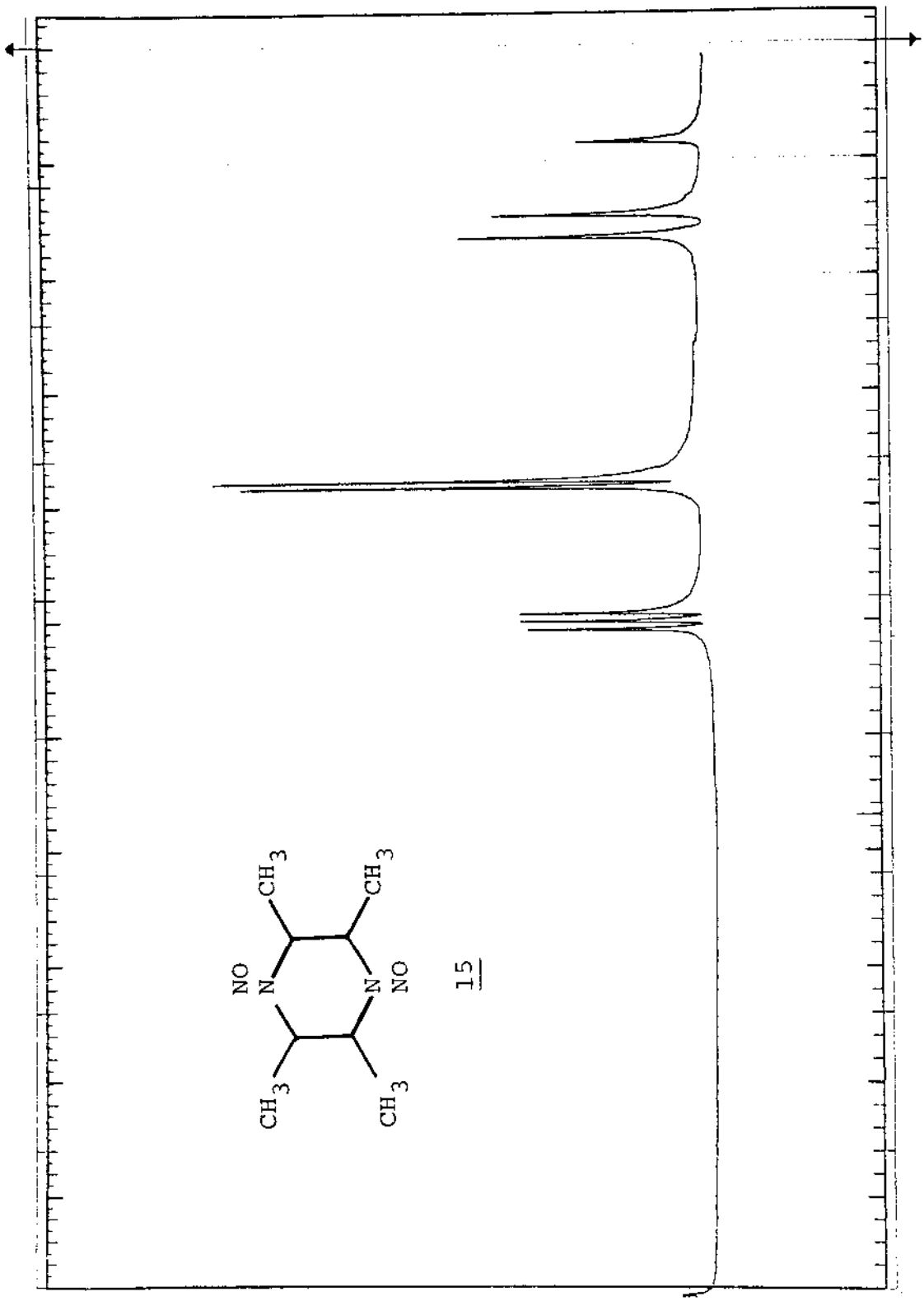


Fig. 12--The NMR spectrum of compound 15.



15

Fig. 13--The ^{13}C NMR spectrum of compound 15.

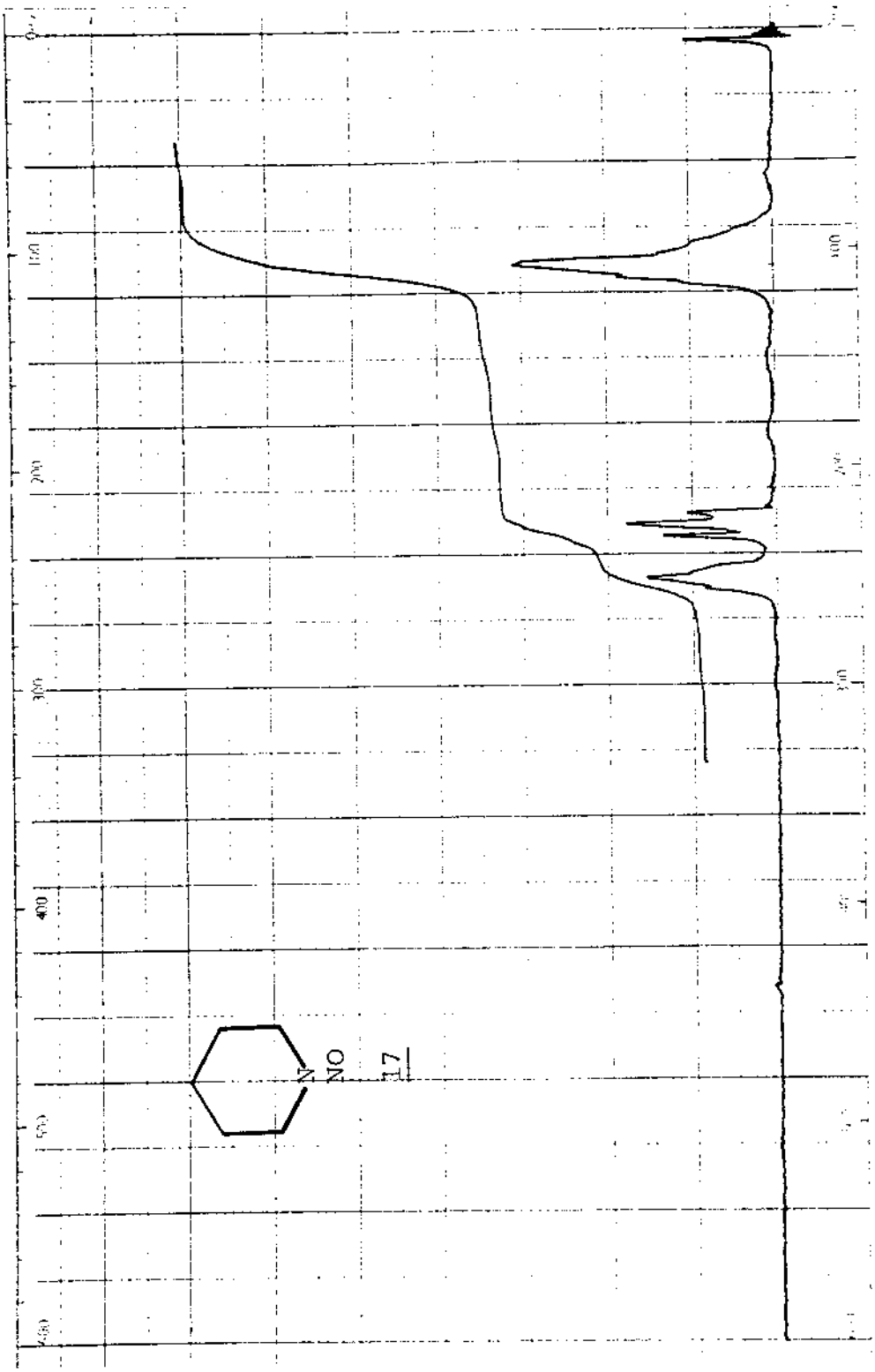


Fig. 14--The NMR spectrumof compound 17.

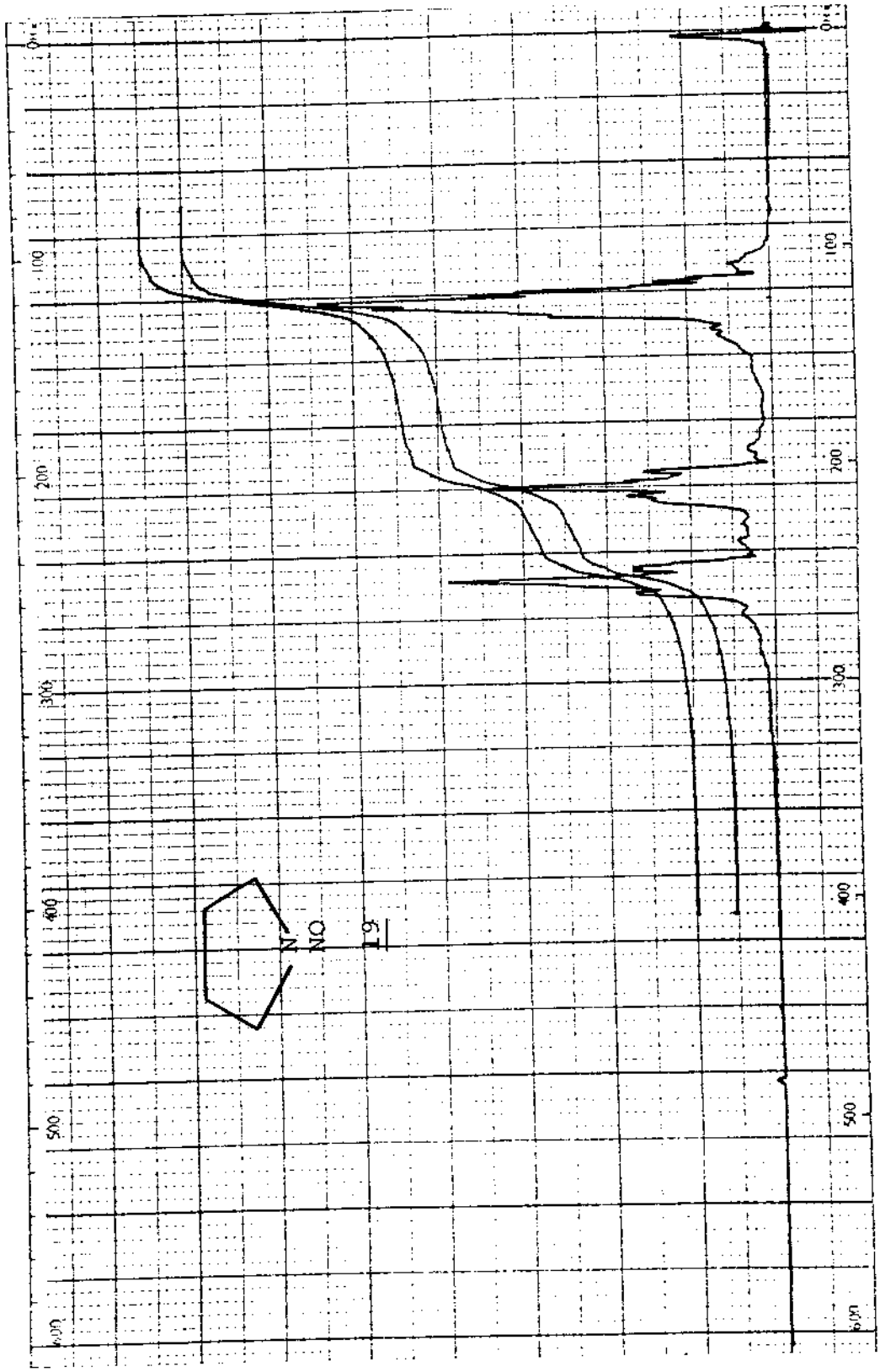


Fig. 15--The NMR spectrum of compound 19.

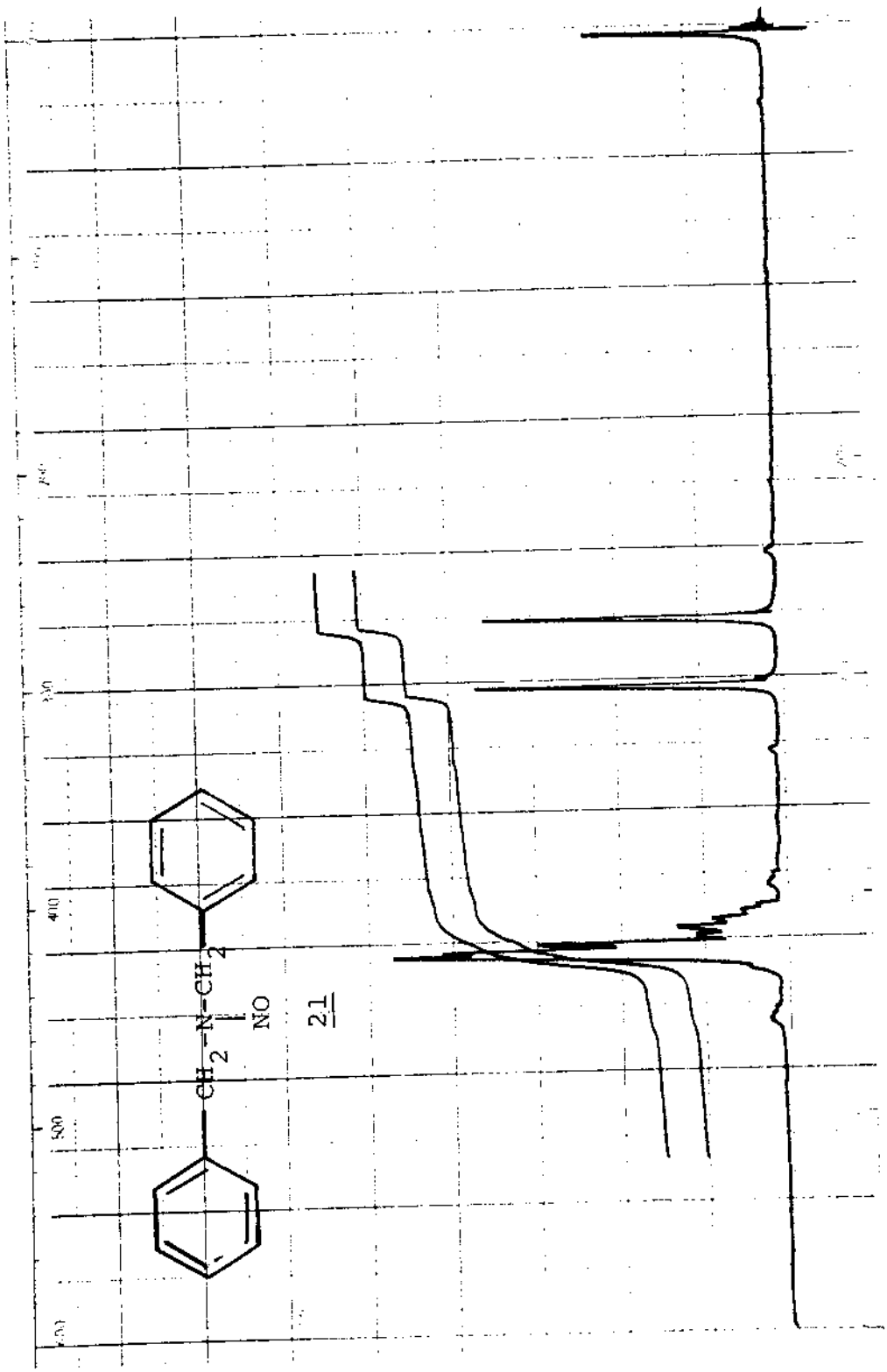
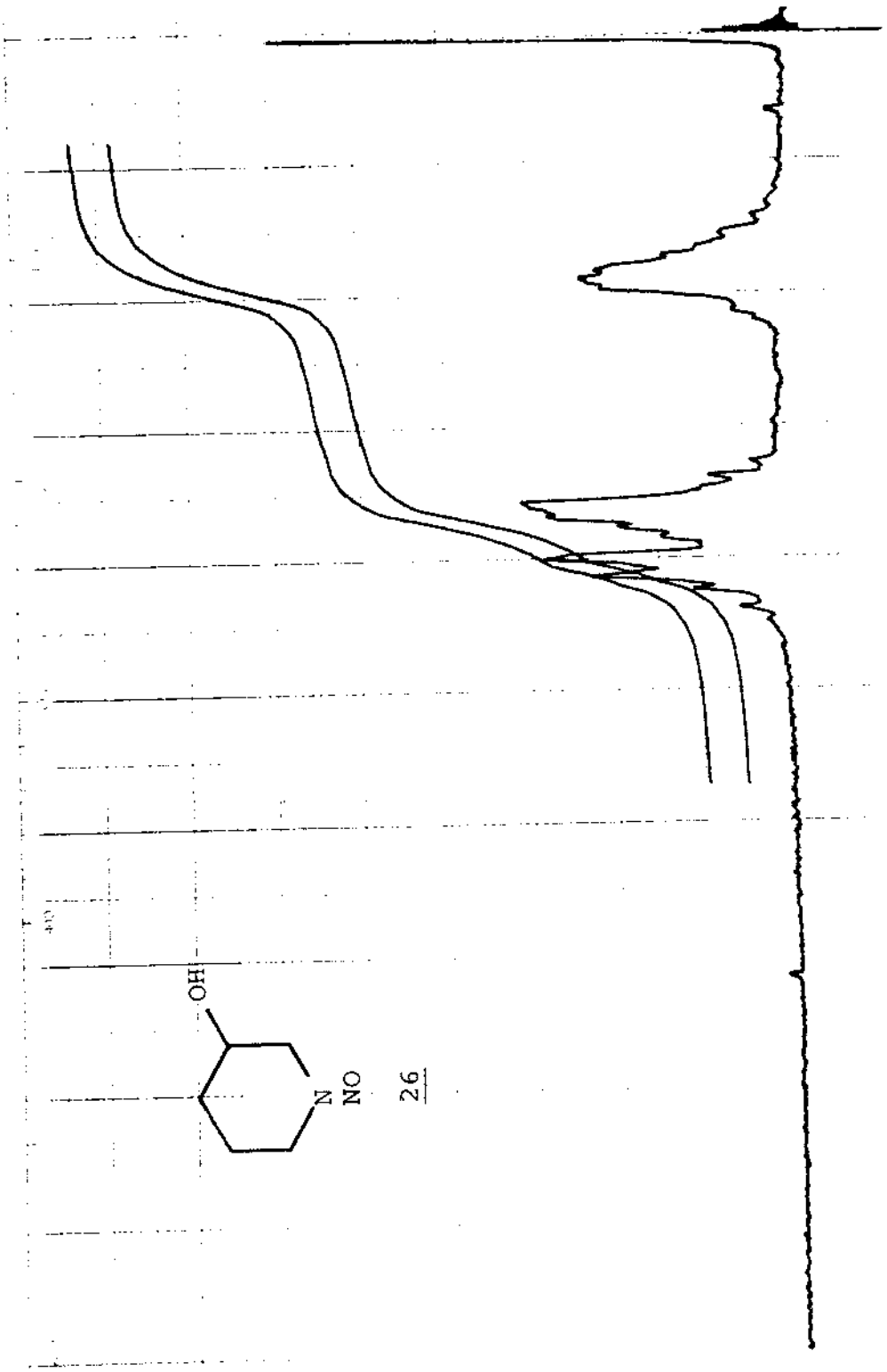


Fig. 16--The NMR spectrum of compound 21.



26

Fig. 17--The NMR spectrum of compound 26.

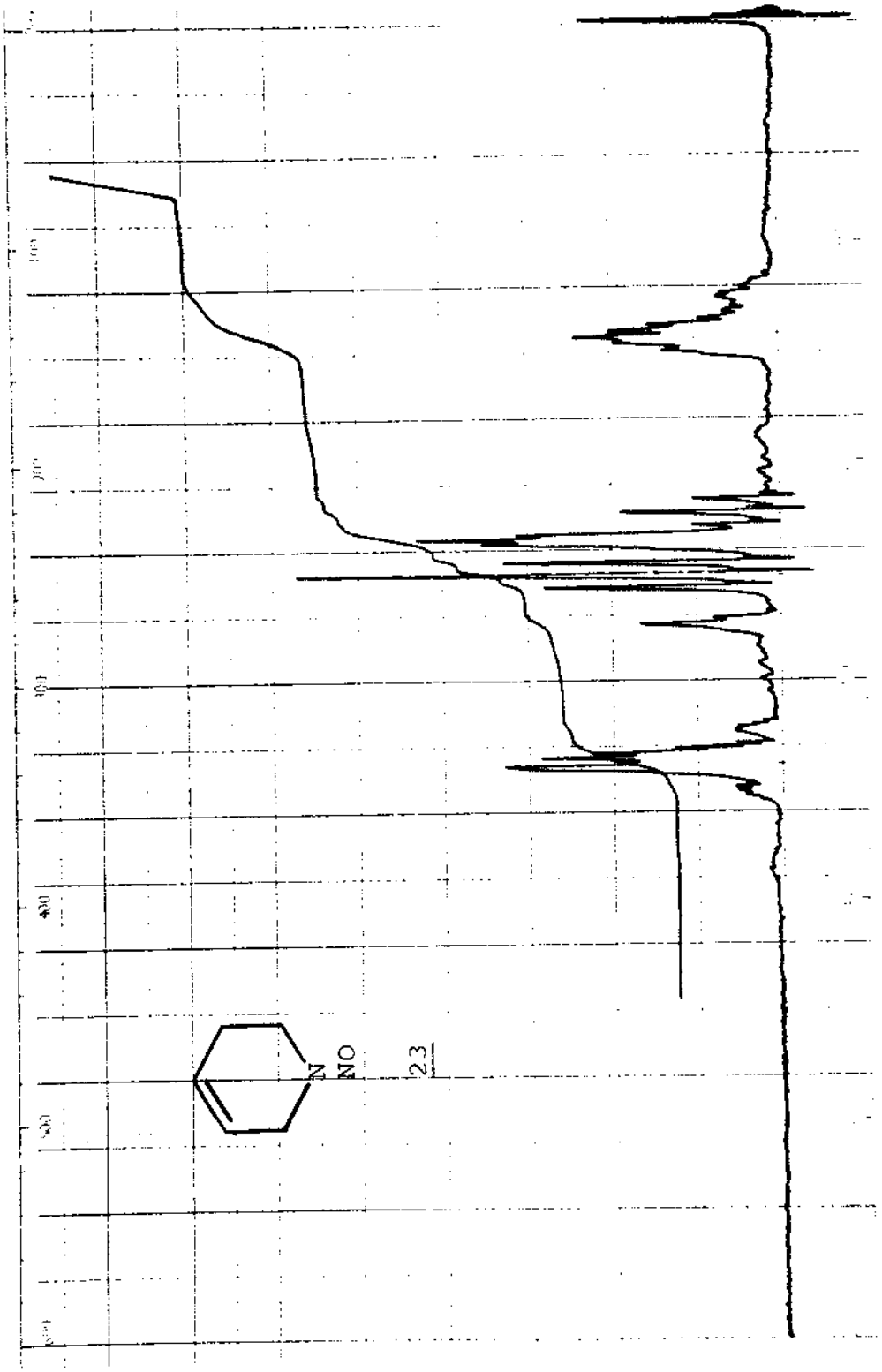
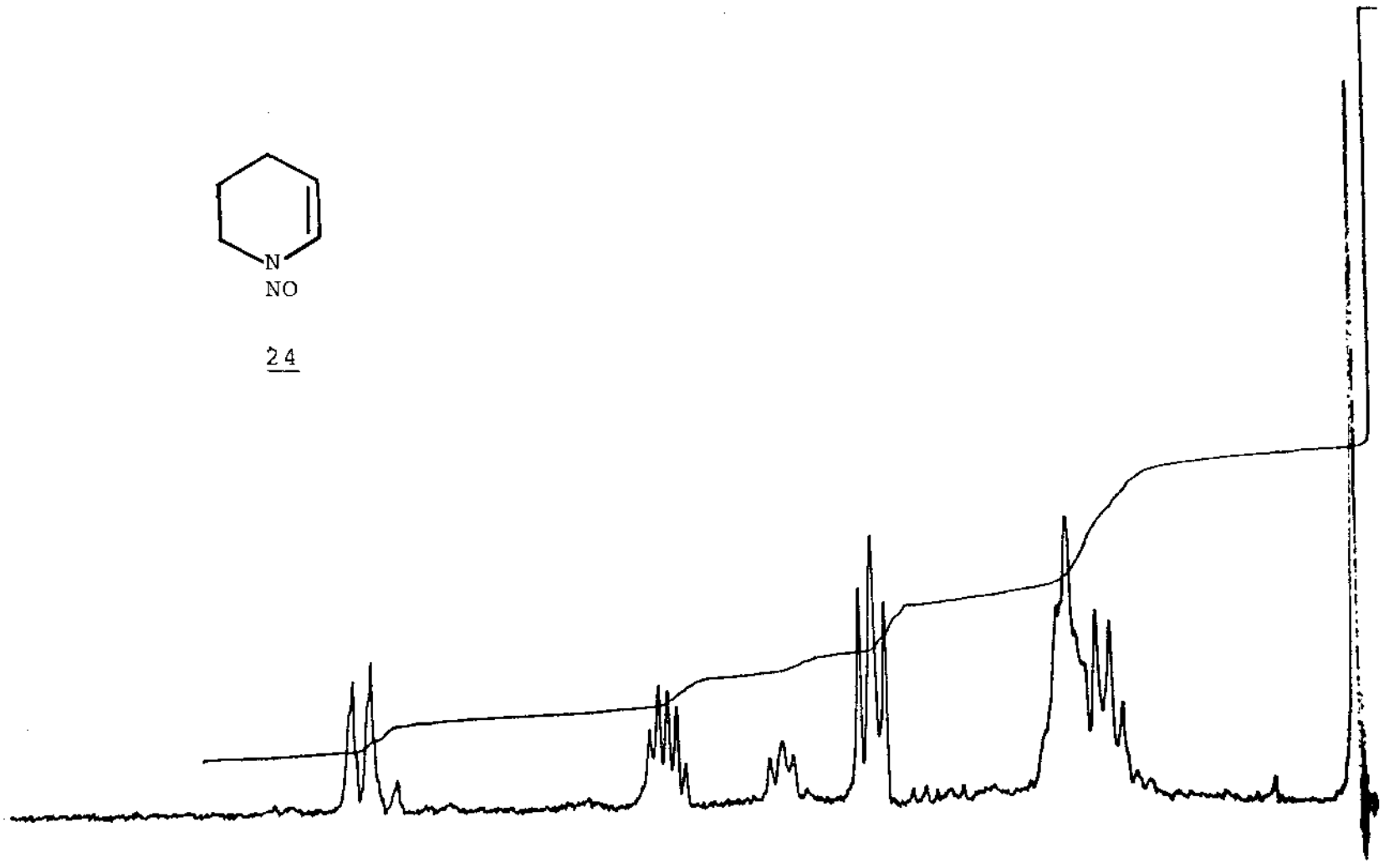


Fig. 18--The NMR spectrumof compound 23.



24

Fig. 19--The NMR spectrum of compound 24.

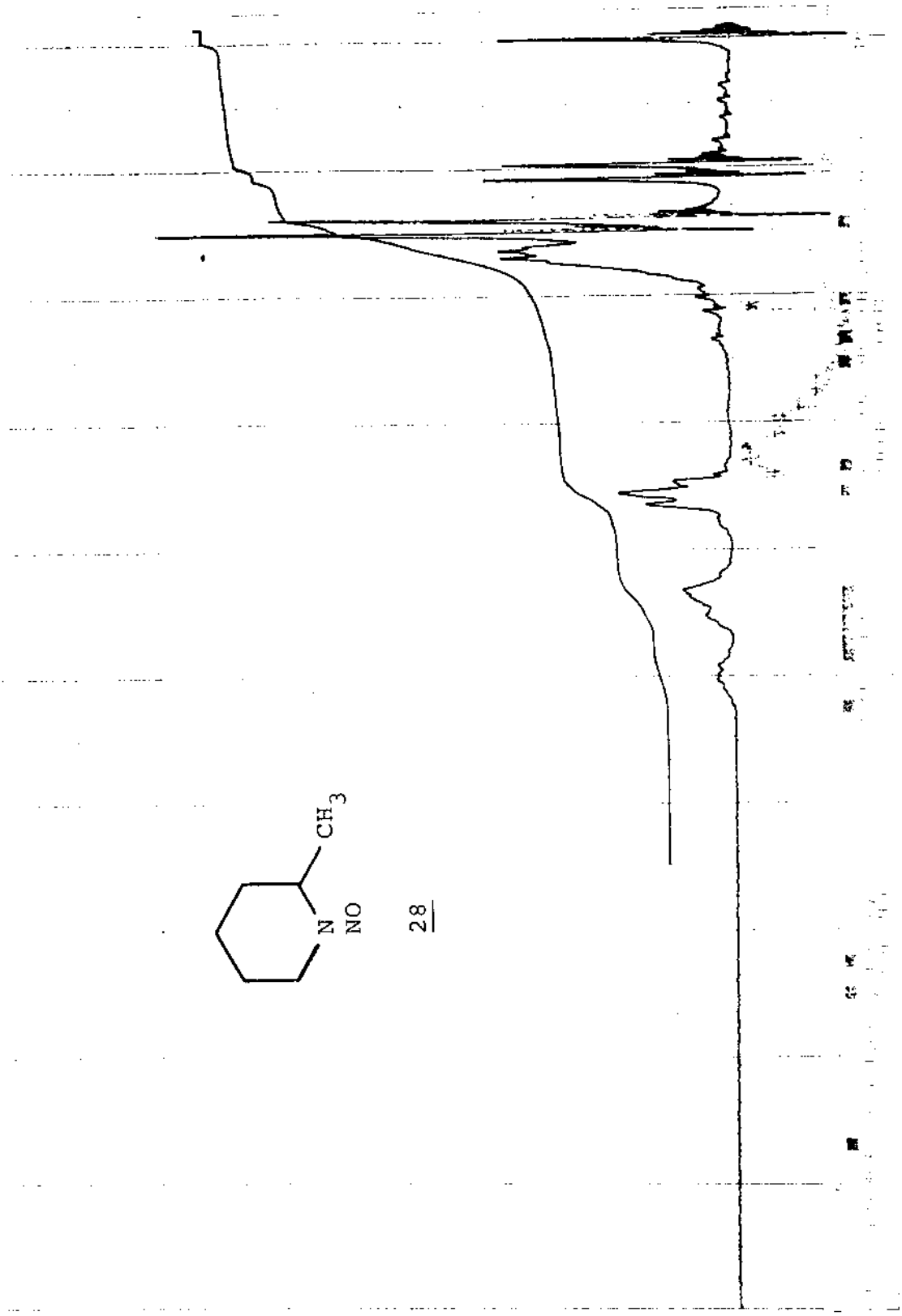


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Fig. 20--The NMR spectrum of compound 28.

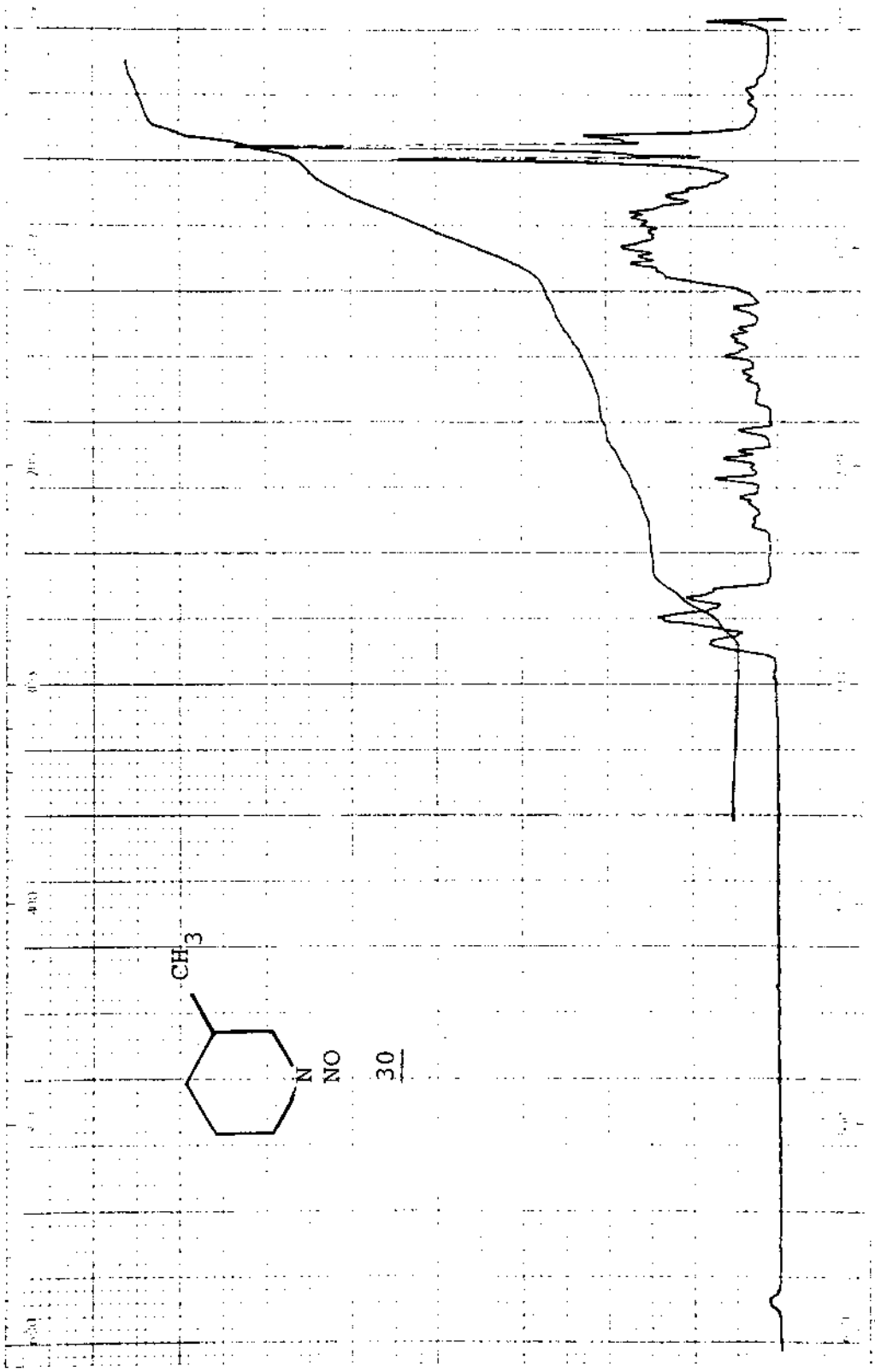


Fig. 21--The NMR spectrum of compound 30.

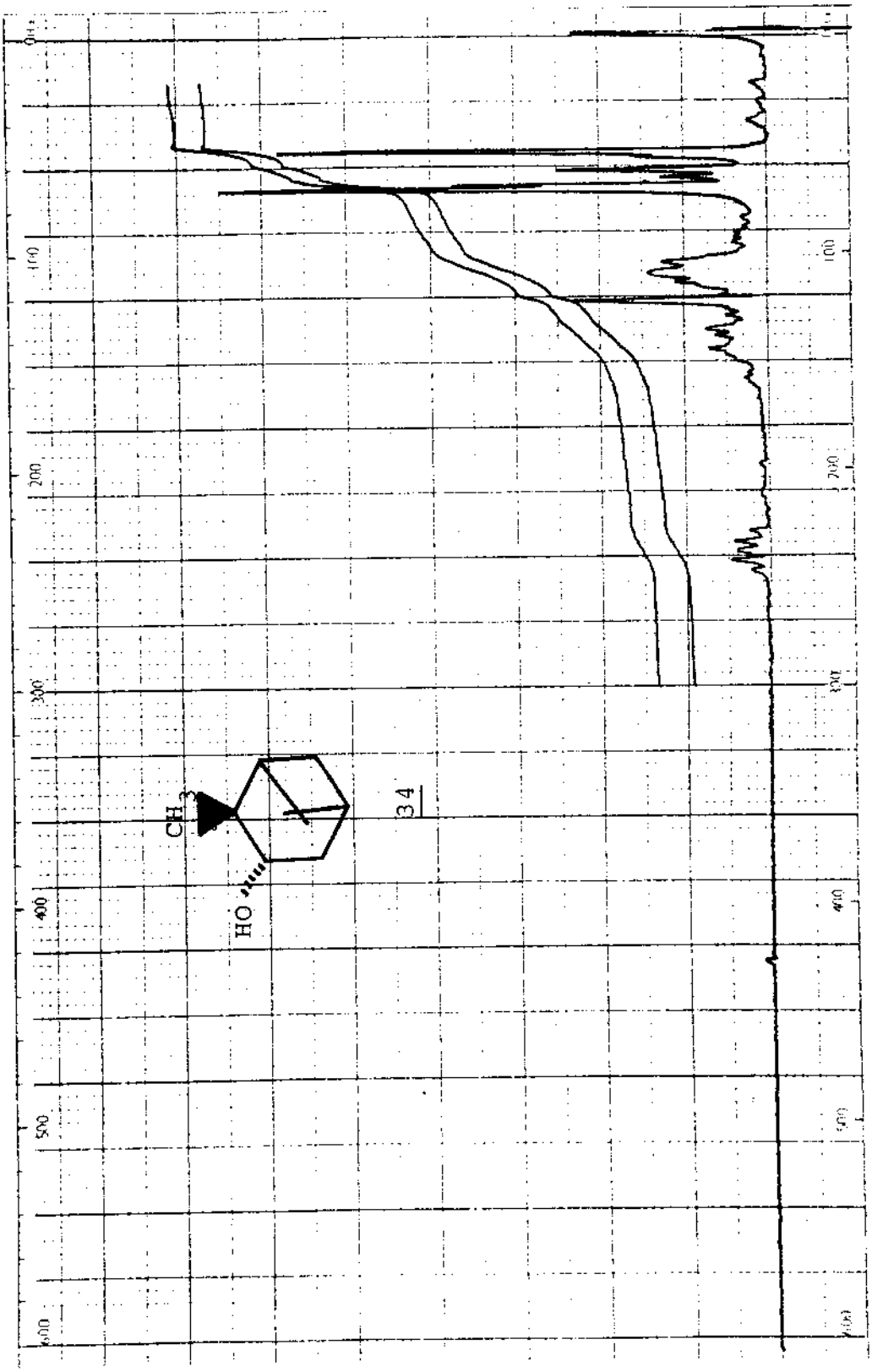


Fig. 22--The NMR spectrum of compound 34.

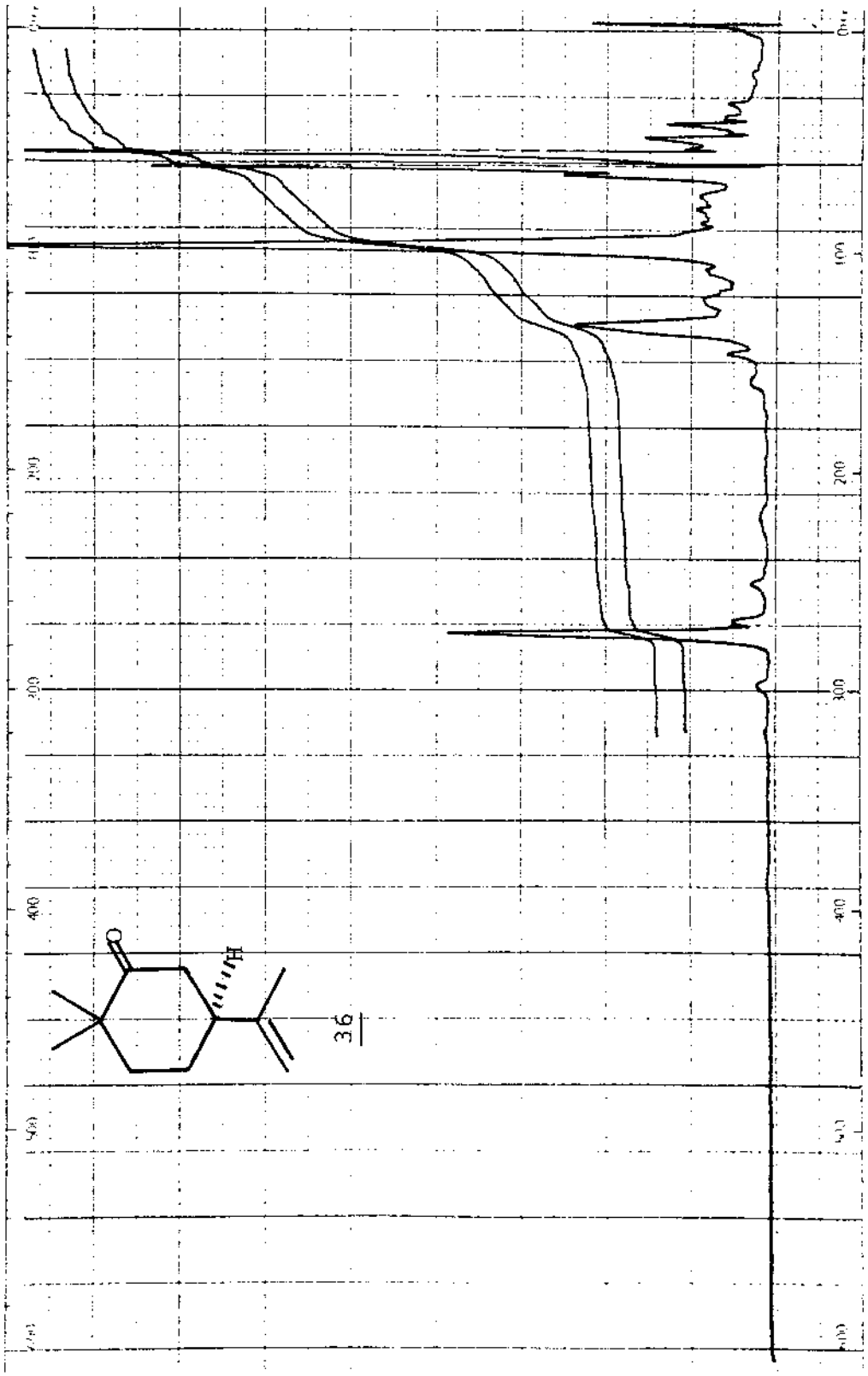


Fig. 23--The NMR spectrum of compound 36.

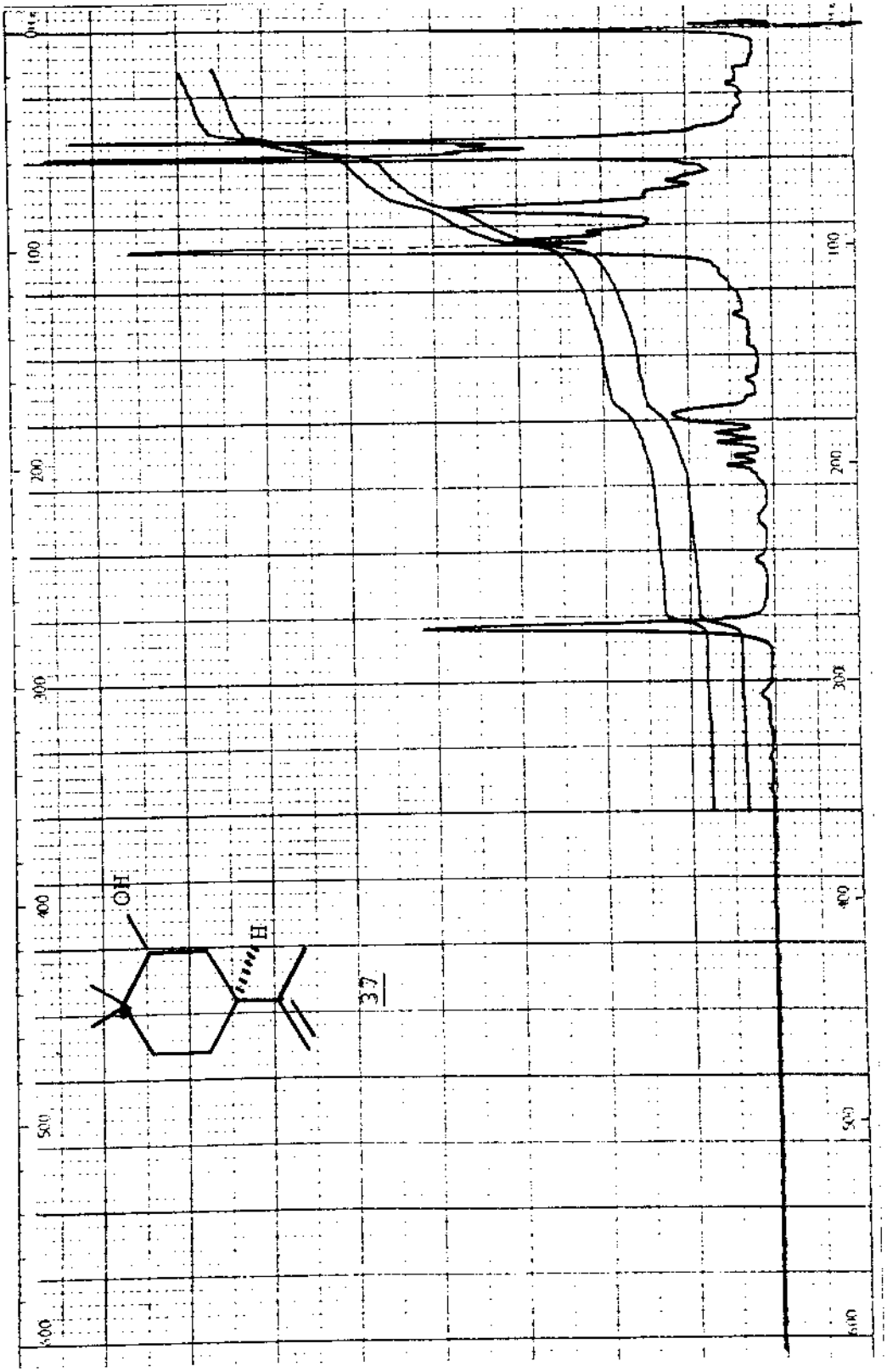


Fig. 24--The NMR spectrum of compound 37.

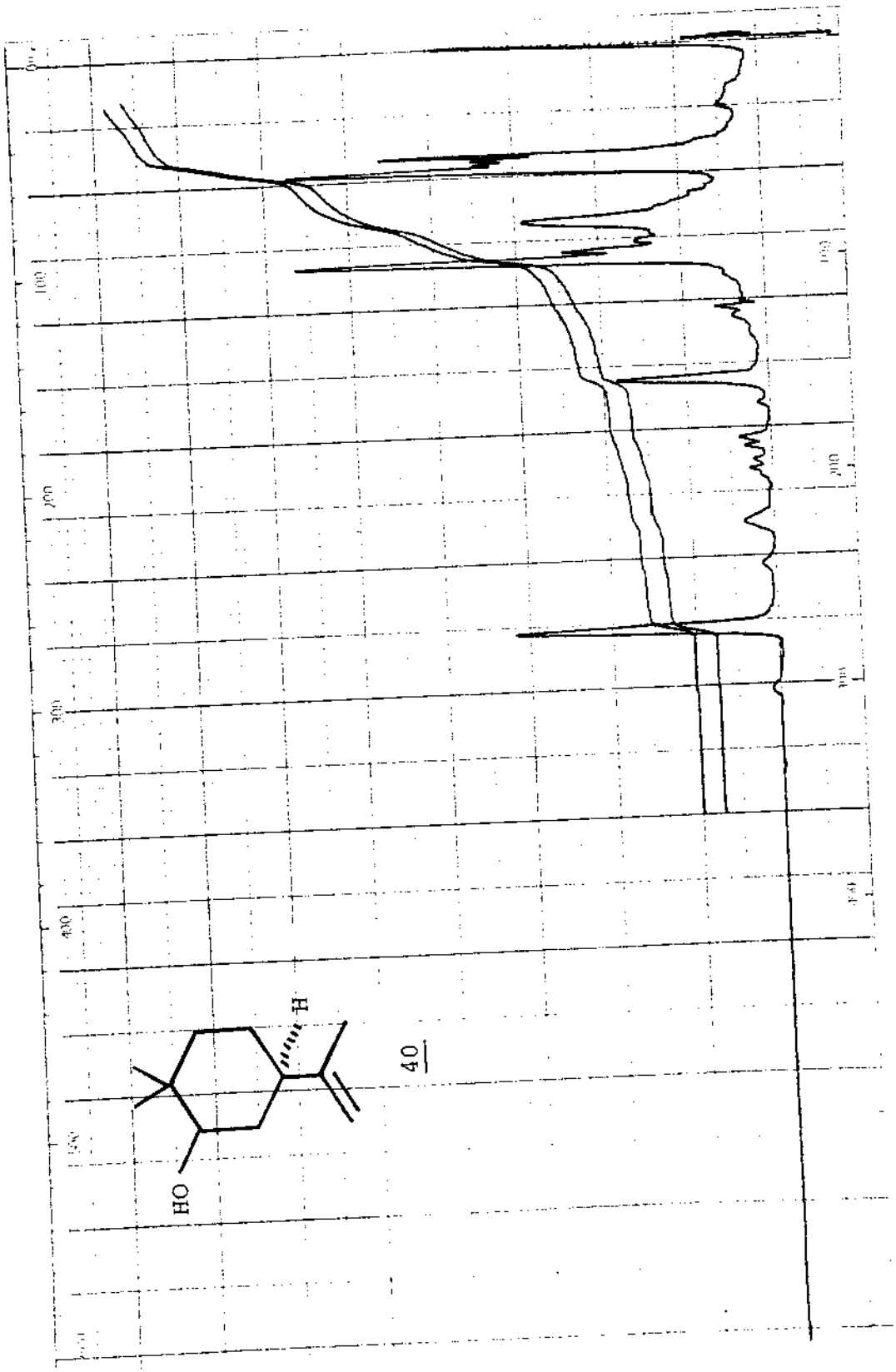


Fig. 25--The NMR spectrum of compound 40.

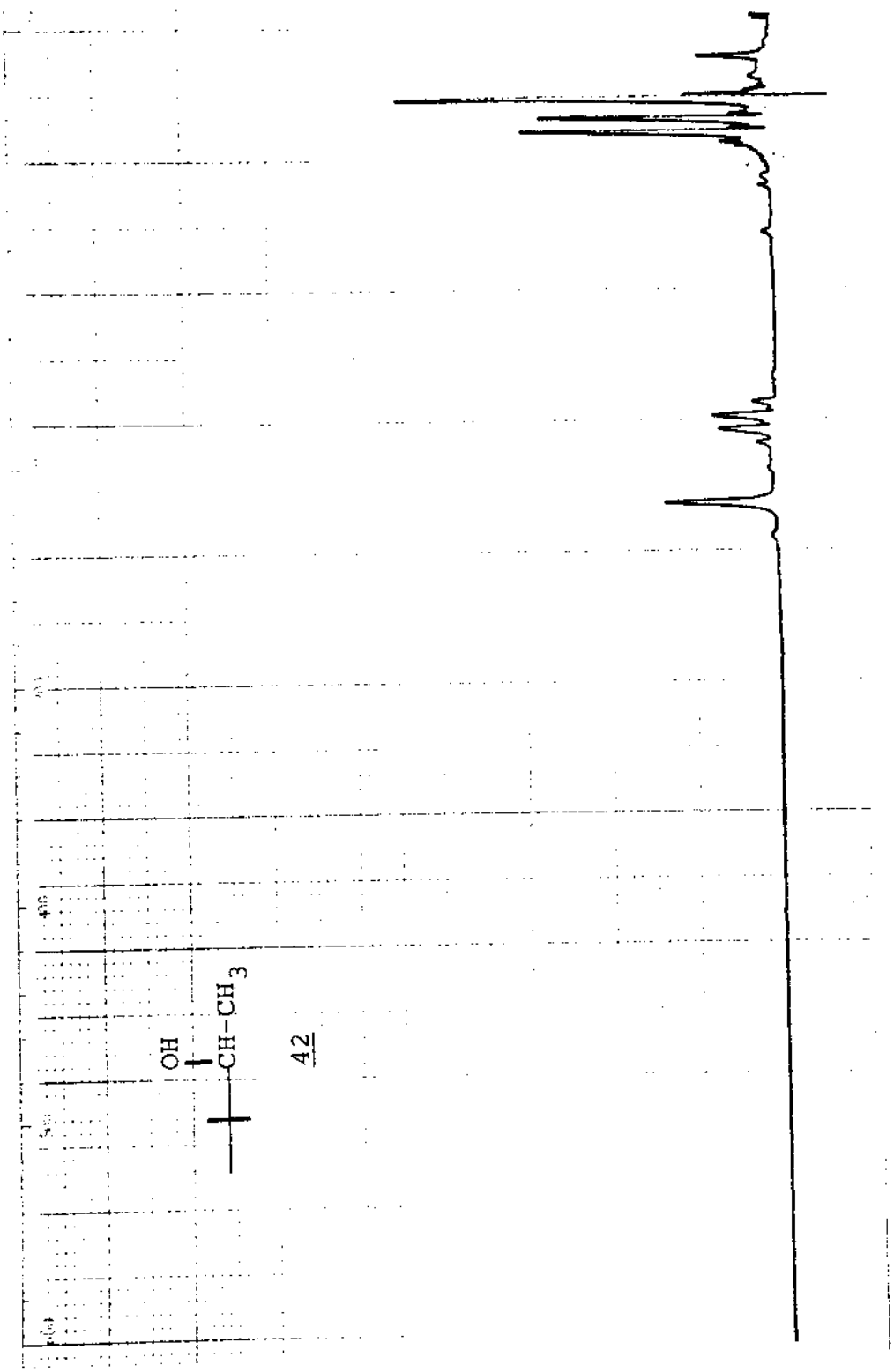


Fig. 26--The NMR spectrum of compound 42.

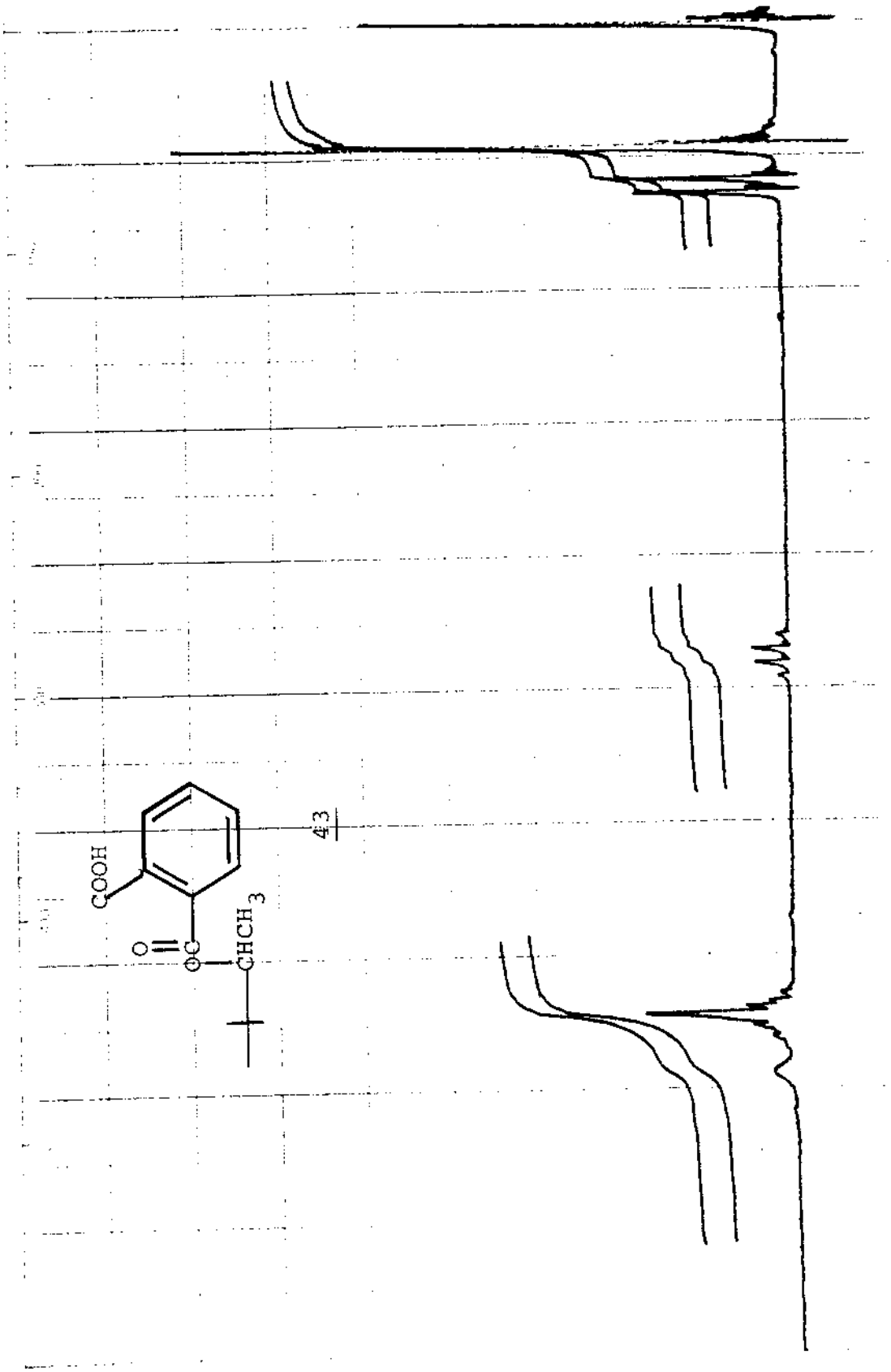


Fig. 27--The NMR spectrum of compound 43.

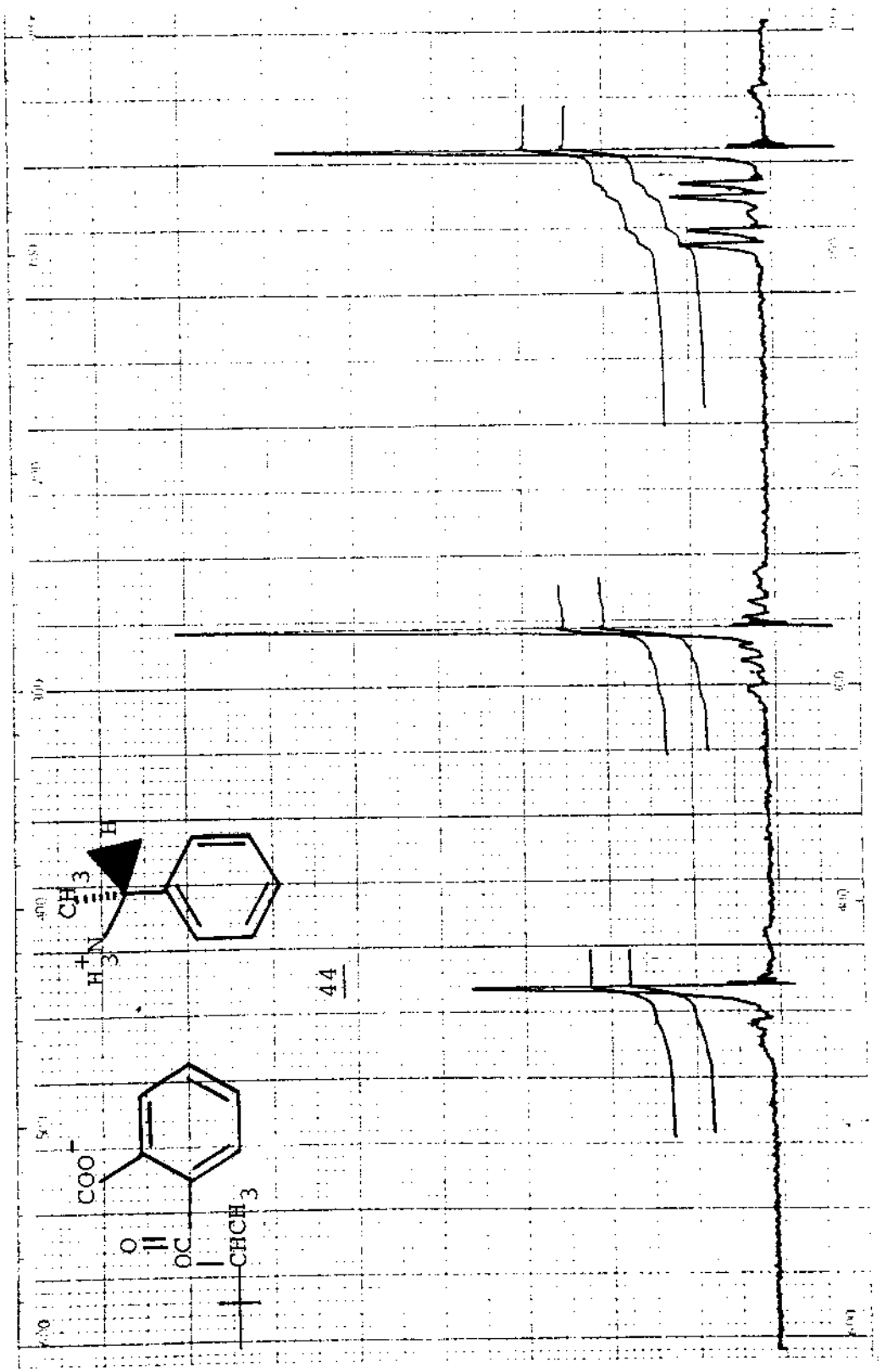


Fig. 28--The NMR spectrumof compound 44.

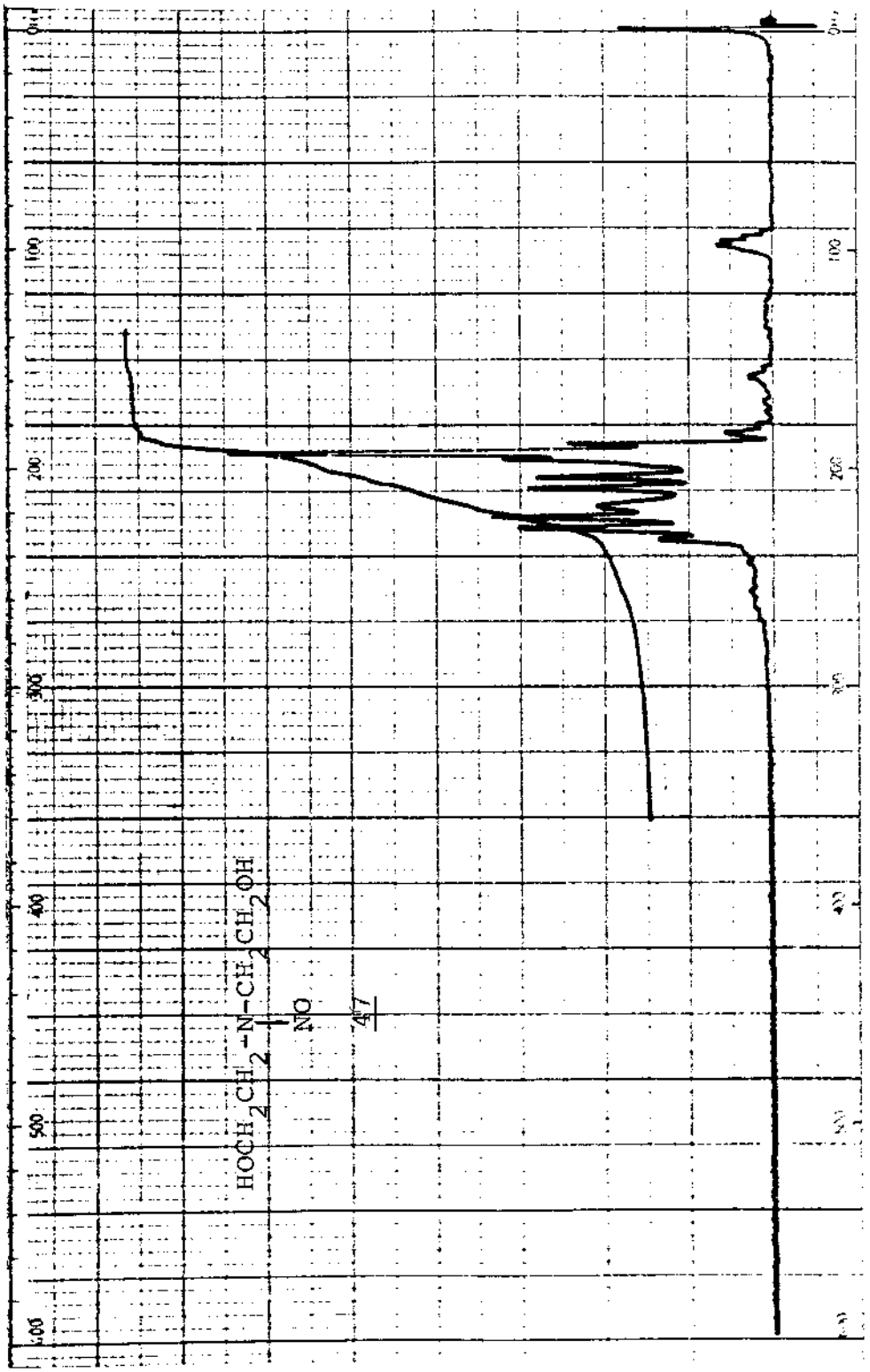


Fig. 29--The NMR spectrum of compound 47.

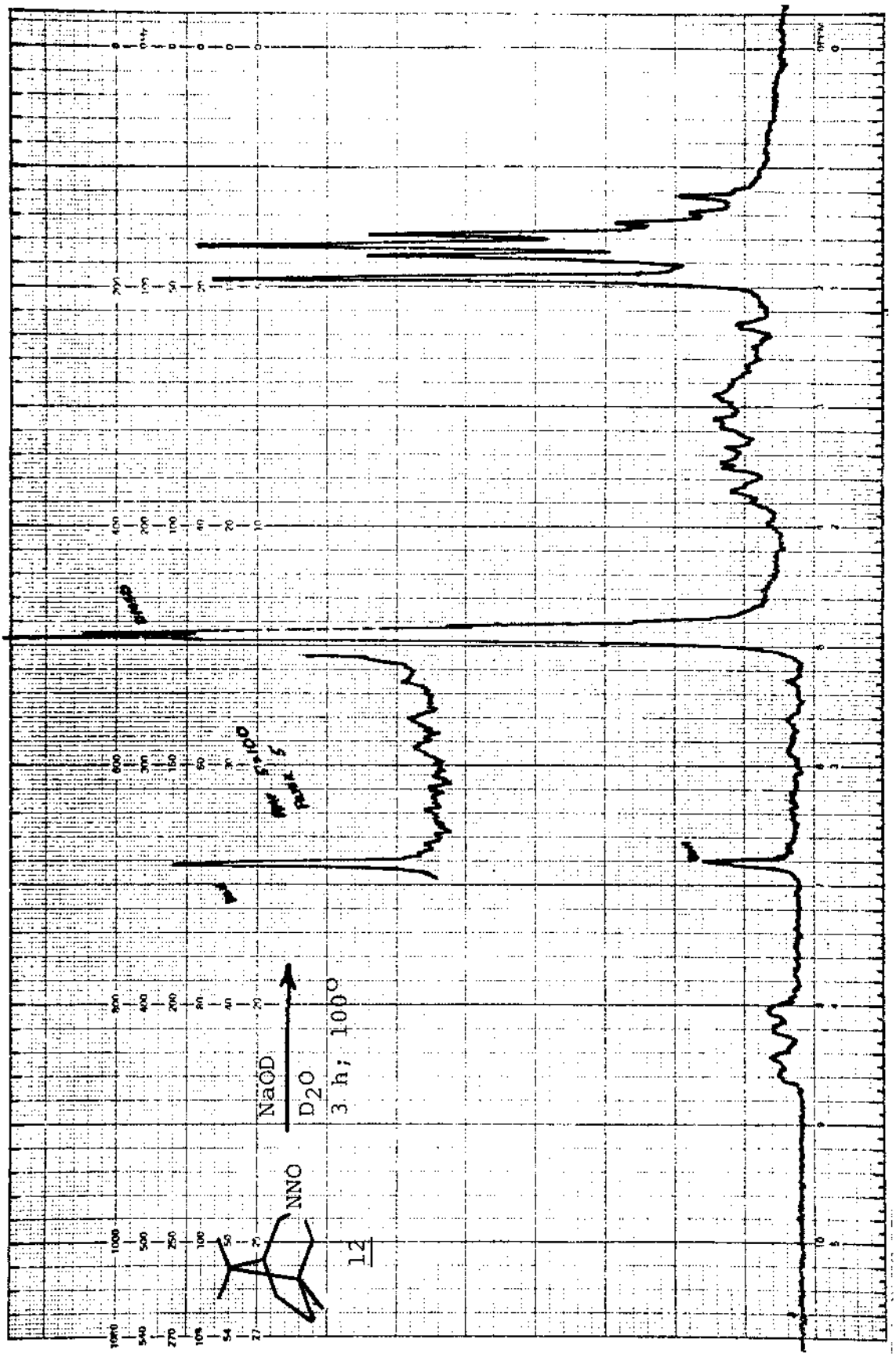


Fig. 30--The NMR spectrum of compound 12 after deuterium exchange (3 hr).

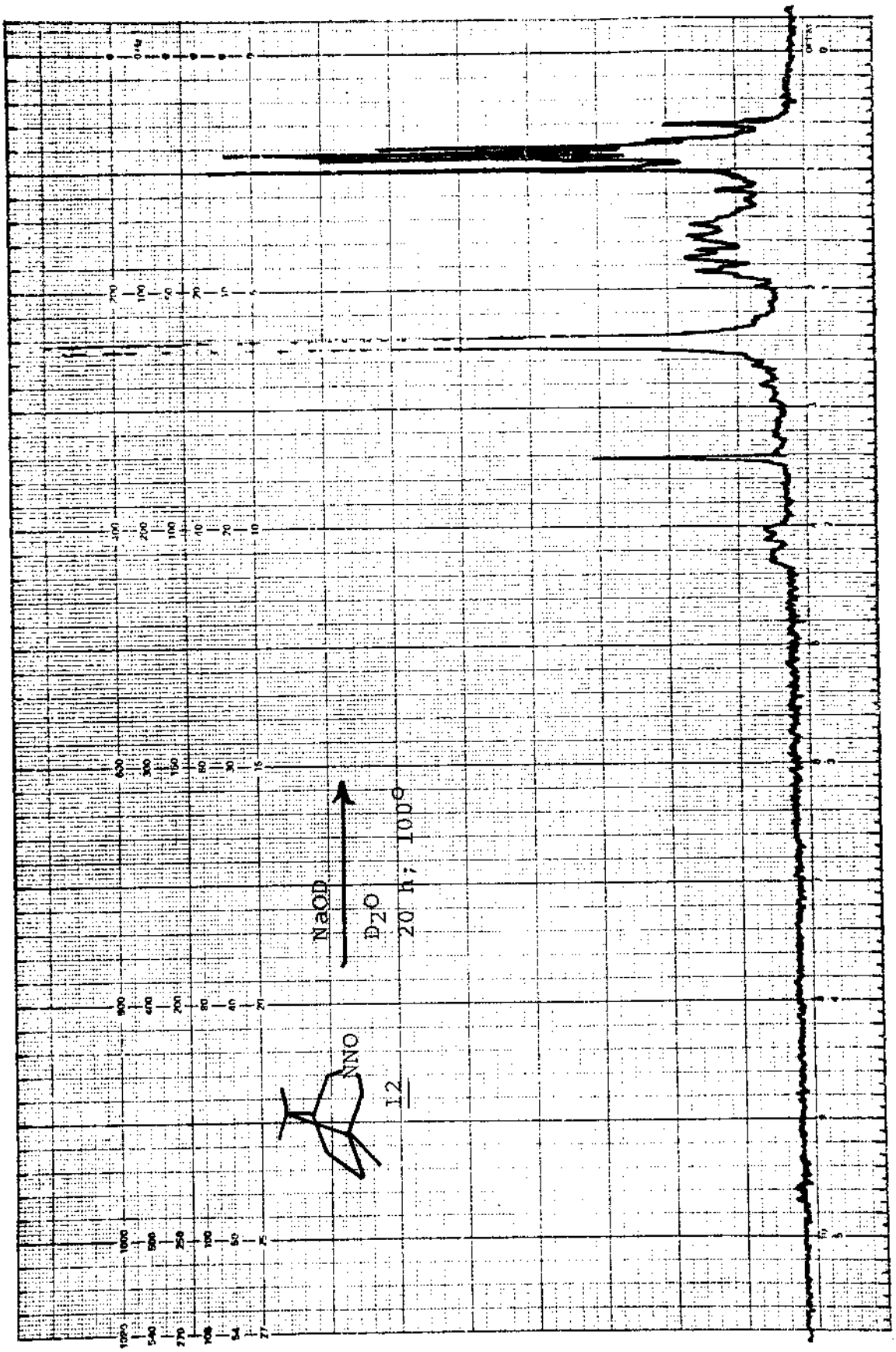


Fig. 31--The NMR spectrum of compound 12 after deuterium exchange (20 hr).

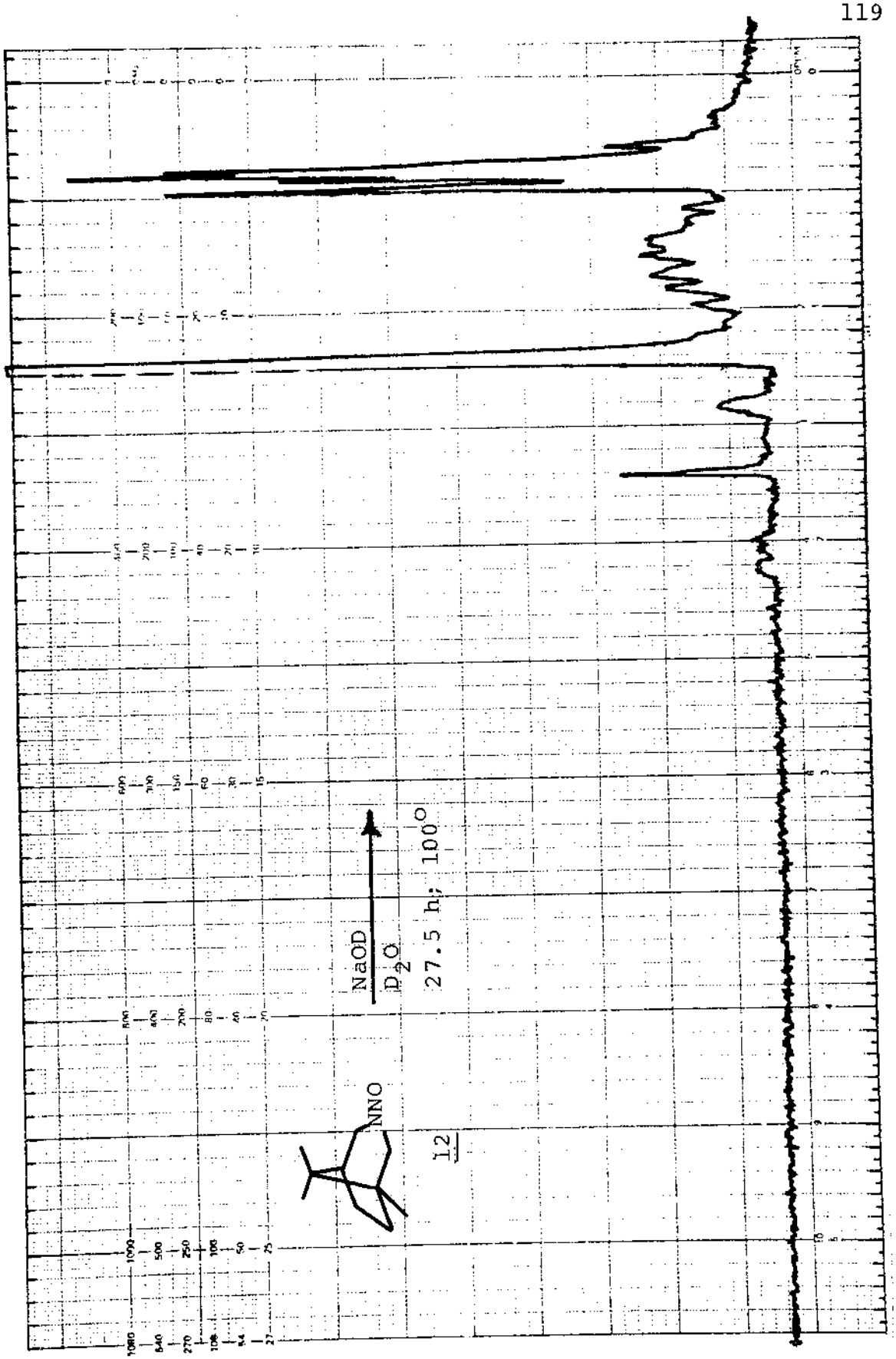


Fig. 32--The NMR spectrum of compound 12 after deuterium exchange (27.5 hr).

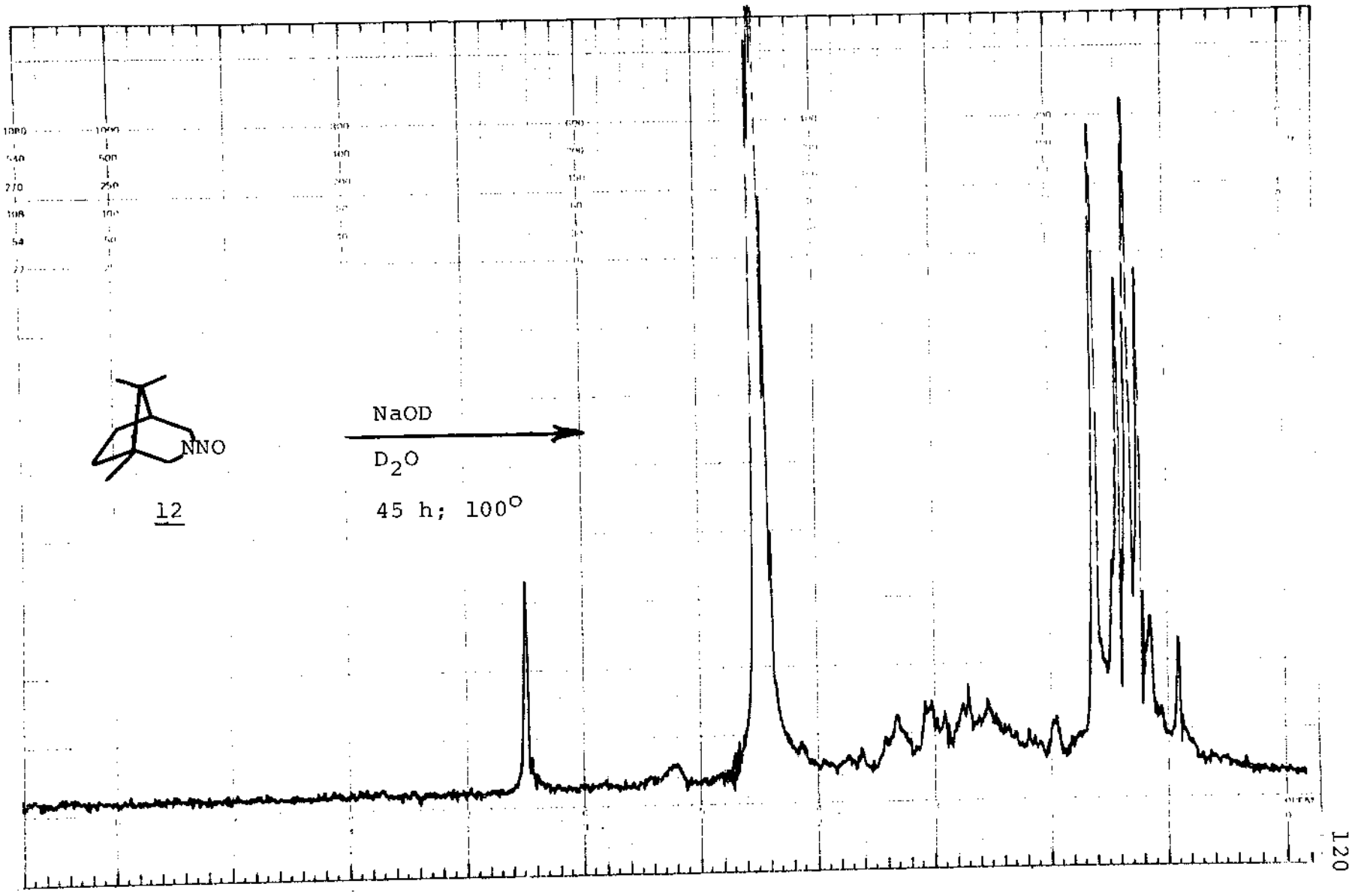


Fig. 33--The NMR spectrum of compound 12 after deuterium exchange (45 hr).

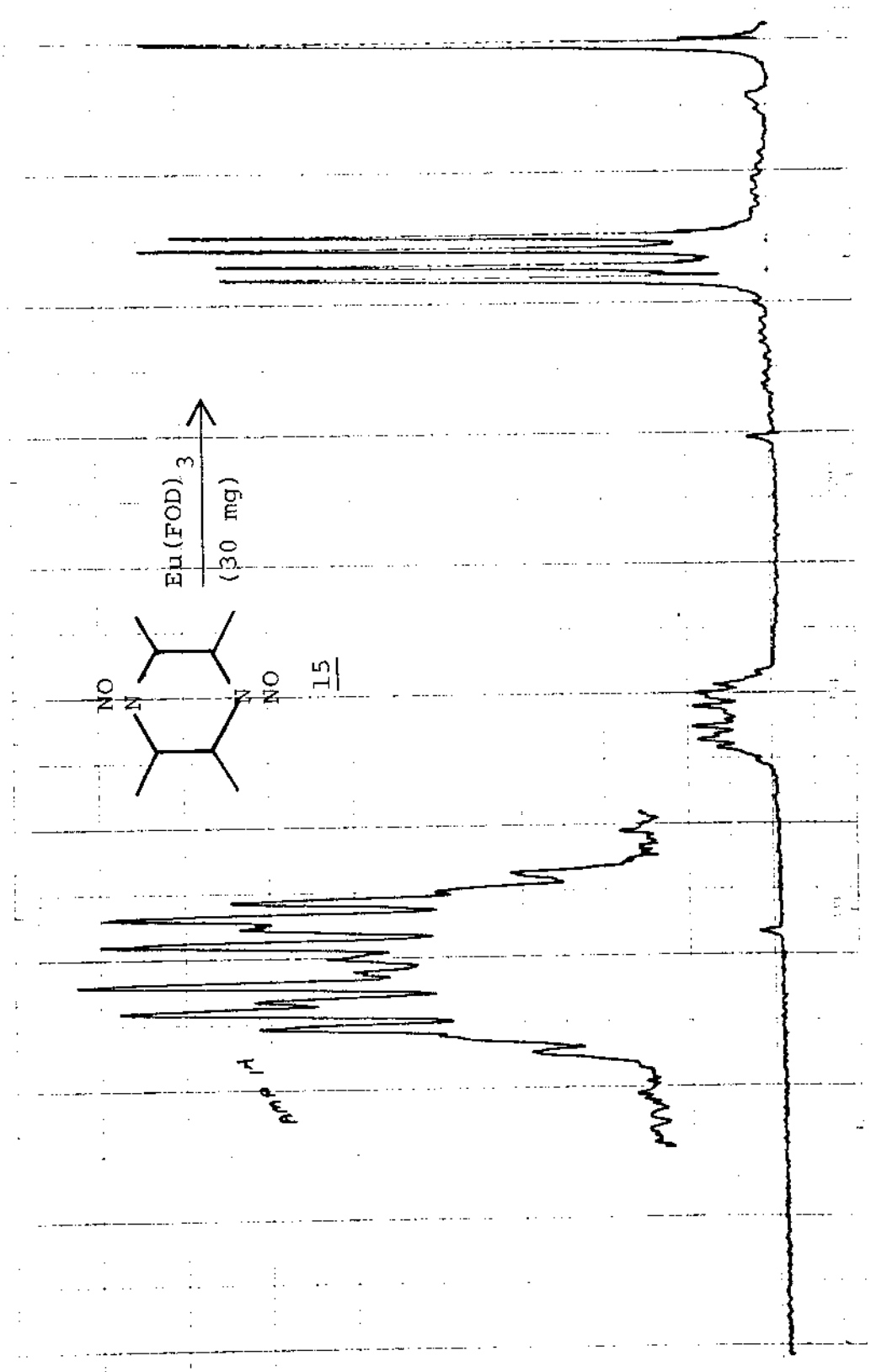


Fig. 34--The NMR spectrum of compound 15 with a shift reagent (30 mg).

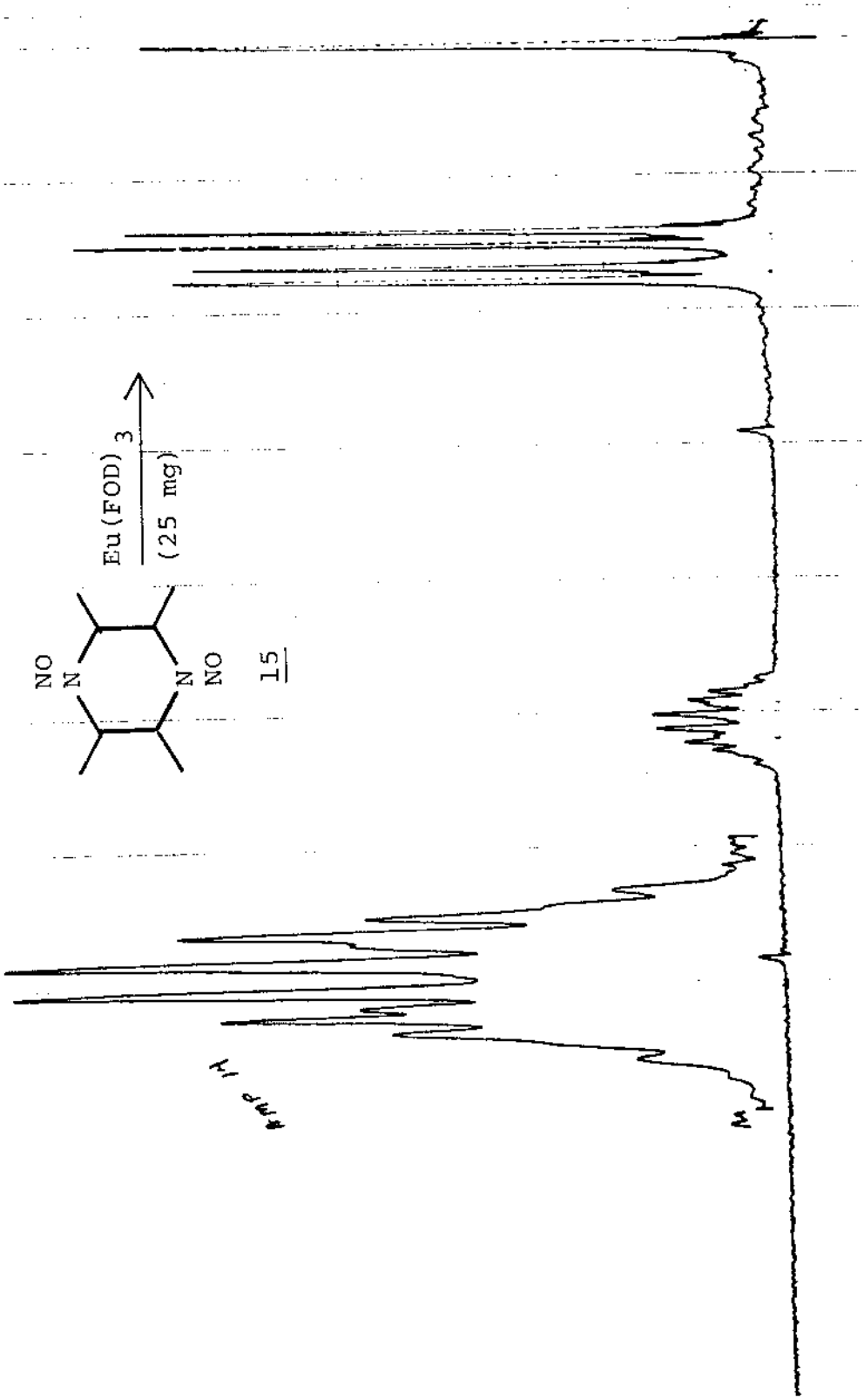


Fig. 35--The NMR spectrum of compound 15 with a shift reagent (25 mg).

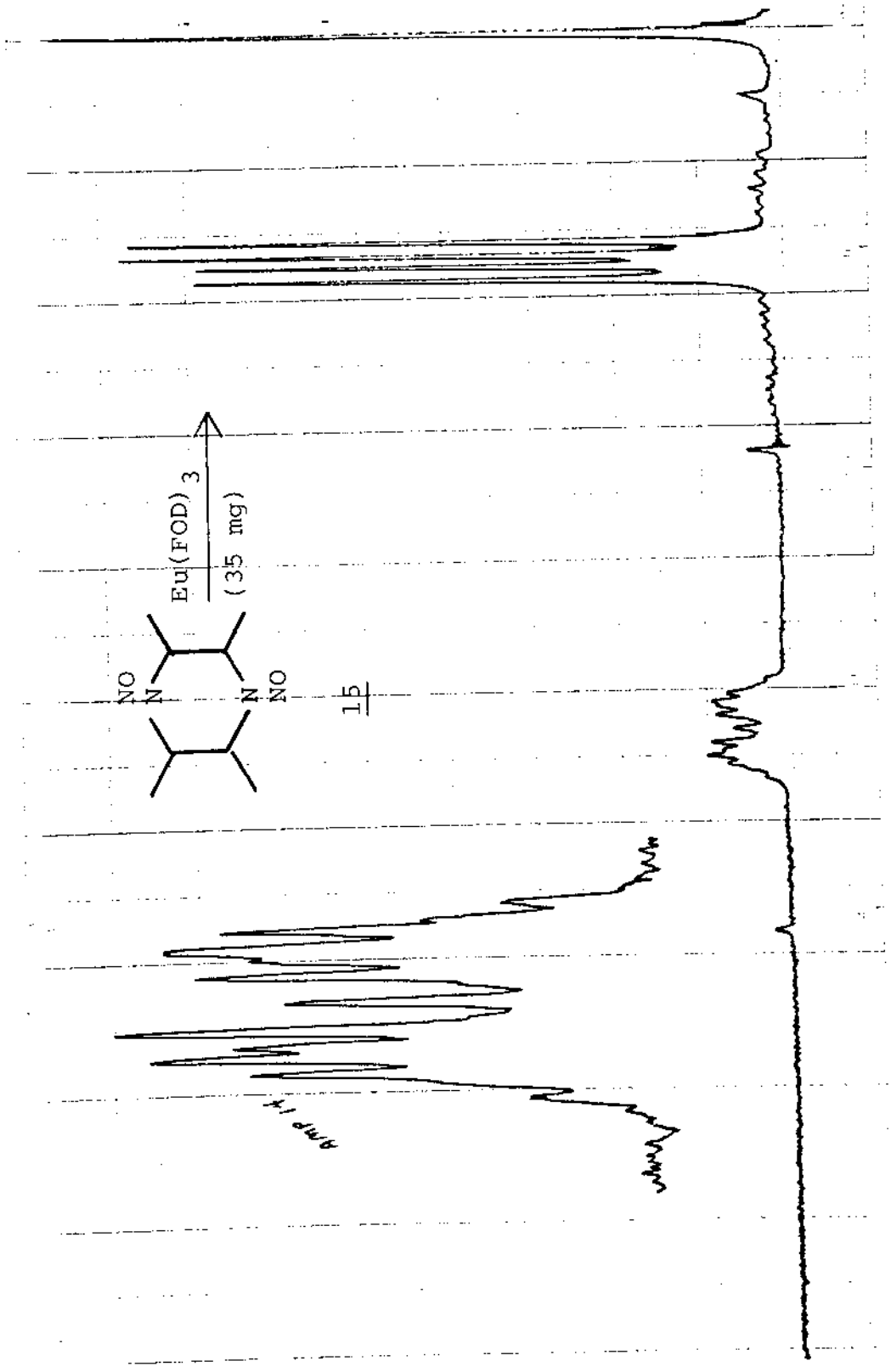


Fig. 36--The NMR spectrum of compound 15 with a shift reagent (35 mg).

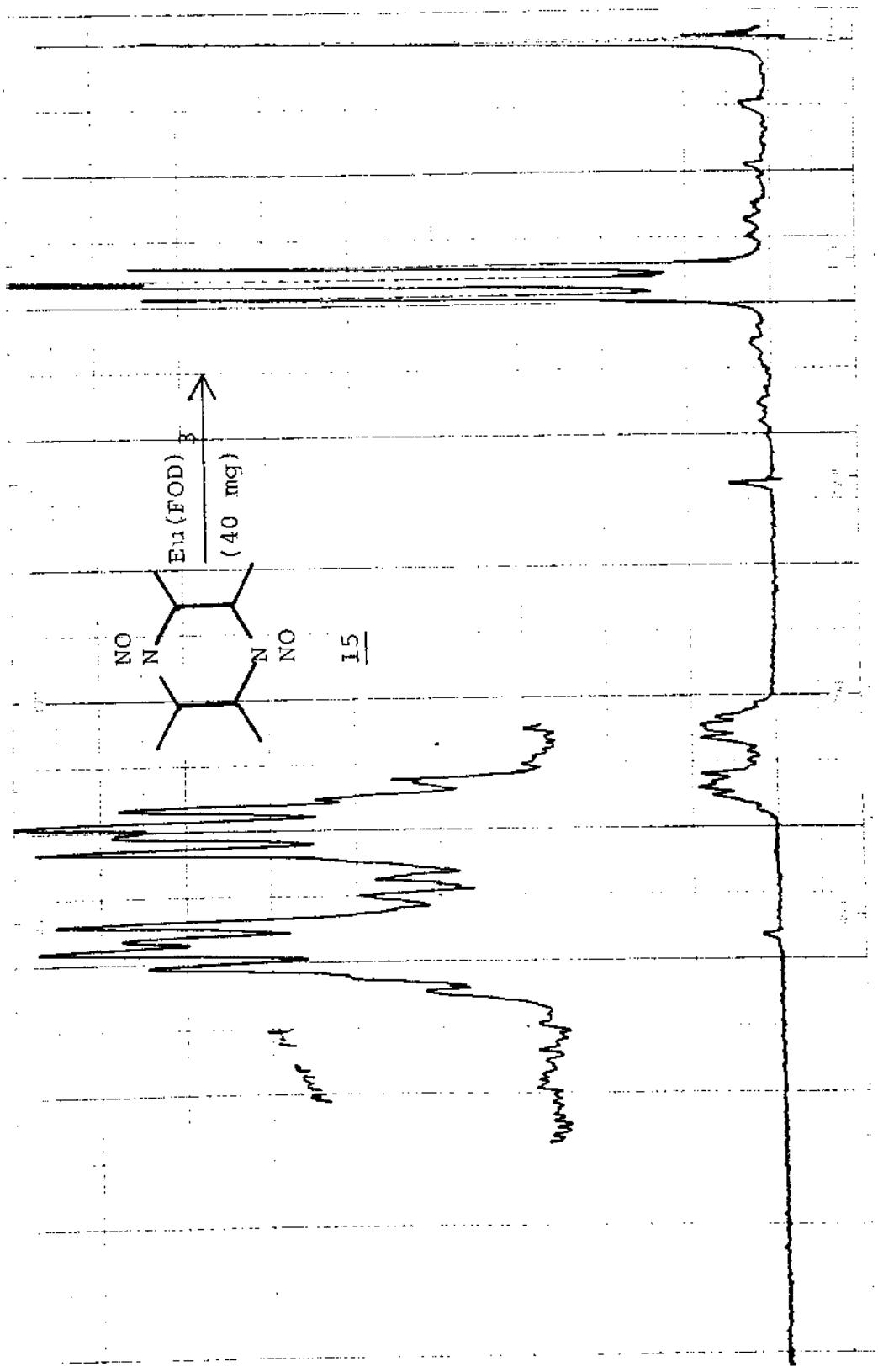


Fig. 37--The NMR spectrum of compound 15 with a shift reagent (40 mg).

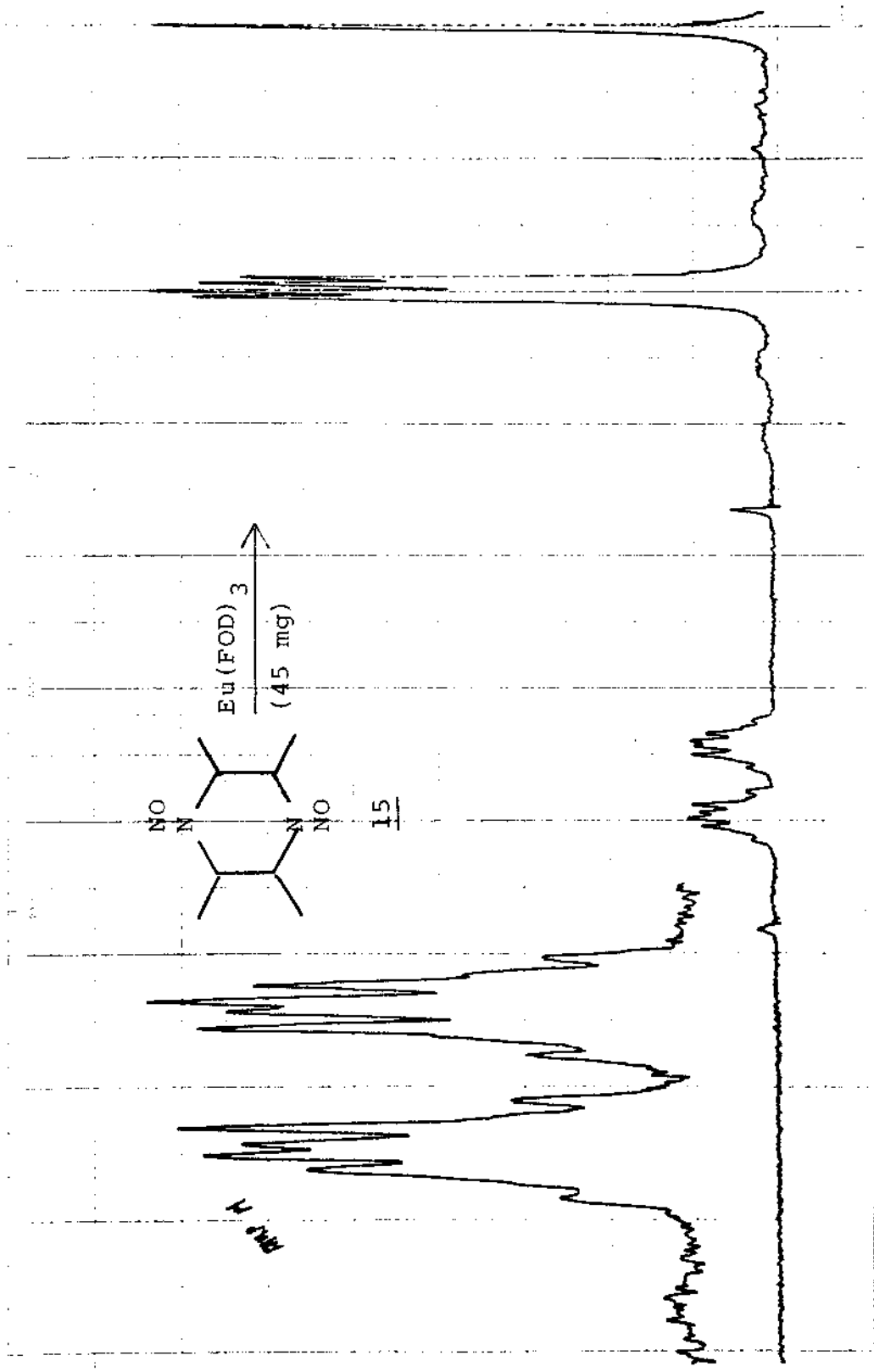


Fig. 38--The NMR spectrum of compound 15 with a shift reagent (45 mg).

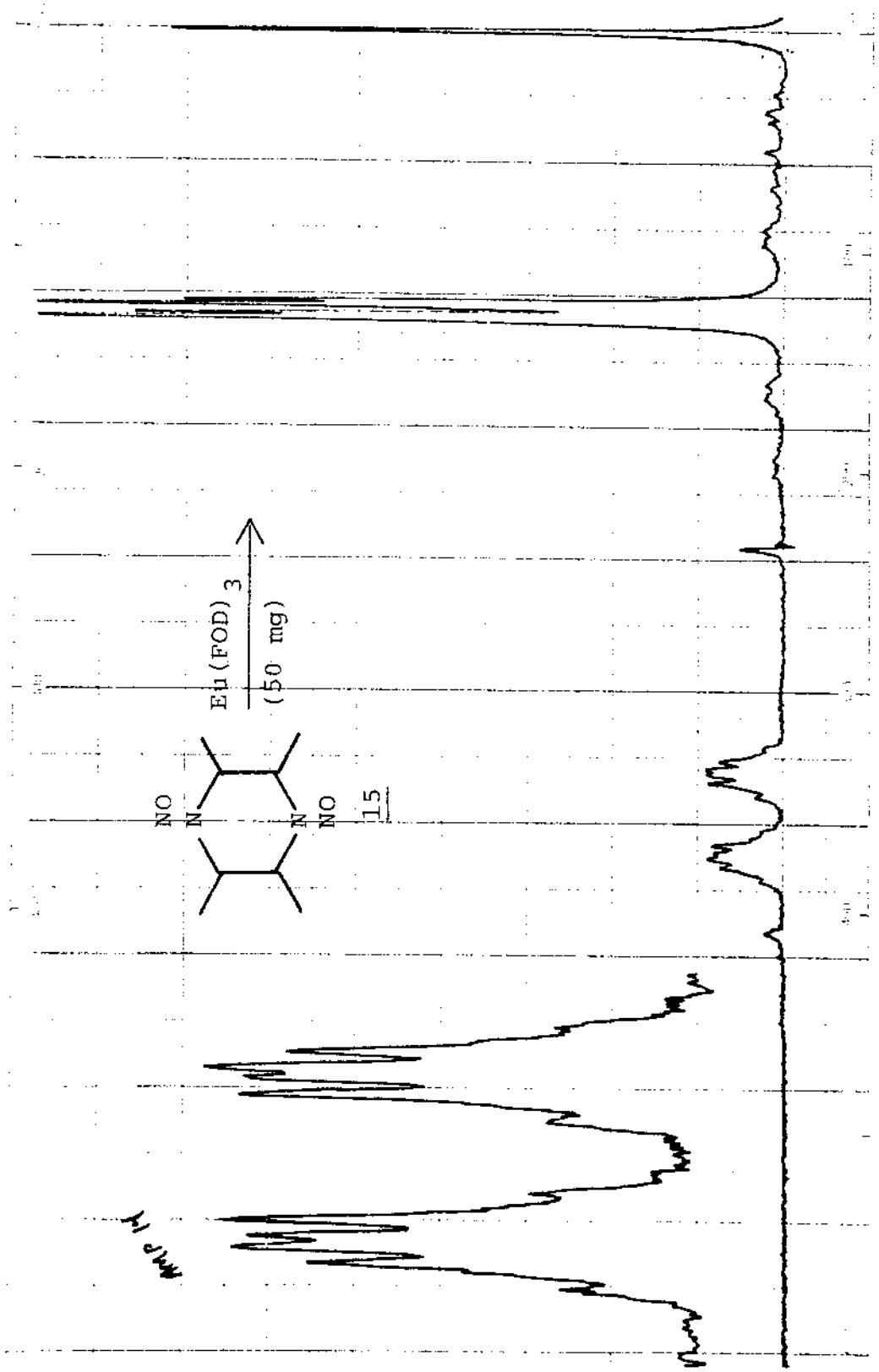


Fig. 39--The NMR spectrum of compound 15 with a shift reagent (50 mg).

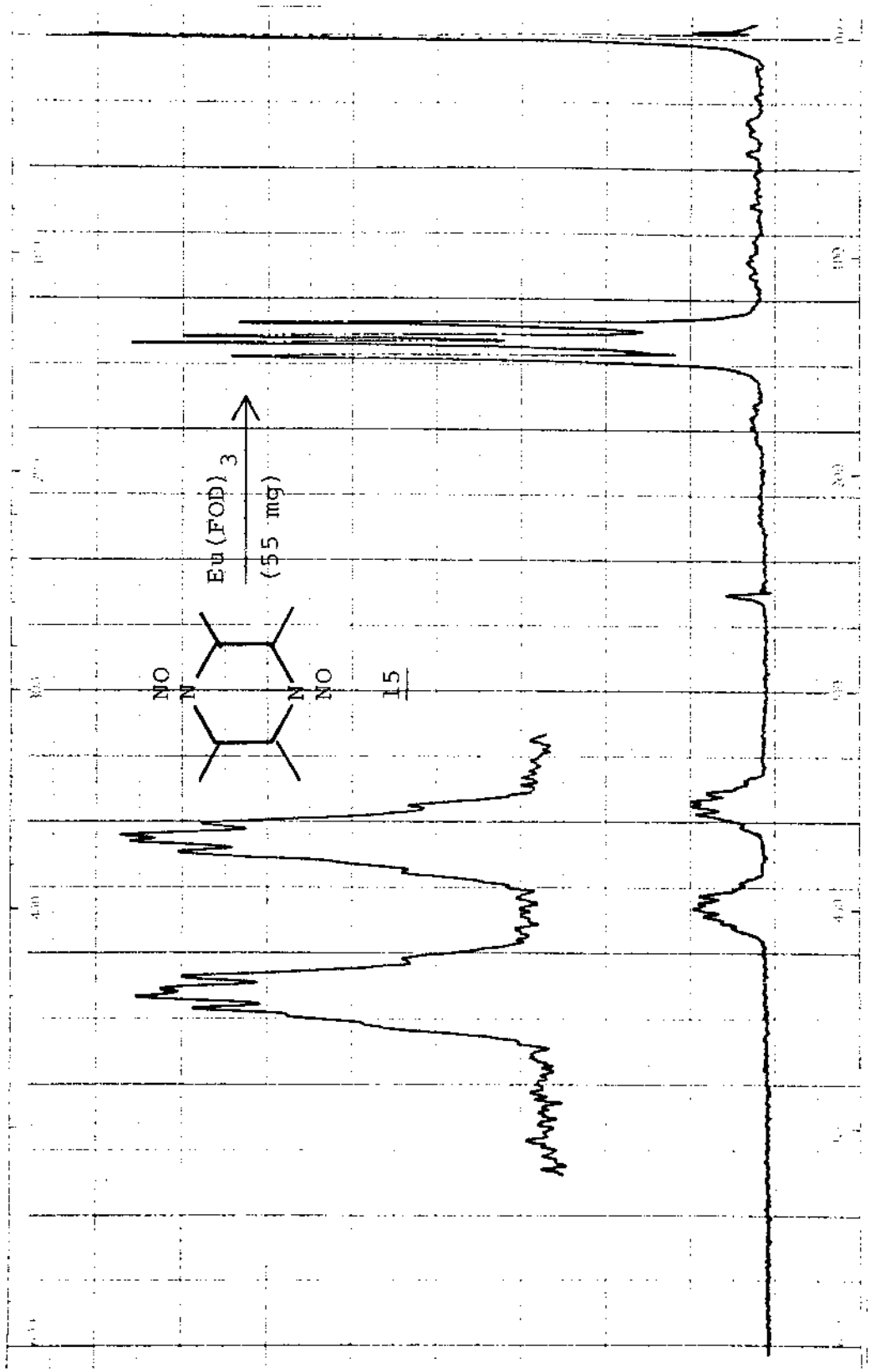


Fig. 40--The NMR spectrum of compound 15 with a shift reagent (55 mg).

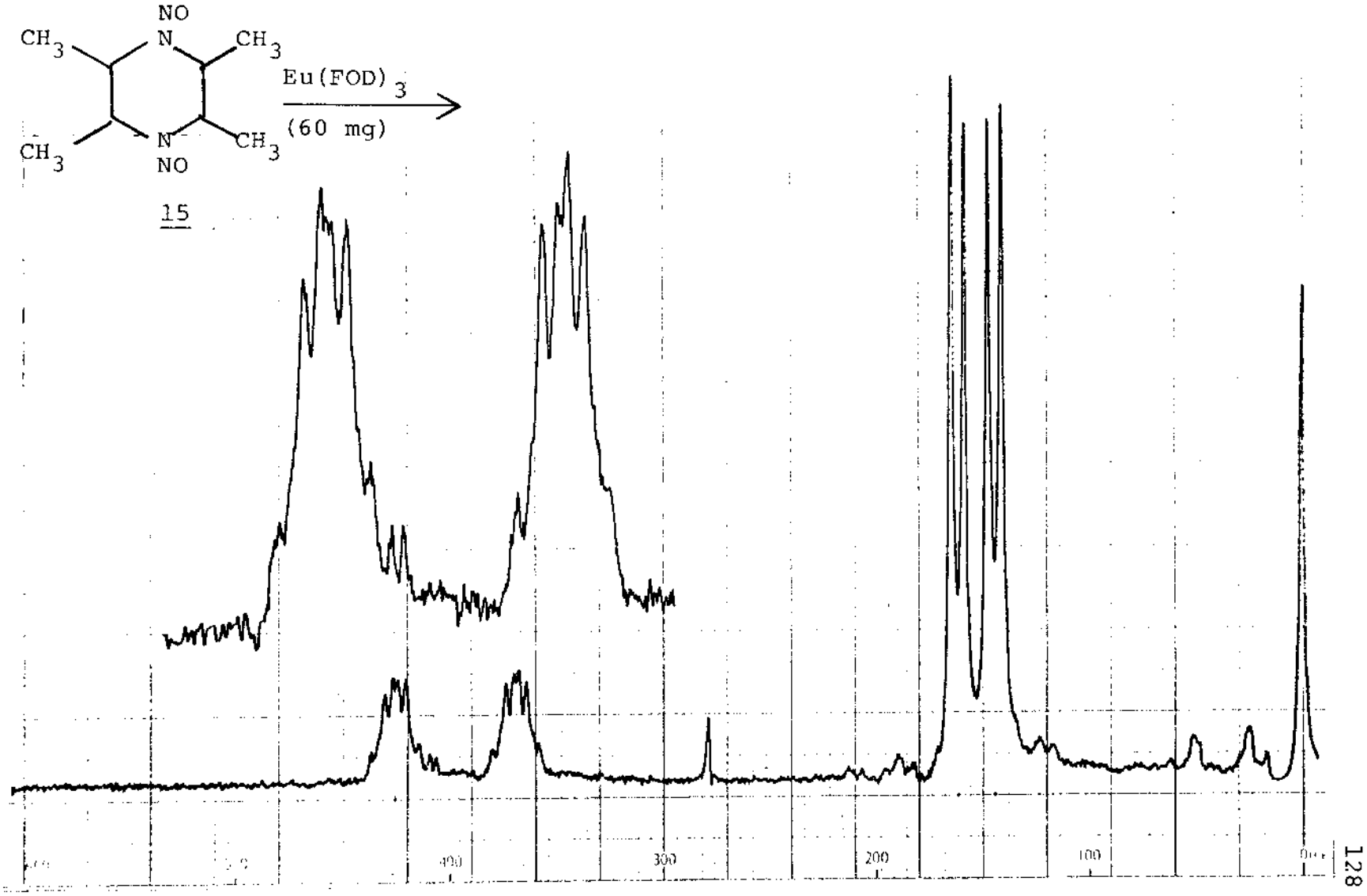


Fig. 41--The NMR spectrum of compound 15 with a shift reagent (60 mg).

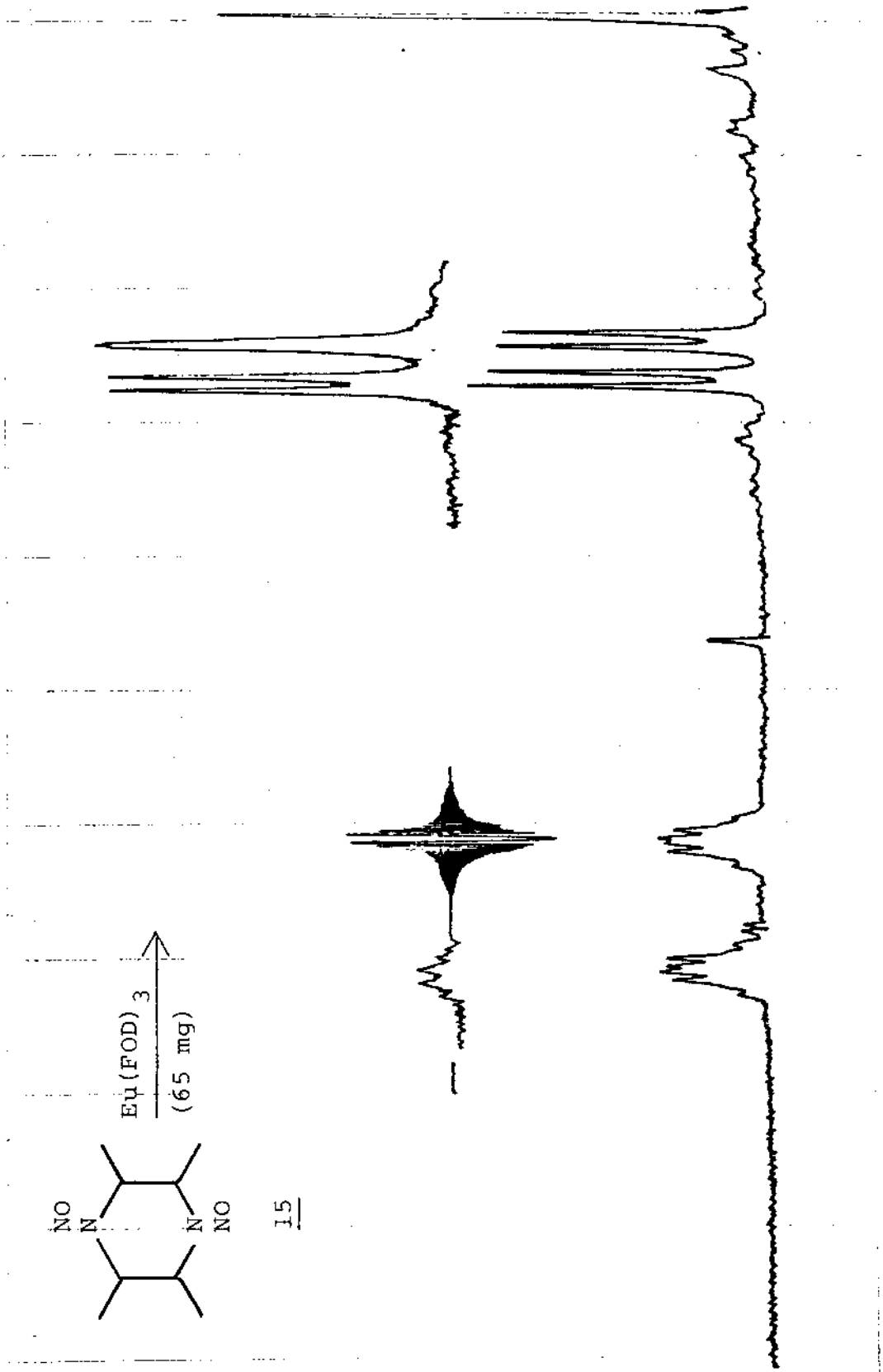


Fig. 42--The NMR spectrum of compound 15 with a shift reagent (65 mg).

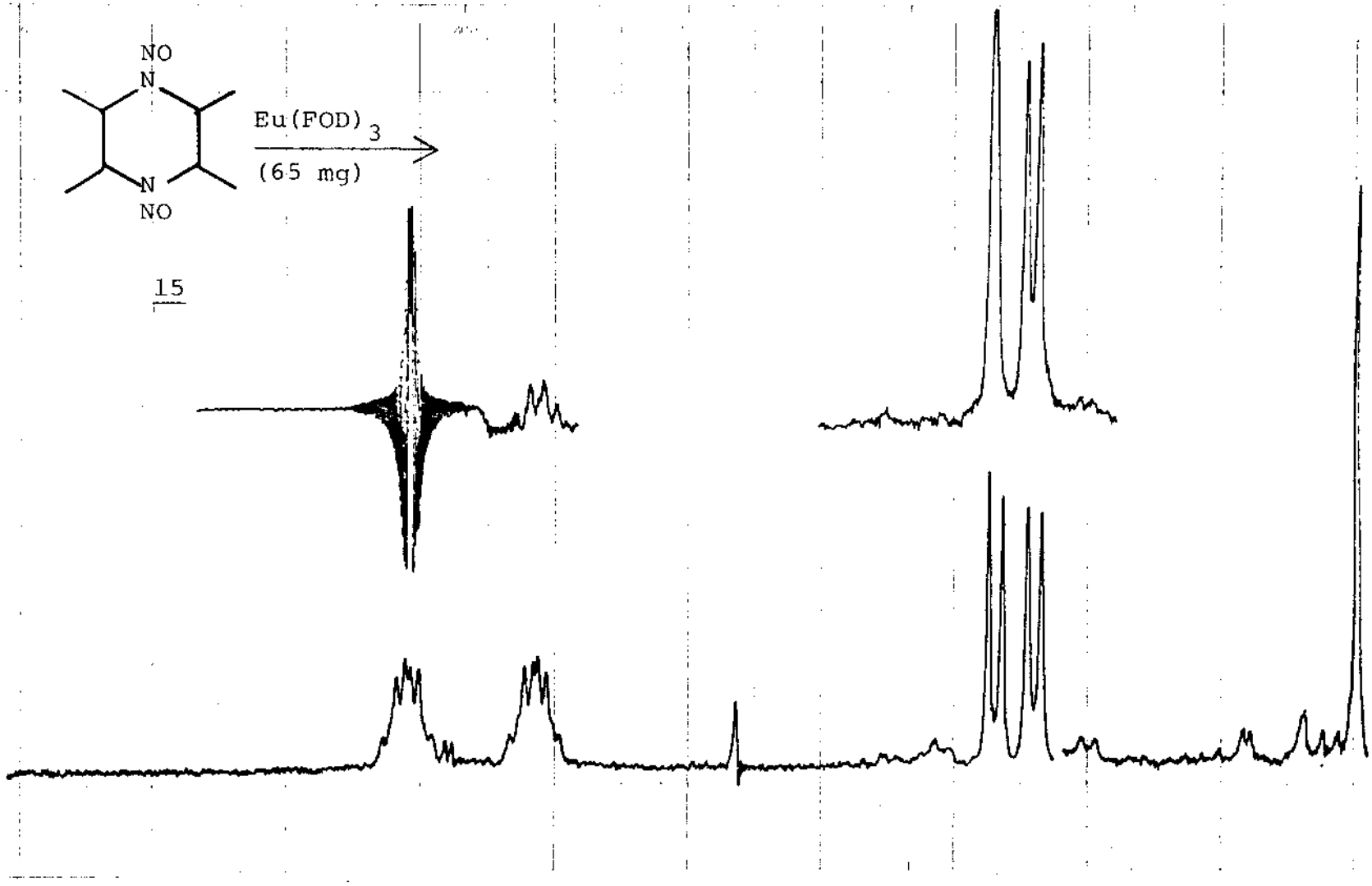


Fig. 43--The NMR spectrumof compound 15 with a shift reagent (65 mg).

APPENDIX B

INFRARED SPECTRA

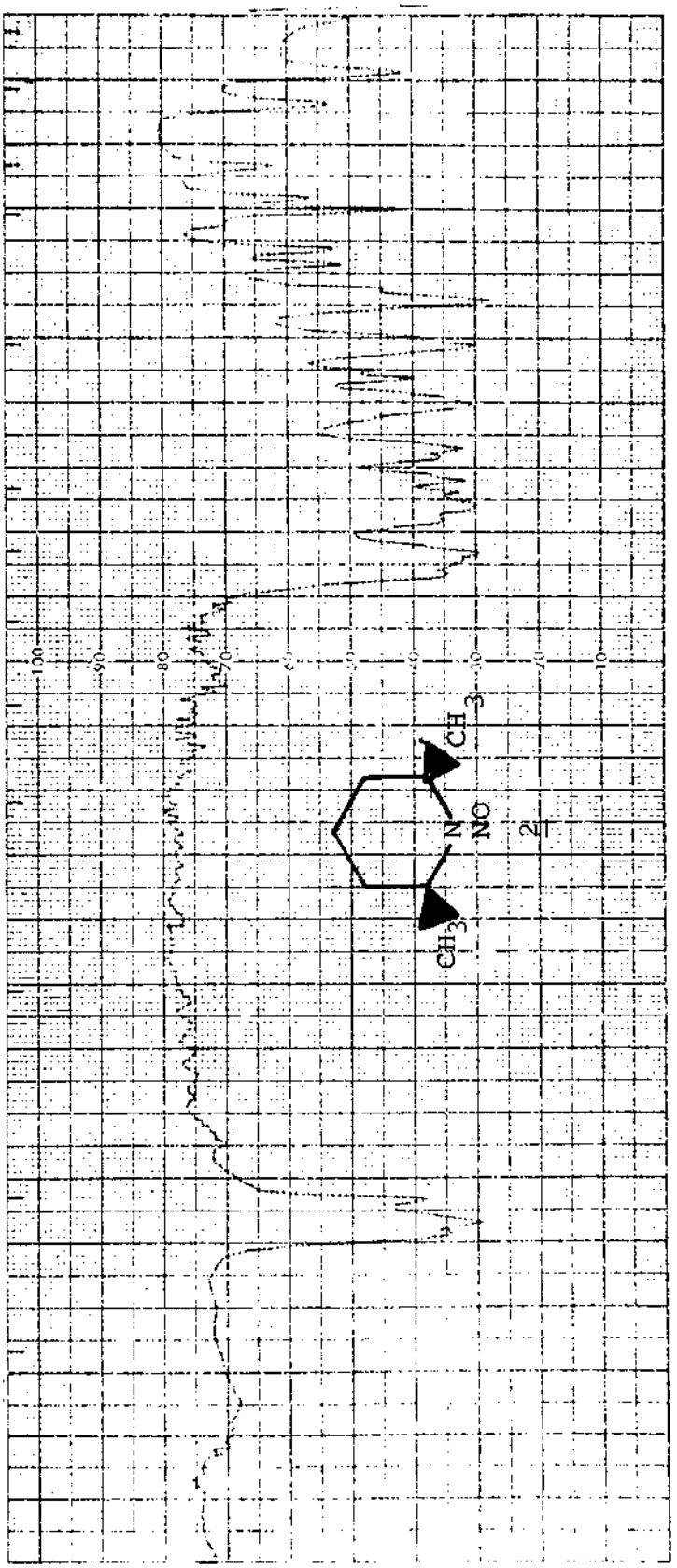


Fig. 1--The IR spectrum of compound 3.

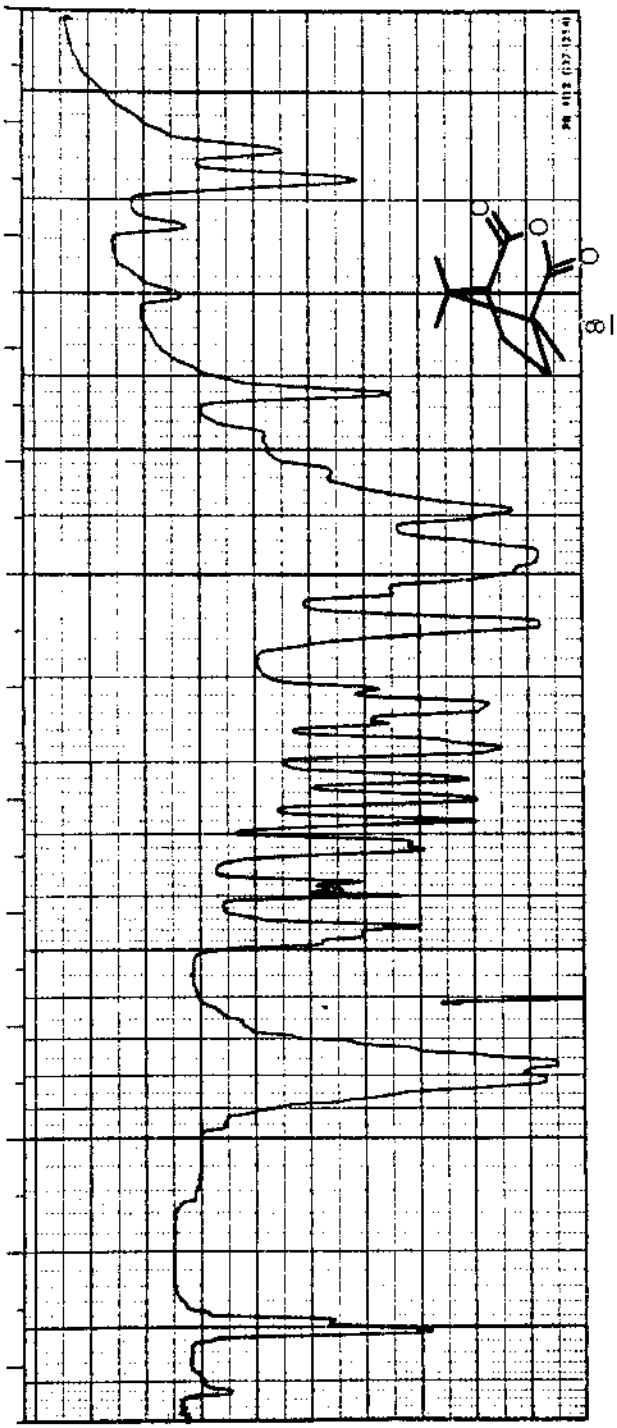


Fig. 2--The IR spectrum of compound 8.

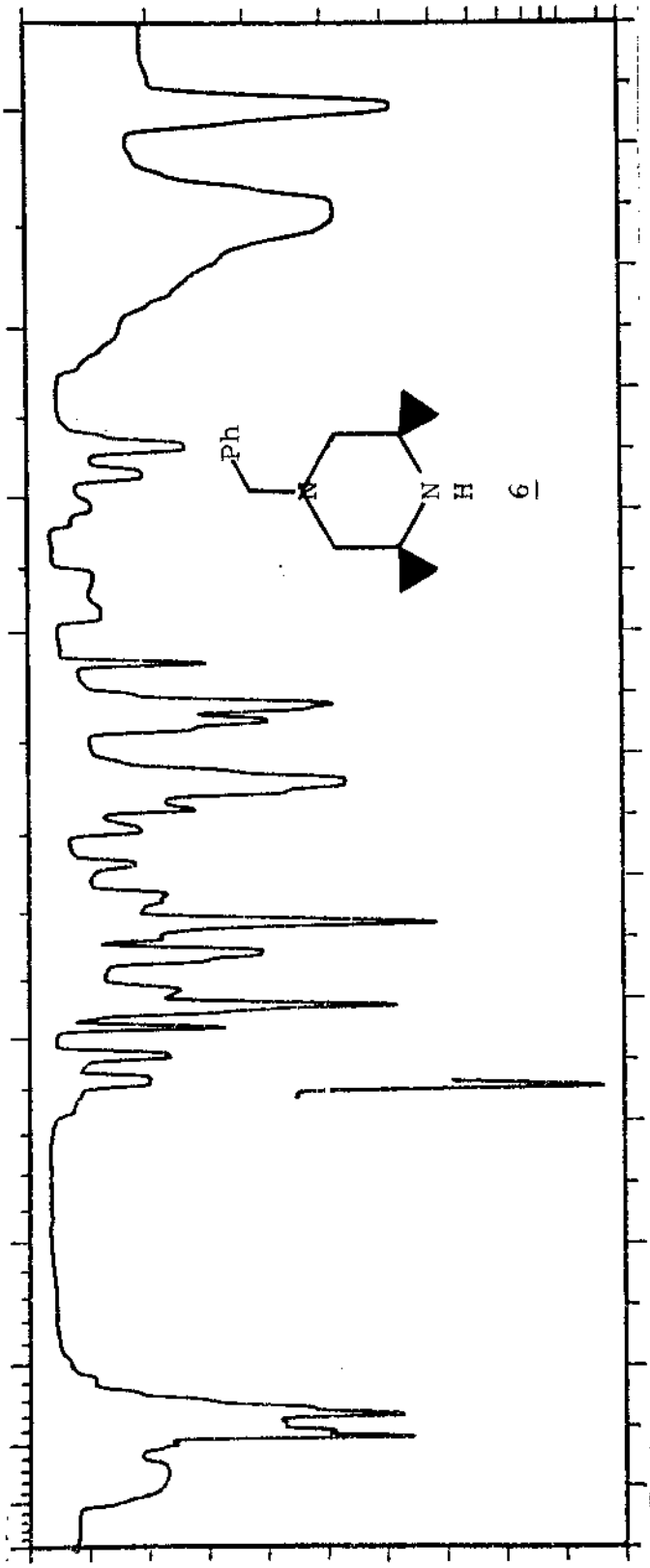
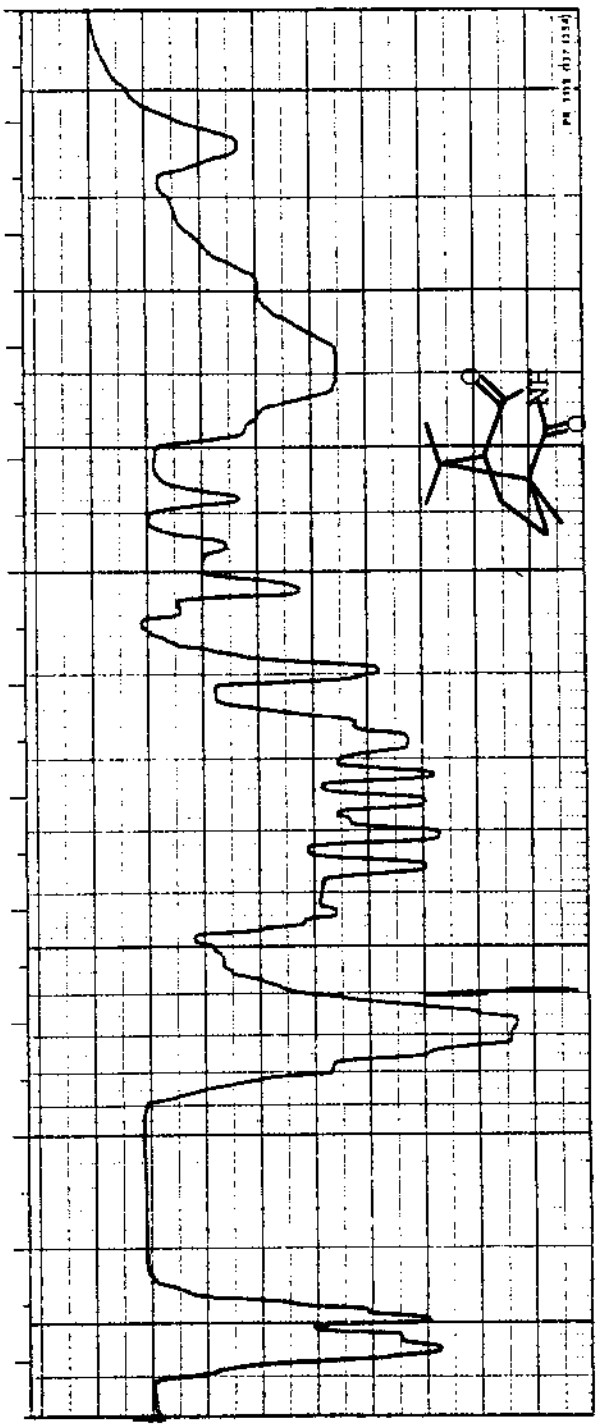


Fig. 3--The IR spectrum of compound 6.



10

Fig. 4--The IR spectrum of compound 10.

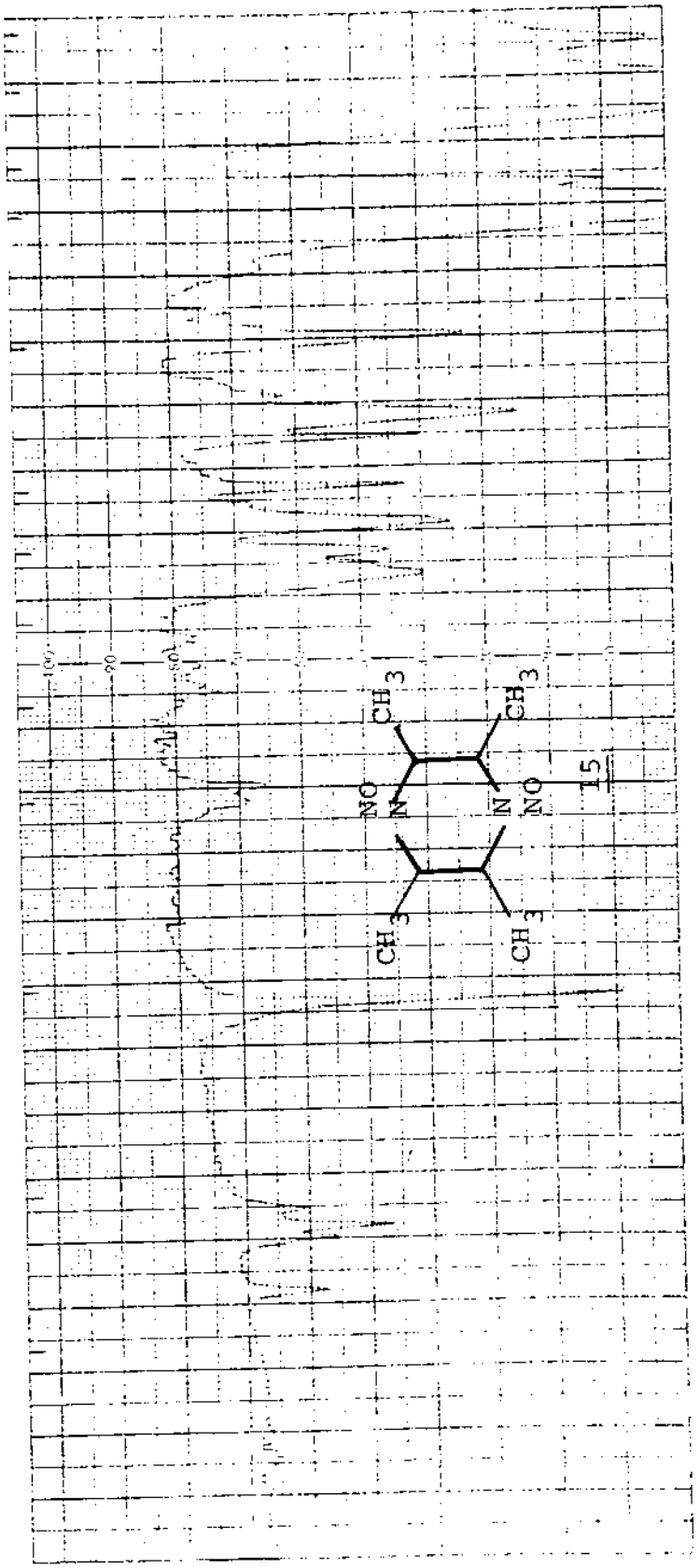


Fig. 5--The IR spectrum of compound 15.

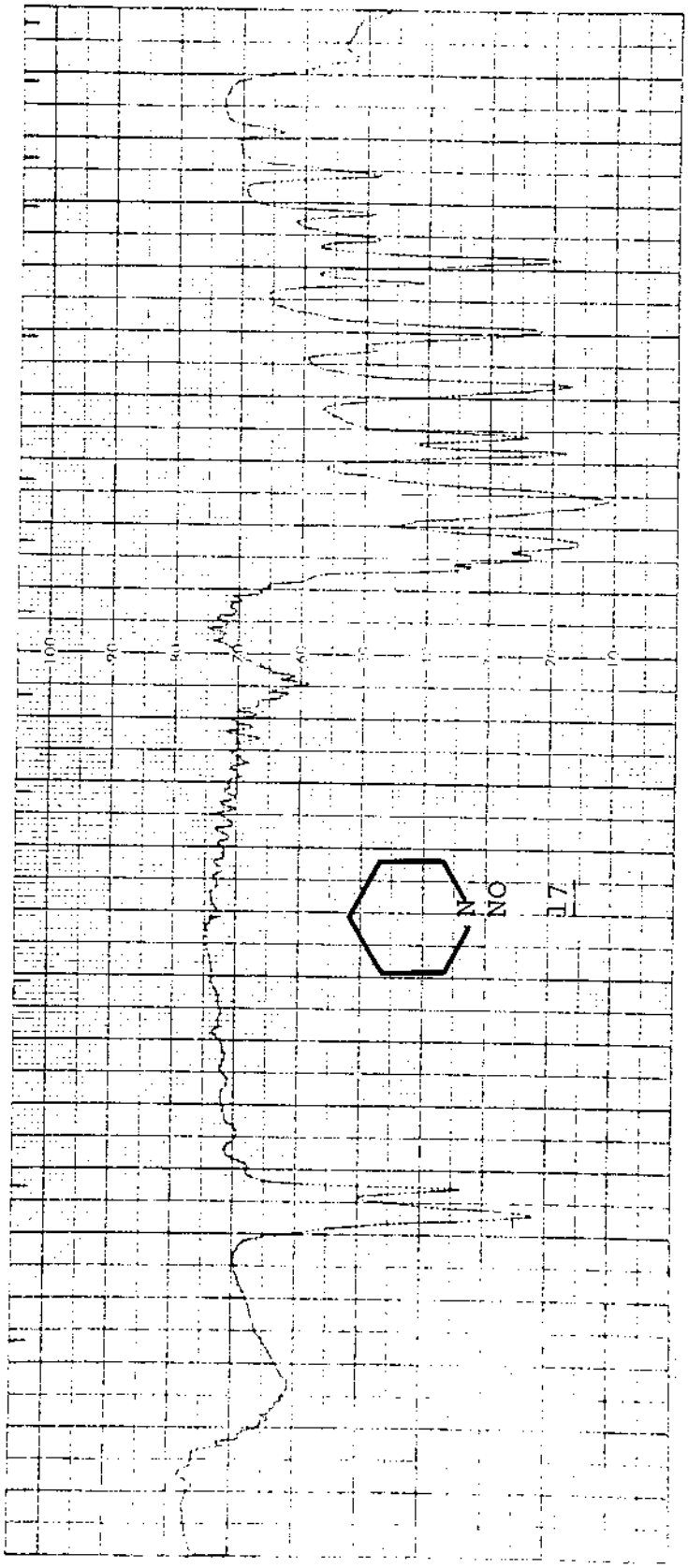


Fig. 6--The IR spectrum of compound 17.

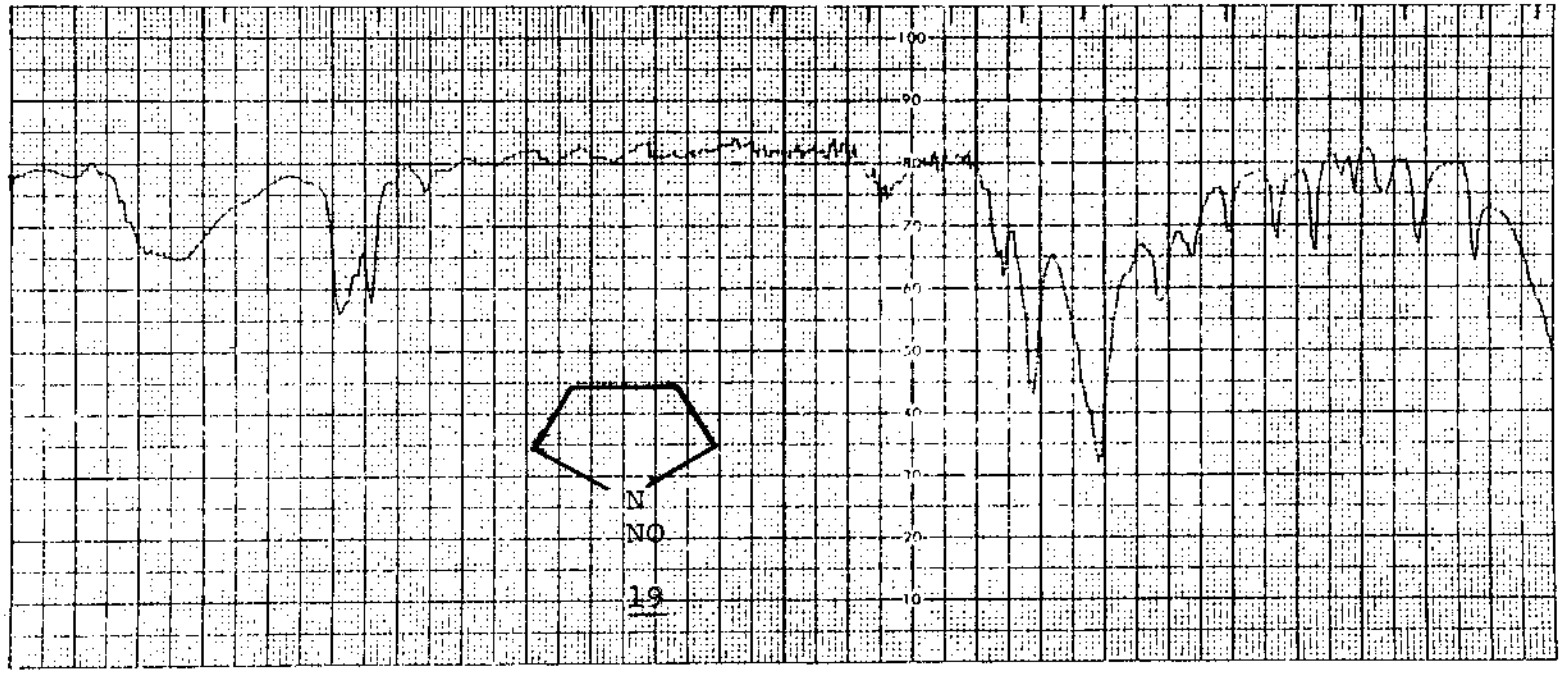


Fig. 7--The IR spectrum of compound 19.

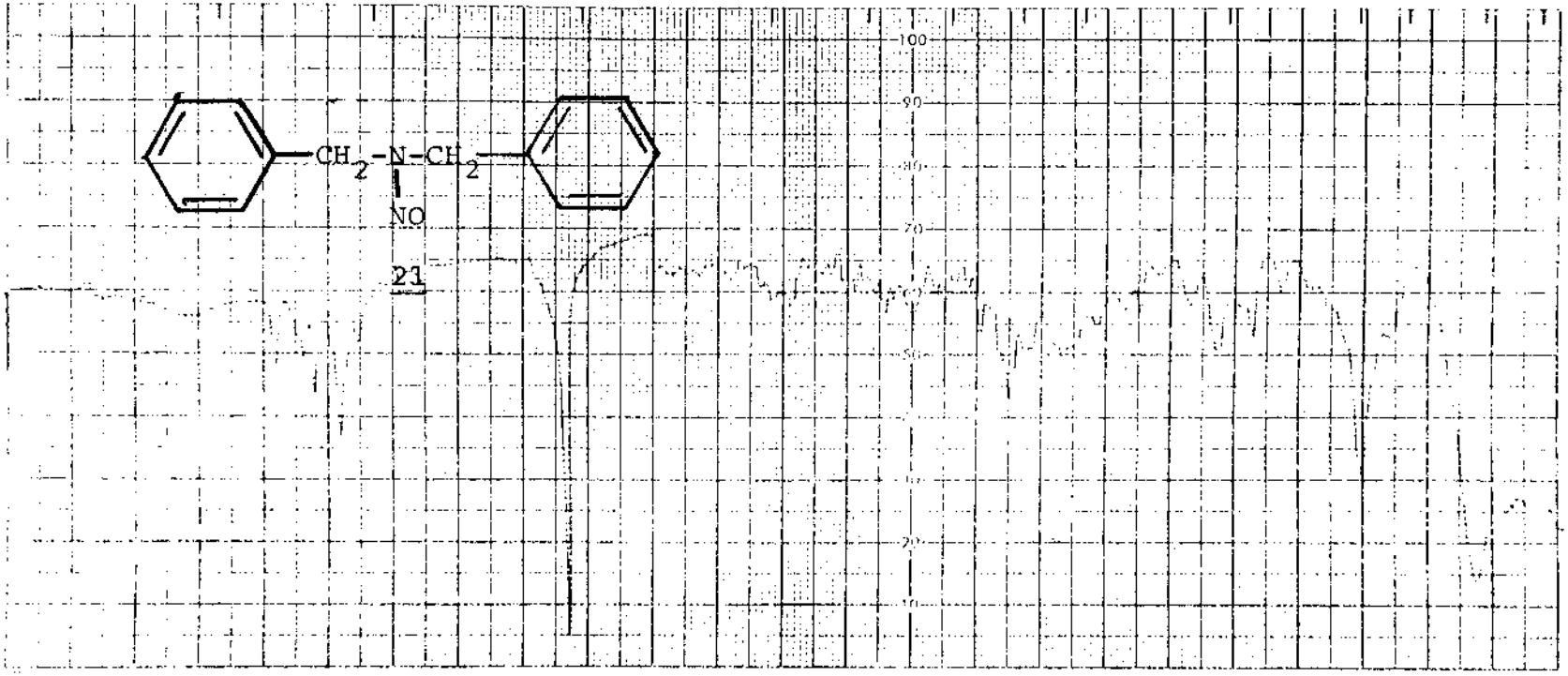


Fig. 8--The IR spectrum of compound 21.

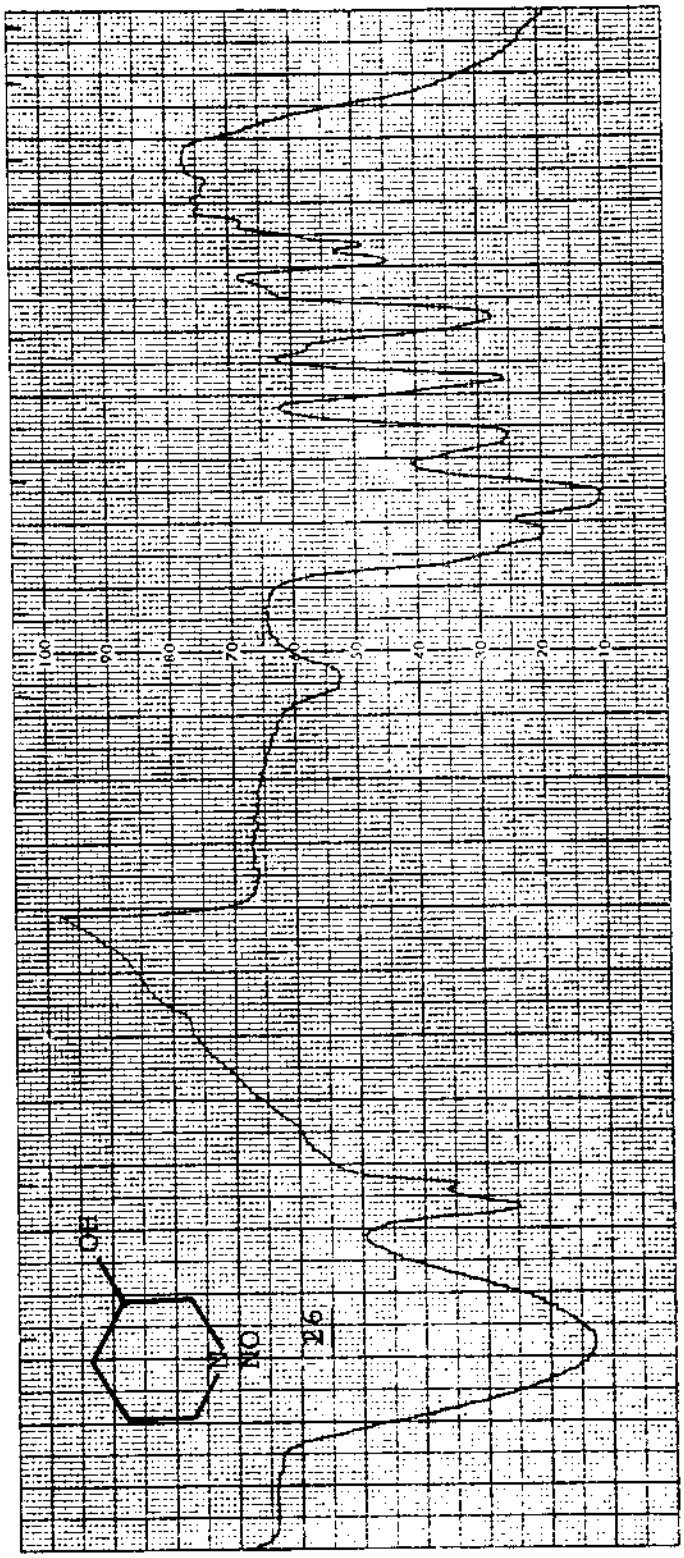


Fig. 9--The IR spectrum of compound 26.

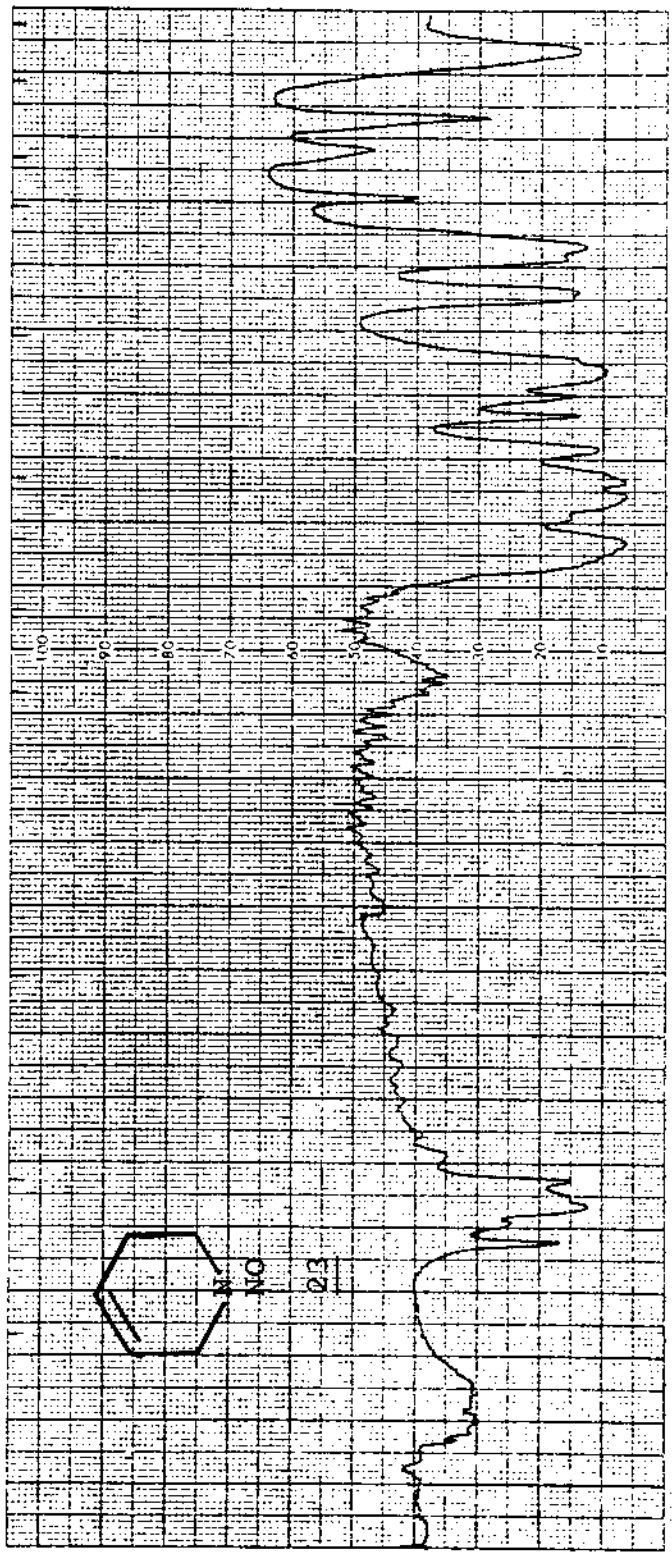


Fig. 10--The IR spectrum of compound 23.

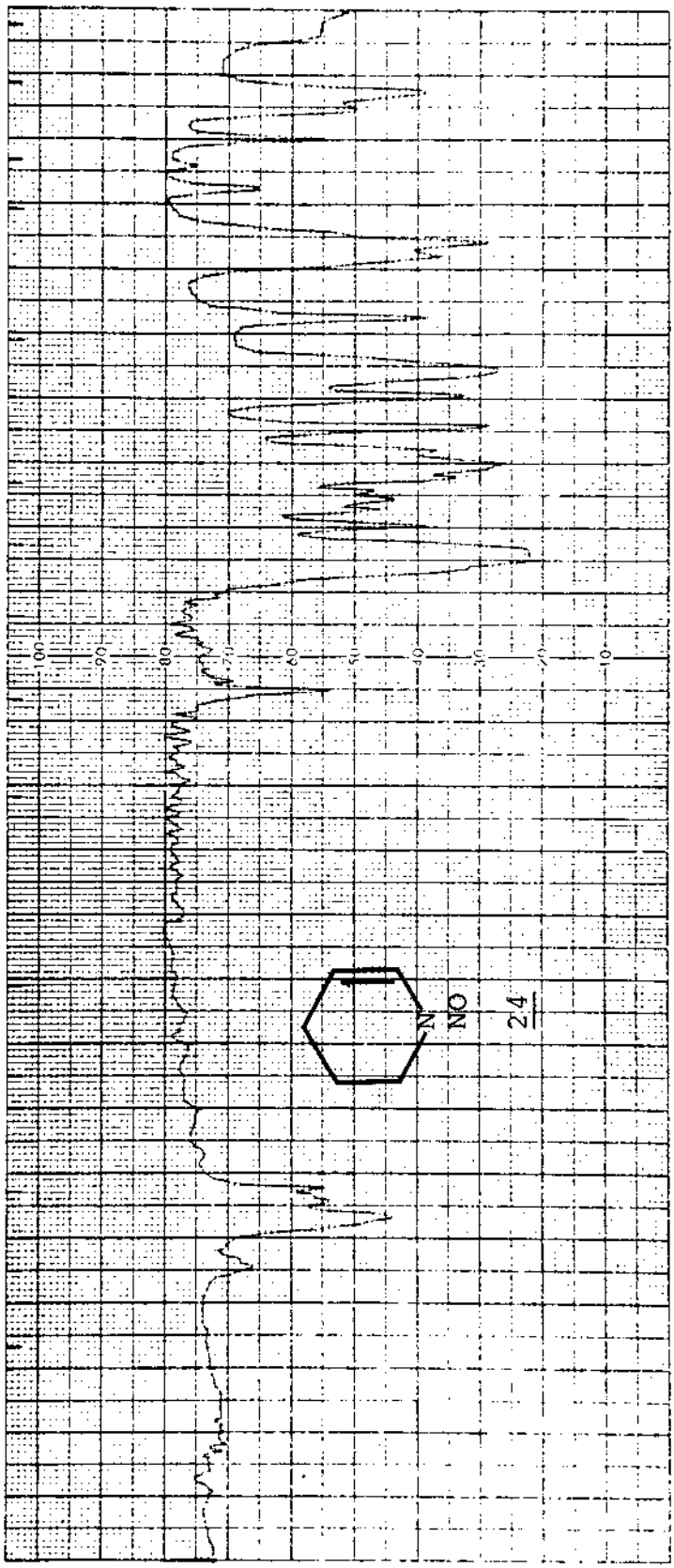


Fig. 11--The IR spectrum of compound 24.

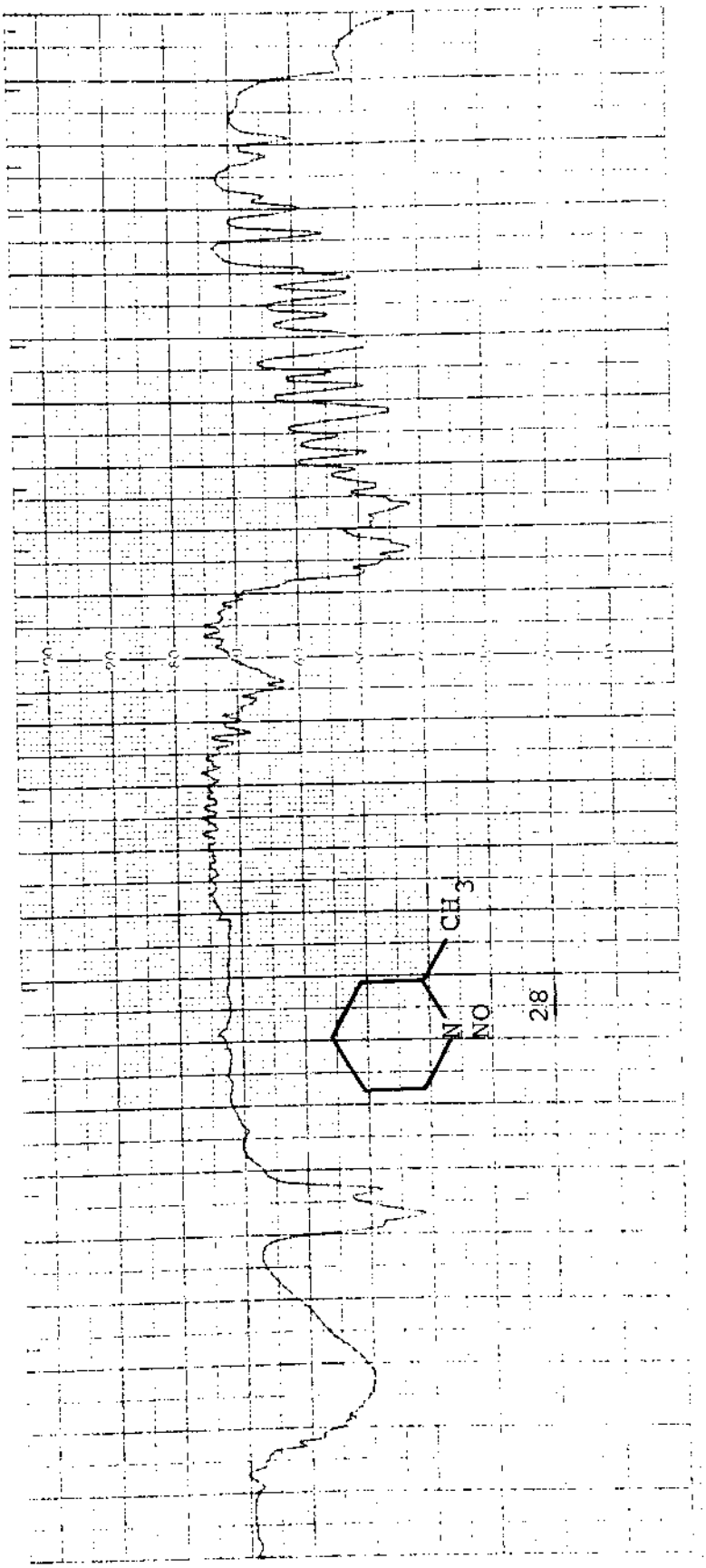


Fig. 12--The IR spectrum of compound 28.

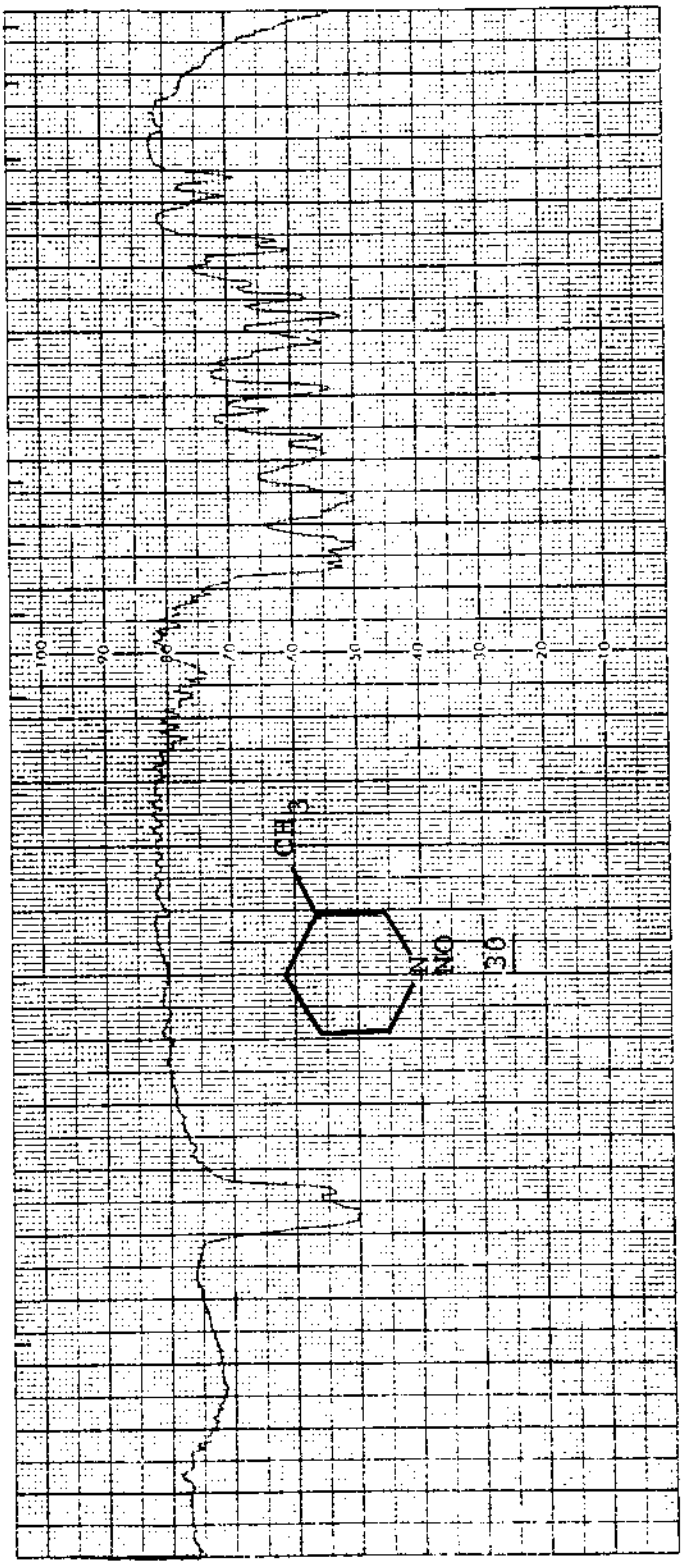


Fig. 13--The IR spectrum of compound 30.

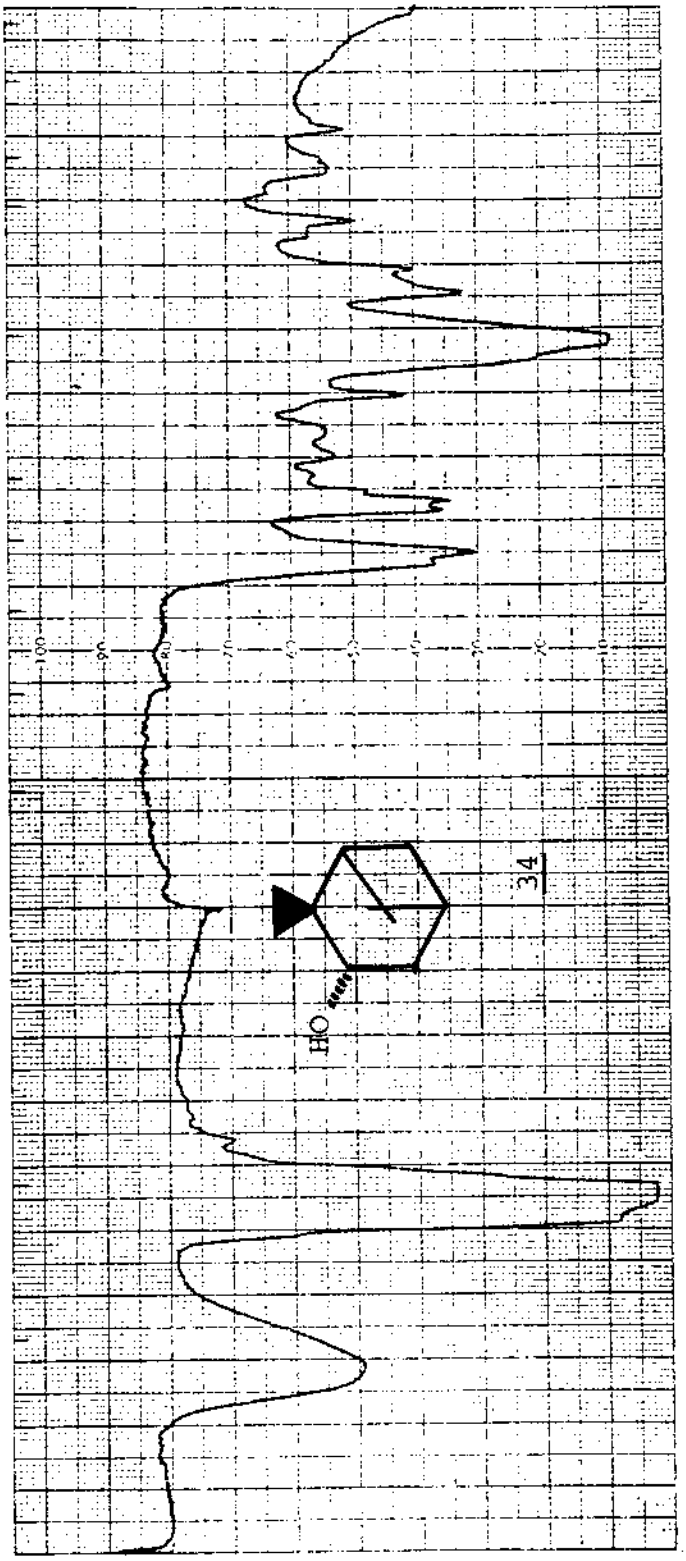


Fig. 14--The IR spectrum of compound 34.

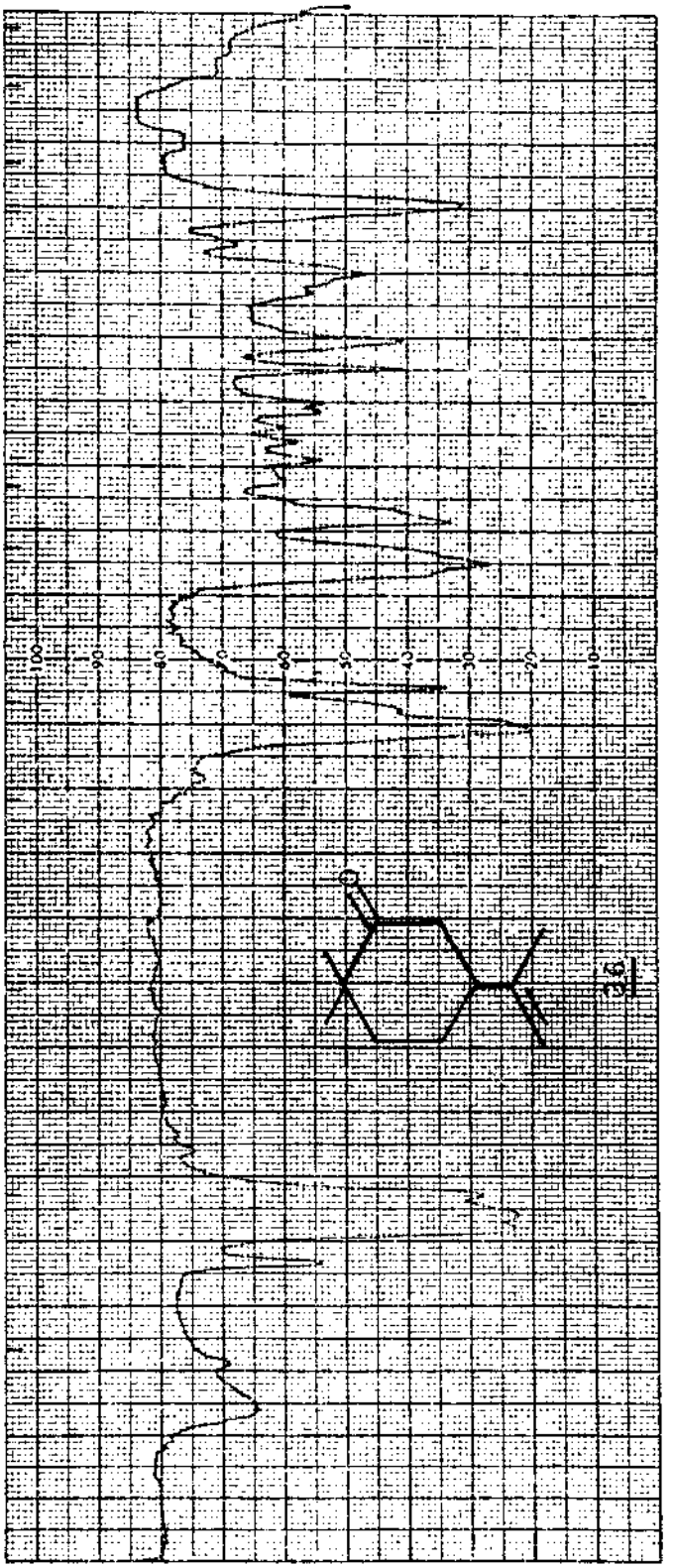


Fig. 15--The IR spectrum of compound 36.

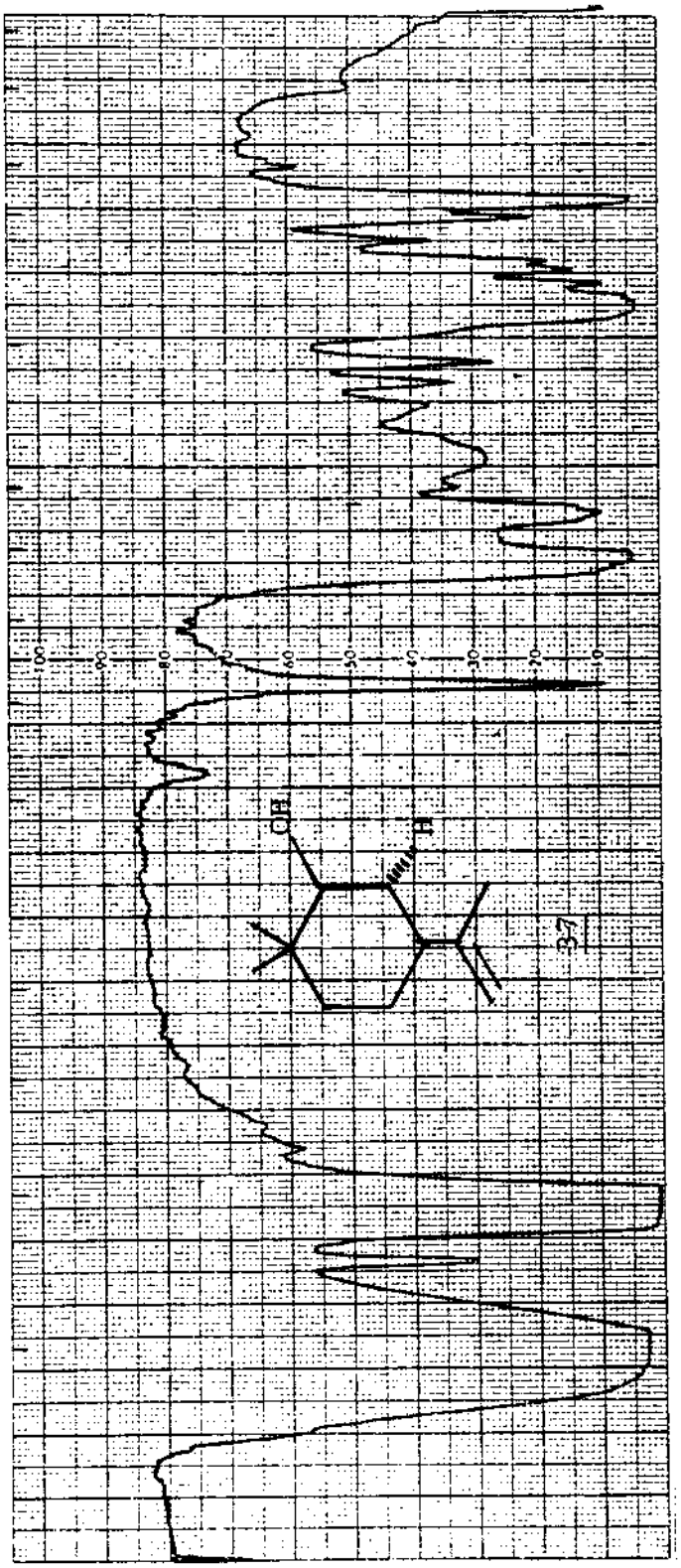


Fig. 16--The IR spectrum of compound 37.

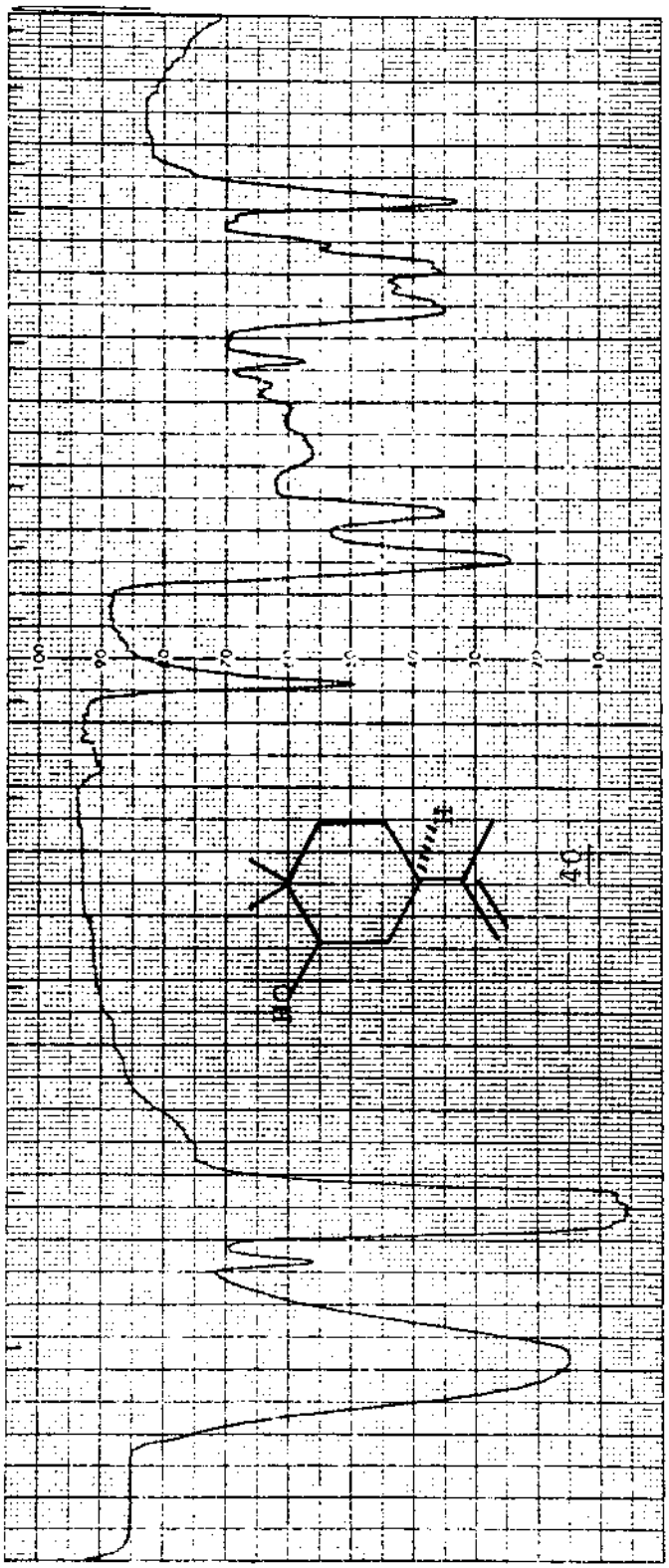


Fig. 17--The IR spectrum of compound 40.

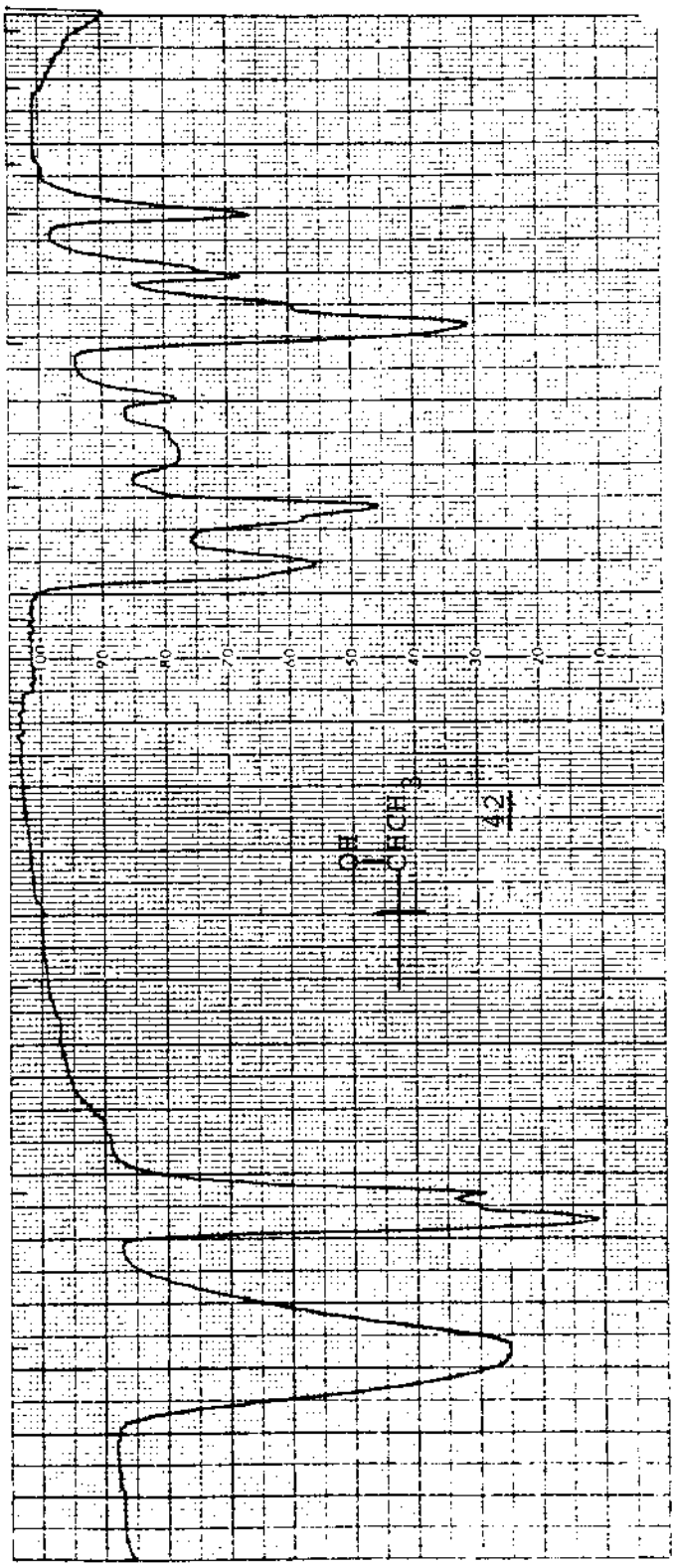


Fig. 18--The IR spectrum of compound 42.

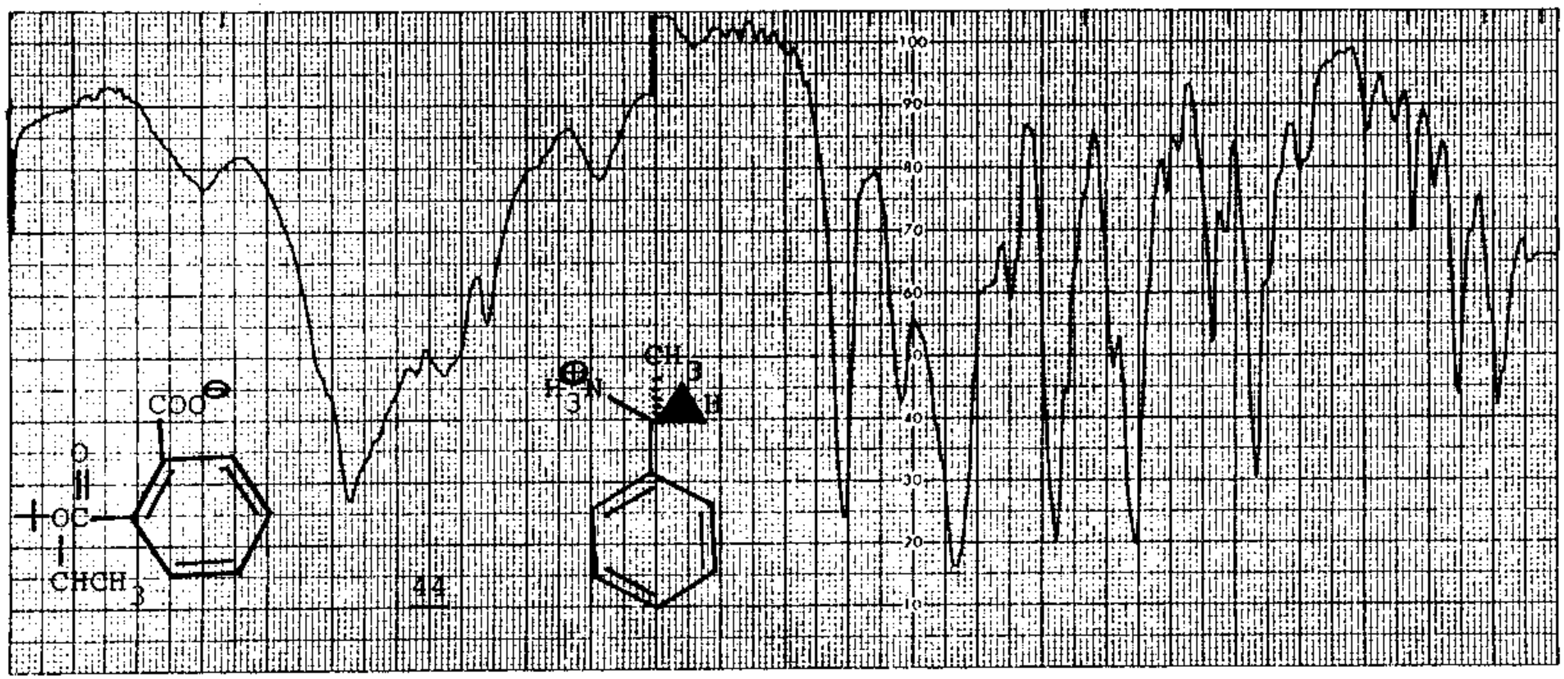


Fig. 19--The IR spectrum of compound 44.

APPENDIX C

CIRCULAR DICHROISM SPECTRA

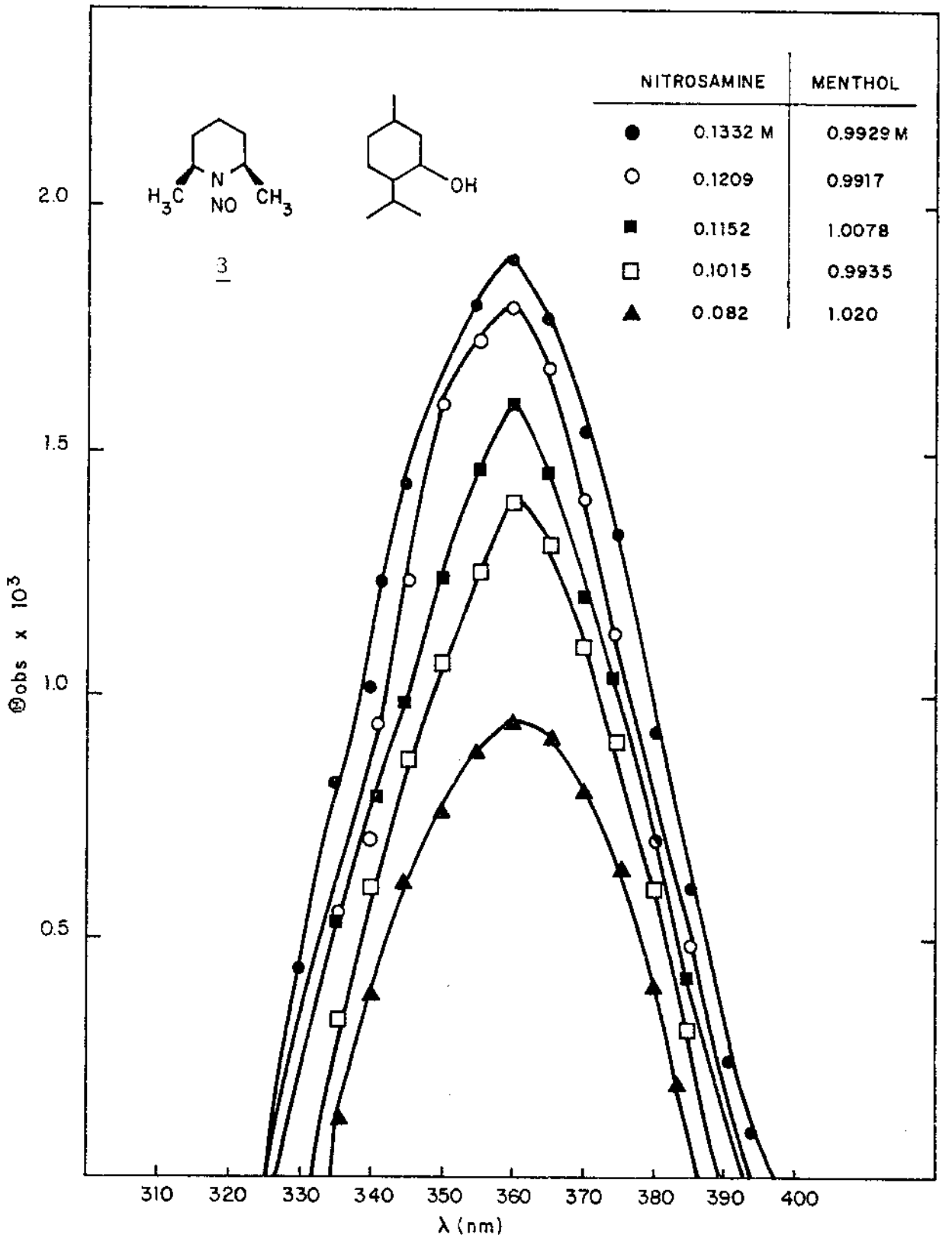


Fig. 1--The ICD spectra of compound 3 with menthol.

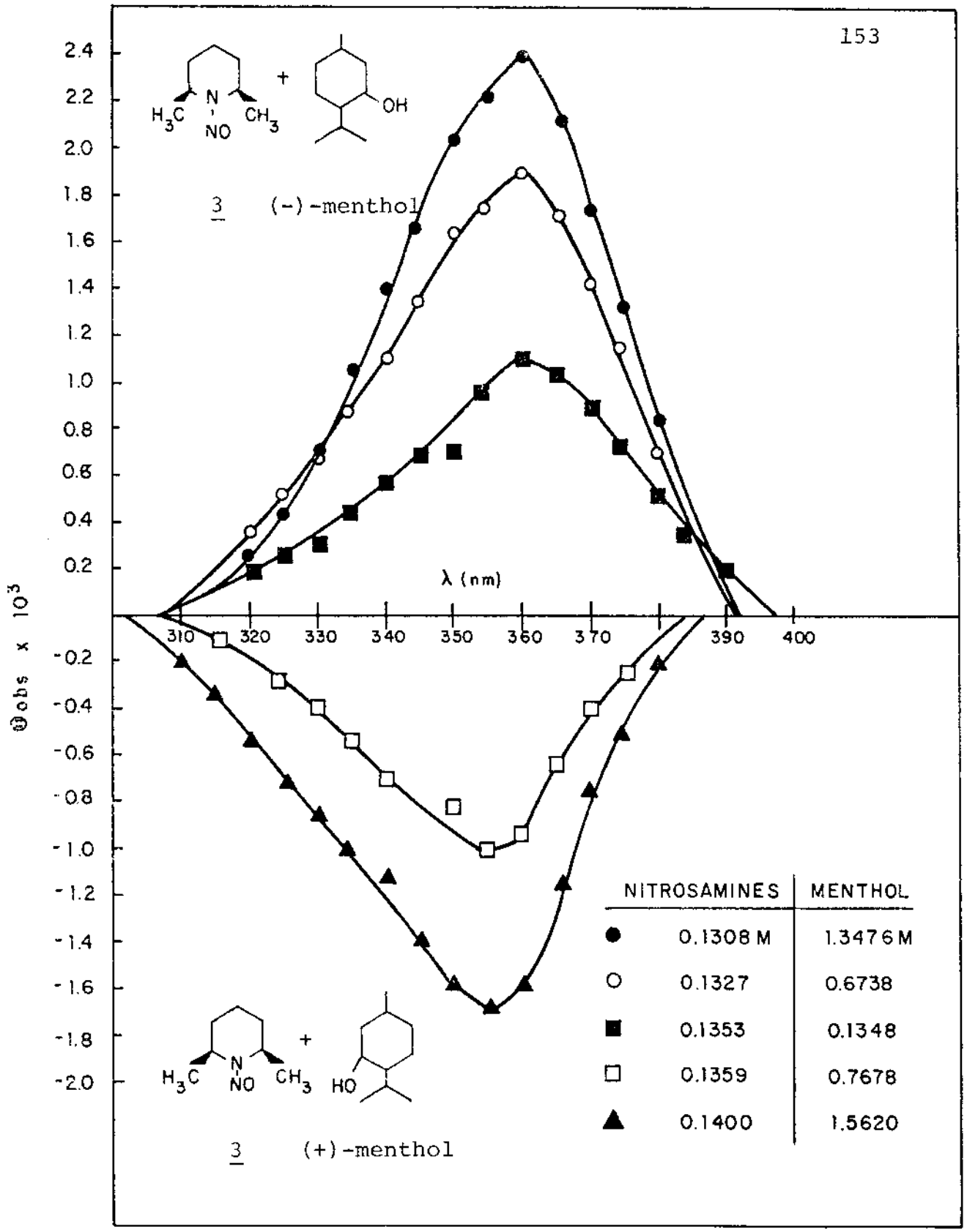


Fig. 2--The ICD spectra of compound 3 with menthol.

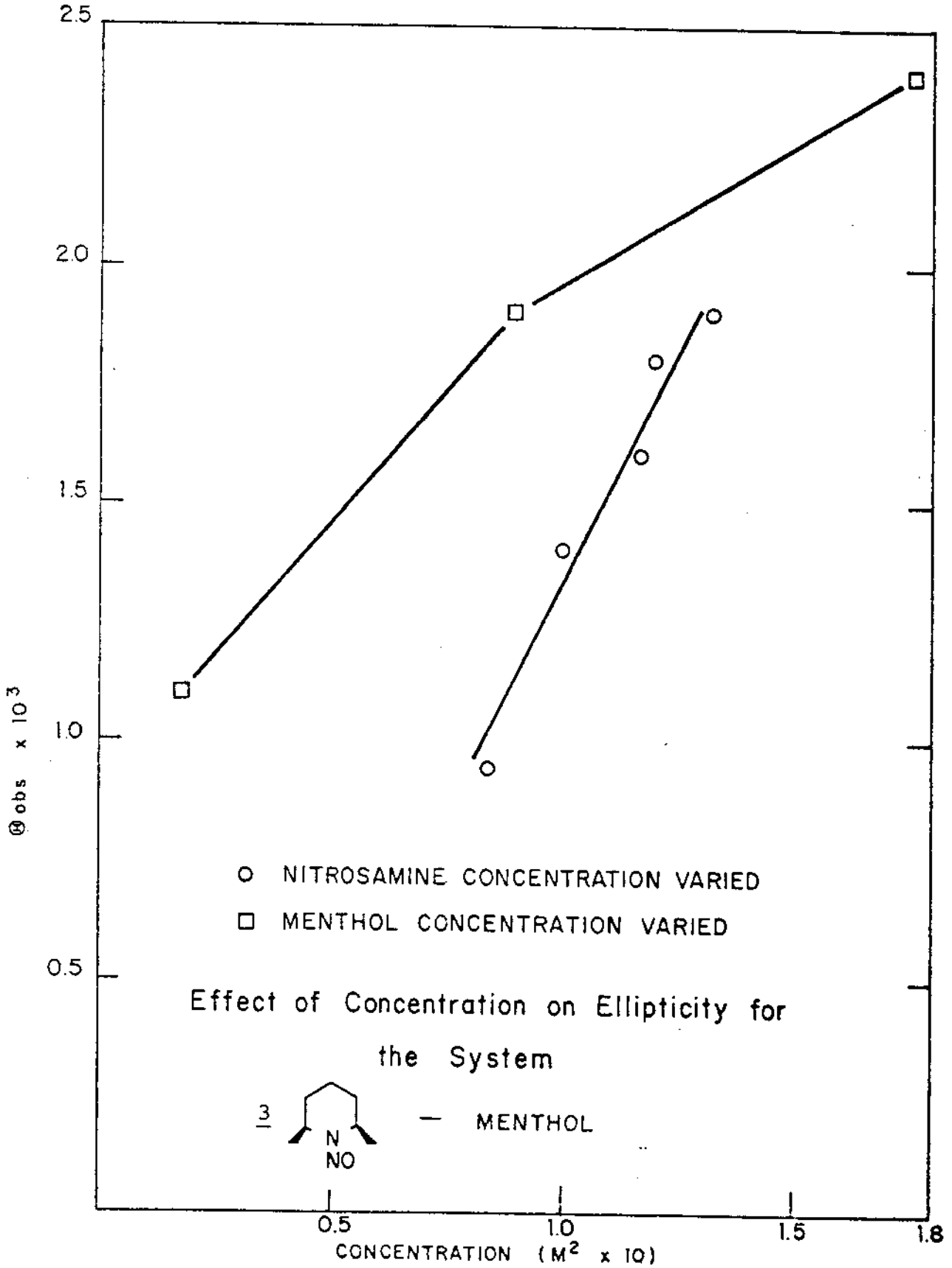


Fig. 3--The effect of concentration of the (-)-menthol-3 system on ellipticity.

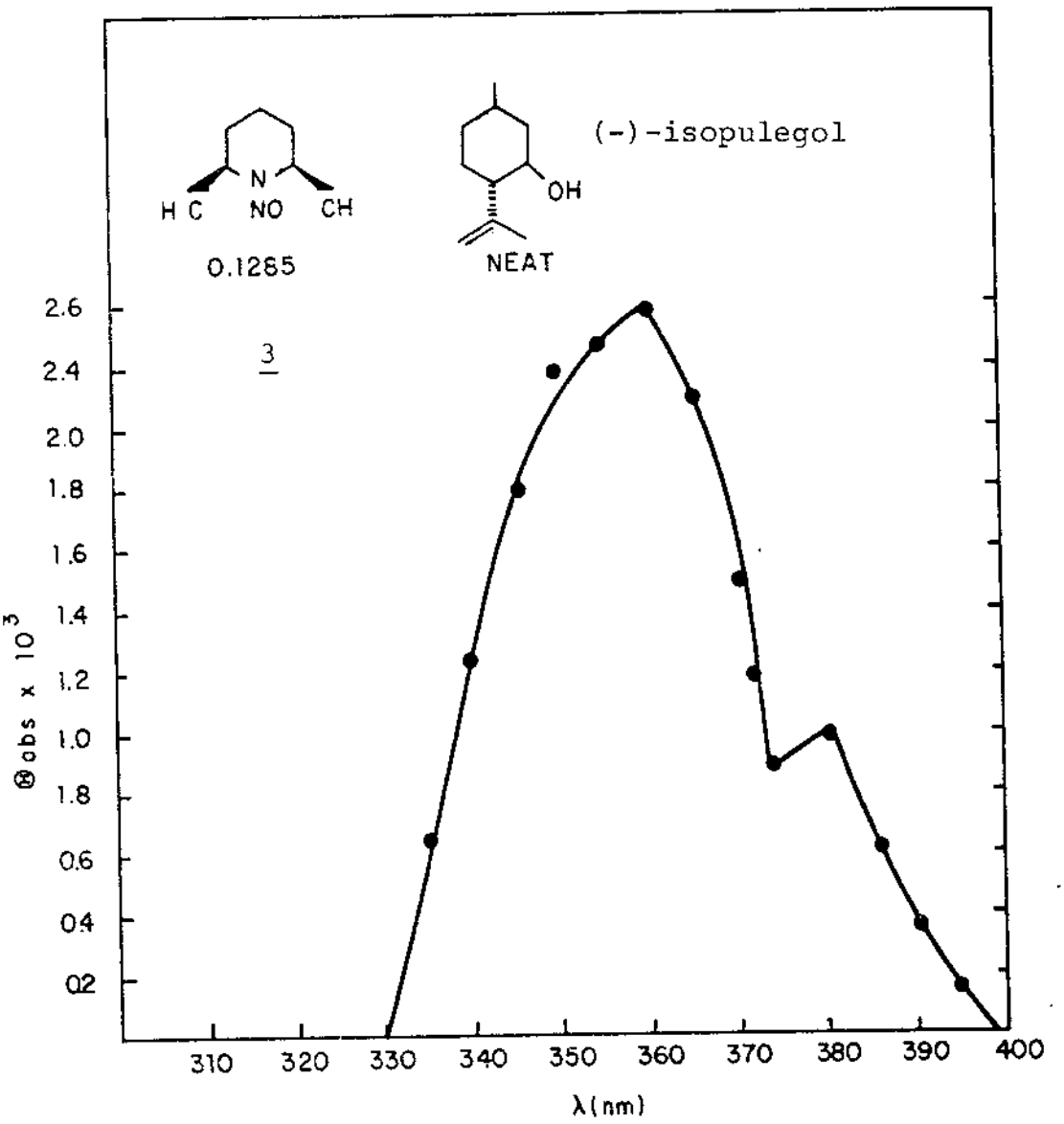


Fig. 4--The ICD spectrum of compound 3 with (-)-isopulegol.

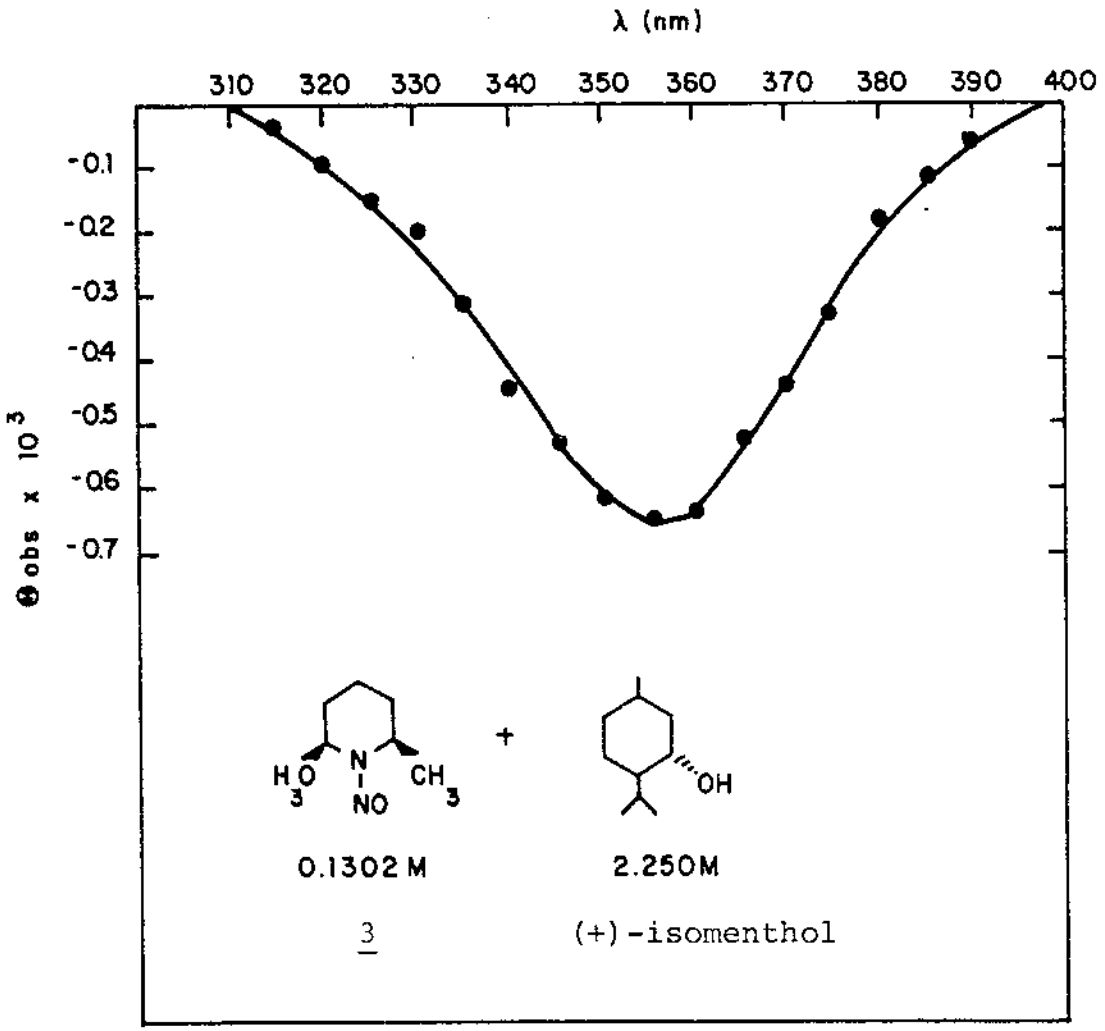


Fig. 5--The ICD spectrum of compound 3 with (+)-isomenthol.

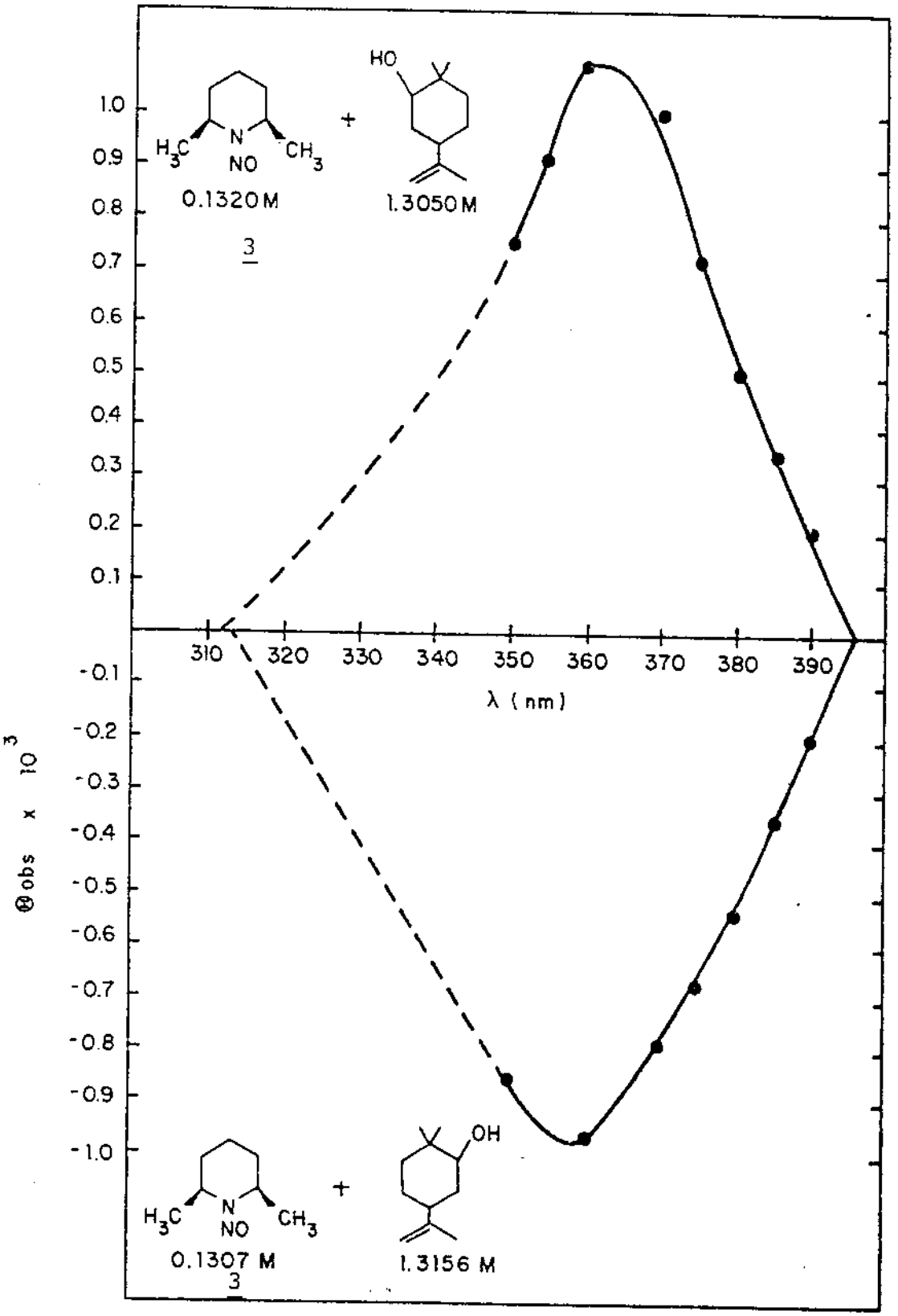


Fig. 6--The ICD spectra of compound 3 with 2-methyldihydrocarveol (dashed lines indicate the spectra are extrapolated to zero due to interference by a ketone impurity).

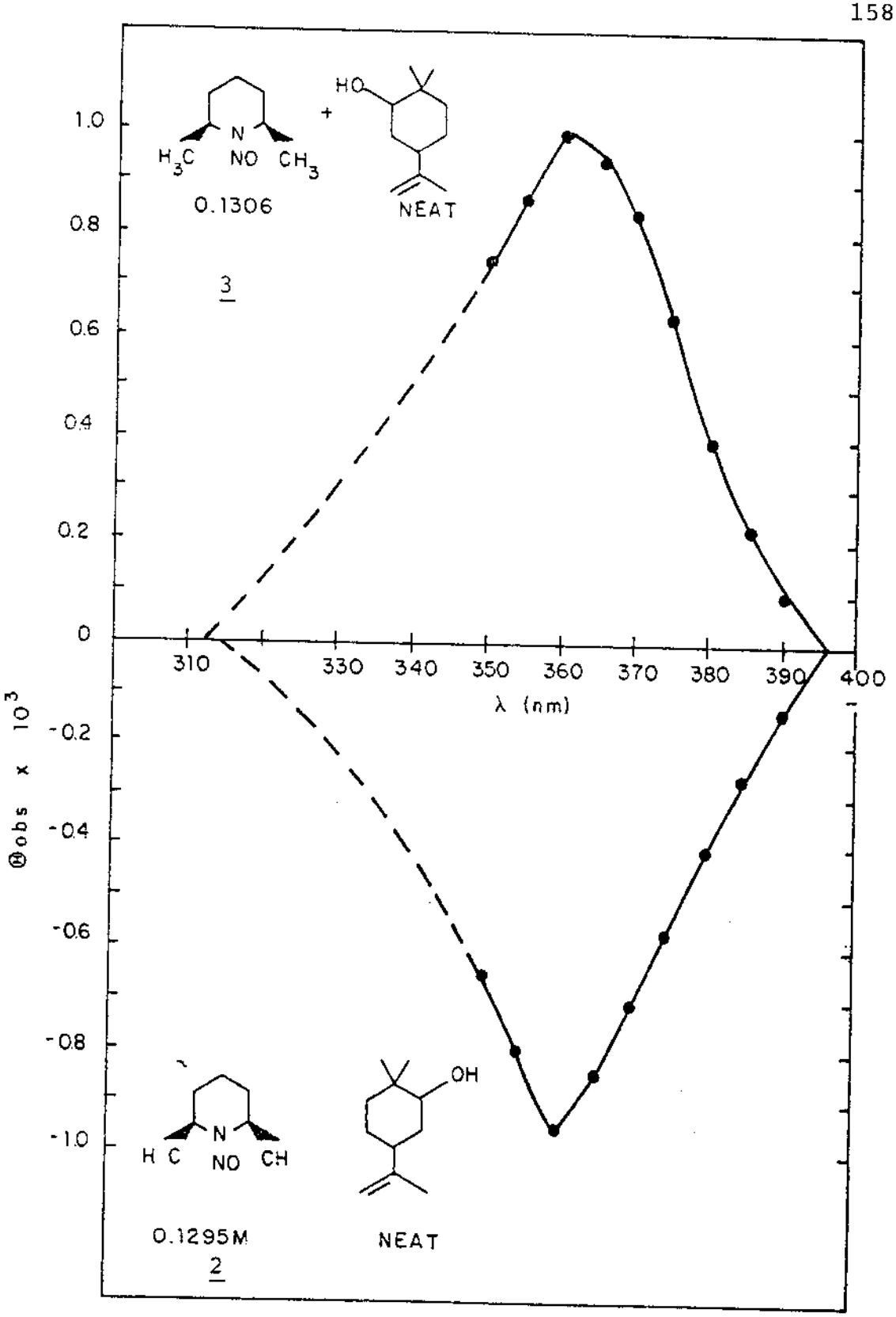


Fig. 7--The ICD spectra of compound 3 with 2-methyldihydrocarveol (dashed lines indicate the spectra are extrapolated to zero due to interference by a ketone impurity).

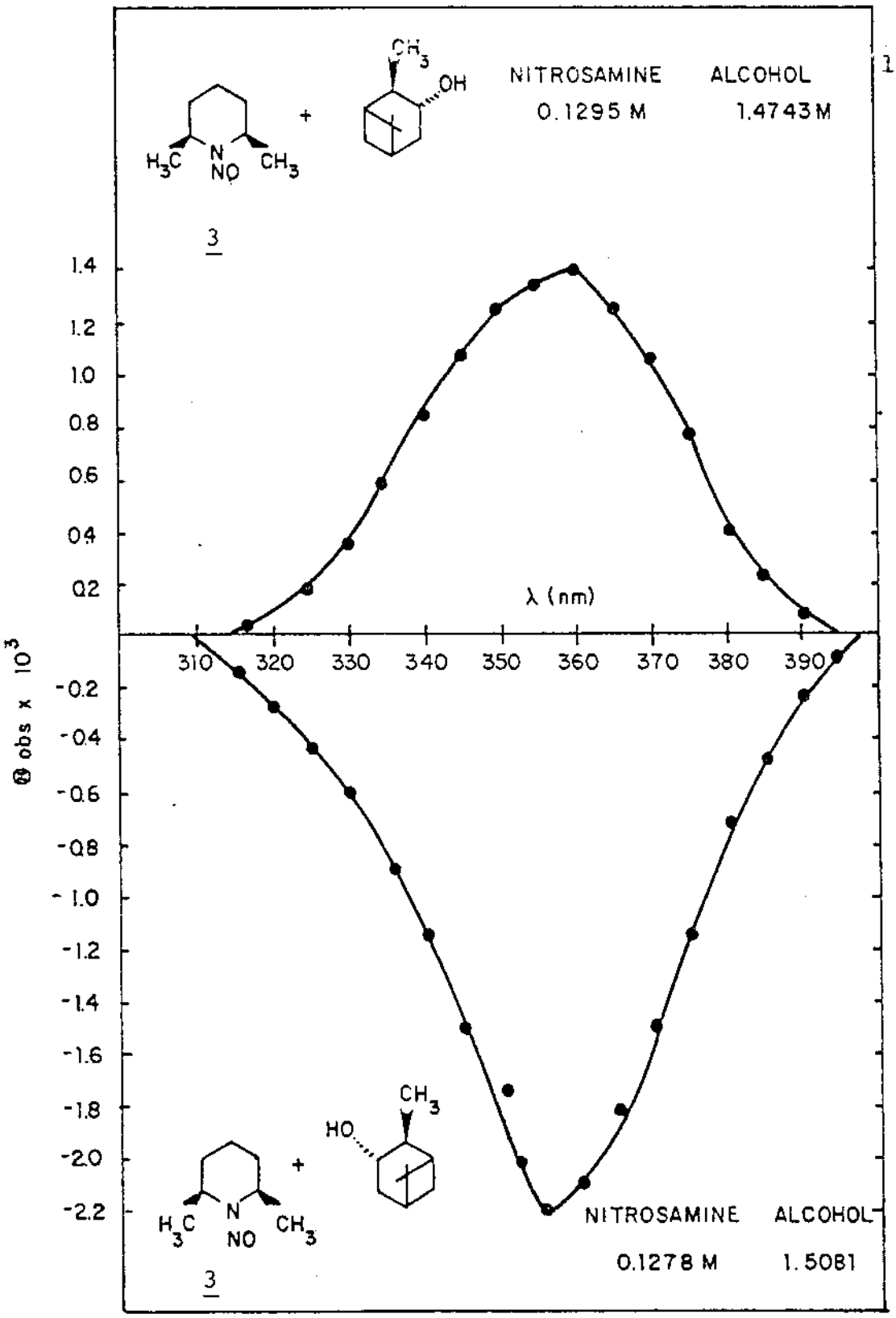


Fig. 8--The ICD spectra of compound 3 with pinanol. (NOTE: Due to the large nitrosamine concentrations the curves do not appear as mirror images.)

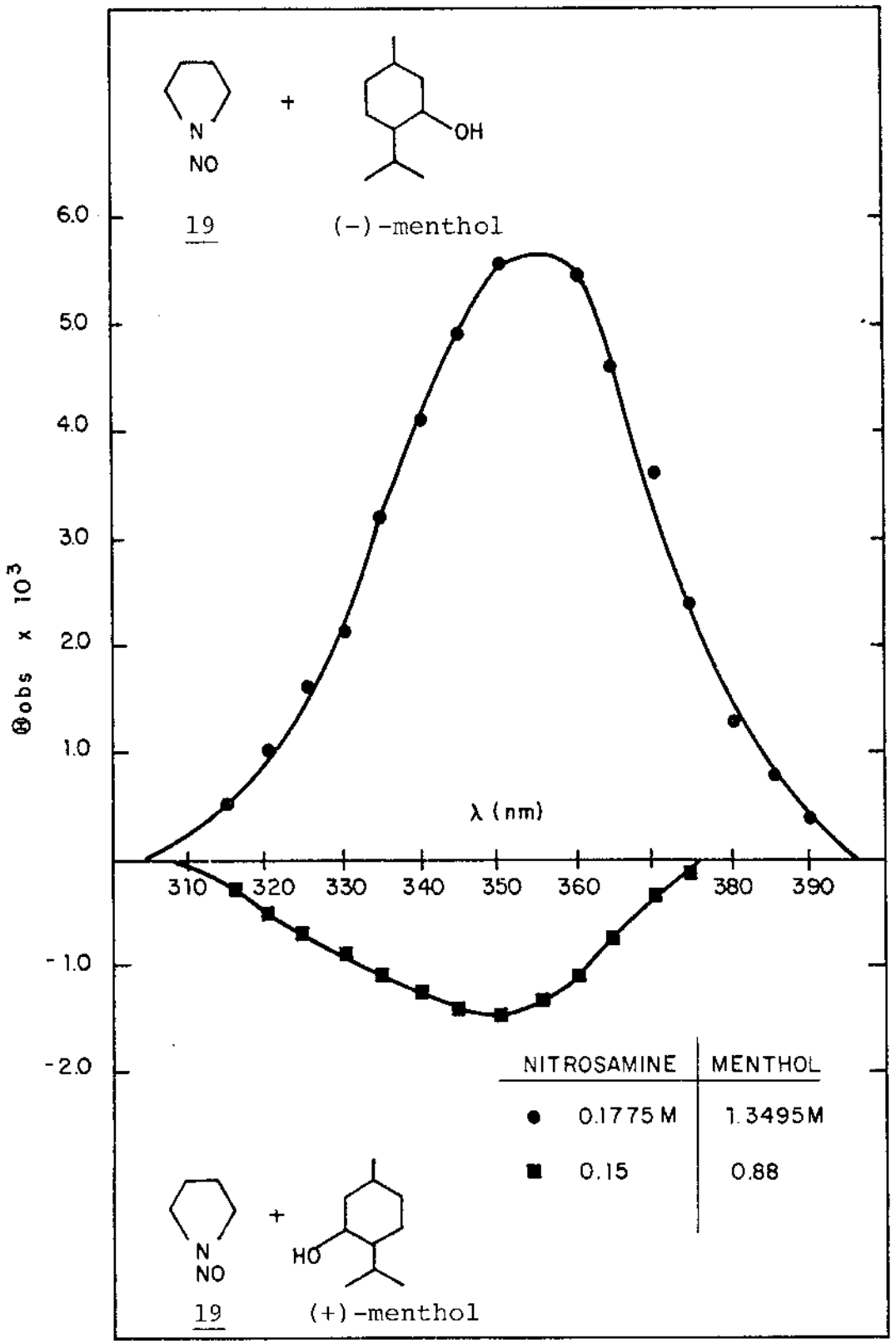


Fig. 9--The ICD spectra of compound 19 with menthol.

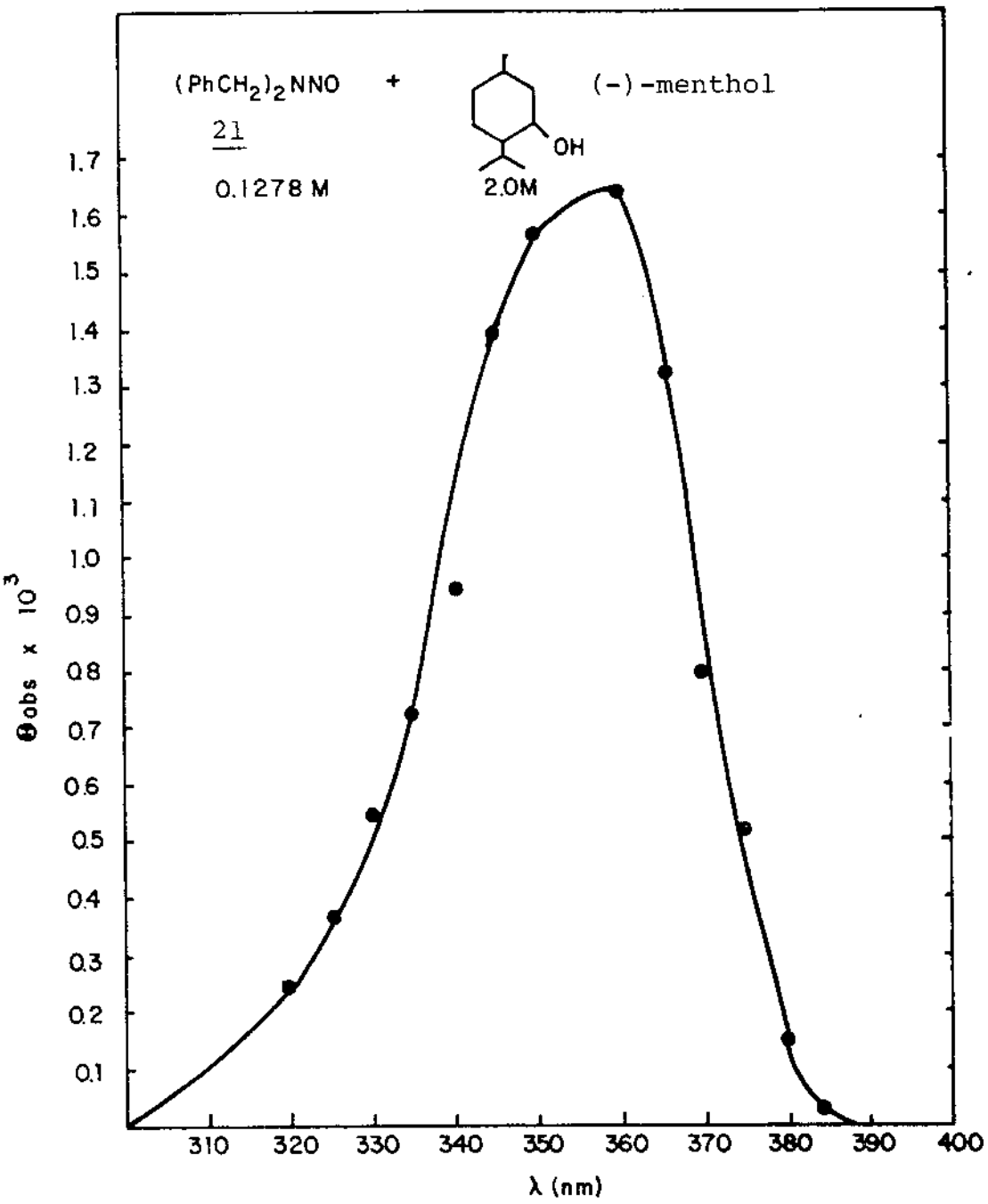


Fig. 10--The ICD spectrum of compound 21 with (-)-menthol.

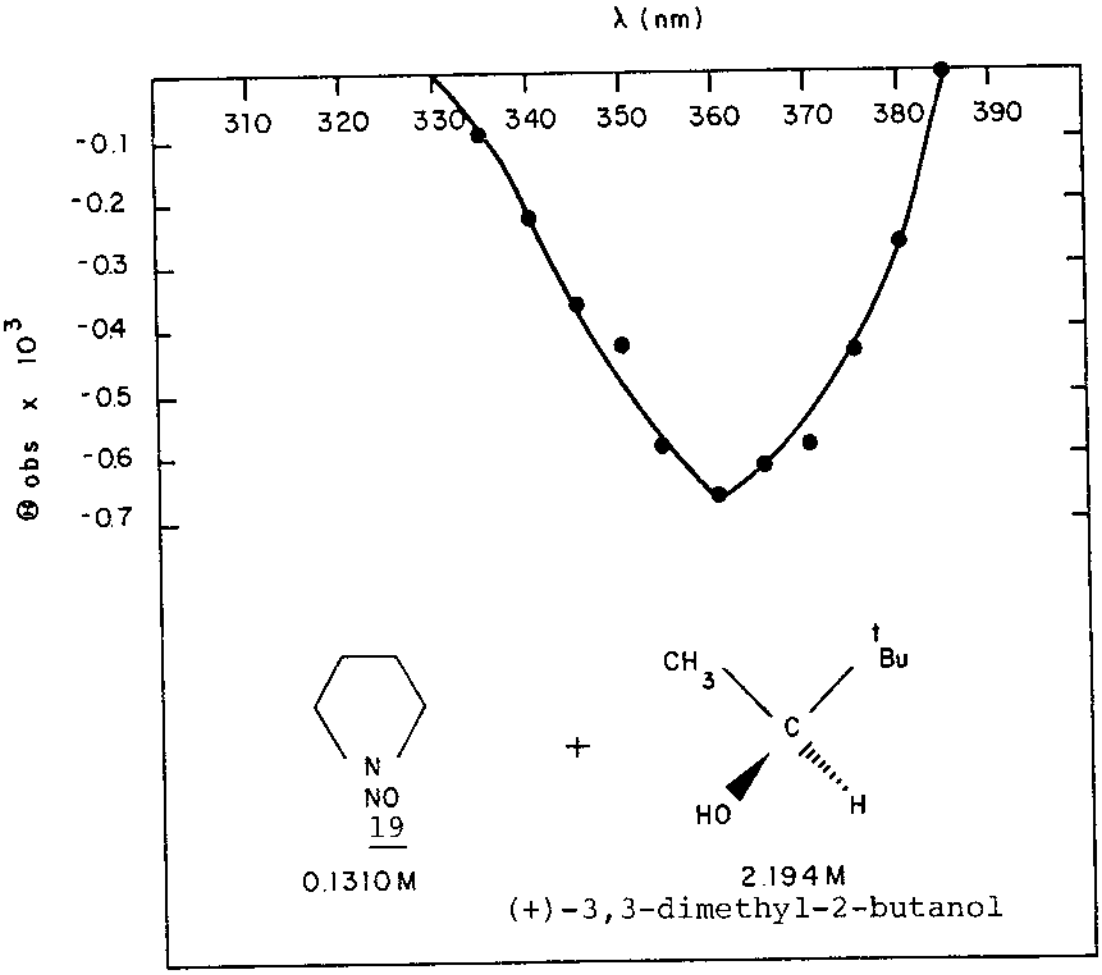


Fig. 11--The ICD spectrum of compound 19 with (+)-3,3-dimethyl-2-butanol.

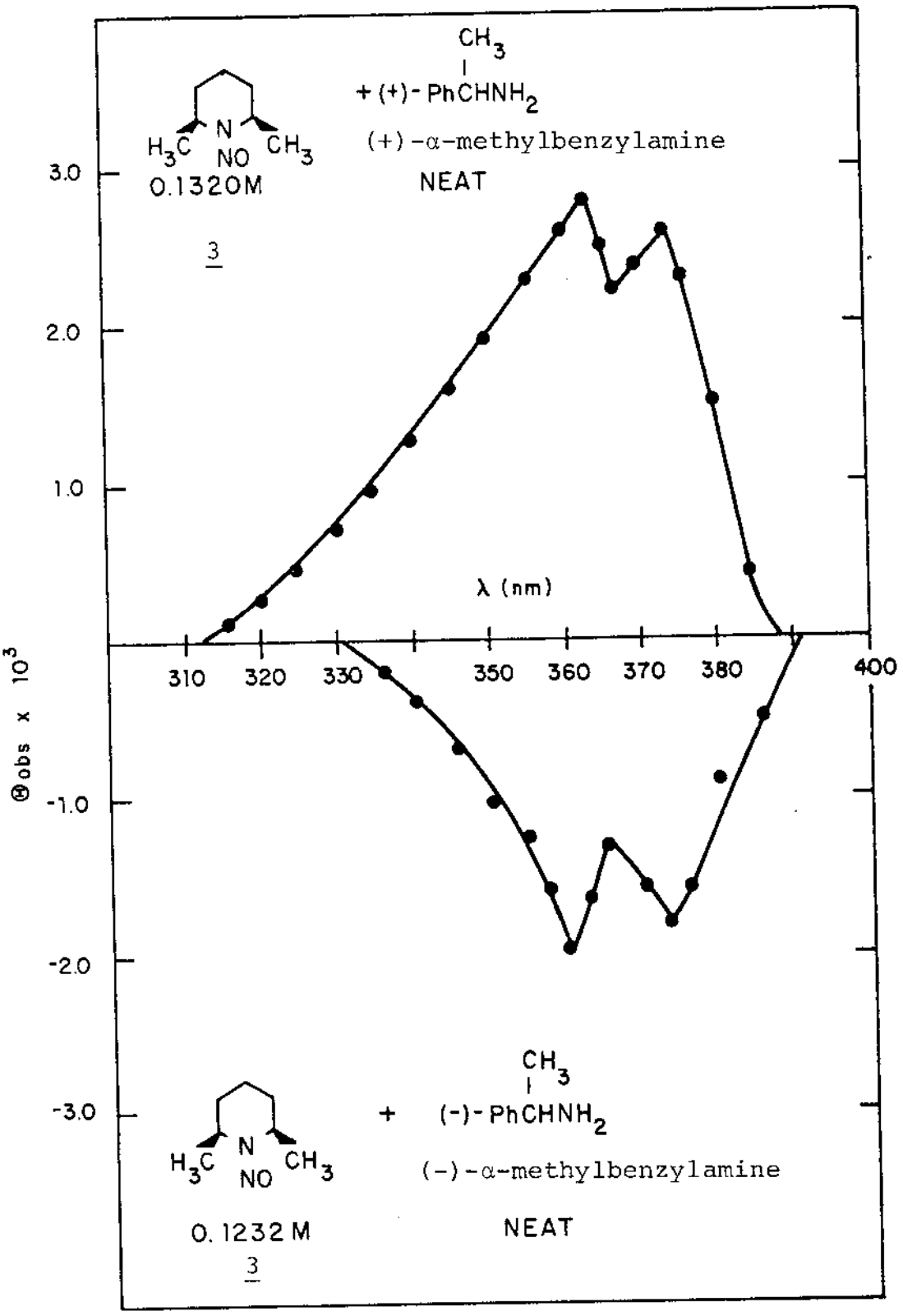


Fig. 12--The ICD spectra of compound 3 with α-methylbenzylamine.

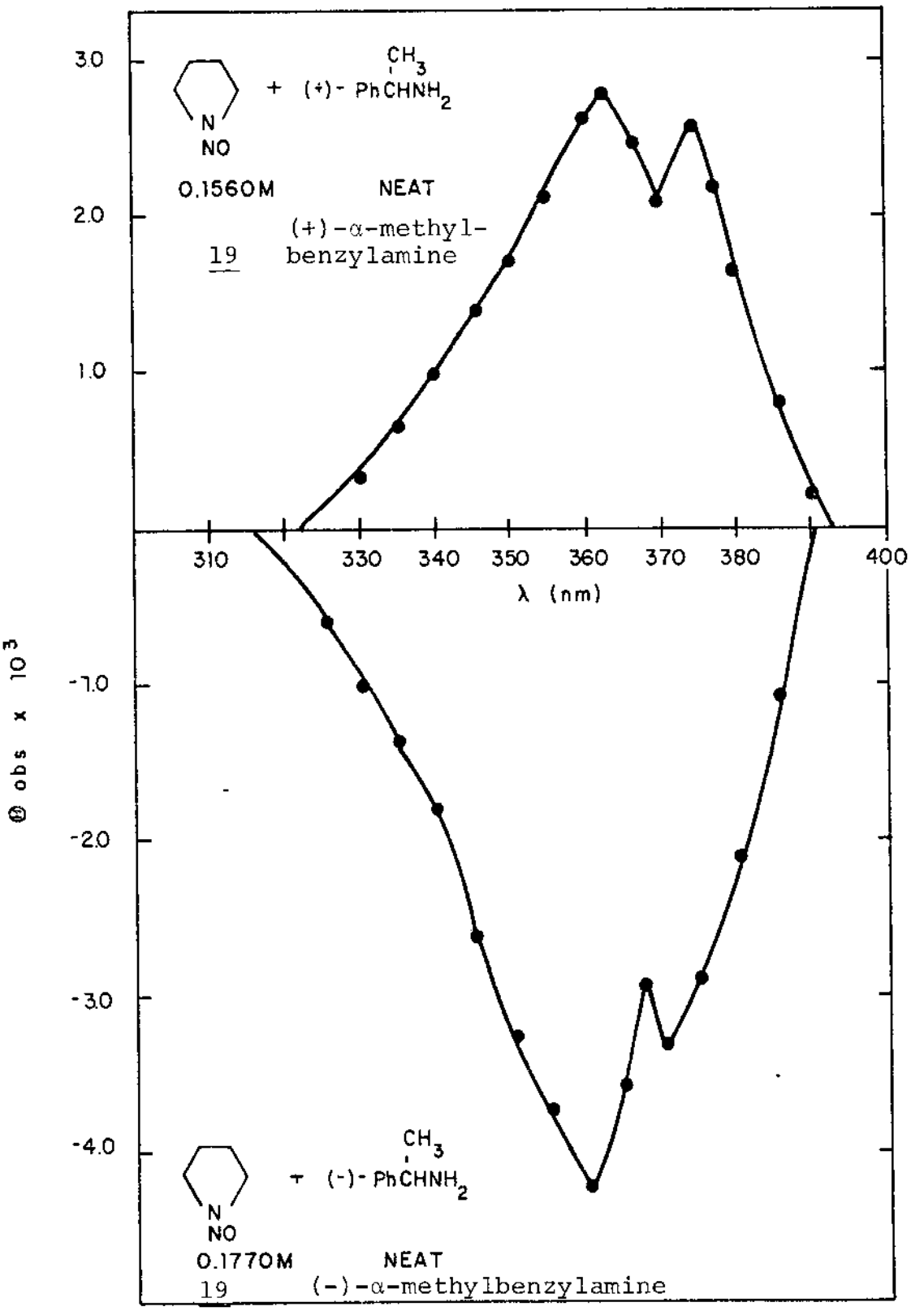


Fig. 13--The ICD spectra of compound 19 with α-methyl-benzylamine.

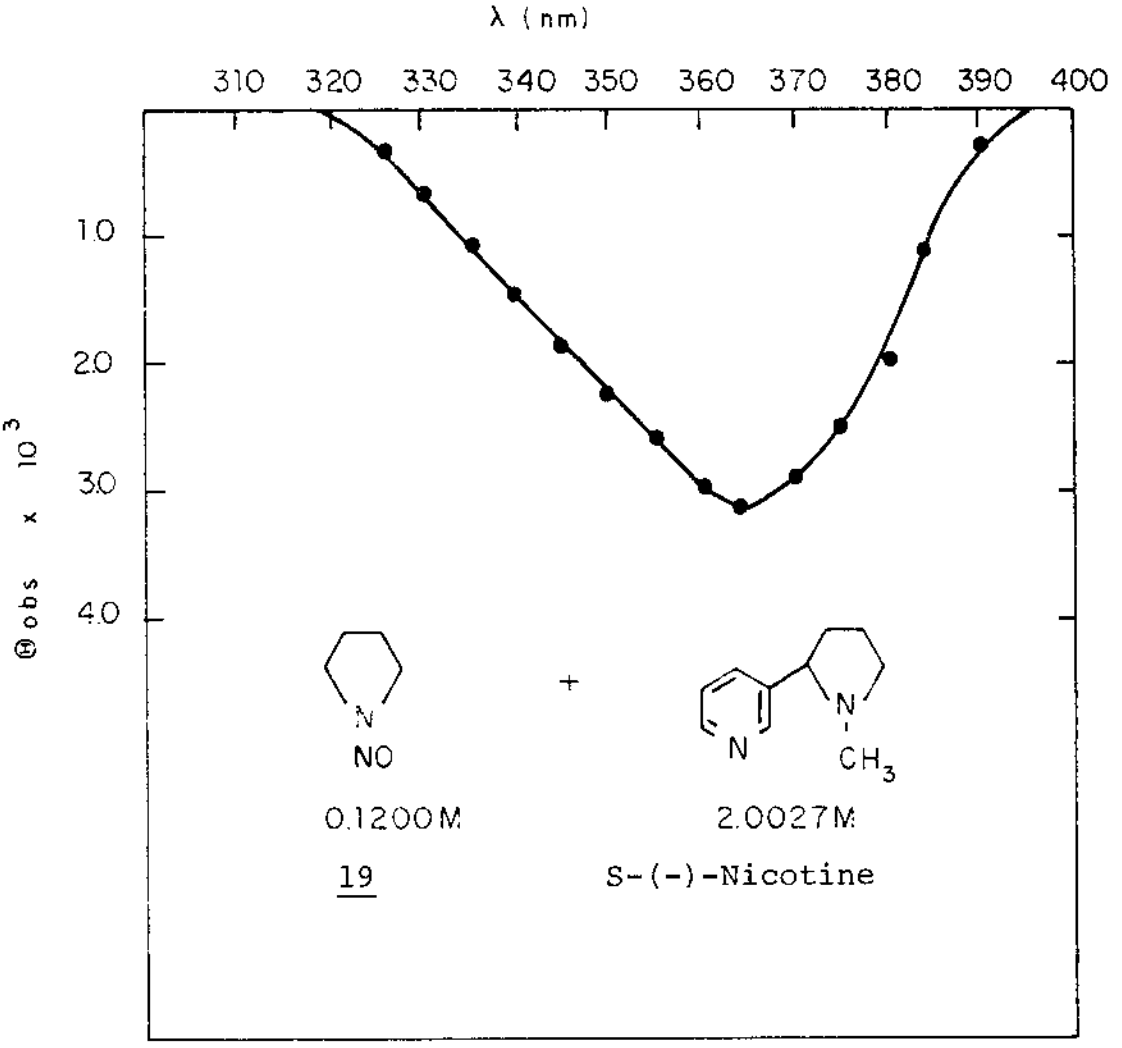


Fig. 14--The ICD spectrum of compound 19 with S-(-)-nicotine.

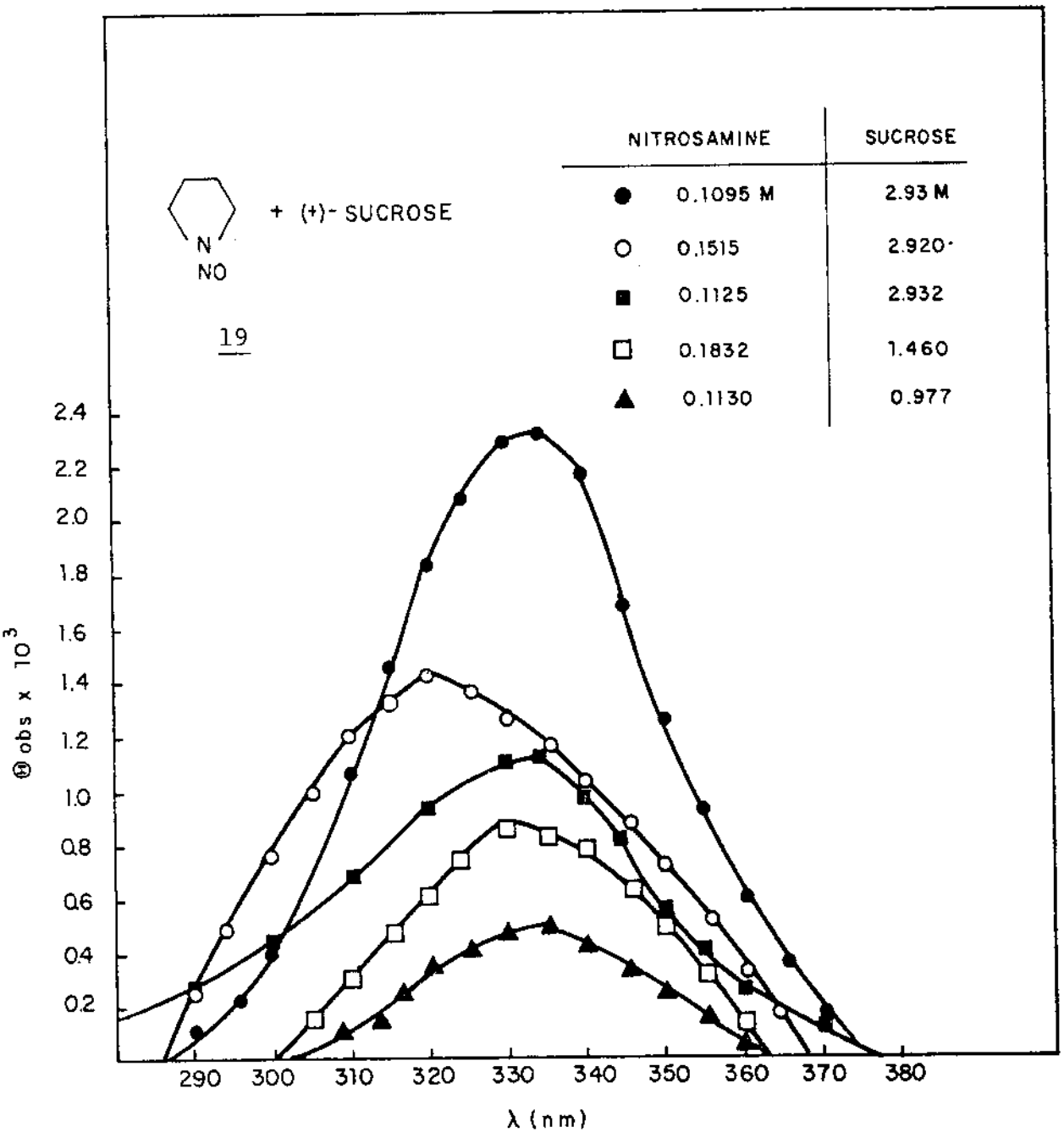


Fig. 15--The ICD spectra of compound 19 with sucrose.

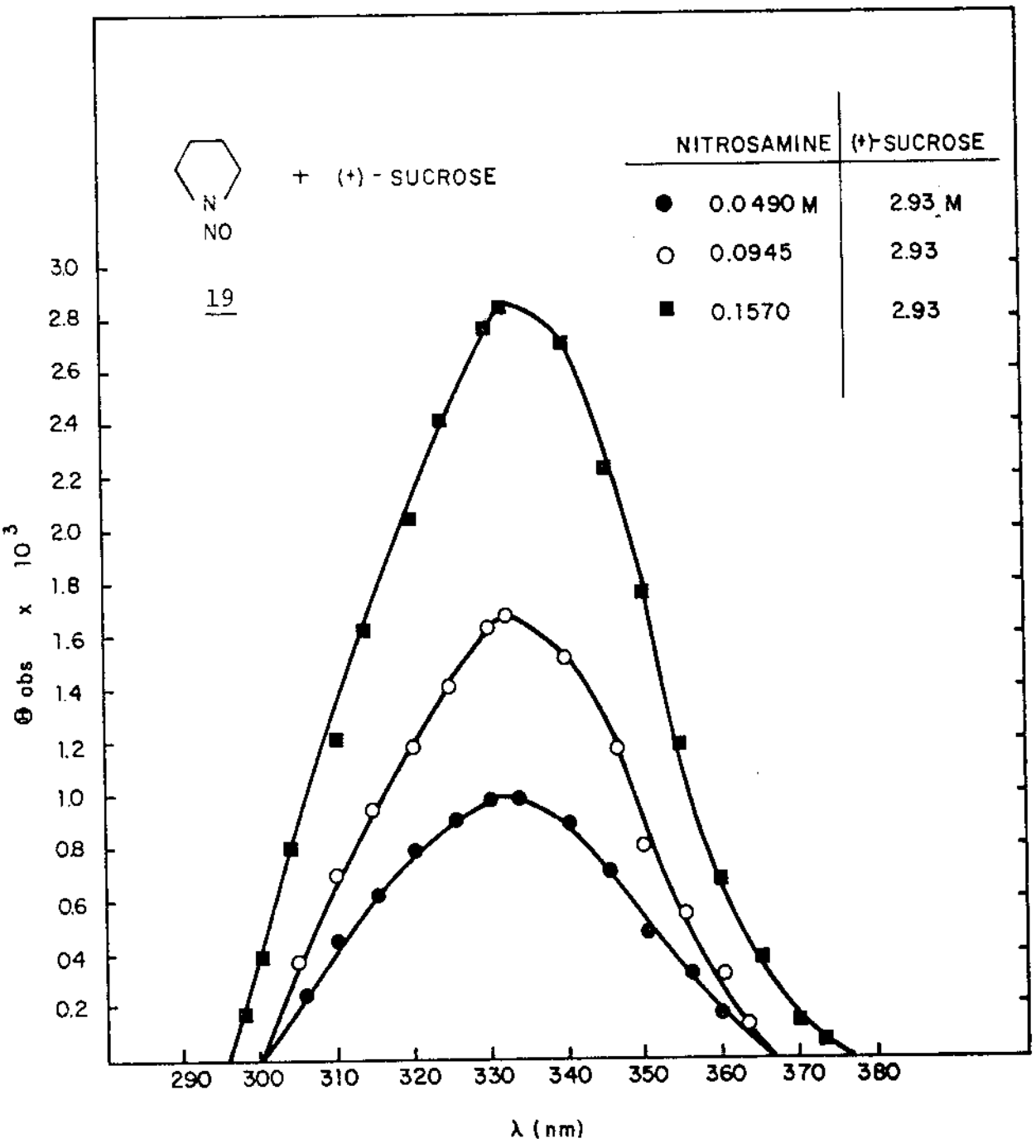


Fig. 16--The ICD spectra of compound 19 with (+)-sucrose.

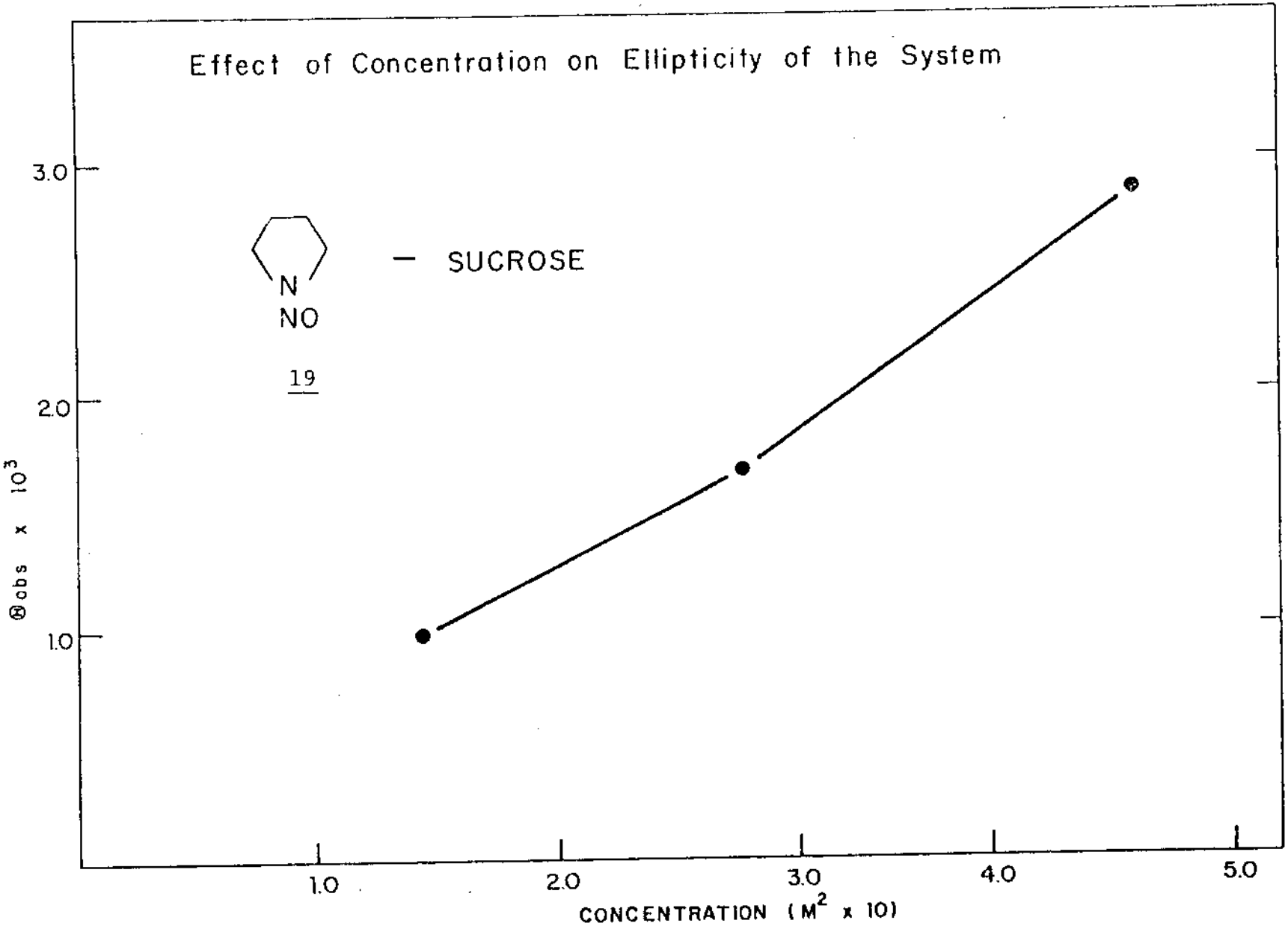


Fig. 17--The effect of concentration of the sucrose-19 system on ellipticity.

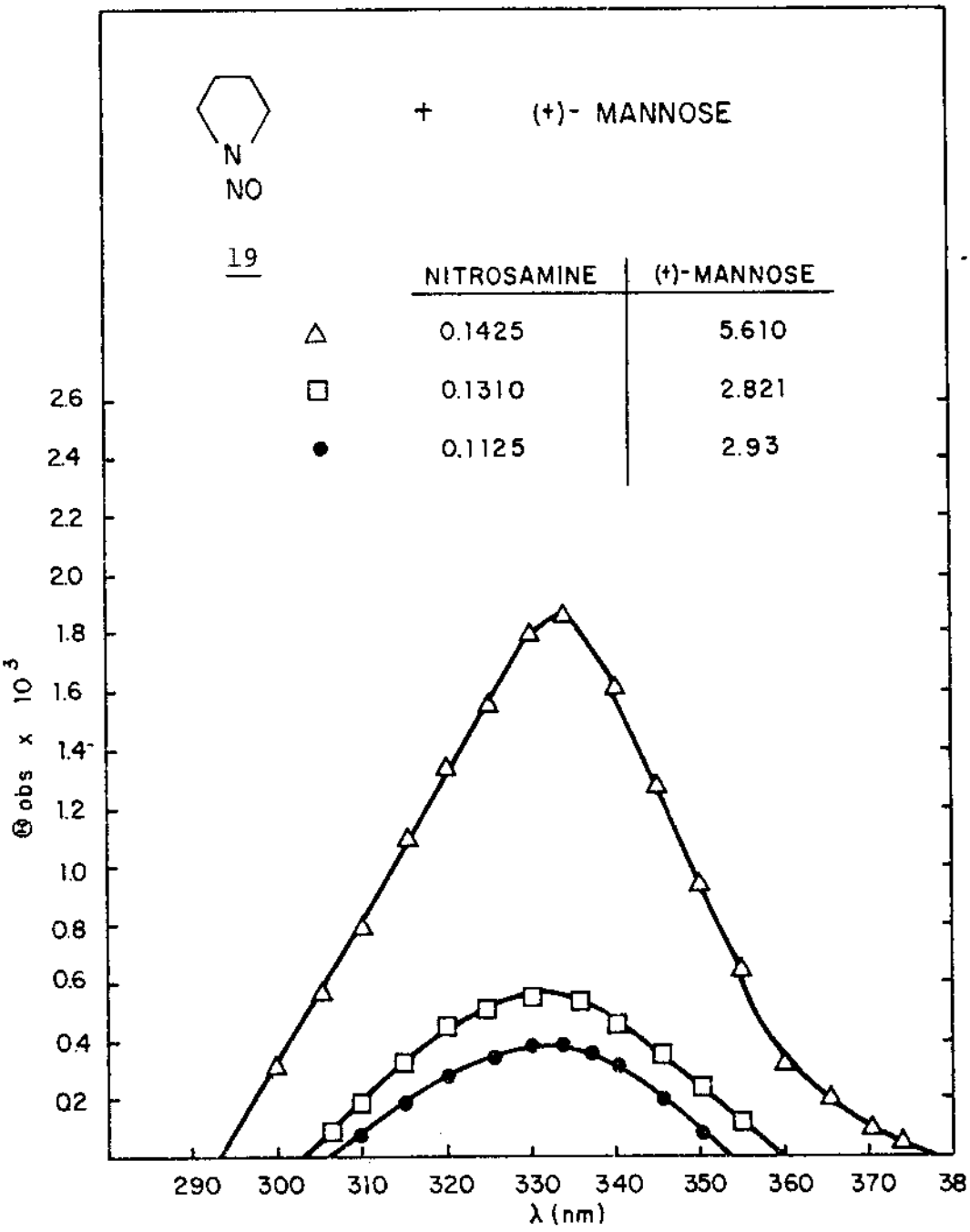


Fig. 18--The ICD spectra of compound 19 with (+)-mannose.

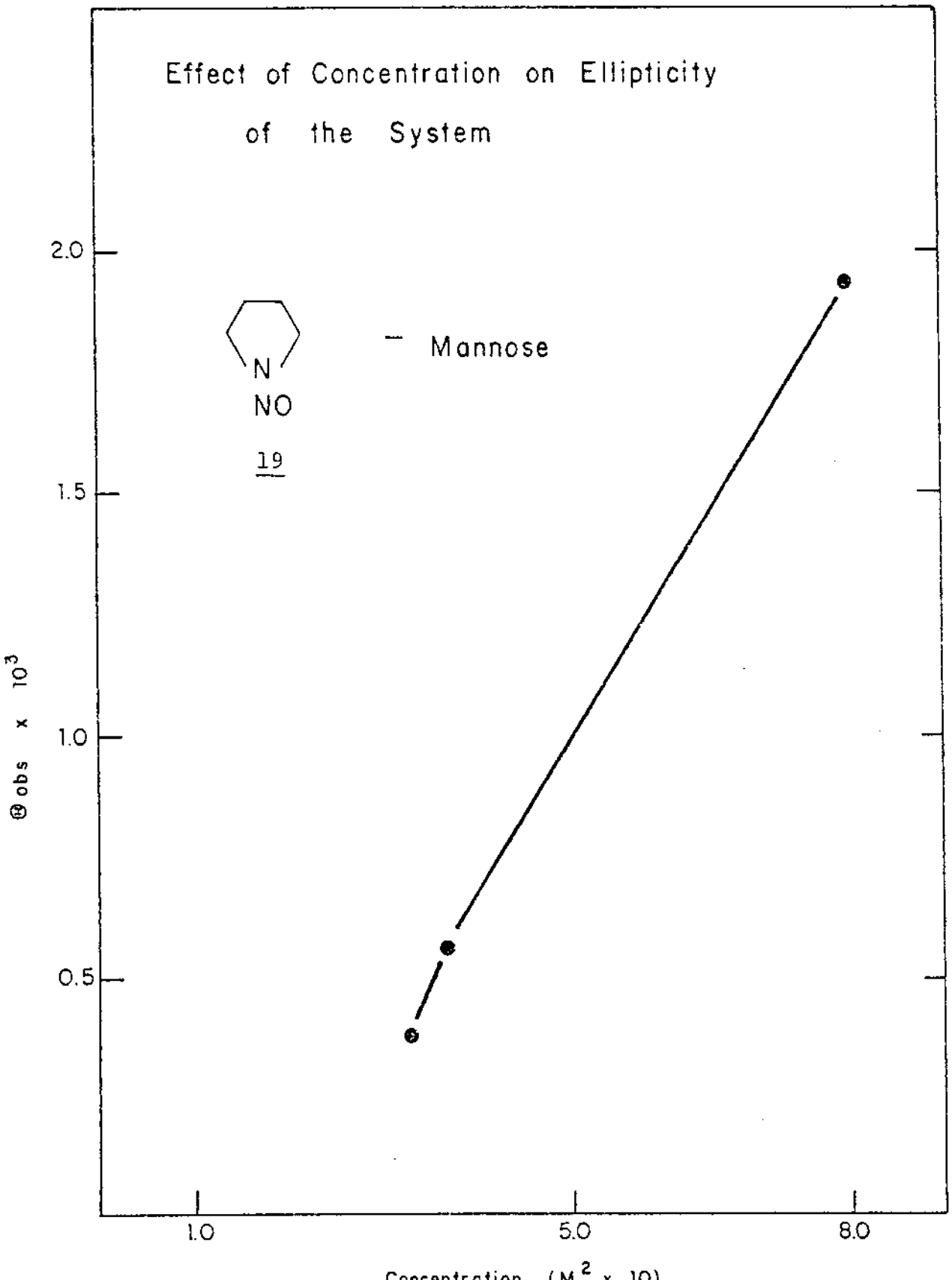


Fig. 19--The effect of concentration of the mannose-19 system on ellipticity.

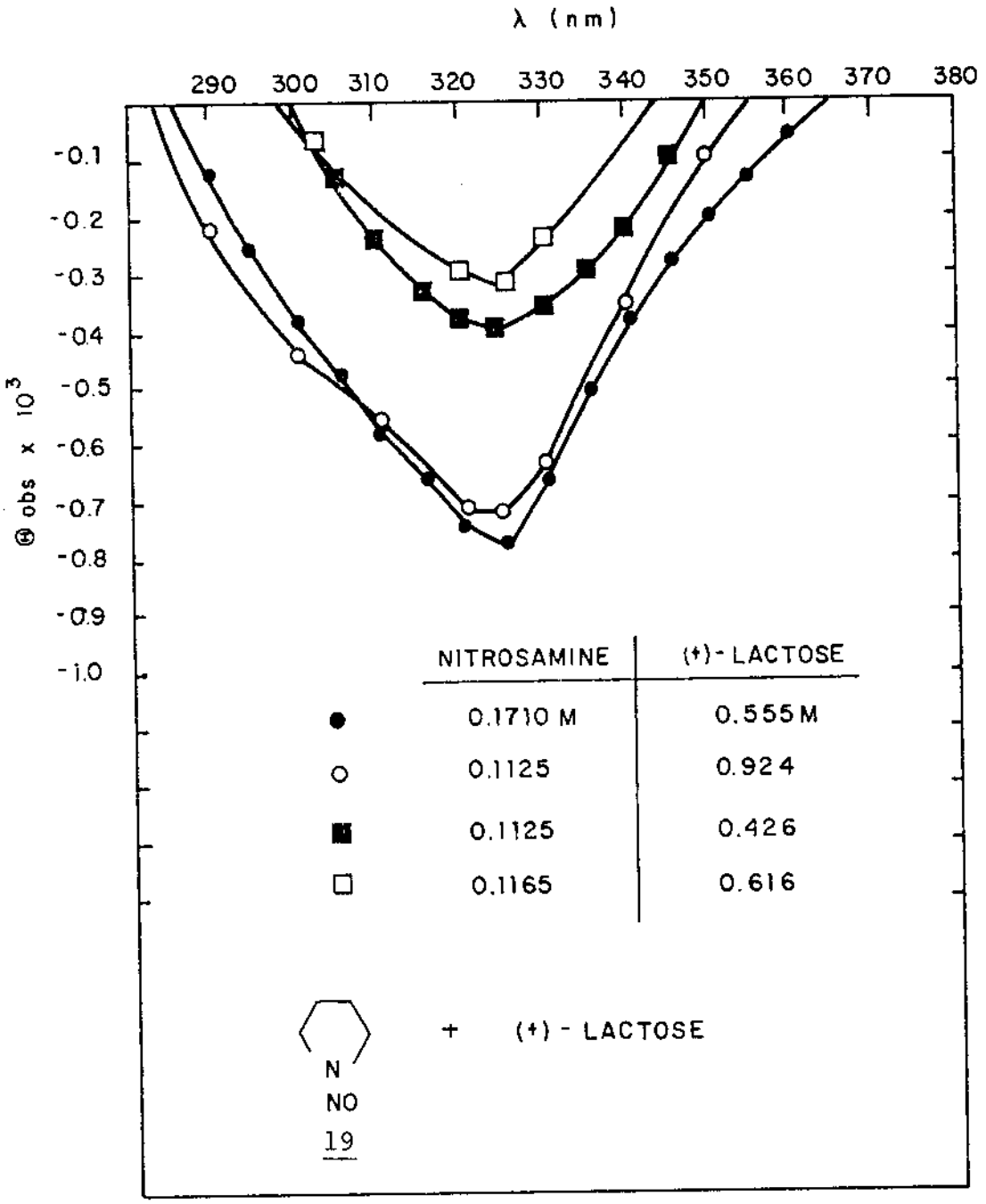


Fig. 20--The ICD spectra of compound 19 with (+)-lactose.

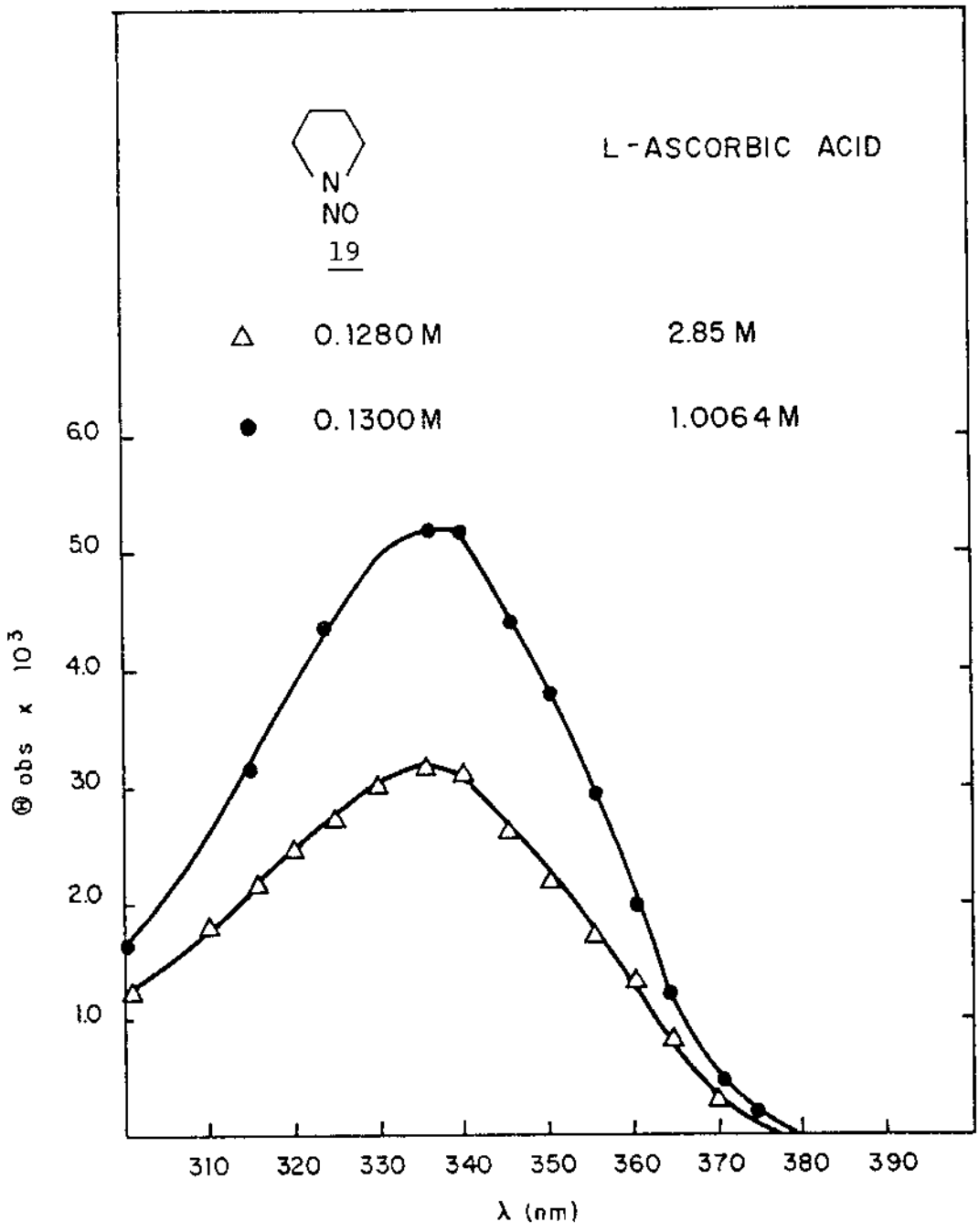


Fig. 21--The ICD spectra of compound 19 with L-ascorbic acid.

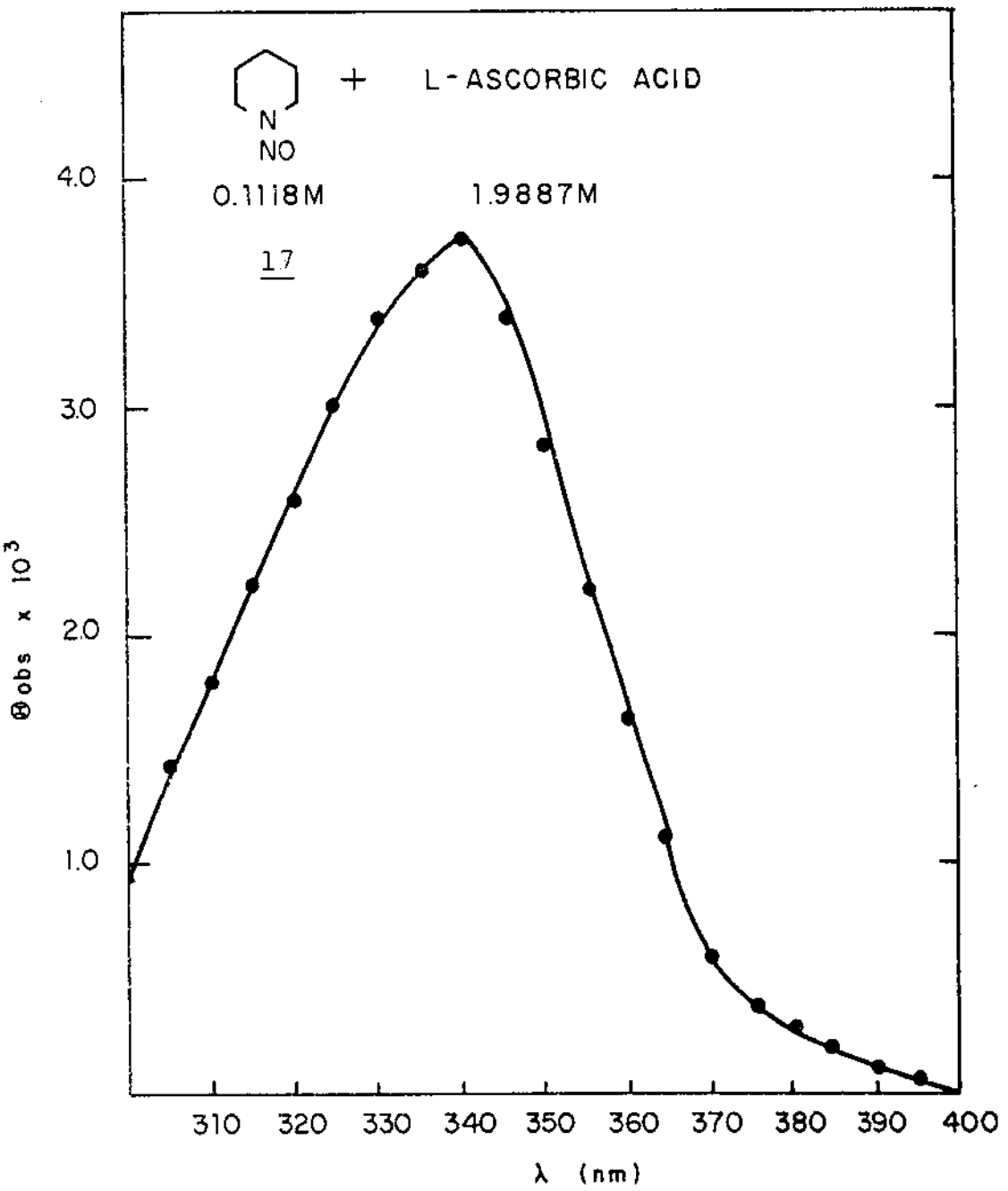


Fig. 22--The ICD spectrum of compound 17 with L-ascorbic acid.

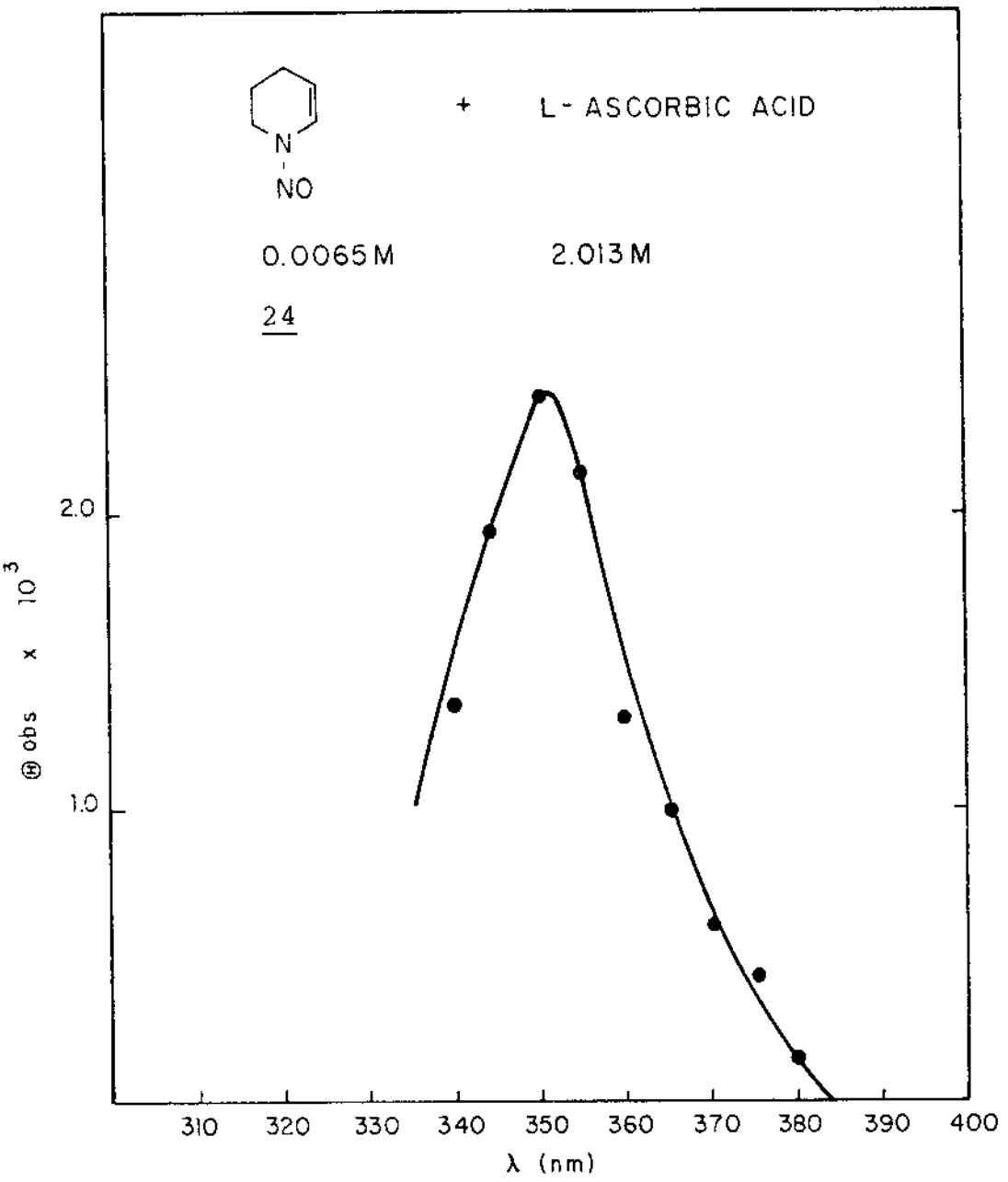


Fig. 23--The ICD spectrum of compound 24 with L-ascorbic acid.

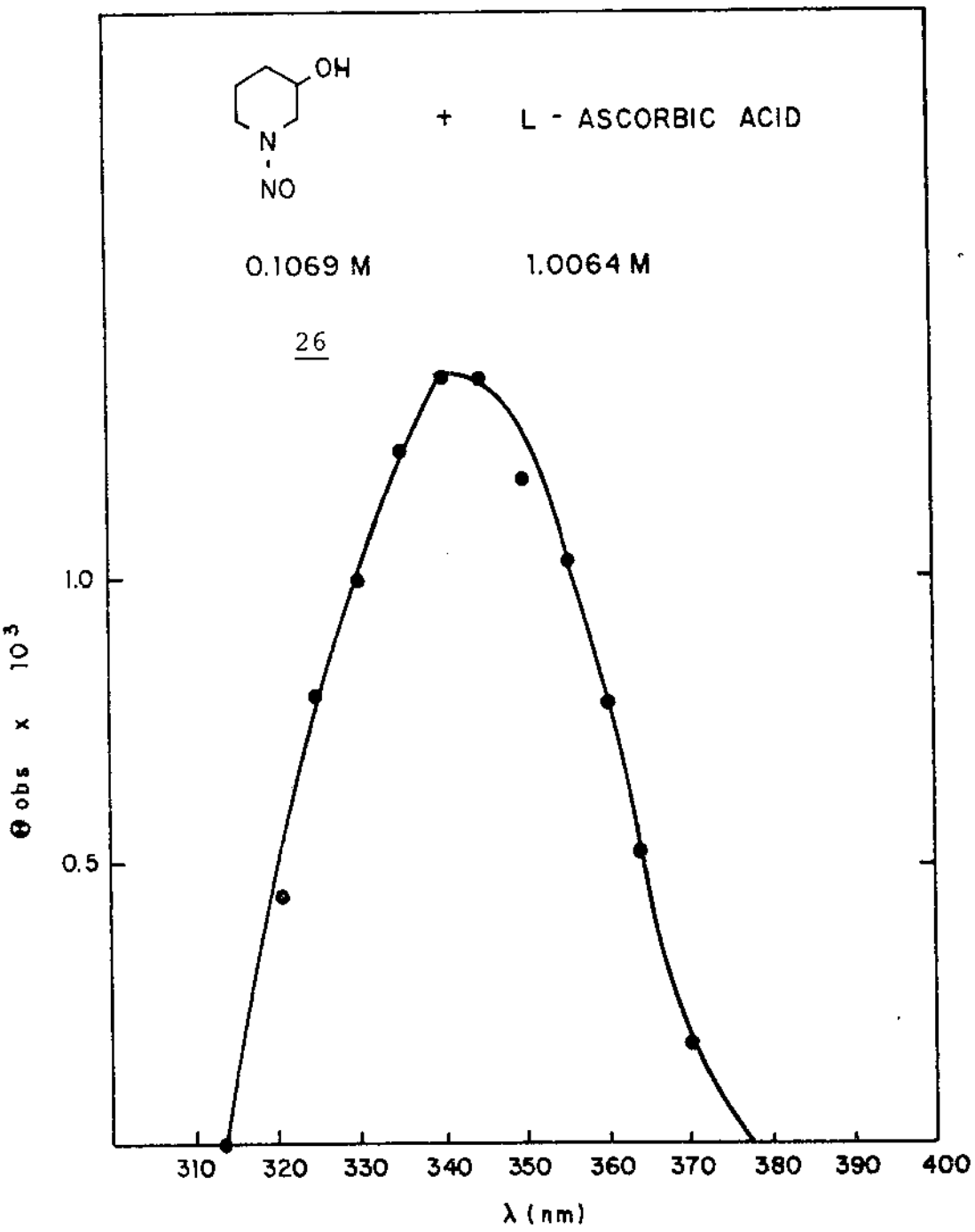


Fig. 24--The ICD spectrum of compound 26 with L-ascorbic acid.

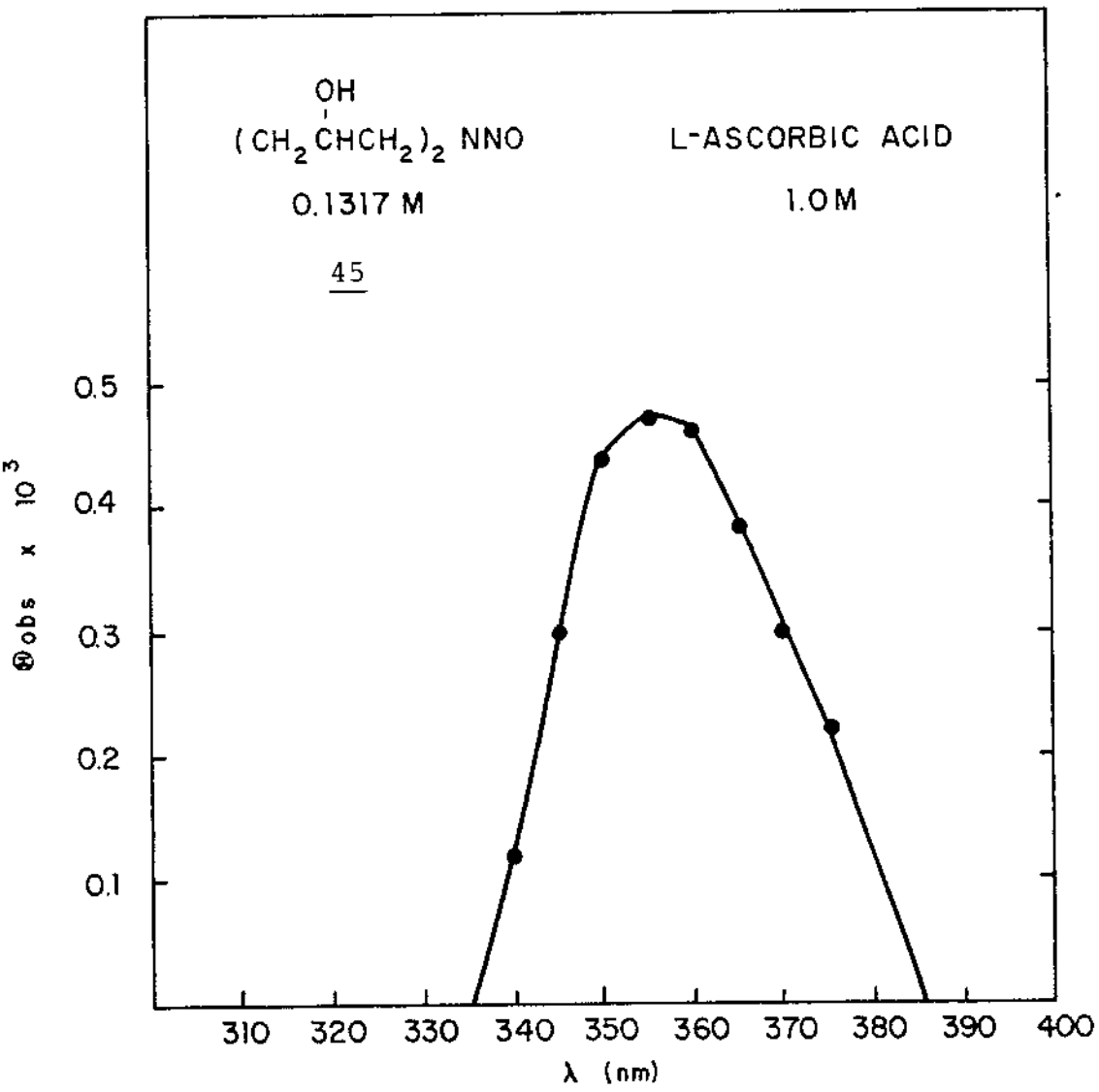


Fig. 25--The ICD spectrum of compound 45 with L-ascorbic acid.

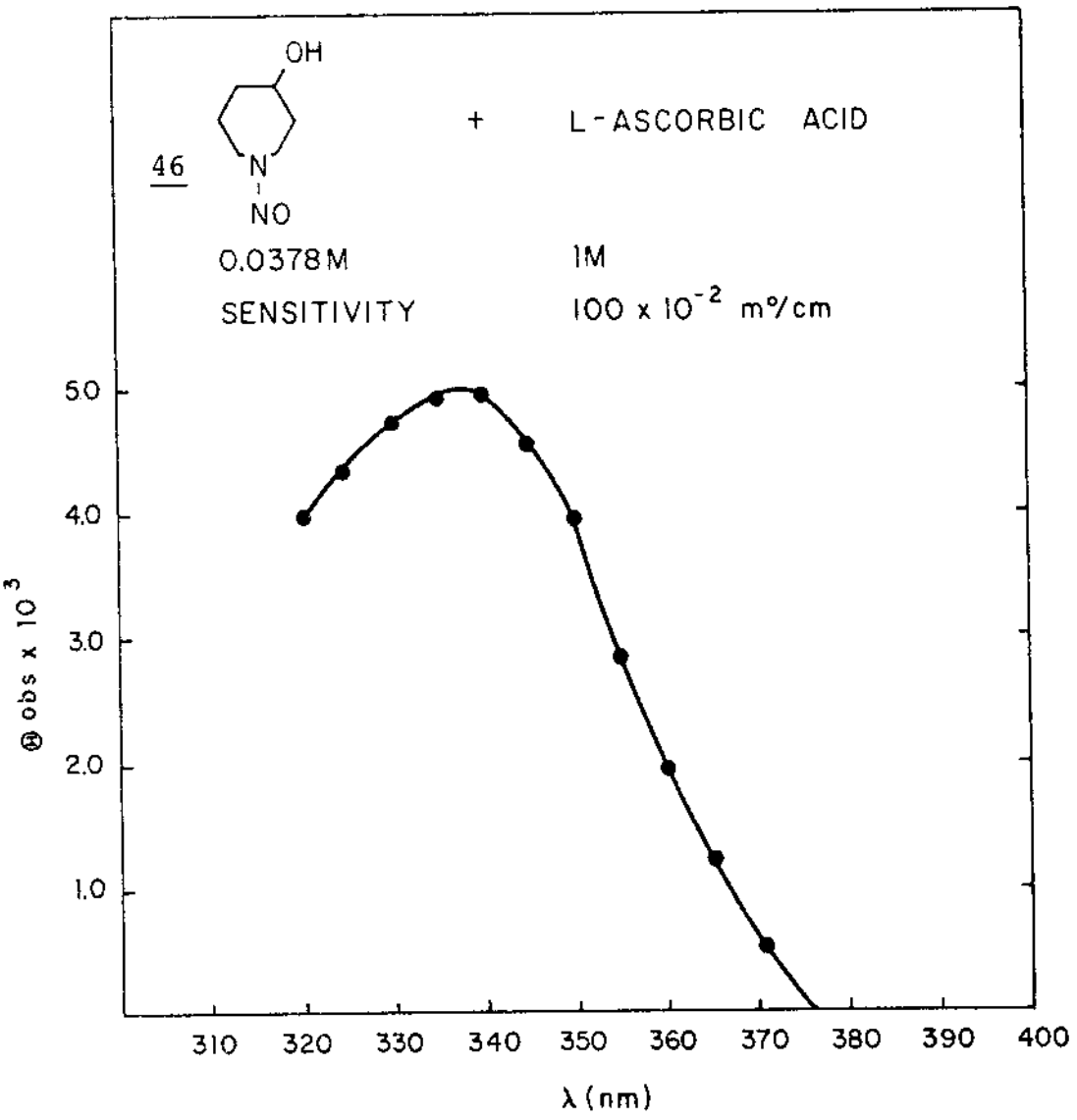


Fig. 26--The ICD spectrum of compound 46 with L-ascorbic acid.

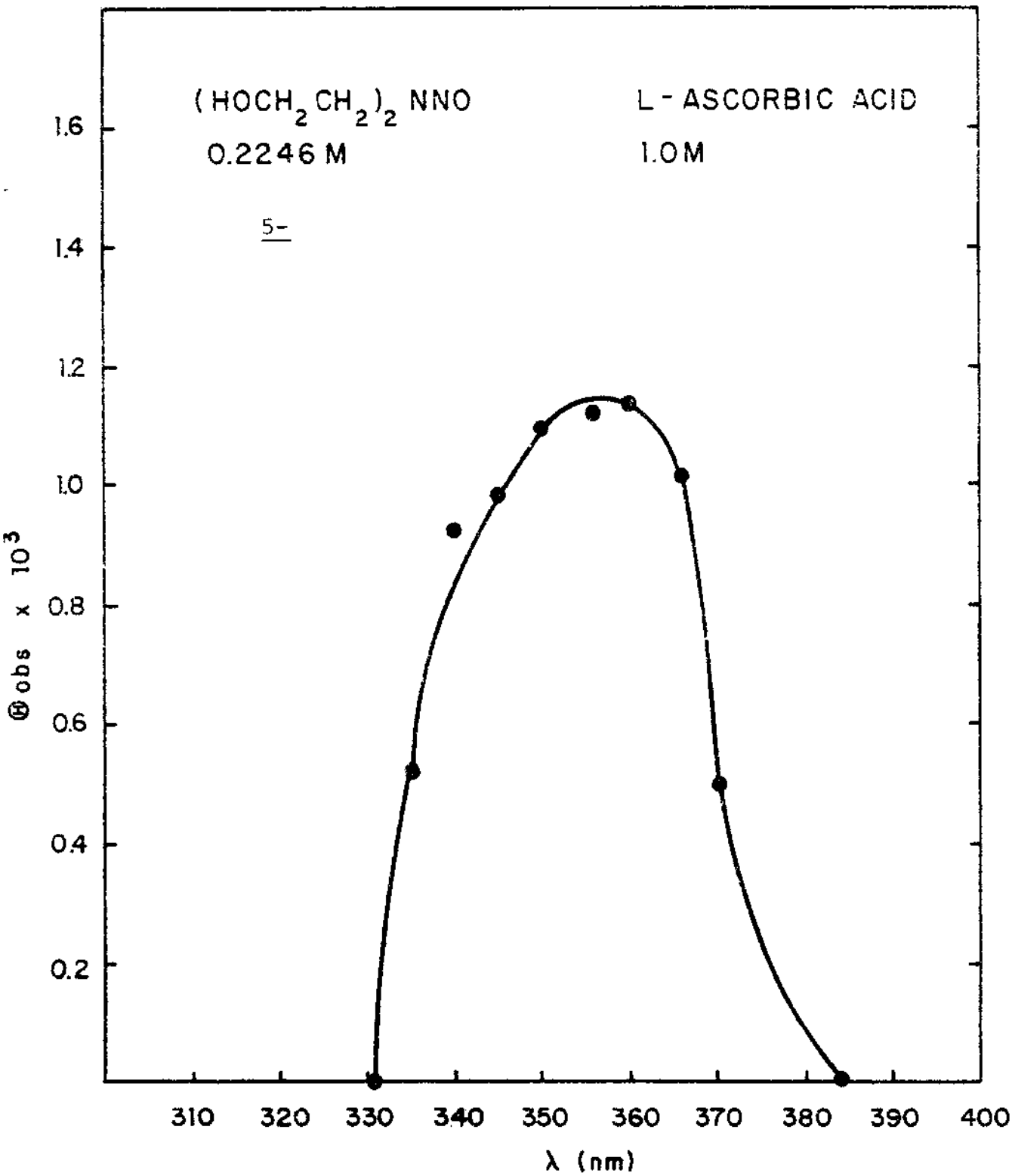


Fig. 27--The ICD spectrum of compound 50 with L-ascorbic acid.

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