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THE ALKALOIDS OF LOBELIA YUCCOIDES HILLEBR.

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
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By

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To my father and mother

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THE ALKALOIDS OF LOBELIA YUCCOIDES HILLEBR.

by Arthur Samuel Goldberg

A thesis submitted to the Graduate School of the
University of Hawaii in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

ABSTRACT

The Lobelieae constitute a tribe of the plant family Campanulaceae and comprise some 22 genera. Six of these are endemic to the Hawaiian Islands. In addition, the cosmopolitan genus Lobelia is represented in Hawaii by five endemic species, one of which is Lobelia yuccoides Hillebr.

Of some 200 species of the genus Lobelia, only 21 have been examined chemically through 1964. As a result of these investigations 43 different alkaloids have been described, 37 of which have been characterized. No Hawaiian species have, however, been investigated chemically.

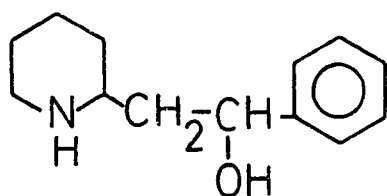
An investigation of an alkaloidal extract of the root and stem bark of Lobelia yuccoides Hillebr. using two-dimensional thin-layer chromatography indicated the presence of 10-14 alkaloids.

One of these tentatively referred to as A-1, was isolated in a yield corresponding to 0.0006% of dry bark through the use of column chromatography. Alkaloid A-1 was shown to be identical with 8-phenylnorlobelol (I), an alkaloid previously isolated from Lobelia inflata.

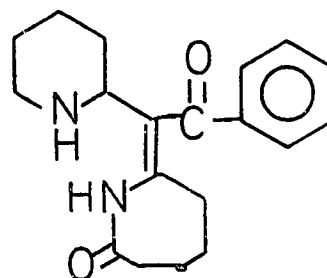
The second alkaloid was isolated in yields of 0.003-0.004% of dry bark, m.p. 208-210° (dec.), $[\alpha]_D^{22}$ 0.00 (water), pK_a 7.2, and is referred to as A-2. Alkaloid A-2 was isolated by extraction of an aqueous solution of the alkaloid hydrochlorides with chloroform, evaporation of the chloroform extract to dryness followed by trituration with chloroform, and then by cooling the resulting solution. This resulted in the selective precipitation of alkaloid A-2.

Elemental analyses, mass spectrum, and potentiometric titration of A-2 led to a molecular formula of $C_{19}H_{25}N_2O_2Cl$. Chemical degradation and spectral evidence pointed to a postulated structure (II) for A-2.

This structure is different from previously characterized Lobelia alkaloids and constitutes, therefore, a new structural type.



I



II

I. INTRODUCTION

A. Botanical

The Lobelieae, a tribe of the plant family Campanulaceae, comprises some twenty-two genera which encompass over 367 species.^{1, 3} Nowhere, with the exception of South America, does this tribe reach such an extensive development as in the Hawaiian Islands.¹ It has the largest number of species of any plant family represented in the Hawaiian Islands.¹ Other island groups of the Western Pacific are devoid of Lobelioideae as for example Fiji and Samoa; this is also true of the Malayan region, the Philippines and New Guinea.² The only Pacific islands which possess Lobelioideae are Tahiti, Raiatea, and Raratonga which have a total of four species belonging to three different genera.¹

The development of the Hawaiian species of Lobelioideae and their great number is undoubtedly due to the extremely varied conditions in these islands, produced by the enormous range of altitudes, and consequently variable climatic conditions.² The Hawaiian Islands contain six endemic genera, Brighamia, Cyanea, Clermontia, Rollandia, Delissea, Trematolobelia and in addition, members of the cosmopolitan genus Lobelia. These genera are represented in Hawaii by more than 149 species, varieties and forms.

The genus Lobelia possesses some 200 species and has a wide geographical distribution.¹ It occurs in the

tropical as well as temperate regions of the world with the exception of Central and Eastern Europe and Western Asia. The largest number of species, however, are found in Africa and South America.¹ They are usually found in the high mountains, but in Hawaii they are commonly found in the middle forest zone.¹

Lobelia yuccoides Hillebr. is one of the five endemic species belonging to the genus Lobelia; it is also known by its Hawaiian name Panaunau.² It belongs to the sub-genus Tupa, Sectio 5, Revalutella E. Wimm, G. Don Wimm, which contains 112 species, ten of which are indigenous to the Hawaiian Islands.³ Lobelia yuccoides Hillebr. is a tall and handsome shrub which was given the specific name yuccoides because of its resemblance to a small yucca plant.² It has a simple erect trunk with a thin woody zone and extensive pith, closely covered with spires of rhomboidal leaf scars and bears a crown of leaves at the end. The leaves are gray beneath and dark green above.² Lobelia yuccoides Hillebr. is monocarpic; that is, it flowers only once in its life. During its flowering state it bears a single flowering spike three feet in length with up to 400 flowers.² It is very similar to a few of the species found in the Abyssinian highlands which are found at elevations up to 14,000 feet.² Lobelia yuccoides Hillebr. inhabits the ridges and canyons of the Waianae mountains of Oahu and the leeward side of Kauai and is commonly found at an elevation

of 3000 feet.

B. Chemical

Of some 200 species of the genus Lobelia, only 21 have been examined chemically to-date (Table I).⁴⁻⁷ As a result of these investigations, 43 different alkaloids have been described.^{8,9} Of these alkaloids, 37 have been characterized and the structures of the remaining six are still unknown.^{8,9} A majority of these alkaloids have been isolated from Lobelia syphilitica L.⁹ (14 alkaloids), and Lobelia inflata⁸ (24 alkaloids).

The presence of alkaloids in the genus Lobelia was first recorded in 1836 by Proctor,¹⁰ who isolated a base from Lobelia inflata which he named lobeline. The base was then synthesized by Lewis¹¹ and isolated as an oil from which Siebert¹² prepared and analyzed a series of salts and then postulated a structure. The plant was re-examined in 1921 by Böhringer and Söhne¹³ who isolated three alkaloids and called them alpha, beta, and gamma-lobeline. In that same year, Heinrich Wieland and his students published the first¹⁴ of a series of papers (1921-1939), which describes the isolation and structural elucidation of Lobelia alkaloids.

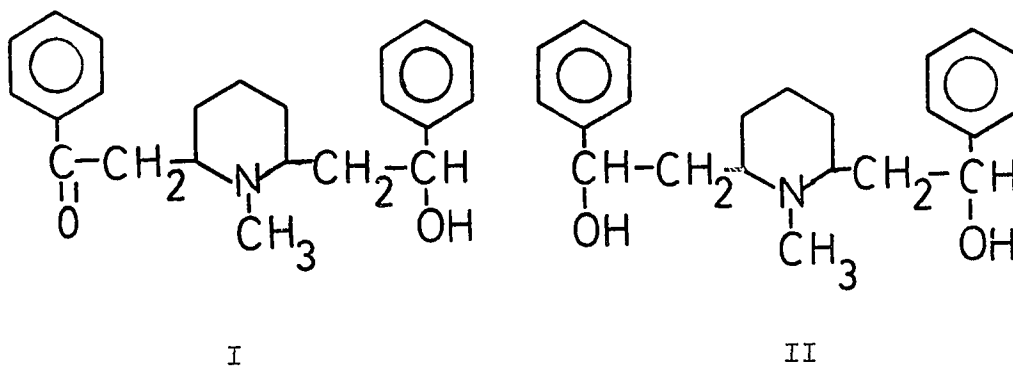
In 1950, Steingegger¹⁵ and Grütter isolated 1-lobeline (I), lobelandine (II), and an unknown alkaloid (lurenine) from Lobelia urens.

TABLE I. LOBELIA SPECIES INVESTIGATED PREVIOUSLY

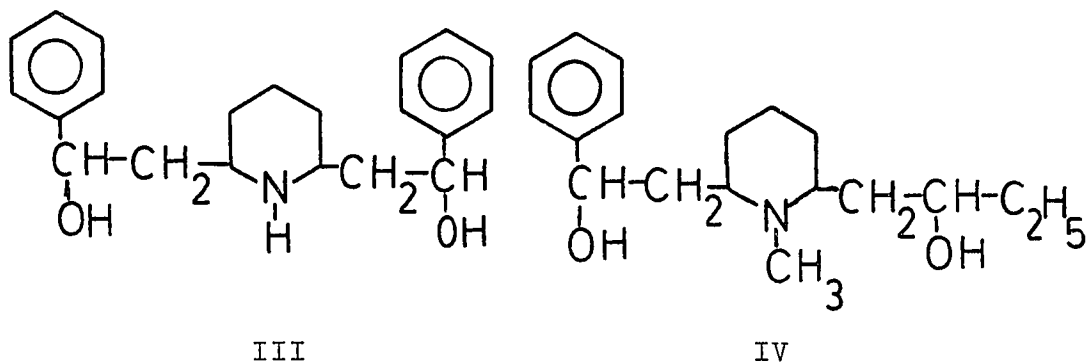
Number	Binomial	Geographic Location	Number of Alkaloids
1	<u>L. cardinalis</u>	N. America and Canada	3
2	<u>L. decurrens</u>	Chile and Ecuador	1
3	<u>L. dortmanna</u>	Canada, N. America, Europe	2
4	<u>L. elongata</u>	Southeast United States	6
5	<u>L. erinus</u>	Capetown, Venezuela, Ecuador	2
6	<u>L. gibberoa</u>	Tropical E. Africa	Unknown
7	<u>L. inflata</u>	N. America and Canada	24
8	<u>Isotoma longifora</u>	Hong Kong, Peru	5
9	<u>L. langeana</u>	Brazil	4
10	<u>L. nicotianaefolia</u>	S. India to Ceylon	4
11	<u>L. purpurascens</u>	Australia	1

TABLE I. (cont.)

Number	Binomial	Geographic Location	Number of Alkaloids
12	<u>L. pyramidelis</u>	Himalayan Territory to Upper Burma	3
13	<u>L. puberula</u>	N. America	6
14	<u>L. sessifolia</u>	Kamt schatha via Japan and S. Siberia to Junnan and Formosa	2
15	<u>L. salicifolia</u>	Chile	8
16	<u>L. suavibracteata</u>	Belgium Congo, Uganoa	Unknown
17	<u>L. radicans</u>	Japan, China, Java	2
18	<u>L. syphilitica</u>	S. Canada and Maine to Alberta	14
19	<u>L. tupa</u>	Chile	8
20	<u>L. urens</u>	Europe	3
21	<u>L. delisseana</u>		Unknown
22	<u>Pratia concolor</u> (<u>L. concolor</u>)		Unknown



In 1955, an investigation of the major alkaloids of Lobelia nicotianaefolia Heyne¹⁶ resulted in the isolation of norlobelanidine (III), 1-lelobanidine II (IV), and a new base, 1-lelobanidine III, an optical isomer of (IV).



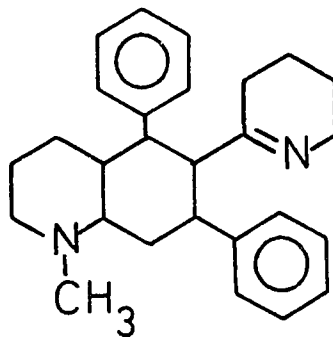
In 1956, work on Lobelia salicifolia Sweet¹⁷ yielded six alkaloids, two of which are of undetermined structure.

Two years later, paper chromatography of an alkaloidal extract of Lobelia tupa L.¹⁸ showed the presence of eight alkaloids.

All of the Lobelia alkaloids characterized through 1960 were of the same structural type, of which

lobeline (I) is typical. These alkaloids all contain a piperidine ring which is sometimes unsaturated in the three or four position, while substitution generally occurs in the two, six positions; the ring is also found monosubstituted in either of the later two positions.^{8,19}

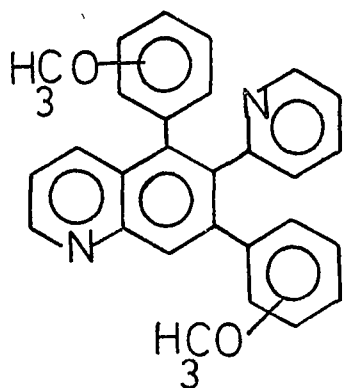
The recent literature contains reports on the structural elucidation of three *Lobelia* alkaloids which constitute new structural types. In 1964, the research group of CIBA Pharmaceutical Company determined the structure of lobinaline (V).²⁰



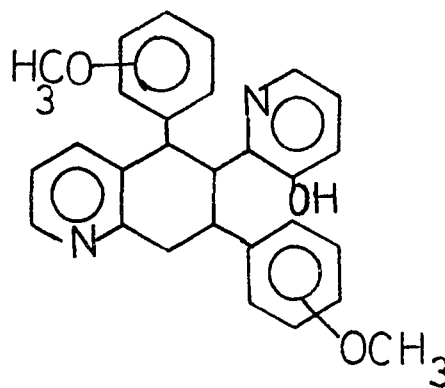
V

This is the major alkaloid of *Lobelia cardinalis* which was first isolated in 1938 by Manske²¹ but whose structure was previously unknown.

Shortly after the structure of lobinaline was determined, Tschesche et al. isolated two new alkaloids, syphilobine A (VI) and syphilobine F (VII), from *Lobelia syphilitica*²² and assigned them the following partial structures.



VI



VII

These two alkaloids are structurally similar to lobinaline and appear to be related biogenetically.²²

In addition to these three new structural types recently elucidated are two alkaloids of unknown structures, lophiline²³ ($C_{28}H_{40}O_3N_2Cl_2$) and an unnamed $C_{19}H_{26}O_3N_2$ alkaloid,²⁴ both of which contain two nitrogen atoms and may therefore constitute novel structural types. A survey for alkaloids in Hawaiian plants²⁵⁻²⁸ has revealed the presence of alkaloids in three of the endemic genera of the family Campanulaceae: Trematolobelia, Clermontia, and Cyanea and also in some endemic species of the genus Lobelia. In the course of field testing of plants for the presence of alkaloids by a procedure developed by Culvenor and Fitzgerald²⁹ it was found that Lobelia yuccoides Hillebr. gave a strong positive test for alkaloids.

A chemical investigation of Lobelia yuccoides Hillebr. seemed desirable in view of the recent elucidation

of new structural types from *Lobelia* plants,^{20,22} and because no chemical investigation had been carried out on any of the endemic Hawaiian species of the plant family Campanulaceae. *Lobelia yuccoides* Hillebr. was chosen because it was the most abundant of the species tested and it is located in an area which is easily accessible.

Isolation of known and/or unknown alkaloids may assist the botanist in the correlation of chemical constituents with plant taxonomy and will aid in the establishment of biosynthetic routes for alkaloids within a particular genus.

The research may be of value to the pharmacologist since the genus *Lobelia* has been known to contain physiologically active alkaloids. Lobeline is known as a potent respiratory stimulant and has been used in the treatment of asphyxia, and in accidents during anesthesia.³⁰ It is also used as smoking deterrent and functions by displacing nicotine in the system.³⁰ Isolobinine is said to be responsible for the emetic and asthma-relieving properties of the crude drug *Lobelia inflata*.³¹ Spasmolytic activity has not been recorded for *Lobelia* alkaloids, but it has been shown that lobelanine has a slight neurotropic and a similar musculotropic activity.³¹

C. Acknowledgements

I wish to thank David Boylan and all others who helped me collect plant material on numerous trips to the

Waianae Mountains.

Grateful acknowledgement is also made to the National Institutes of Health and to Abbott Laboratories for supporting this research through grants to the University of Hawaii.

II. EXPERIMENTAL

A. General Information

All melting point were determined on a Fisher-Johns melting point apparatus and are uncorrected.

Elemental analyses were performed by Berkeley Analytical Laboratory, Berkeley, California.

Ultraviolet (UV) spectra were measured with a Cary 14 Recording Spectrophotometer.

Infrared (IR) absorption spectra were determined with a Beckman IR-5 automatic recording spectrophotometer either as potassium bromide pellets or in chloroform solution. Infrared absorption maxima are designated as strong (s), medium (m), weak (w), broad (b), and shoulder (sh).

Nuclear magnetic resonance (NMR) spectra were obtained with a model A-60 (60mc.) Analytical NMR Spectrometer of Varian Associates. Absorption peaks are recorded in delta values (δ), using tetramethylsilane (TMS) as an internal standard, referring to TMS as $\delta = 0$.

Optical rotations were taken using an ETL-NPL automatic Polarimeter Type 143 A.

The pK_a measurement was performed by Eli Lilly Research Laboratory.

The mass spectrum was determined by Prof. Carl Djerassi.

Aluminum oxide G and silica gel G were used for thin-layer chromatography (TLC) as supplied by the

manufacturers. Thin-layer plates were prepared using the Desaga/Brinkmann standard and variable applicator (Brinkmann Instruments Inc.) according to the method of Stahl.³²

The following anion exchange resins used were all identical in type and performance:³³ Bio-Rad AG 1-X4, 50-100 mesh, Dowex 1-X4, 50-100 mesh, Dowex 1-X8, 100-200 mesh, all in the chloride form.

The presence of alkaloids was determined by testing with Mayer's and Dragendorff's reagents.³⁴ All tests using Dragendorff's reagent were performed by spraying the reagent on a TLC plate spotted with the material to be tested.

B. Procurement and Preparation of Plant Material

All collections of plant material were made on the island of Oahu in the Waianae Mountains on the Palikea trail and in the valleys adjacent to the trail. Since this research was mainly concerned with alkaloids occurring in the bark, root and stem bark were collected. Taxonomic identification was made by Dr. Charles H. Lamoureux of the Department of Botany, University of Hawaii. A summary of the collections along with other pertinent data is presented in Table II.

The combined bark, root and stem, was dried in a forced draft oven for 68 hr. at 50° and then ground in a Wiley mill to pass a 1-mm screen.

TABLE II. SUMMARY OF PLANT COLLECTIONS

Collection	Location	Amount of Dried Material (kg.)	Date	Results
1	Palikea Trail, Oahu	4.2	August, 1963	Preliminary Work
2	Palikea Trail, Oahu	6.0	December, 1963	8-Phenylnorlobelol, 35 mg.
3	Palikea Trail, Oahu	8.8	January, 1965	A-2, 272 mg.
4	Palikea Trail, Oahu	9.0	March, 1965	A-2, 339 mg.
5	Palikea Trail, Oahu	10.0	June, 1965	A-2, 420 mg.

C. Large Extraction I

1. Separation of the Crude Alkaloids

This first extraction scheme appeared to be the most promising of a series of pilot extraction procedures and the entire extraction scheme is summarized in Fig. 1.

A total of 6 kg. of plant material collected on December 6, 1963, was extracted with petroleum ether (30-60°) in a soxhlet for 24 hr. to remove fats and waxes. The petroleum ether extract gave a negative test for alkaloids and was discarded.

The plant material was next extracted with ethanol for 3 d. when a sample of the solvent gave a very weak Mayer's test.

The alcoholic extract was concentrated in vacuo until a viscous dark brown syrup resulted. The syrupy residue was mixed with 5 l. of 1 N. sulfuric acid solution and then filtered under suction using diatomaceous earth as a filter aid. The residue was still strongly alkaloidal and was placed in a column and eluted with 1 N. sulfuric acid until a weak Mayer's test was obtained.

The combined acidic extracts were placed in a separatory funnel and extracted with ethyl acetate until the organic layer was almost colorless. The ethyl acetate fraction gave a negative test for alkaloids and was discarded.

The acidic extract was then cooled in an ice-water bath and solid sodium carbonate was added under constant

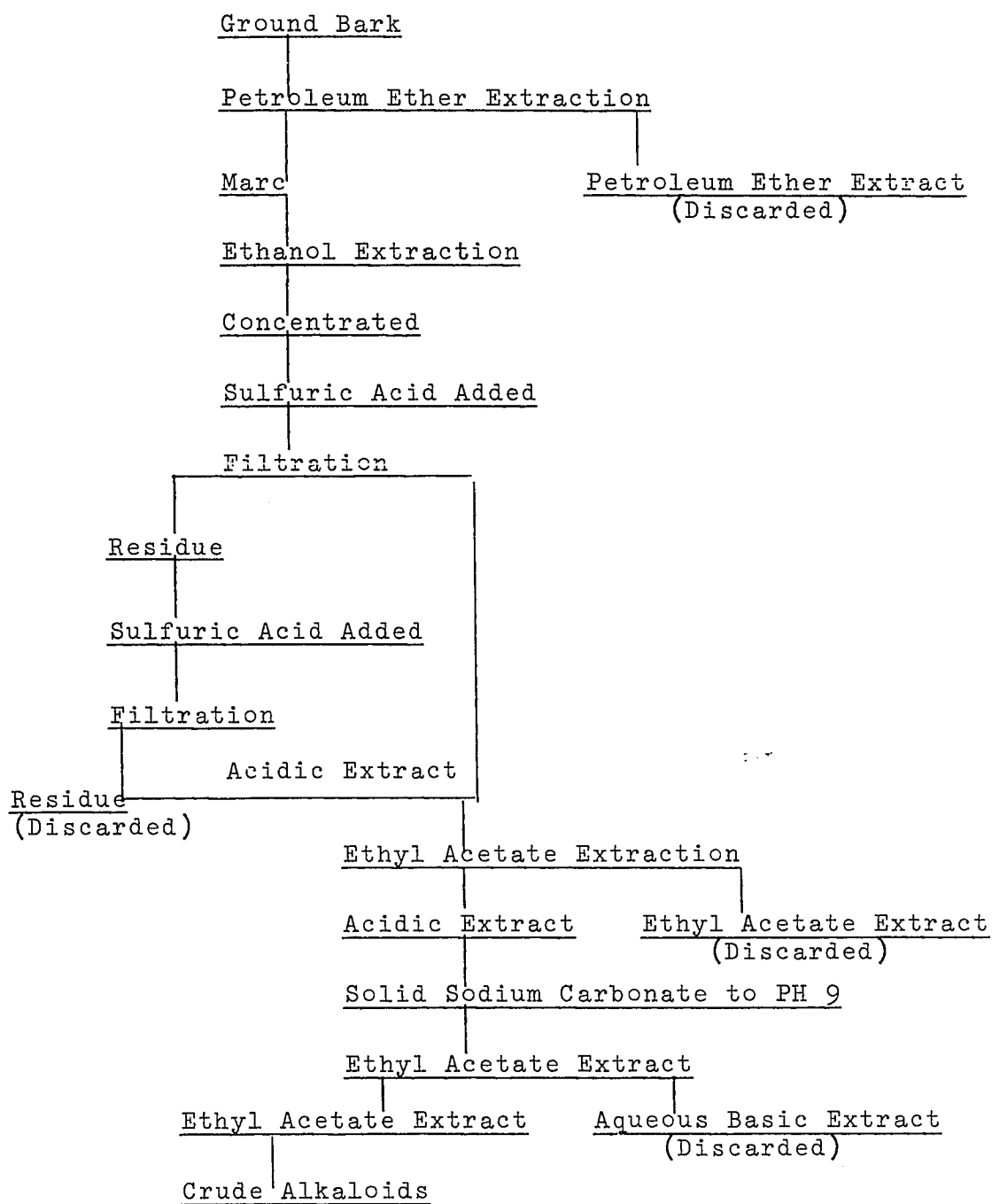


Fig. 1. Scheme for Large Extraction I.

stirring until a pH of nine was reached. The basic solution was extracted with ethyl acetate in a separatory funnel until the ethyl acetate layer appeared almost colorless. The basic solution still contained alkaloids and was exhaustively extracted with ethyl acetate in a liquid-liquid extractor. The ethyl acetate extract was washed with water to remove the last traces of base and was evaporated in vacuo to dryness. The total weight of the crude alkaloidal extract was 48.7 g.

A sample of the crude extract was subjected to two-dimensional TLC. Solvent mixtures of chloroform and methanol were found to give the best separations. By using chloroform-methanol systems of 40:1, 30:1, 20:1, and 10:1 by volume, 10-14 alkaloids were detected after developing the plates with Dragendorff's reagent. When these solvent systems were employed in pilot columns using neutral alumina, the separations were not as good and usually resulted in a large overlap of alkaloids.

2. Chromatography of the Crude Alkaloidal Extract

The crude fraction (48.7 g.), which also contained a large amount of non-alkaloidal material, was dissolved in methanol and then mixed with diatomaceous earth. The solvent was then evaporated leaving a brown powder. The powder was mixed with chloroform and placed on a column of 906 g. of acidic alumina. This column was intended to achieve a crude separation, and this was the

reason for the low absorbant to material ration (20:1). The results from the column chromatography are summarized in Table III.

TABLE III. CHROMATOGRAPHIC SEPARATION OF THE CRUDE ALKALOIDS

Fraction Number	Solvent System	Mayers Test	Weight (g.)
1	100% Chloroform	(-)	0.30
2	100% Chloroform	(+)	8.3
3	100% Chloroform	(-)	0.15
4	100% Chloroform	(+)	24.8

Fraction 1 was greenish in color and was eluted just before the first major band. It gave a negative alkaloid test and was discarded. Fraction 2 came off as a broad band with considerable tailing. Upon continued elution with chloroform a negative Mayer's test resulted (Fraction 3) and this fraction was discarded.

Because of the high degree of tailing, it appeared that no significant separation would be achieved by eluting the column with chloroform-methanol mixtures; therefore, the column was eluted with 100% methanol to remove the remaining alkaloids. A considerable quantity of non-alkaloidal material remained on the column.

3. Chromatography of Fraction 4

The material from fraction 4 (21 g.) was dissolved in chloroform and placed on a column of 900 g. of neutral alumina. The column was eluted with 100% chloroform until a negative alkaloid test resulted. Thin-layer chromatography showed that all of the fractions were identical and they were combined totalling 4 g. Continued elution of the column using mixtures of chloroform-methanol gradually increasing the methanol content resulted in a large overlap of the fractions collected as shown by TLC. The combined fractions weighed 6.2 g. Elution of the column with 100% methanol produced a fraction containing 7 g. of a mixture of alkaloids plus an appreciable amount of non-alkaloidal material.

a. Purification of the 100% Methanol Fraction

The methanol fraction (7 g.) was dissolved in 20 ml. of methanol and then added to 200 ml. of a 5% solution of hydrochloric acid. The solution was filtered under suction using diatomaceous earth as a filter aid. The residue was discarded as it gave a negative Mayer's test. The acidic solution was cooled in an ice-water bath and solid sodium carbonate was added until the solution was rendered basic. The basic solution was extracted with chloroform until it was no longer alkaloidal. The chloroform extract was washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness, furnished 1.9 g. of

material.

b. Chromatography of the Purified Methanol Fraction

The material (1.9 g.) was dissolved in a mixture of benzene-absolute ethanol (5:1) and placed on a column of 80 g. of neutral alumina. The chromatographic results are summarized in Table IV. Fractions 5-8 appeared identical

TABLE IV. CHROMATOGRAPHY LEADING TO ALKALOID A-1

Fraction Number	Mayers Test	Wt. (mg.)	Solvent System
1	(+)	124	Benzene:Ethanol 5:1
2	(-)	70	Benzene:Ethanol 5:1
3	(-)	20	Benzene:Ethanol 4:1
4	(+)	100	Benzene:Ethanol 4:1
5	(+)	175	Benzene:Ethanol 4:1
6	(+)	138	Benzene:Ethanol 4:1
7	(+)	97	Benzene:Ethanol 3:1
8	(+)	40	Benzene:Ethanol 2:1
9	(+)	630	100% Ethanol

and were combined. Further work on fraction 1 and fractions 5-8 proved unsuccessful. Fraction 9 (630 mg.) was rechromatographed on 63 g. of neutral alumina using a solvent system of benzene-absolute ethanol (5:1). Twenty 10-ml. fractions were collected. Thin-layer chromatography of fractions 1-5 showed the presence of two minor alkaloids along with a

small quantity of the major alkaloid. Fractions 6010 (130 mg.) contained the major alkaloid along with trace quantities of the two minor alkaloids. Fractions 11-20 (173 mg.) showed only trace quantities of the minor alkaloids and were discarded.

D. Isolation and Purification of Alkaloid A-1

The residue from fraction 2 was dissolved in a minimum of boiling iso-octane and left at room temperature resulting in 15 mg. of crystalline alkaloid. Recrystallization from the same solvent afforded 12 mg. of pure alkaloid. All attempts to crystallize additional material from the mother liquors were unsuccessful. The mother liquors were combined and evaporated to dryness in a sublimation tube and then sublimed at 55° at ca. 0.05 mm. pressure. A yellow-white solid appeared on the cold finger and TLC of this material showed the presence of two spots with very similar R_f -values. The material was triturated with ethanol leaving behind a small quantity of white solid which was resublimed yielding 20 mg. of a white crystalline solid.

E. Characterization of Alkaloid A-1

1. Physical Properties

a. Melting Point

The material obtained by crystallization from iso-octane melted at $87-88^{\circ}$. The resublimed material melted at $100-101^{\circ}$.

b. Elemental Analysis

Found: C, 75.75; H, 9.10; N, 6.87%

Calculated for $C_{13}H_{19}NO$: C, 76.04; H, 9.35;
N, 6.82%

c. Ultraviolet Absorption Spectrum

In methanol (Fig. 2) : λ max 207 m μ (ϵ , 12,126),
250 (472)

d. Infrared Absorption Spectrum

The infrared spectrum of a 3 mg. sample in a potassium bromide pellet (250 mg.) showed the following absorption bands: 3175 cm^{-1} (m), 2933(s), 2841(s), 2370(w), 1600(w), 1490(w), 1453(sh), 1439(s), 1333(w), 1330(s), 1300(w), 1285(w), 1266(w), 1203(m), 1156(w), 1136(m), 1121(m), 1098(w), 1083(m), 1064(m), 1030(w), 1017(m), 974(w), 943(m), 922(w), 901(w), 836(m), 805(w), 780(m), 757(s), 701(s). Fig. 3 shows the infrared spectrum of A-1 and of a synthetic sample of 8-phenylnorlobelol.

e. Nuclear Magnetic Resonance Spectrum

The NMR spectrum was taken in deuteriochloroform and absorption occurred at the following delta values (Fig. 4) : 7.41(singlet, 7.35(chloroform peak), 5.02-5.20 (quartet), 3.79, 3.15-2.70, 1.91-1.25.

f. Optical Rotation

The readings were taken in a 0.1 dm. cell of ca. 0.9 ml. capacity. A total of 10 reference and 10 sample readings were taken. A sample of 5.12 mg. of compound was

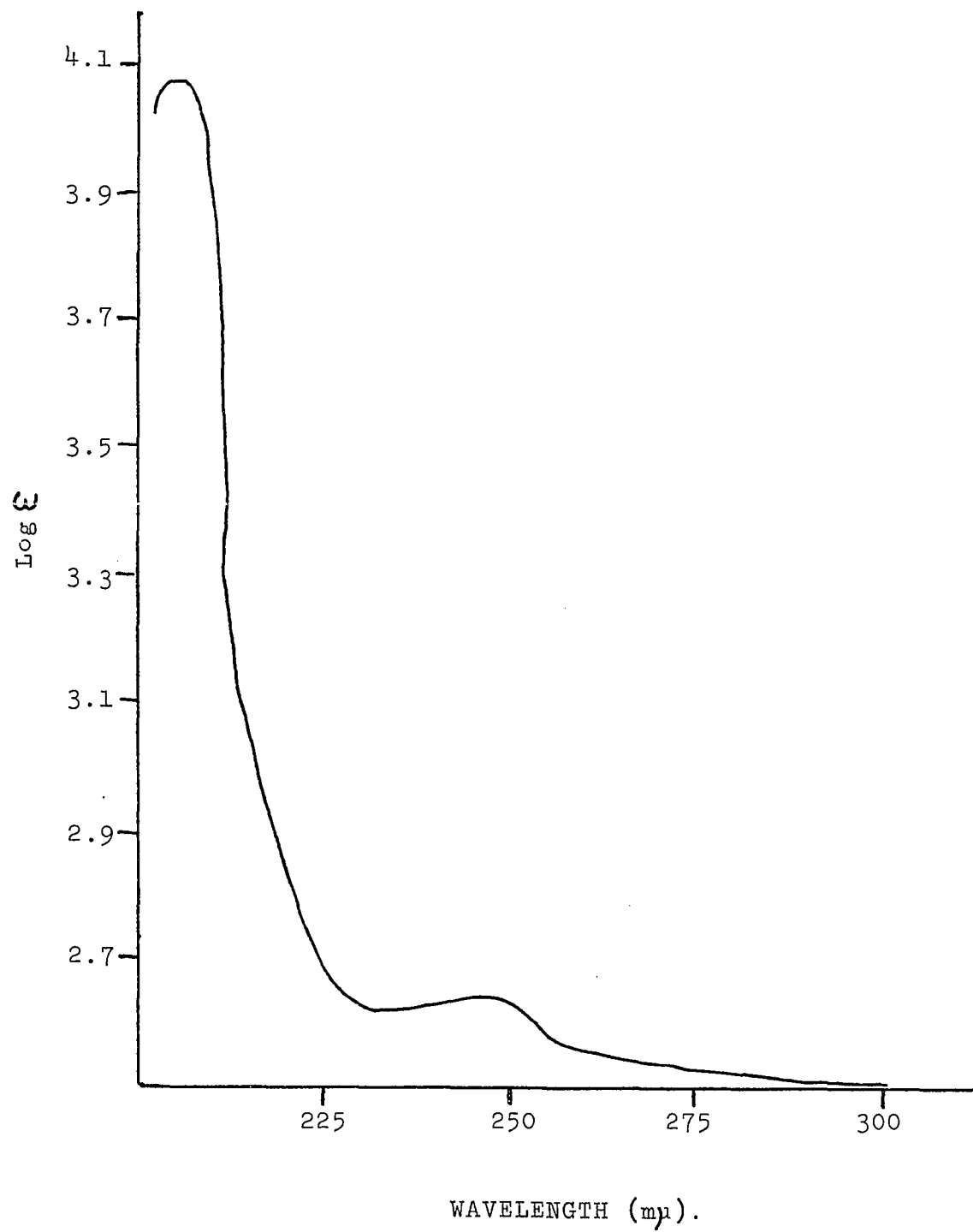


Fig. 2. Ultraviolet Spectrum of A-2 in methanol.

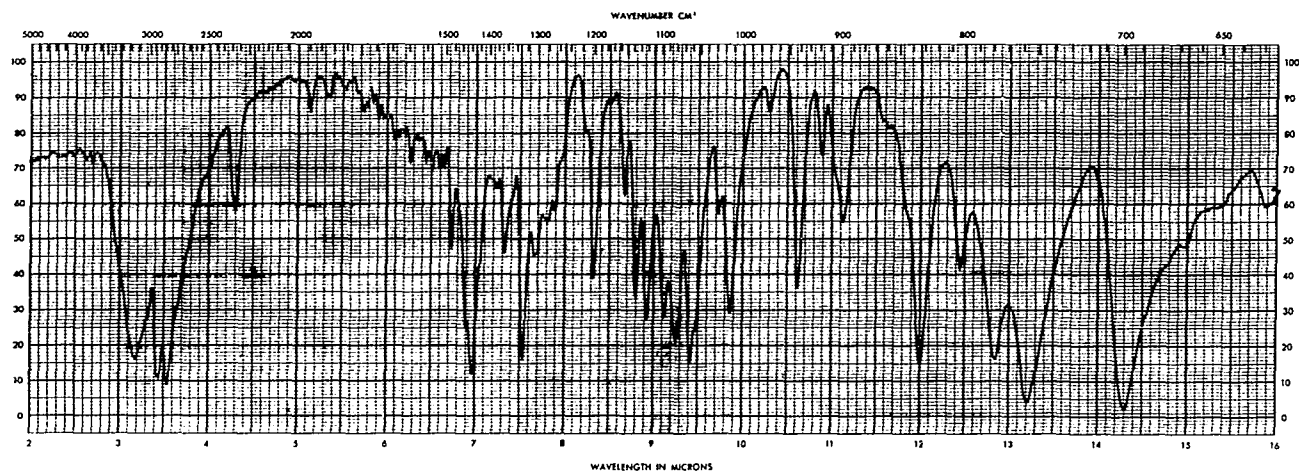
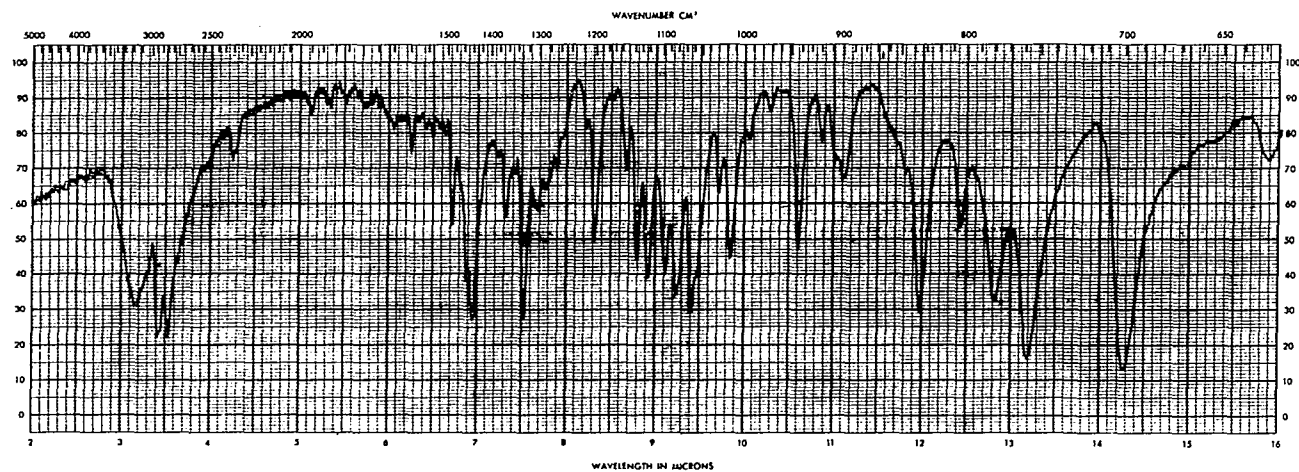


Fig. 3. Infrared Spectra (potassium bromide) of:
 a. (top) A-1.
 b. (bottom) Synthetic 8-phenylnorlobelol.

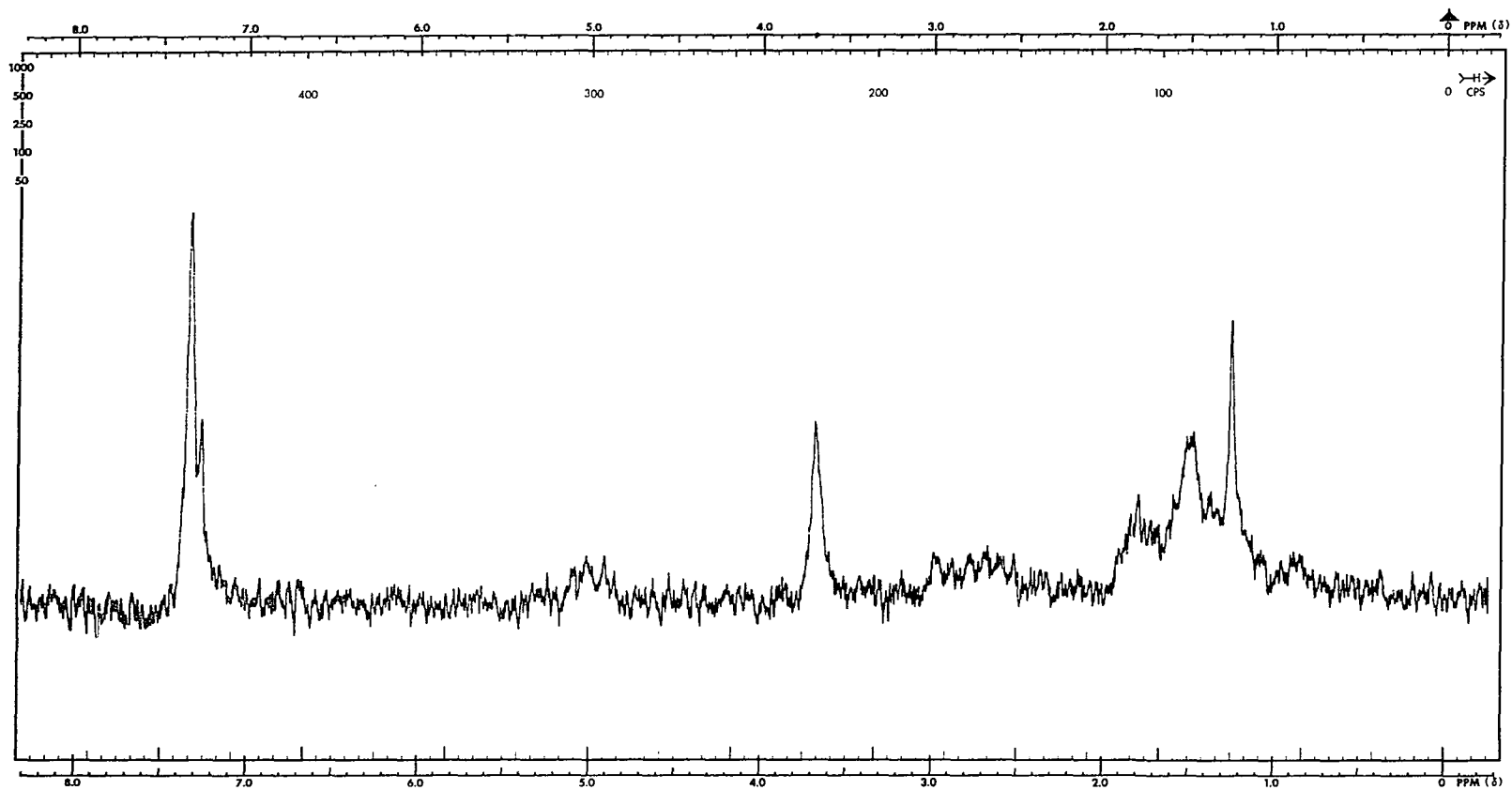


Fig. 4. Nuclear Magnetic Resonance Spectrum of A-1 in deuteriochloroform.

dissolved in 1 ml. of absolute ethanol, α +0.024,
 $[\alpha]_D^{22} +46.9$.

2. Derivatives

a. The Attempted Hydrochloride

A sample of 12 mg. was dissolved in a minimum of absolute methanol and hydrogen chloride gas was gently bubbled through the solution for 5 min. All attempts to crystallize the hydrochloride from a variety of solvents were unsuccessful.

b. The Chloroaurate

The material (12 mg.) used in the attempted preparation of the hydrochloride was dissolved in a minimum of 2 N. hydrochloric acid and a few drops of a 10% solution of chloroauric acid³⁴ ($\text{AuCl}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$) were added producing a turbidity. Upon cooling the solution, 14 mg. of golden yellow needles crystallized which melted at 137-138°. Anal. : Found: C, 28.88, 29.06; H, 4.03, 4.15.

Calculated for $\text{C}_{13}\text{H}_{19}\text{NO} \cdot \text{AuCl}_3 \cdot \text{HCl}$: C, 28.63;
 H, 3.70.

F. Large Extraction II

1. Preliminary Work

The procedure used for the isolation of 8-phenylnorlobelol was extremely tedious and time-consuming. The use of numerous alumina columns suffered from the disadvantages of a large hold-up of material and of pronounced tailing. No additional alkaloids could be isolated from any

of the remaining fractions.

It seemed desirable at this point to develop a more efficient isolation procedure.

A variety of new pilot extractions and columns were examined and the results of these investigations will briefly be summarized. Chromatography using neutral alumina or silica gel produced undesirable results. Silica gel was too strong of an adsorbent, and the alkaloids migrated only when solvent systems of high polarity were used. The use of basic alumina appeared to produce decomposition in some of the alkaloids. Separation by extraction with solvents of increasing polarity, and extraction of the alkaloids at different pH-values using a variety of organic solvents met with no success.

Similar findings were reported in an investigation of *Lobelia salicifolia* Sweet.¹⁷

2. Separation of the Crude Alkaloids

The following scheme, which is summarized in Figs. 5 and 6, appeared to be the most promising of those investigated. The dried ground plant material (10 kg.) was extracted with ethanol in a soxhlet for 96 hr. The alcoholic extract was cooled below room temperature and filtered with suction using diatomaceous earth as a filter aid. The use of diatomaceous earth as a filter aid was found to save considerable time during filtrations and it was employed in all subsequent filtrations throughout this isolation scheme.

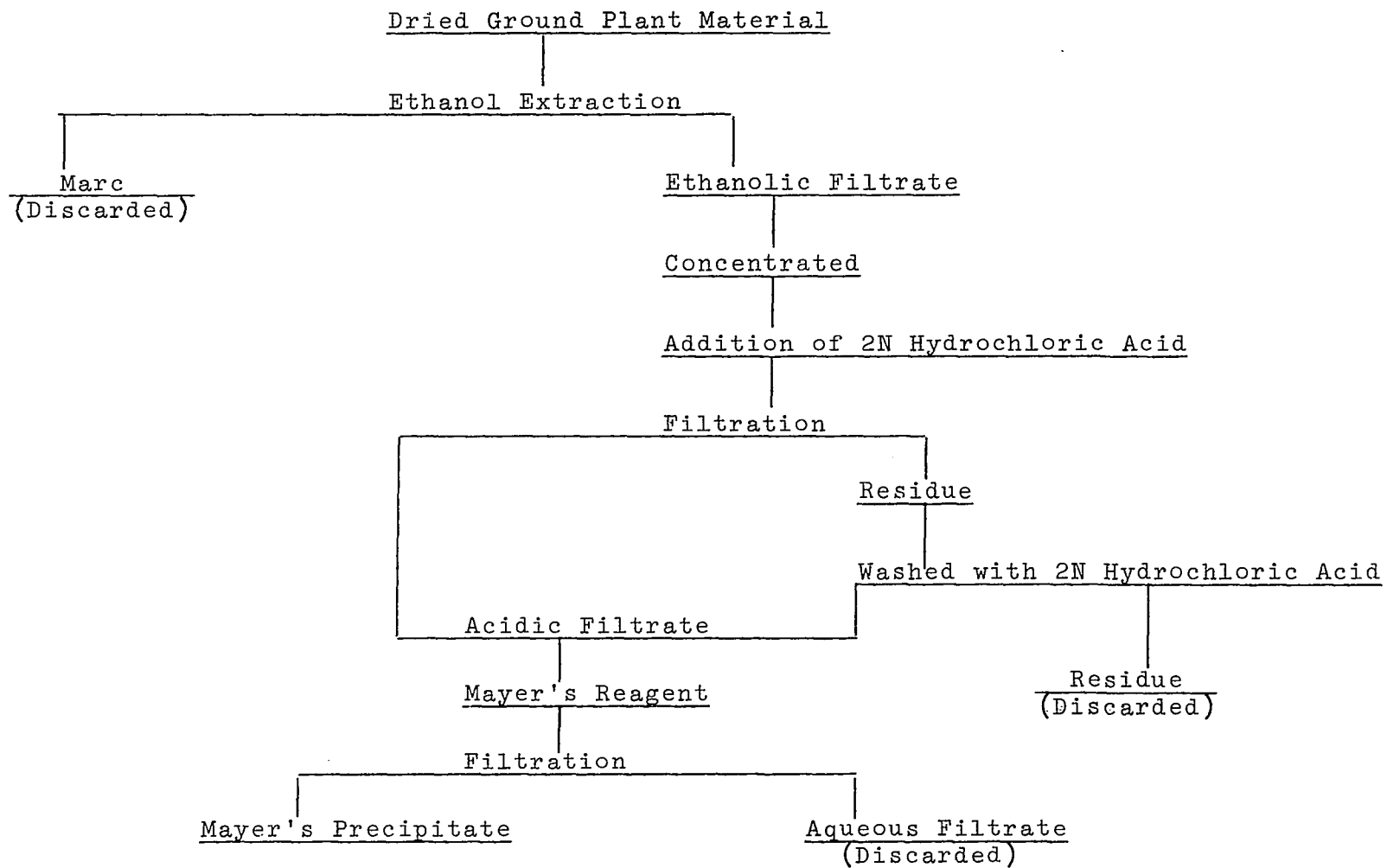


Fig. 5. Scheme for Large Extraction II.

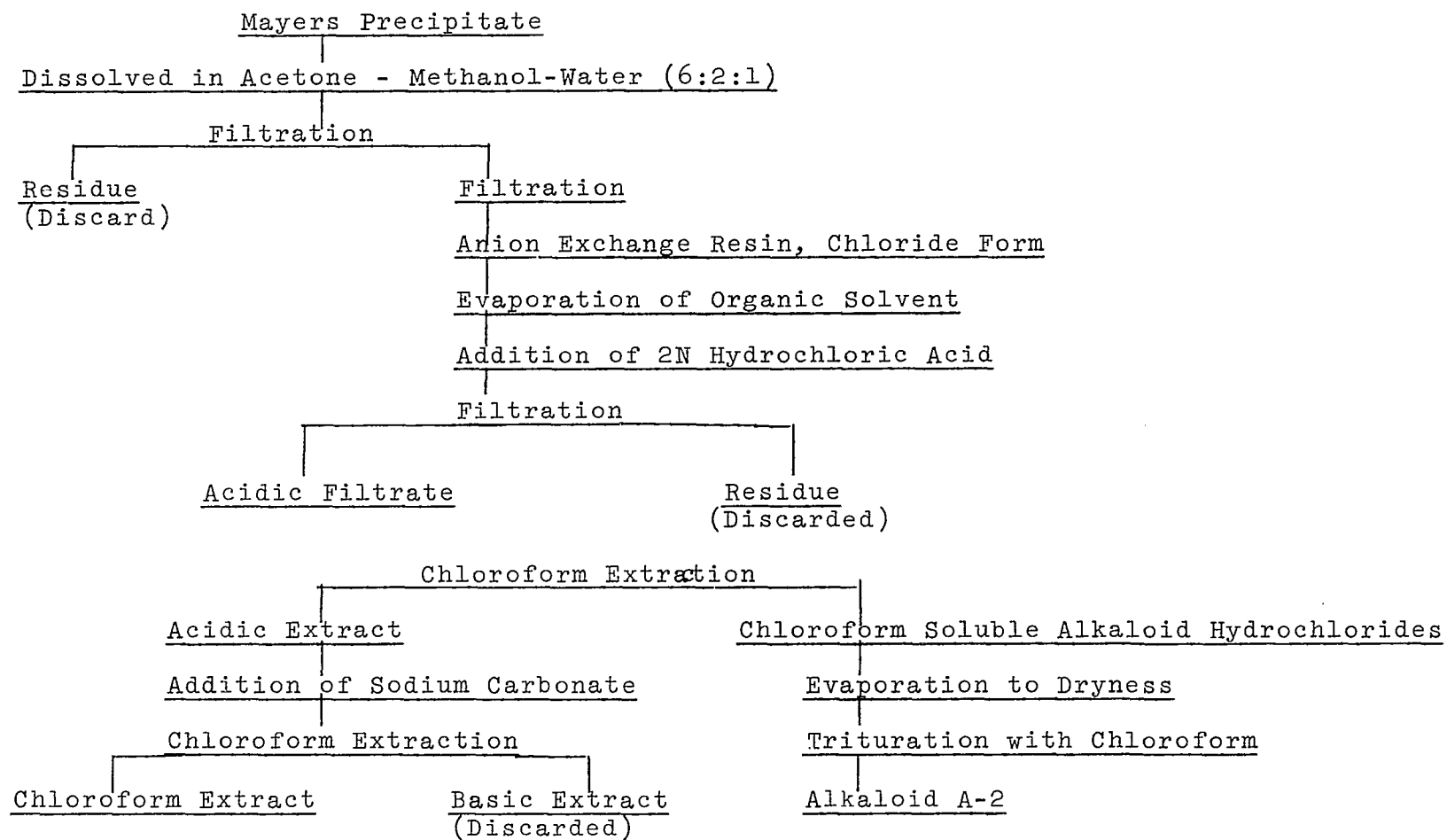


Fig. 6. Scheme Leading to Isolation of Alkaloid A-2.

The solid residue was washed with petroleum ether to remove any fats and waxes, and the petroleum ether was discarded as it gave a negative alkaloid test. Washing with 2 N. hydrochloric acid solution was carried out until the residue no longer gave a positive alkaloid test. The acid wash was saved for further use.

The ethanolic filtrate was concentrated by evaporation in vacuo producing a dark brown syrupy liquid. The liquid was mixed with 2 N. hydrochloric acid; for each kilogram of plant material extracted, ca. 2.5 l. of acid was used. The acidic solution now contained a large quantity of suspended material and was filtered under suction. The residue was still strongly alkaloidal and was washed with a solution of 2 N. hydrochloric acid until it gave a negative Mayer's test. All of the acidic extracts were combined and refiltered. A solution of Mayer's reagent, modified to double strength, was added to the acidic filtrate.

A yellow flocculent precipitate formed and was allowed to settle. Additional reagent was then added until no further precipitation occurred. The precipitate was isolated by filtration and the resulting filtrate discarded. The solid residue (Mayer's precipitate plus diatomaceous earth) was washed with distilled water until the eluate gave only a weakly positive test for chloride ions using a few drops of a 5% solution of silver nitrate. The solid material was next stirred with a solution of acetone-methanol-water

(6:2:1) and filtered.

A column containing 1 lb. (wet weight) of anion exchange resin in the chloride form was washed with 1 l. of distilled water followed by 1 l. of a solution containing acetone-methanol-water (6:2:1). The filtrate containing the Mayer's precipitate was placed on the column, which was then eluted with the same solvent mixture until the eluate no longer gave a positive test for alkaloids. The eluate (brown liquid) was evaporated in vacuo until the major part of the organic solvent was removed. Hydrochloric acid (2 N.) was added to the solution to increase the solubility of the alkaloid hydrochlorides. Addition of chloroform to the acidic solution, followed by evaporation in vacuo removed the last traces of acetone and methanol. (Chloroform forms azeotropes with both acetone and methanol.³⁵) The resulting solution now contained a dark brown non-alkaloidal resinous material which stuck to the sides of the flask. The solution was purified by filtration and the filtrate (4 l.) was extracted with chloroform in a liquid-liquid extractor for 7 d. Evaporation of the chloroform yielded 47.9 g. of a brown amorphous solid. The solid residue constituted the chloroform-soluble alkaloid hydrochlorides along with non-alkaloidal material, which might have become impregnated in the Mayer's precipitate.

The acidic fraction was cooled below room temperature and sodium carbonate was slowly added while the

solution was constantly stirred. (Solid sodium carbonate was used in order to keep the volume of the solution at a minimum.) A large quantity of white precipitate formed when the solution turned basic. The basic solution was exhaustively extracted of its alkaloids with chloroform in a liquid-liquid extractor. The chloroform extract was washed with distilled water to remove any remaining sodium carbonate and was then evaporated to dryness yielding 14.2 g. of a brown solid. This material contained the free bases of the chloroform-insoluble alkaloid hydrochlorides.

G. Isolation and Purification of Alkaloid A-2

The residue containing the chloroform soluble alkaloid hydrochlorides was triturated with chloroform leaving an insoluble white powder. Filtration of this material followed by washing with cold chloroform resulted in 420 mg. of a dry white powder. This material was designated alkaloid A-2. The IR spectrum and the TLC results indicated that A-2 was isolated in a high degree of purity. A small quantity of the solid was, however, recrystallized from ethanol producing fluffy white needles.

H. Characterization Studies of Alkaloid A-2

1. Physical and Chemical Properties

a. Melting Point

The crystalline compound turned orange-brown at 202° and melted at 208-210° with decomposition.

b. Solubility

The crystals were insoluble in petroleum ether, ether, benzene, acetone, and ethyl acetate; slightly soluble in hot chloroform; and soluble in hot ethanol, methanol, and water.

c. Thin-layer Chromatography

Thin-layer chromatography on alumina using a solvent system of chloroform-benzene (1:1) showed one spot at R_f 0.30 while 100% chloroform produced an R_f -value of 0.60.

d. Halide Test³⁶

The alkaloid was dissolved in a minimum of dilute nitric acid and two drops of 5% silver nitrate solution were added. A white precipitate formed immediately.

e. Alkaloid Tests

The compound was dissolved in a minimum of 5% hydrochloric acid and a drop of Mayer's reagent was added. A yellow-white precipitate formed indicating a positive test. Positive tests were also obtained with Dragendorff's, Wagner's, and Hager's reagents. These are common alkaloid reagents,³⁴ but they are less selective than Mayer's reagent.

f. Test for Secondary Amines³⁶

A small quantity of alkaloid was dissolved in distilled water and a 1% solution of sodium nitroprusside (1 drop) in acetaldehyde-water (1:9) was added. Addition of 1 drop of a 4% sodium carbonate solution produced a pink

color after 1 min., which became more intense on standing.

g. Baeyer's Test for Unsaturation³⁸

Alkaloid A-2 (6 mg.) was dissolved in 0.4 ml. of water and one drop of Baeyer's reagent (1% potassium permanganate solution) was added. The solution turned from violet to a light orange-brown color within ca. one min. After standing for two min., a brown precipitate appeared. A blank sample treated under identical conditions remained unchanged.

h. Titration

A potentiometric titration of 2.31 mg. of sample dissolved in 33% dimethylformamide-water was performed by the Eli Lilly Research Laboratory. The alkaloid gave an initial pH of 6.1 in solution, had a pK_a value of 7.2, and had a molecular weight of 347 (found value).

i. Elemental Analysis

Found: C, 65.05, 65.11; H, 7.50, 7.31; N, 7.91, 7.96; Cl, 9.85, 9.88%. Calculated for $C_{19}H_{25}N_2O_2Cl$ (M. Wt. 348.9) : C, 65.41; H, 7.21; N, 8.03; Cl, 10.16%.

j. Ultraviolet Absorption Spectrum

In methanol (Fig. 7) : λ max 203 $m\mu$ (ϵ , 26,743), 248(11,136), 281(1265); λ min 223 $m\mu$ (ϵ , 2847), 278(1202).

k. Infrared Absorption Spectrum

The infrared spectrum of 2 mg. of the hydrochloride in 250 mg. of potassium bromide showed the following

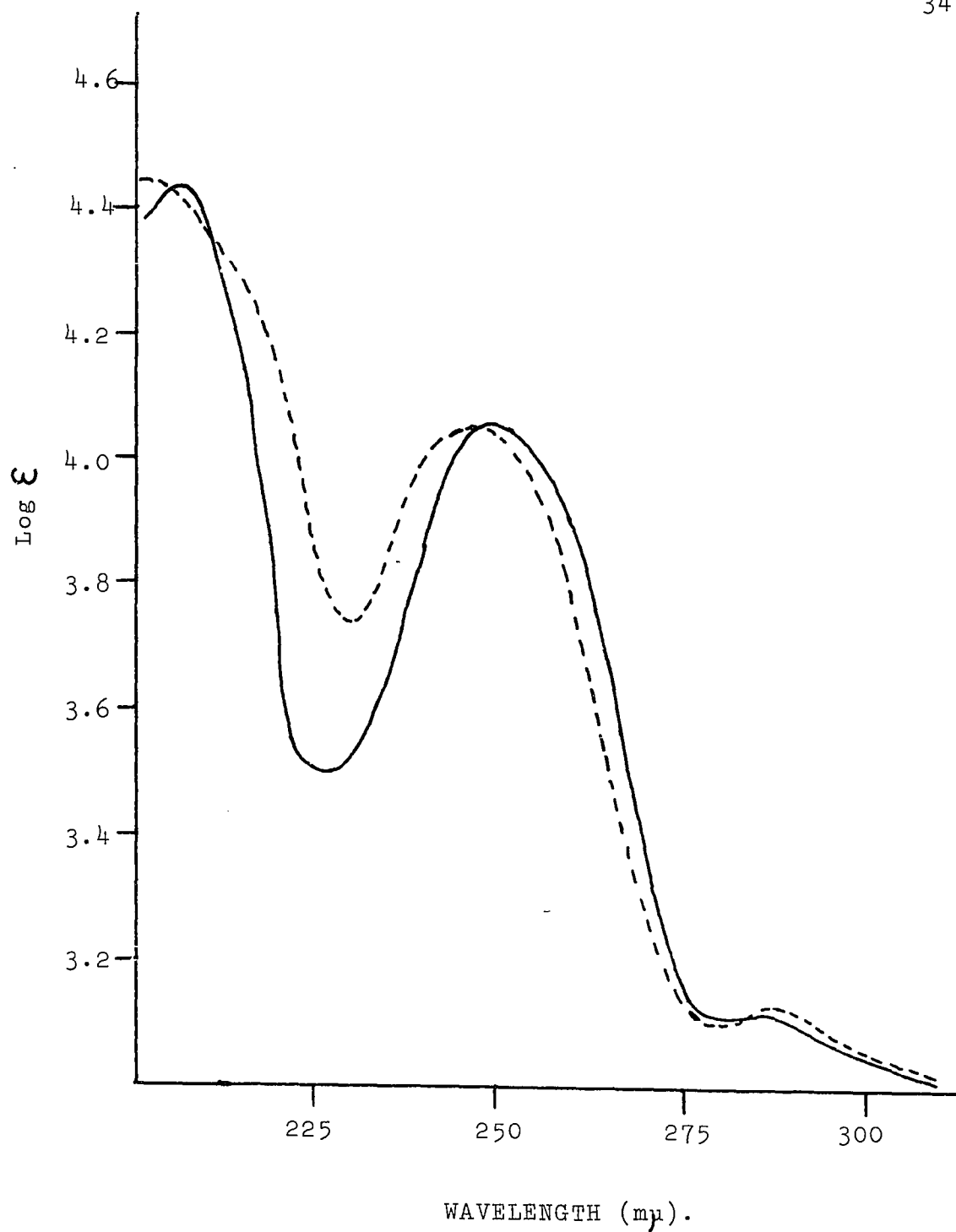


Fig. 7. Ultraviolet Spectra (methanol) of: a, _____, A-2; b,, Free Base of A-2.

absorption bands (Fig. 8) : 3425 cm^{-1} (b,m), 2959(sh,w), 2899(s), 2778(b,w), 2703(s), 2695(s), 2564(sh), 2500(sh), 2457(sh), 2398(sh), 2128(w), 2020(w), 1684(s), 1637(s), 1595(sh), 1575(sh), 1439(s), 1370(m), 1314(m), 1279(m), 1259(m), 1235(w), 1215(m), 1202(sh), 1185(w), 1161(w), 1149(m), 1129(w), 1088(w), 1073(sh), 1038(m), 1020(m), 984(w), 926(m), 895(m), 889(m), 873(w), 848(w), 830(w), 796(w) 752(s), 687(s), 656(s).

1. Nuclear Magnetic Resonance Spectrum

The only suitable solvent in which A-2 was soluble was deuterium oxide. The spectrum of A-2 in this solvent showed absorption at the following delta values (omitting solvent peaks): 1.2-2.4, (broad multiplet), 3.0-3.4, 3.88, 4.05, 4.25, 7.68-7.78, 8.04-8.18.

m. Mass Spectrum

The mass spectrum, taken at Stanford University, showed a small molecular ion peak at 312, its strongest signal at 207(100%), and further significant peaks at 105(33%), 84(44%), and 77(38%). A complete list of m/e values appears in Table V.

n. Optical Rotation

All readings were taken in a 0.1 dm. cell of ca. 0.9 ml. capacity. A total of 8 reference and 8 sample reading were taken with each sample used.

A 20.23 mg. sample was dissolved in 1 ml. of distilled water, $\alpha + 0.0019$, $[\alpha]_D^{22} + 0.9$.

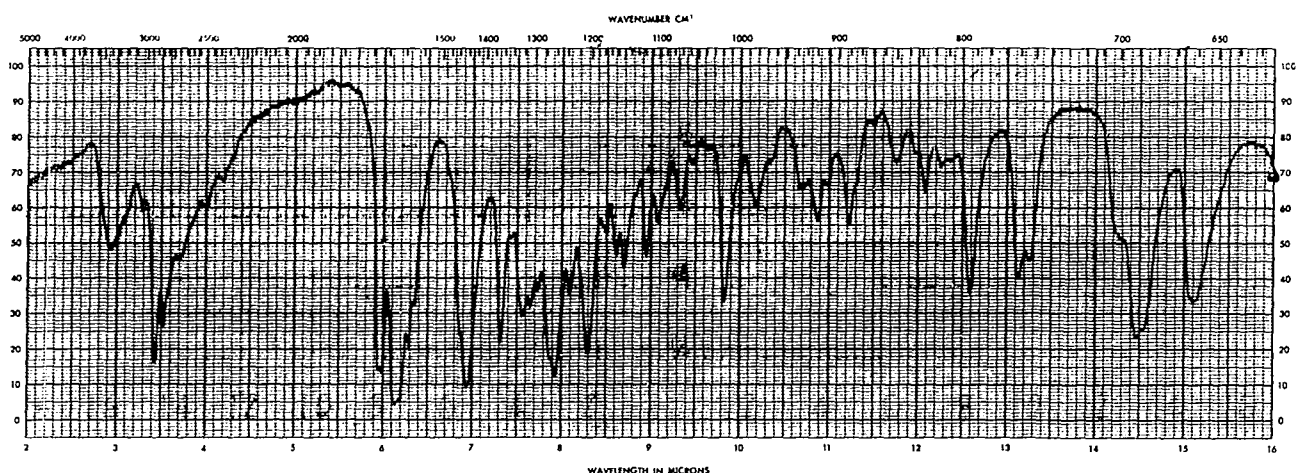
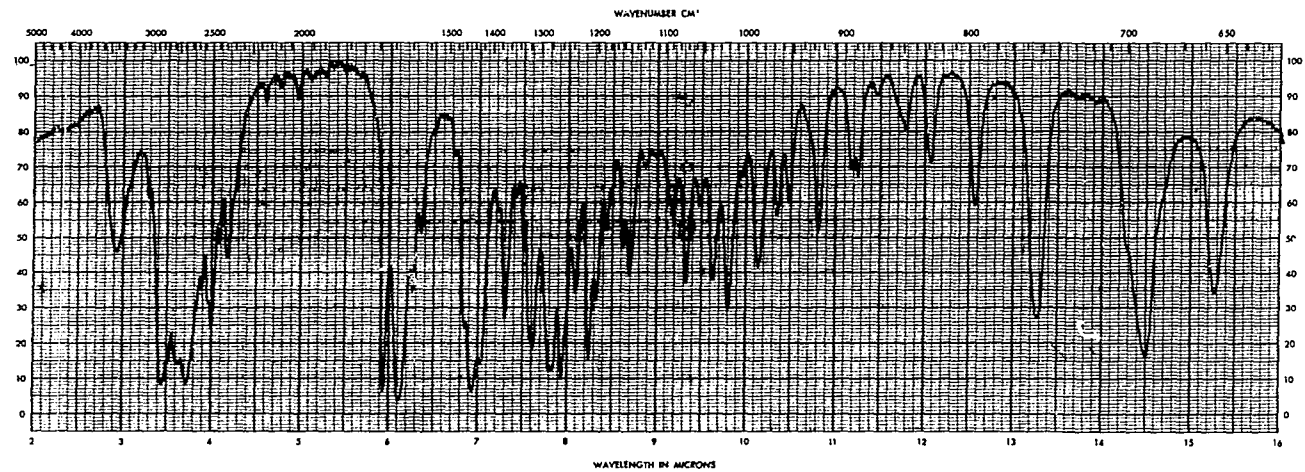


Fig. 8. - Infrared Spectra (potassium bromide) of:
 a. (Top) A-2.
 b. (Bottom) Free base of A-2.

TABLE V. MASS SPECTRUM OF ALKALOID A-2

<u>m/e</u>	<u>%</u>	<u>m/e</u>	<u>%</u>	<u>m/e</u>	<u>%</u>
50	1	95	4	163	2
51	8	96	7	164	4
52	2	97	3	165	6
53	6	98	2	166	2
54	9	103	1	167	1
55	26	104	1	172	2
56	13	105	33	176	2
57	9	106	4	177	1
58	1	107	2	178	2
59	1	108	3	179	1
60	5	109	3	188	1
61	2	110	3	189	1
63	1	111	2	190	1
65	8	112	2	191	1
66	2	113	1	192	2
67	8	115	1	193	2
68	100	117	1	198	1
69	15	120	2	200	7
70	6	121	2	201	3
71	4	122	6	202	1
73	5	123	4	205	1
74	1	124	7	207	100
75	1	125	2	208	15
76	1	129	2	209	2
77	38	131	1	212	1
78	5	134	2	214	1
79	4	135	1	228	1
80	5	136	3	230	1
81	7	137	3	231	1
82	13	138	1	232	1
83	18	139	1	254	4
84	44	147	2	255	1
85	4	148	2	256	1
87	1	149	4	269	1
91	3	150	3	293	1
92	2	151	1	311	2
93	3	152	1	312	4
94	4	162	1	313	2

For the second measurement 10 mg. of alkaloid was dissolved in 1 ml. of distilled water, α 0.000, $[\alpha]_D^{22}$ 0.0.

2. Derivatives

a. The Picrate

Ten milligram of alkaloid was dissolved in a minimum of ethanol and a few drops of a saturated solution of picric acid in ethanol were added. The solution was warmed in a water bath and 12 mg. of picrate crystallized by allowing the solution to stand at room temperature. The picrate was recrystallized from methanol yielding 8 mg. of small yellow needles melting at 205-207°.

b. The Perchlorate

The alkaloid readily formed a perchlorate derivative by the following procedure: A few milligram of alkaloid was dissolved in a minimum of distilled water and 4 drops of a 20% solution of perchloric acid were added. A white turbidity resulted. After standing for 1 hr., the perchlorate crystallized as small, white radiating needles. The crystals turned yellow at 195° and melted at 207-209.5°.

I. Preparation of the Free Base of Alkaloid A-2

Attempts to prepare the free base by addition of base to an aqueous solution of A-2 usually resulted in poor yields. Two of these reactions are described below.

Eleven milligram of alkaloid was dissolved in 1 ml. of water and the solution cooled in an ice-water bath.

Addition of three drops of an 5% sodium hydroxide solution produced no precipitation although the solution was basic to litmus. One milligram of crystalline alkaloid was isolated from the solution after remaining in the ice box for three days.

Three drops of 5% sodium hydroxide solution were added to a cold solution of 20 mg. of hydrochloride dissolved in 1 ml. of water, resulting in the immediate precipitation of a white solid. The solid was centrifuged from the solution producing 3 mg. of base.

Because of the poor yields in the above reactions, two new methods for converting the hydrochloride to the free base were investigated. Lobeline hydrochloride was used as a trial compound. The methods are described below.

An anion exchange column containing 40 ml. of wet resin (chloride form) was washed with 200 ml. of distilled water and converted to the hydroxide form by washing with 1 l. of 1 N. sodium hydroxide solution. The effluent was tested for completeness of conversion by acidification with concentrated nitric acid followed by addition of a drop of a 1% silver nitrate solution.³³ The column was washed with distilled water until a P^H of less than nine was obtained. The washing was completed using 300 ml. of methanol. A sample of lobeline hydrochloride (66 mg.) dissolved in methanol was placed on the column which was eluted with methanol until the effluent no longer gave a positive

alkaloid test. The eluate was evaporated furnishing 59 mg. of a light yellow oil.

The second method for converting the hydrochloride to the free base consisted of passing the alkaloid (66 mg.) through a column containing 25 g. of neutral alumina. A yield of 55 mg. of white crystalline base resulted.

Alkaloid A-2 was converted to the free base using the second method which afforded a purer product than the first method. A sample of 55 mg. of A-2 dissolved in a minimum of methanol was placed on a column containing 25 g. of neutral alumina. The column was eluted with chloroform until the resulting solution gave a negative Mayer's test. Evaporation of the chloroform produced 53 mg. of a yellow-white solid.

1. Physical Properties

a. Melting Point

The free base melted at 115-117°.

b. Solubility

The base was insoluble in petroleum ether and ether and readily soluble in benzene, chloroform, ethanol, and methanol.

c. Thin-Layer Chromatography

Thin-layer chromatography on alumina using a system of chloroform-benzene (2:1) gave an R_f -value of 0.30 while 100% chloroform resulted in an R_f of 0.70.

d. Sublimation

The free base sublimed at 125° and ca. 0.1 mm pressure.

e. Ultraviolet Absorption Spectrum

In methanol (Fig. 7): λ max 200 μ (ϵ , 27,275), 248(10956), 281(1386); λ min 223 μ (ϵ , 4471), 278 (1252).

f. Infrared Absorption Spectrum

The infrared spectrum of 1 mg. of the base in 250 mg. of potassium bromide showed the following major absorption bands (Fig. 8): 3460 cm^{-1} (b,w), 3040(w), 2924 (s), 2841(sh), 1675(s), 1637(s), 1590(w,sh), 1575(w,sh), 1439(s), 1368(m), 1325(w), 1307(w), 1294(w), 1263(m), 1236 (w), 1205(m), 1176(w), 1161(w), 1149(w), 1119(s), 1103(w), 1073(w), 1019(m), 982(w), 919(w), 892(w), 851(w), 827(w), 816(w), 794(s), 762(s), 749(sh), 692(s), 662(s).

g. Nuclear Magnetic Resonance Spectrum

The NMR spectrum taken in deuteriochloroform (Fig. 9) showed absorption at the following delta values: 1.24-2.00, 2.15, 2.61, 2.82-2.92, 3.10, 3.29, 3.45, 4.28, 4.44, 4.69, 4.91, 7.45-7.65, 8.08-8.18.

J. Hydrogenation of Alkaloid A-2

A sample of 8 mg. of alkaloid was dissolved in 10 ml. of water containing 0.5 ml. of 5% hydrochloric acid and 5 mg. of a 5% palladium-on-carbon powder as a catalyst. The mixture was vigorously stirred for 2 hr. at room

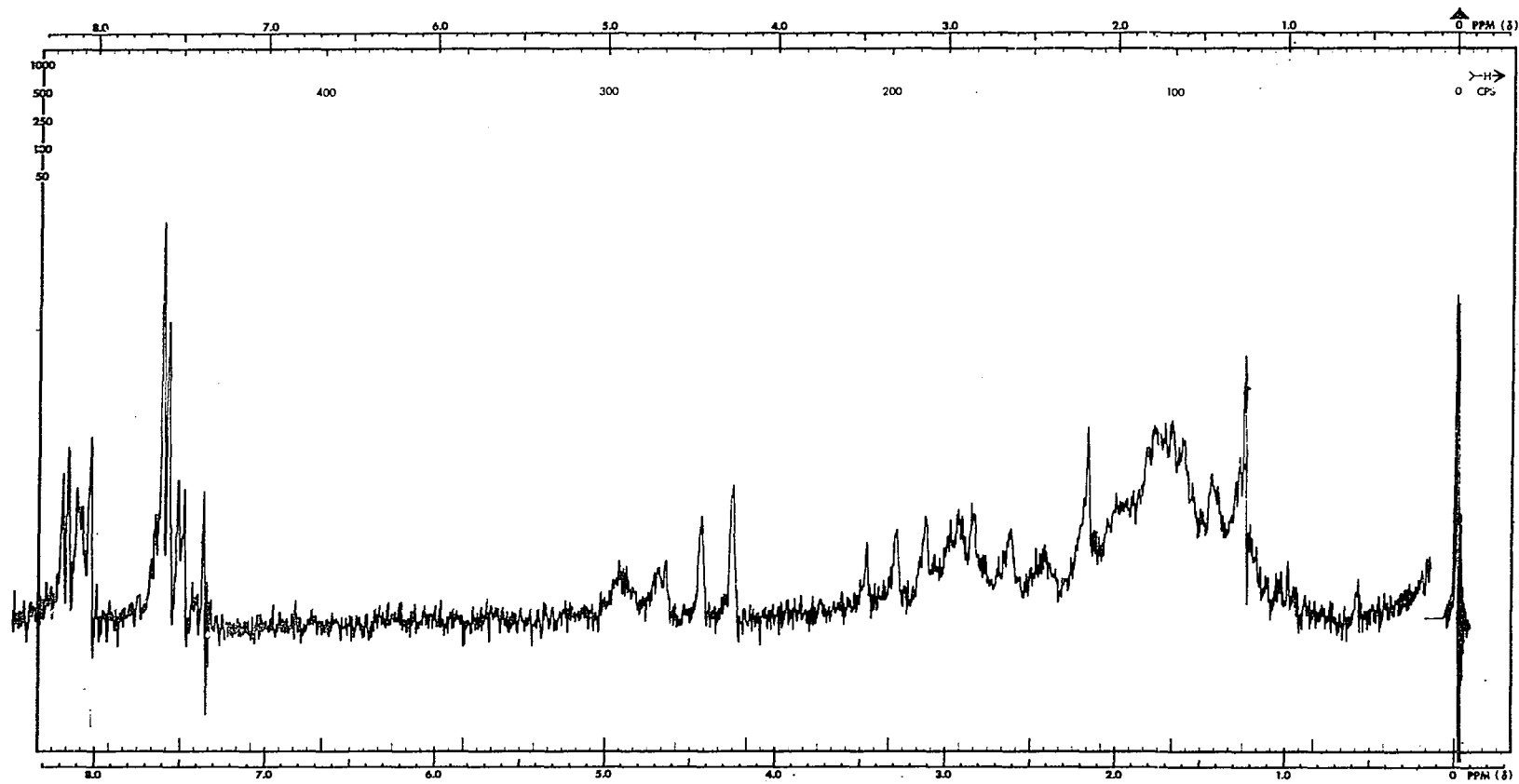


Fig. 9. Nuclear Magnetic Resonance Spectrum of the Free Base of A-2 in deuteriochloroform.

temperature at a slight overpressure of hydrogen. A comparison of the ultraviolet and infrared spectra with the starting material along with the TLC results indicated that hydrogenation had not taken place.

Six milligram of A-2 was dissolved in a mixture of ethanol (15 ml.) and glacial acetic acid (0.5 ml.) and 5 mg. of 10% palladium-on-charcoal was added. The mixture was stirred for 2 hr. at room temperature at a slight overpressure of hydrogen. The results of TLC, ultraviolet, and infrared spectroscopy all showed that no hydrogenation had occurred.

A sample of 9.8 mg. of alkaloid dissolved in a mixture of 15 ml. of distilled water and 0.5 ml. of 5% hydrochloric acid was hydrogenated for 2 hr. with vigorous stirring using 7 mg. of platinum dioxide catalyst ($\text{PtO}_2 \cdot \text{XH}_2\text{O}$, 83.8%) at a slight overpressure of hydrogen. The catalyst was removed by filtration and the aqueous solution extracted with chloroform in a liquid-liquid extractor for 15 hr. Thin-layer chromatography of the chloroform extract showed two spots both having lower R_f -values than A-2. The infrared and ultraviolet spectrum of the mixture revealed the disappearance of the original carbonyl group.

A sample of 30 mg. of alkaloid was hydrogenated by dissolving it in a mixture of ethanol-water(6:9), adding 13 mg. of platinum dioxide catalyst, and vigorously stirring the mixture for 7 hr. at room temperature at a slight

overpressure of hydrogen. Thin-layer chromatography of the product on alumina using 100% chloroform furnished the following R_f -values: 0.15(major spot), 0.30(major spot), 0.65 (minor spot), and 0.70(minor spot). Alkaloid A-2 gave an R_f -value of 0.70 using the above solvent system. An attempt to isolate the individual compounds using preparative TLC proved unsuccessful.

Hydrogenation of 40 mg. of A-2 in a solution of 15 ml. of ethanol and 15 drops of glacial acetic acid was performed under the conditions described above. Thin-layer chromatography of the product showed six spots and NMR spectroscopy revealed the absence of the original aromatic protons. No further investigation was attempted with this material.

K. Reduction of Alkaloid A-2 with Sodium Borohydride

A cold solution of 32 mg. (5 x 0.018 m.) of sodium borohydride dissolved in 10 ml. of deionized, deoxygenated water was slowly added to a solution of 64 mg. (0.018 m.) of alkaloid A-2 dissolved in 10 ml. of the above solvent. The reaction flask was immersed in an ice-water bath and the solution was stirred magnetically throughout the reaction. After addition of sodium borohydride was completed the mixture was stirred for 30 min. while immersed in an ice-water bath.

The solution was allowed to stand at room temperature for 2 hr. and was then exhaustively extracted with

chloroform in a separatory funnel. The chloroform fraction was evaporated in vacuo at room temperature furnishing 64 mg. of white crystalline material.

1. Physical Properties of the Reduction Product

a. Melting Point

A sample of the crude material melted at 162-163°.

b. Thin-Layer Chromatography

Thin-layer chromatography on alumina using 100% chloroform revealed one spot at R_f 0.00, with a solvent system of chloroform-methanol (20:1) an R_f of 0.63 was observed.

c. Ultraviolet Absorption Spectrum

In methanol, λ max 254 m μ (ϵ , 719).

d. Infrared Absorption Spectrum

The infrared spectrum taken in chloroform showed the following major absorption peaks: 3311 cm⁻¹(b,m), 2985 (sh), 2941(s), 2857(sh), 1629(sh), 1445(sh), 1368(w), 1335 (w), 1302(w), 1266(s), 1164(w), 1117(w), 1105(w), 1087(w), 1047(m), 1031(w), 909(w), 699(m-s), 662(w).

e. Nuclear Magnetic Resonance Spectrum

The NMR spectrum in deuteriochloroform (Fig. 10) showed peaks at the following delta values (omitting peaks due to impurities): 1.05-2.22 (broad multiplet), 2.40, 2.59, 2.90, 3.70, 4.87, 5.69-5.75, 7.43.

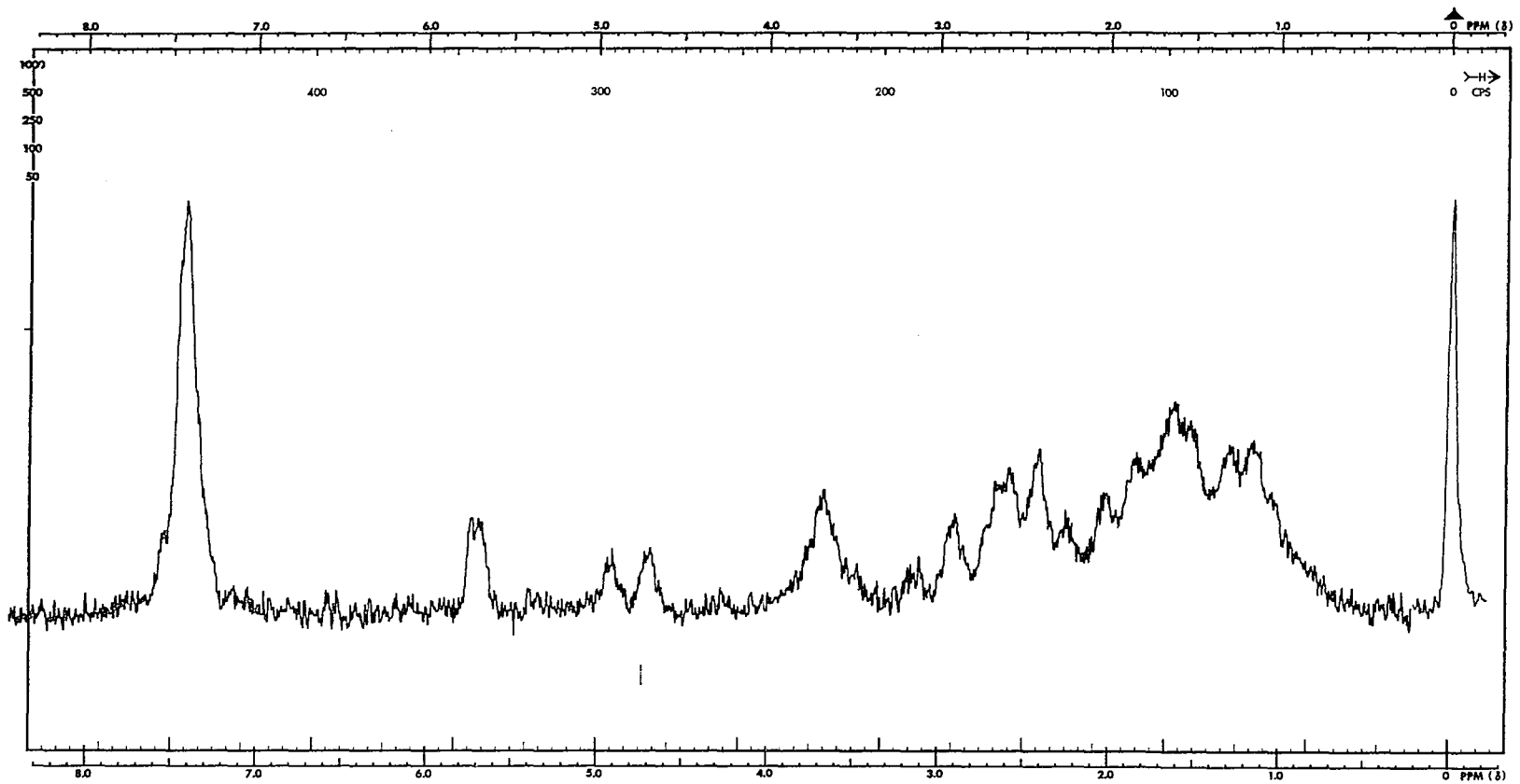


Fig. 10. Nuclear Magnetic Resonance Spectrum of Reduction Product of A-2 in deuteriochloroform.

L. Dehydration of the Sodium Borohydride Reduction Product

The sodium borohydride reduction product was dehydrated using a method described by Bernstein.³⁹ A sample of 70 mg. (0.19 mmole.) of the reduction product dissolved in 10 ml. of distilled, dried pyridine was cooled to below zero degrees in a bath containing crushed ice, salt, and water. The flask was fitted with a reflux condenser through which five drops (ca. 1.30 mmole.) of thionyl chloride was slowly added while the solution was stirred magnetically. The color of the solution gradually darkened and turned brown after standing at room temperature for 3 hr. Evaporation of the solvent in vacuo at room temperature furnished 55 mg. of a brown, viscous oil.

1. Characterization of the Products

Thin-layer chromatography on alumina using 100% chloroform showed four spots having the following R_f -values: 0.00 and 0.20 (two minor spots), 0.60 and 0.70 (two major spots). Using a solvent system of chloroform-methanol (10:1) afforded the following R_f -values: 0.0-0.4 (streaking), 0.85 and 0.95 (major spots). An attempt was made to separate the two major spots using preparative TLC on 1 mm. alumina plates and a solvent system of 100% chloroform. Two fractions were collected. Thin-layer chromatography of these fractions showed that only a partial separation was achieved. The results of TLC of these two fractions are shown in Fig. 11.

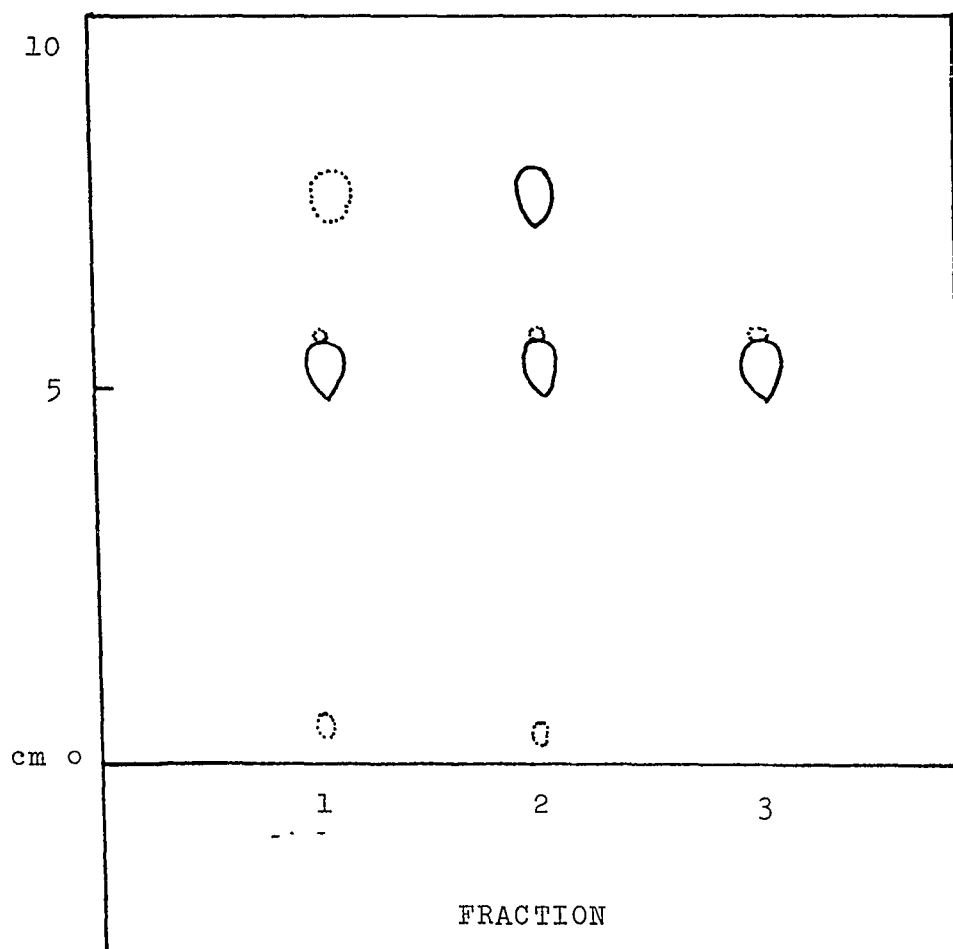


Fig. 11. TLC using Chloroform-Methanol (20:1) of the Initial Reduction Product (3), and of the Dehydration Products after Attempted Separation using Preparative TLC.

A sample of the initial reduction product (No. 3, refer to Fig. 11) and run on a TLC plate along with the two fractions collected, and served as a reference. Fraction 1 (refer to Fig. 11) apparently contained more unreacted material than dehydration product, while Fraction 2 seemed to contain more of the dehydration product and only a small amount of unreacted material.

a. Ultraviolet Absorption Spectra

(In ethanol) Fraction 1; λ_{max} 257 m μ (ϵ , 946); (In ethanol) Fraction 2; λ_{max} 263 m μ (ϵ , 3032).

The UV spectra of Fractions 1 and 2 along with that of the initial reduction product are shown in Fig. 12.

b. Infrared Absorption Spectrum

The infrared spectrum of the crude dehydration product in chloroform showed the following major absorption peaks: 3344 cm⁻¹(w), 2985(sh), 2941(s), 2857(sh), 1631(s), 1445(s), 1368(w), 1335(w), 1316(w), 1300(w), 1266(m), 1164(w), 1124(w), 1001(w), 976(b,w), 858(w), 917(w), 897(w), 832(w), 699(m), 600(w).

c. Nuclear Magnetic Resonance Spectrum

The NMR spectrum in deuteriochloroform showed the following peaks downfield from 4 delta: Fraction 1 (refer to Fig. 11) : 4.40, 4.70, 4.87, 5.69-5.75, 7.43; Fraction 2 (refer to Fig. 11) : 4.70, 4.87, 5.69-5.75, 7.43.

M. Ozonolysis of Alkaloid A-2

A flask containing 75 mg. of A-2 dissolved in

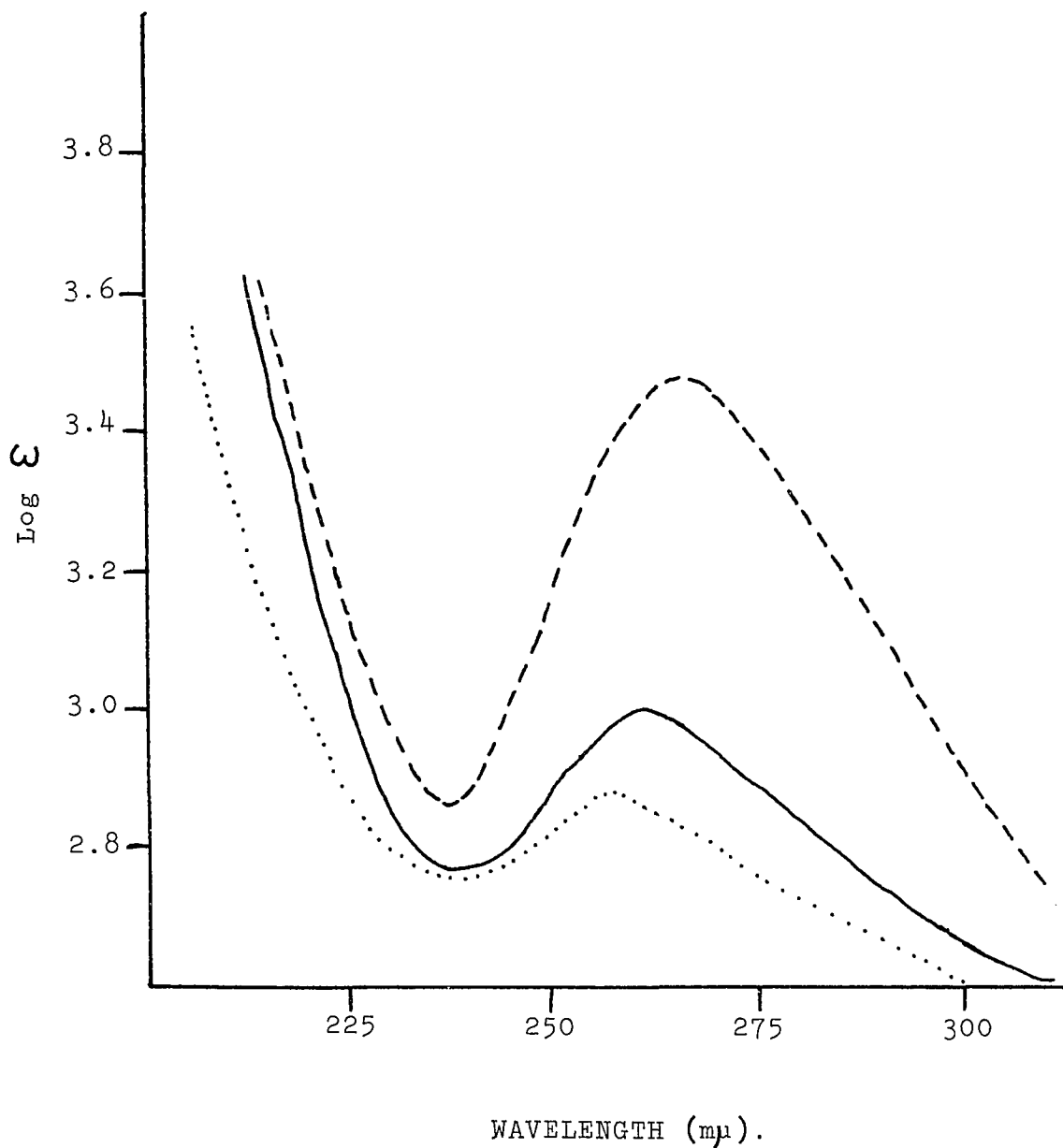


Fig. 12 Ultraviolet Spectra (ethanol) of: a,, Reduction Product; b, _____, Fraction 1; c, ----, Fraction 2 (Fr 1 and 2 are the Dehydration Products after Attempted Separation using Prep. TLC.).

15 ml. of a mixture of acetone-water (2:1) was immersed in a cooling bath of dry ice and acetone immediately before ozonolysis. Ozone was bubbled through the solution for 1 min. A strong smell of ozone persisted in the reaction flask for 10 min. after completion of the reaction. The contents of the flask were frozen by continued immersion in the dry ice and acetone bath and then evaporated in vacuo to remove excess ozone. Evaporation was continued until the solvent mixture had started to liquefy. The reaction mixture was then added to 10 ml. of 30% hydrogen peroxide and the resulting solution heated to 65° for 10 hr. Evaporation of this solution resulted in a yellow liquid, which smelled faintly of hydrogen peroxide. Continued evaporation of the liquid produced a viscous yellow oil which no longer smelled of hydrogen peroxide. The oil was triturated with 15 ml. of distilled water and extracted with chloroform for 25 hr. in a liquid-liquid extractor. The chloroform extract was dried over anhydrous magnesium sulfate, and evaporated in vacuo furnishing 31 mg. of a brown residue. Evaporation of the aqueous fraction yielded 31 mg. of brown residue.

1. Characterization of the Products

The two ozonolysis fractions along with a sample of A-2 were subjected to a series of spot tests. All of these tests were carried out on alumina TLC plates. The results of these tests are summarized in Table VI. The ultraviolet spectrum of the aqueous fraction showed an

TABLE VI. COMPARISON OF THE TWO FRACTIONS OBTAINED
FROM OZONOLYSIS OF A-2 BY MEANS OF SPOT TESTS

Test Number	Test Reagent	A-2	Chloroform Fraction	Aqueous Fraction	Test Characterization
1	Mayer's Reagent ³⁴	(+)	(-)	(+)	Alkaloids
2	Dragendorff's Reagent ³⁴	Strong (+)	doubtful	weak (+)	Alkaloids (Not Specific)
3	Alcoholic solution of 2.4 Dinitrophenylhydrazine ⁴⁰	Yellow spot (+)	Yellow spot (+)	Faint Yellow (Doubtful)	Aldehydes and Ketones
4	A solution (1:1 by volume) of 4% Triphenyltetrazonium chloride in methanol plus a 4% Sodium Hydroxide solution in Methanol ⁴¹	Pink Spot (+)	Pink Spot (+)	Weakly (+)	Aldehydes and Ketones
5	A 0.1% Ninhydrin solution in citrate buffer ⁴²	(-)	(-)	Light Purple Spot (+)	Amino Acids, Primary and Secondary Amines

apparent maximum at 255 μ while the UV spectrum of the chloroform fraction showed a shoulder at 272 μ and strong end absorption. Both spectra were taken in methanol and are qualitative. The infrared spectrum of the chloroform-soluble fraction in chloroform showed a peak at 3390 cm^{-1} and very broad and strong absorption in the carbonyl region. Both fractions apparently contained impurities. An attempt to isolate a pure compound from the aqueous fraction by preparation of a picrate proved unsuccessful.

N. Hydrolysis of Alkaloid A-2

Hydrolysis of A-2 was carried out three times. For the first two reactions, quantities under 100 mg. were used. Hydrochloric acid was used in all of the reactions; only the work-up procedure and the isolation methods were modified. All attempts to isolate the hydrochloride or the free base met with little success. The free base appeared to be unstable and began to undergo decomposition after remaining in solution for a short time. The material gradually darkened and TLC revealed three to four spots. The results of the three reactions appeared to be the same and only the last hydrolysis reaction will be described in detail.

A solution containing 205 mg. of alkaloid A-2 dissolved in 25 ml. of 20% hydrochloric acid was refluxed for 10 hr. by heating in a silicon oil bath kept at a temperature of 125°. The solvent was evaporated to dryness

in vacuo leaving a yellowish-white solid. The solid was dissolved in 20 ml. of distilled water and extracted with chloroform for 24 hr. in a liquid-liquid extractor. The chloroform extract was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness yielding 9 mg. of residue. The residue gave negative tests with Mayer's, Dragendorff's, and ninhydrin reagents.

Evaporation of the aqueous solution resulted in 195 mg. of a yellow white solid. Thin-layer chromatography of this material on alumina using 100% chloroform showed one spot at R_f 0.00; a system of chloroform-methanol (10:1) gave an R_f -value of 0.80. The residue was tested for the presence of amino acids by spotting a sample on a cellulose plate, which was then developed along with a series of known amino acids with a solvent mixture of isopropanol-water-concentrated ammonia (7:2:1). The plate was allowed to dry and then sprayed with a 0.1% ninhydrin solution in citrate buffer and placed in the dark overnight. The residue gave no spot while the amino acids all produced purple spots (positive test).³⁹

The aqueous residue was triturated with 15 ml. of distilled water and cooled in a bath of ice and water. To this solution was added a cold saturated solution of sodium carbonate until a P^H of nine was reached. The basic solution was extracted with chloroform in a separatory funnel and the chloroform extract was immediately extracted

with four 10 ml. portions of 2N. hydrochloric acid. Evaporation in vacuo of the acidic extract furnished 185 mg. of a yellow, white solid. All further attempts to purify the material were unsuccessful.

1. Characterization of the Product

a. The Picrate

The residue (ca. 185 mg.) was dissolved in a minimum of hot ethanol and a saturated solution of picric acid in ethanol was added. The solution was heated in a water bath and allowed to cool to room temperature where upon 240 mg. of the picrate crystallized. The crude picrate melted at 177-180°. Recrystallization of 10 mg. of the picrate from a mixture of methanol-ethyl acetate (5:1) afforded 8 mg. of picrate melting at 182-184°. Twenty more milligram of picrate was isolated from the mother liquor and kept separate.

Anal.: Found: C, 49.68, 50.06; H, 4.13, 4.02; N, 15.15, 15.13.

Calculated:	<u>Formula</u>	<u>%C</u>	<u>%H</u>	<u>%N</u>
	$C_{15}H_{14}N_4O_7$	49.73	3.90	15.47
	$C_{15}H_{16}N_4O_7$	49.45	4.43	15.38
	$C_{30}H_{30}N_8O_{14}$	49.59	4.16	15.41

b. The Free Base of the Hydrolysis Product

An anion exchange column was prepared using 25 ml. of wet resin in the chloride form. The column was washed with 200 ml. of distilled water followed by 1 l. of a 1N.

solution of sodium hydroxide. A sample of the eluate was tested for the presence of chloride ions and found to be negative. Washing was then continued with distilled water until the eluate had a P^H of less than nine.³³ A final washing was made using 200 ml. of 95% ethanol, followed with 200 ml. of absolute ethanol. Application of the picrate to the column proved to be a problem. The picrate was very difficultly soluble in water and all organic solvents. Absolute ethanol appeared to be the most promising solvent although a considerable amount of the picrate did not go into solution and had to be applied as a slurry. The resin and the solid picrate were intermixed on the column to facilitate exchange. The column was eluted with absolute ethanol until the eluate no longer gave a positive alkaloid test. Evaporation of the eluate furnished 77 mg. of a yellowish solid. Thin-layer chromatography on alumina using chloroform-methanol (10:1) produced the following R_f -values: 0.70 (major spot), 0.35(minor spot).

b. Test for Secondary Amines³⁶

Addition of 1 drop of a 1% solution of sodium nitroprusside in a mixture of acetaldehyde-water (1:9) to a solution of the hydrolysis product in water, followed by a drop of 2% sodium carbonate solution resulted in the immediate production of a pink color which turned violet on standing. A pink or violet color indicates a positive test.

c. Ultraviolet Absorption Spectrum

In methanol (Fig. 13): λ max 220 m μ (ϵ , 13,560), 267(8135) : λ min 213 m μ (ϵ , 12,930), 258(6030). After addition of base (5% sodium hydroxide): λ max 220 m μ (ϵ , 17,400), 283(5710): λ min 217 m μ (ϵ , 17,140), 262(4478). After reacidification (5% hydrochloric acid): λ max 267 m μ (ϵ , 10,500); λ min 235 m μ (ϵ , 2834). Extinction coefficients were calculated using a molecular weight of 339 ($C_{18}H_{24}N_2Cl_2$). This is based on the assumption that the hydrolysis product forms a dipicrate ($C_{18}H_{24}N_2 \cdot 2C_6H_3N_3O_7$).

d. Infrared Absorption Spectrum

The infrared spectrum in chloroform showed the following significant peaks (Fig. 14): 3333 cm^{-1} (b,w), 2933(s), 2849(sh), 1626(m), 1550(b,w), 1443(m), 1370(w), 1328(w), 1261(w), 1247(w), 1136(w), 1111(w), 1105(w), 1070(w), 699(m), 659(b,m).

An attempted hydrogenation of the hydrolysis product using 10% palladium-on-charcoal resulted in a change of the peak at 1629 cm^{-1} from medium to weak intensity and the appearance of a new peak at 1724 cm^{-1} .

e. Nuclear Magnetic Resonance Spectrum

The NMR spectrum in deuteriochloroform showed peaks at the following delta values (Fig. 15): 1.15-2.10, 2.40-2.80, 3.10, 3.25, 4.47, 7.39.

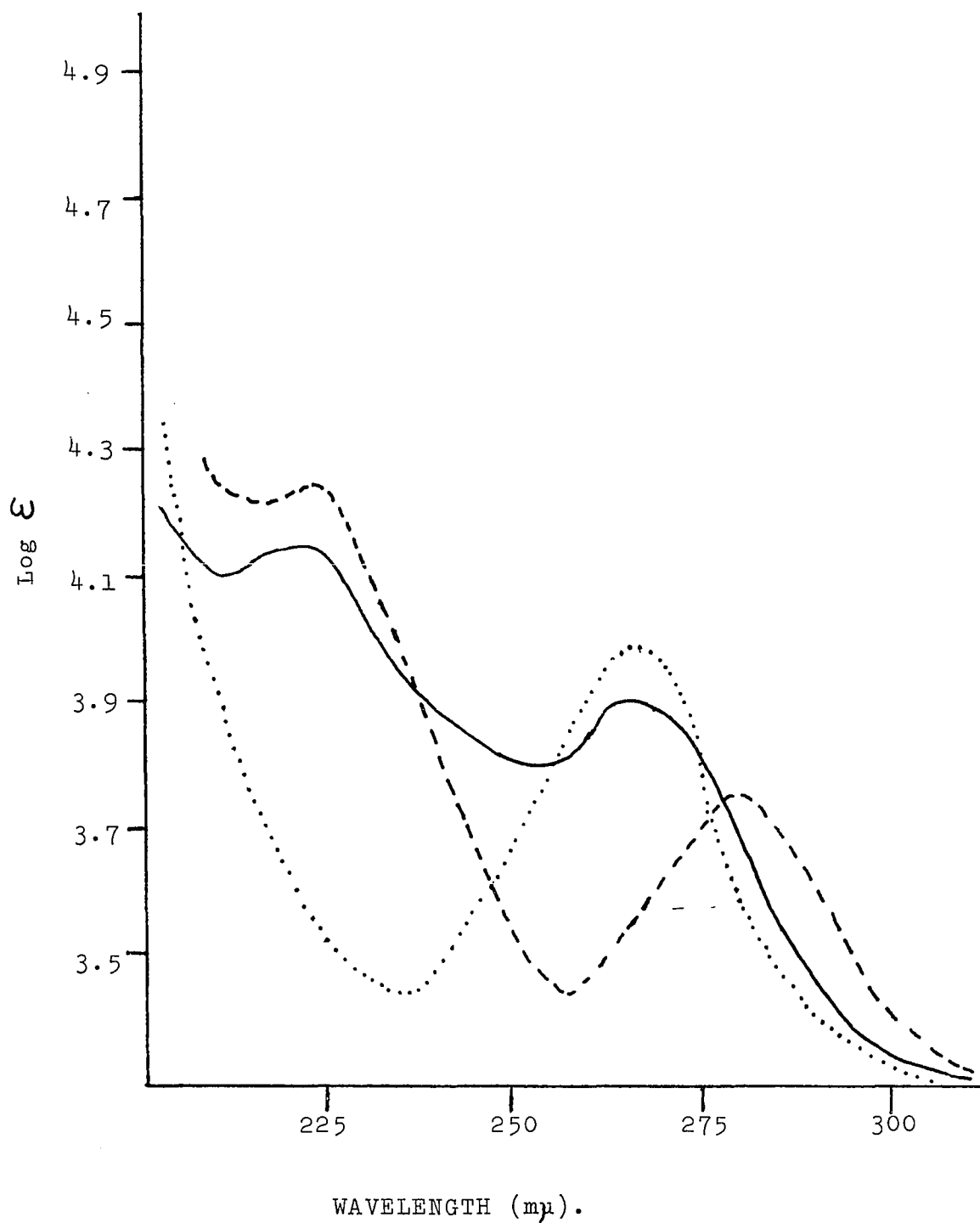


Fig. 13. Ultraviolet Spectra of the Hydrolysis Product of A-2; a, _____, in methanol; b, ----, after Addition of Base (5% sodium hydroxide); c,, after Acidification (5% hydrochloric acid).

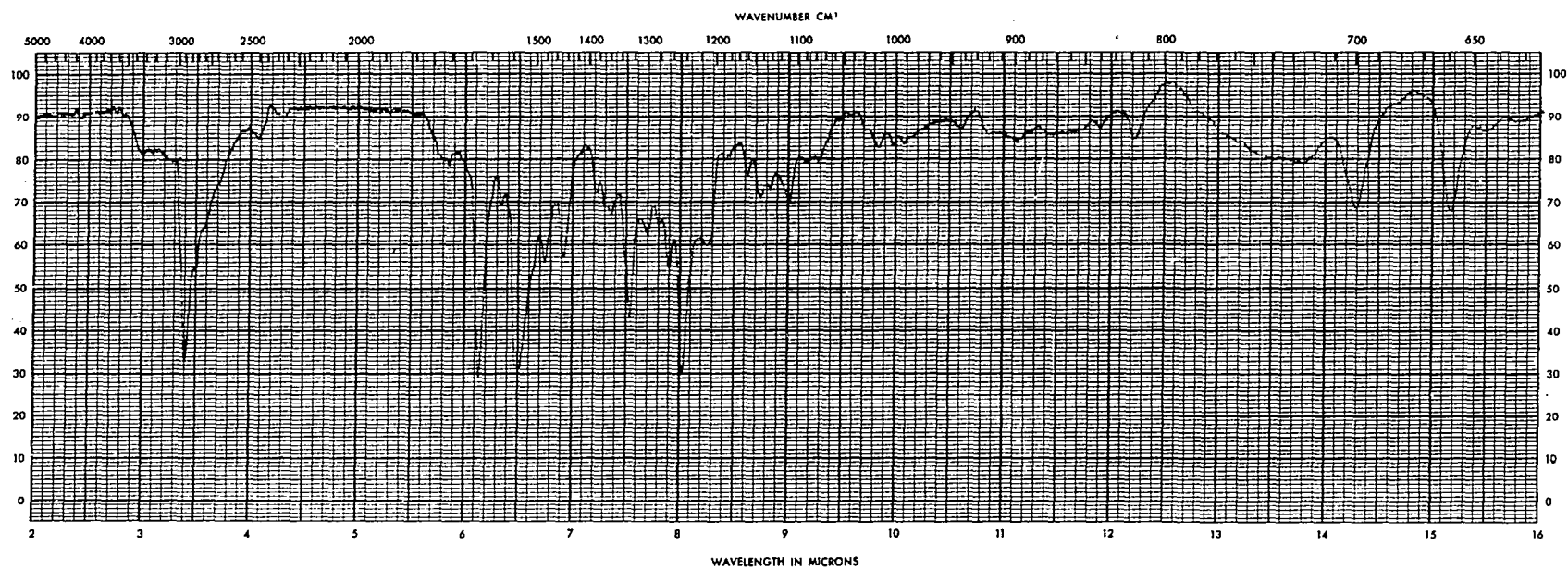


Fig. 14. Infrared Spectrum of Hydrolysis Product of A-2 in chloroform.

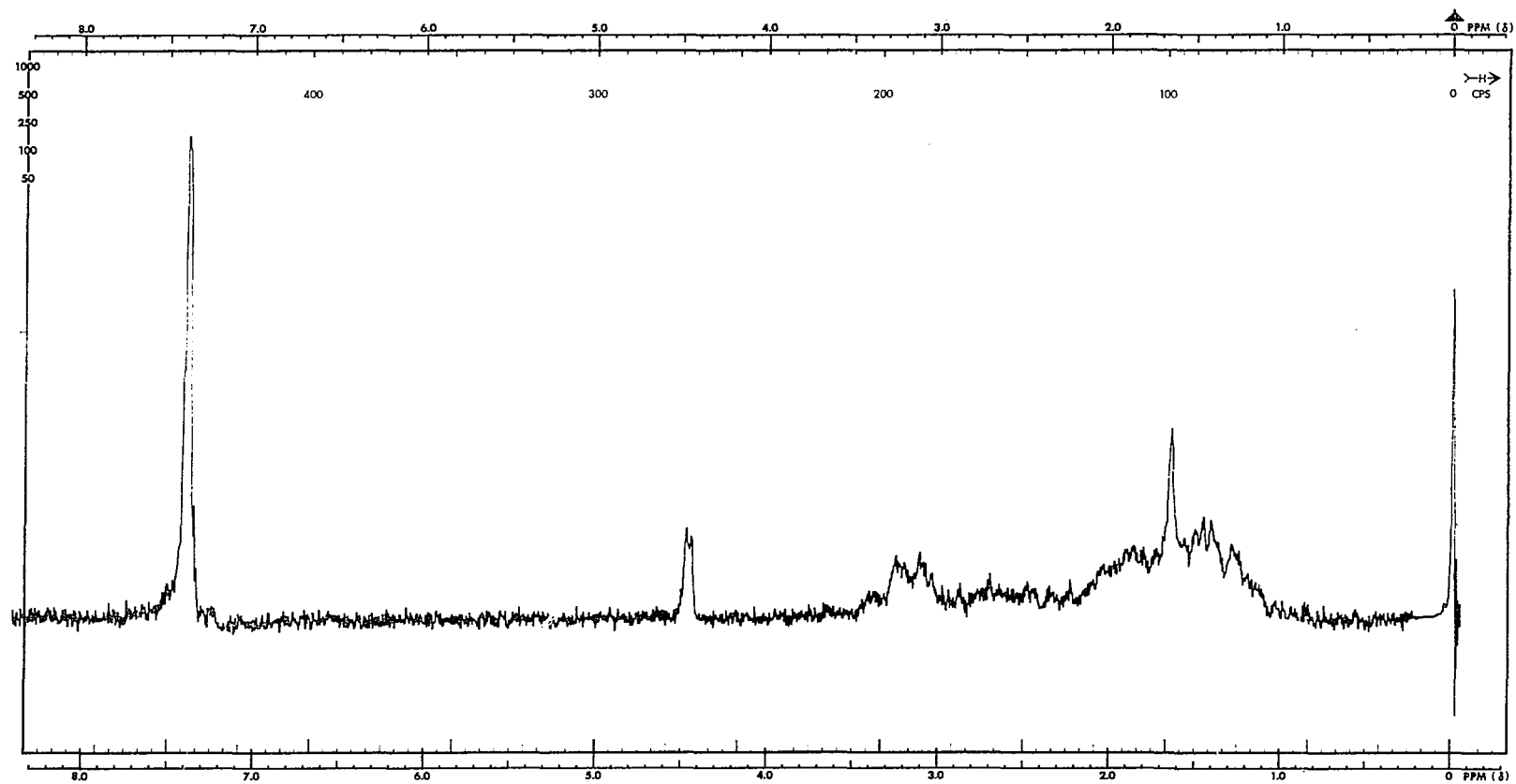


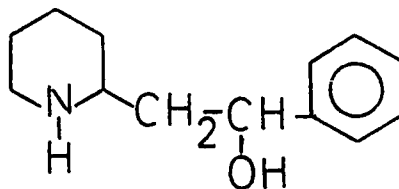
Fig. 15. Nuclear Magnetic Resonance Spectrum of the Hydrolysis Product of A-2 in deuteriochloroform.

III. DISCUSSION OF RESULTS

A. Alkaloid A-1 (8-phenylnorlobelol)

Combustion data of A-1 best fitted a molecular formula of $C_{13}H_{19}NO$. The ultraviolet spectrum of A-1 showed a maximum at 255 m μ of low intensity (ϵ , 472). This was attributed to an unconjugated phenyl ring.⁴³ Bands in the IR spectrum (Fig. 3) at 699 and 758 cm^{-1} and a singlet at 7.35 δ in the NMR spectrum of A-1 both indicated the presence of a monosubstituted benzene ring.^{44,45} The IR spectrum also showed the absence of any absorption in the carbonyl region.

Both the spectral evidence and the molecular formula suggested that alkaloid A-1 might be 8-phenylnorlobelol (VIII).



VIII

The melting point of A-1 (100-101°) agreed closely with that of 8-phenylnorlobelol (102-103°).⁴⁶ A comparison of the melting point of the chloroaurate derivative of A-1 (137-138°) with the chloroaurate derivative of 8-phenylnorlobelol (142-143°)⁴⁶ showed close agreement.

Alkaloid A-1 showed a rotation $[\alpha]_D^{22} +46.9$

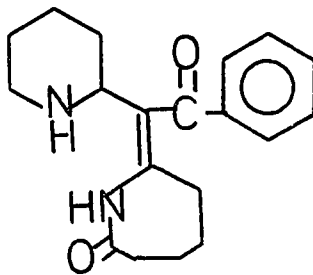
(absol. ethanol) which was very close with the reported rotation $[\alpha]_D^{23} +49 \pm 2$ (absol. ethanol) reported for 8-phenyl-norlobelol.⁴⁶

A synthetic sample of 8-phenylnorlobelol was kindly supplied Professor C. Schöpf. Comparison of A-1 with the synthetic sample by parallel infrared spectroscopy showed both materials to be identical in all respects.

B. Alkaloid A-2

1. Postulated Structure

The results of chemical degradations and of spectral evidence have led to a postulated structure (IX) for alkaloid A-2. This structure is consistent with the major portion of the physical and chemical data, but does not completely explain all of the data. Evidence which



IX

led to the postulation of structure IX will be discussed in the following sections along with some of the weak points of this structure.

2. Characterization as an Alkaloid Hydrochloride

The characterization of A-2 as an alkaloid hydrochloride was attributed to positive tests with Mayer's,

Dragendorff's, and Hager's reagents.³⁴ Evidence that A-2 was isolated as an alkaloid hydrochloride lies in the fact that it was extracted from an aqueous solution containing hydrochloric acid; that it gave a positive test for halide; and that it showed absorption in the region of 2778-2398 cm^{-1} , which is characteristic for alkaloid hydrochlorides.⁴⁷

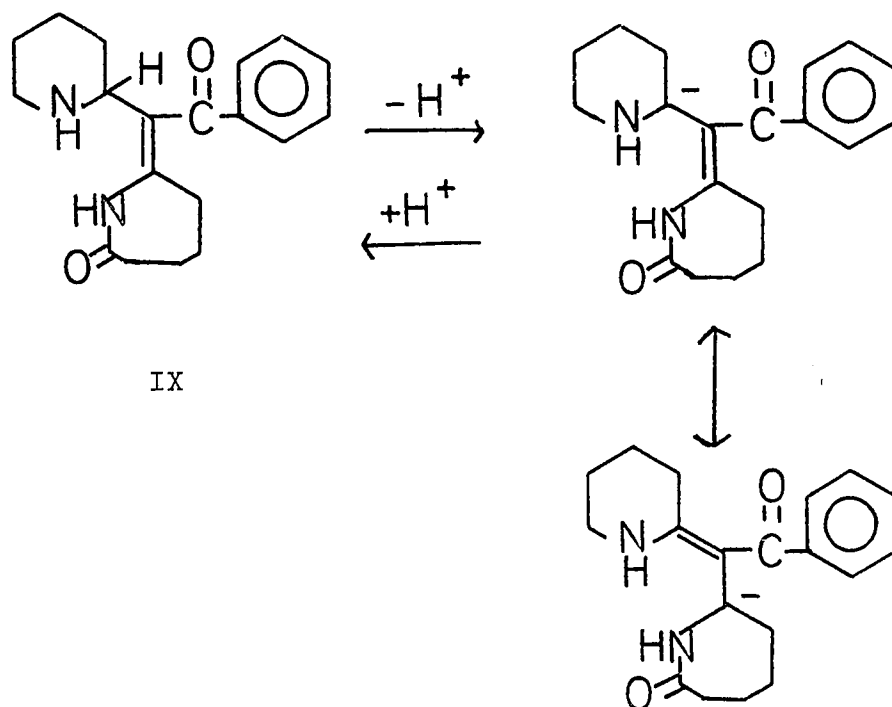
3. Molecular Formula

Combustion data permitted calculation of the best fitting molecular formula $\text{C}_{19}\text{H}_{25}\text{N}_2\text{O}_2\text{Cl}$. A molecular weight of 348.86 for the above formula was in close agreement with a calculated molecular weight of 347 resulting from a potentiometric titration of A-2. Additional support for the correct molecular formula was obtained from the presence of a small peak at m/e 312 in the mass spectrum of A-2. This peak inferred that the molecule first lost hydrogen chloride (m/e 36) before it underwent ionization. Addition of these two masses (312 + 36) indicate a molecular weight of 348 for the alkaloid hydrochloride.

4. Optical Activity

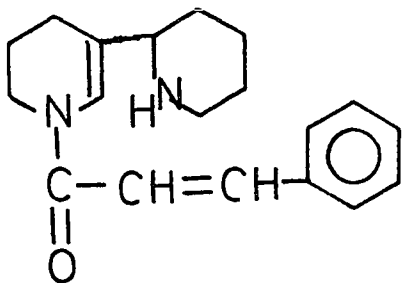
The optical rotation of A-2 was measured on two samples of different concentrations. The results of both measurements indicated that A-2 was optically inactive. One usually would expect optical activity from a naturally occurring compound containing one asymmetric center. The lack of optical activity in A-2 could be attributed to

racemization of the alkaloid either during biosynthesis, or in the process of isolation. In the presence of base, e.g. piperidine alkaloids, the carbon-carbon double bond could easily shift to an alternate position, which is also an α , β -position with respect to the carbonyl. The change is shown below.

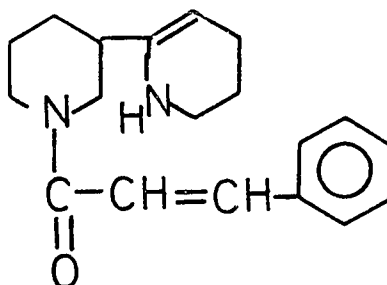


A-2 was not, however, the only alkaloid containing an optically active center allylic to a carbon-carbon double bond, which was found to be optically inactive. Two alkaloids, orensins (X) and isoorensins (XI), isolated from Adenocarpus commutatus, both contain this structural feature and were found to be optically inactive.⁸ The lack of activity in these compounds could also be explained in the same manner

as shown above for IX.



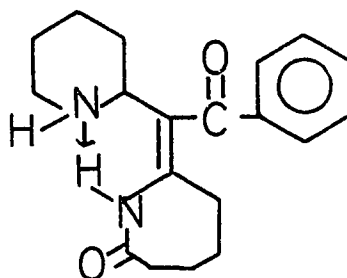
X



XI

5. Basicity

A potentiometric titration of alkaloid A-2 resulted in a single pK_a -value of 7.2, which indicated that the molecule was monobasic. The basicity was attributed, therefore, to the piperidine nitrogen. This pK_a -value was surprisingly low when compared with that of piperidine (11.2), which is four pK - units higher. The steric influence on basicity could be seen by a comparison of the pK_a -value of N-methylpiperidine⁴⁸ (10.1) with that of lobinaline²⁰ (V, 8.2 for the N-methylpiperidine nitrogen). This is difference of 1.9 pK -units. A molecular model of A-2 showed that steric hindrance of the piperidine nitrogen was not great enough to account for the pronounced lowering of the basicity. Structure IX, however, allows the electrons of the piperidine nitrogen to partake in hydrogen bonding by formation of a six-membered ring. This could account for the abnormally low basicity of A-2.



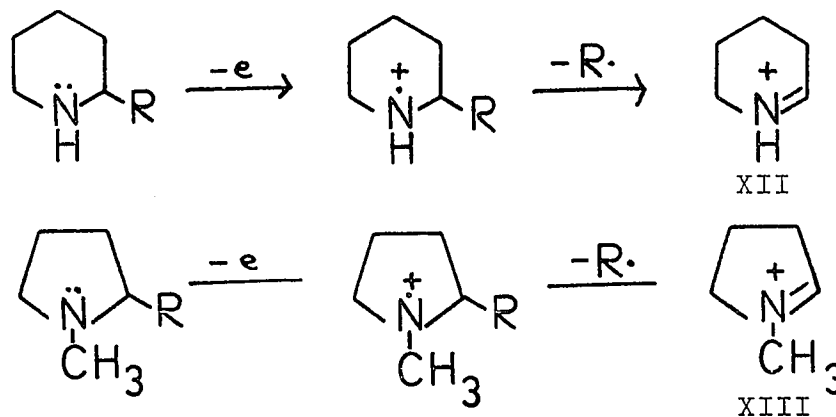
IX

Another factor might be the mobility of the carbon-carbon double bond, which was cited earlier to explain the absence of optical activity. In the alternate position the double bond provides a conjugate link between the nitrogen atom and the electron withdrawing carbonyl group.

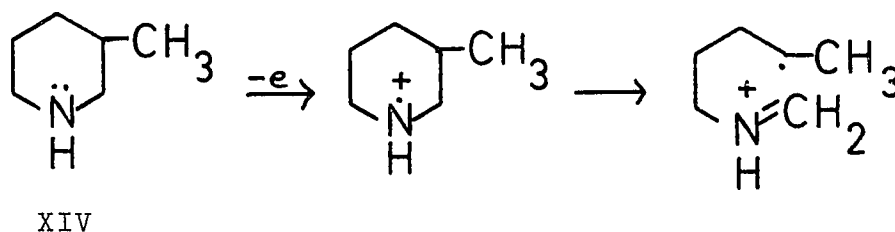
6. Structural Features

a. A Piperidine Ring

All Lobelia alkaloids characterized to date contain a piperidine ring. A peak at m/e 84 in the mass

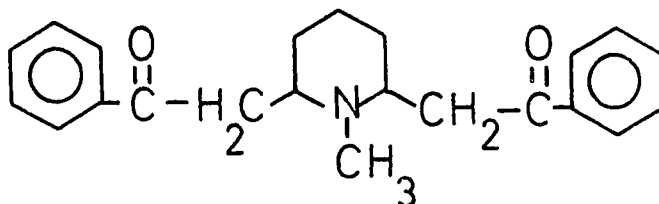


spectrum of A-2 was attributed to either a piperidine cation (XII), or to an N-methylpyrrolidine cation (XIII).^{49,50} The absence of an N-methyl peak in the NMR spectrum of the free base of A-2 eliminated an N-methylpyrrolidine as a structural feature. The piperidine cation (XII) stemmed from the cleavage of an alpha-substituent on the ring.^{49,50} Substitution at other positions on the ring causes a different course of fragmentation as shown in the case of 3-methylpiperidine (XIV).⁵⁰



A sharp peak at 1.24δ in the NMR spectrum of A-2 was assigned to N-H absorption. This was supported by N-H assignments of 1.34δ and 1.47δ for 2- and 3-methylpiperidine, respectively.⁵¹

The IR spectrum (potassium bromide) of A-2 showed a broad medium band at 3425 cm^{-1} which could be attributed to an N-H stretching mode. This band, however, also appeared in the IR spectrum of lobelanine (XV) (in potassium bromide), which contains no N-H group and in the spectrum of potassium bromide itself. Locock⁵² has also observed a band in this region while studying the IR spectra



XV

of lobelia alkaloids and has attributed it to the presence of a small amount of water in potassium bromide. The IR spectrum of the free base of A-2 in chloroform showed; however, a broad weak band at 3460 cm^{-1} which could be attributed to the N-H stretching mode of either the amine or the lactam or both.⁵³

Marion has reported⁵⁴ that the N-H stretching vibration in saturated aliphatic amines and particularly azacyclic structures such as piperidine, was exceedingly weak and difficult to detect. This might account for low intensity of the N-H band in the IR spectrum of A-2.

Alkaloid A-2 also gave a positive test for secondary amines which gave additional support to the presence of a piperidine ring.

b. A Conjugated Phenylcarbonyl Group

Peaks at m/e 77, 105, and 207 in the mass spectrum of A-2 supported the presence of a phenylcarbonyl

The UV spectrum of A-2 showed a maximum at 248 μ (ϵ , 11, 136) and a broad weak peak at 281 (1265). This type of absorption was similar to a number of compounds, all of which contain a phenylcarbonyl moiety. The UV data of a few such compounds are shown in Table VII.

The NMR spectrum of the free base of A-2 exhibited two sets of multiplets at 7.45-7.65 δ and 8.03-8.18 δ . The NMR spectrum of lobelanine (XV) under the same conditions showed an identical absorption pattern in this region. The absorption in this region was attributed to a phenylcarbonyl group.

c. A Lactam Ring

A lactam ring structure was supported by the presence of a neutral nitrogen atom, by potentiometric titration and analytical data, by the appearance of a strong peak at 1637 cm^{-1} in the IR spectrum, and by the isolation of only one product when A-2 was hydrolyzed.

A band at 1637 cm^{-1} in the IR spectrum of A-2 was attributed to a secondary or tertiary lactam; both types of compound have been reported to absorb in this region.⁵³

In all hydrolysis reactions carried out on alkaloid A-2 only one major product was detected. The integrated NMR spectrum of the hydrolysis product showed 23 protons (based on five aromatic protons), which was further evidence for the presence of only one product.

TABLE VII. ULTRAVIOLET ABSORPTION SPECTRA OF SOME COMPOUNDS CONTAINING A PHENYLCARBONYL MOIETY

Compound	max (m μ)		Solvent	Ref.
	246 281	15,560 1,085	methanol	52
	240 278	13,000 1,100	ethanol	43
	248	11,000	ethanol	56
	256	17,000	ethanol	56
A-2	248 281	11,136 1,265	methanol	--

Combustion data of the picrate of the hydrolysis product pointed to two equally possible formulas $C_{15}H_{14}N_4O_7$ or $C_{15}H_{16}N_4O_7$. If picric acid ($C_6H_3N_4O_7$) is subtracted from these formulas, compositions of $C_9H_{12}N$ or $C_9H_{14}N$ result for the formula of the hydrolysis product. These formulas suggest the formation of two hydrolysis products rather than one. If, on the other hand, the hydrolysis product formed a dipicrate, a best fitting molecular formula of $C_{30}H_{30}N_8O_{24}$ was calculated. This corresponds to a formula of $C_{18}H_{24}N_2$ for the hydrolysis product. The NMR spectrum of the base of the hydrolysis product showed a singlet at 7.69δ , and the IR spectrum revealed the absence of the original carbonyl band at 1684 cm^{-1} . This inferred that hydrolysis of A-2 was not merely a simple cleavage of a lactam, but also involved subsequent interaction with the phenylcarbonyl group.

d. Size of the Lactam Ring

The number of unassigned carbon atoms provides a limit for the size of the lactam ring. Twelve carbon atoms have been utilized in the piperidine ring and the phenylcarbonyl group, leaving seven of the original 19 atoms unassigned. This allows the lactam ring to contain from three to seven carbon atoms. Four- and five-membered lactams have been reported⁵³ to absorb in the region of 1760 and 1700 cm^{-1} , respectively. Both of these lactam structures absorb at a much higher frequency range than

does A-2 and were considered improbable.

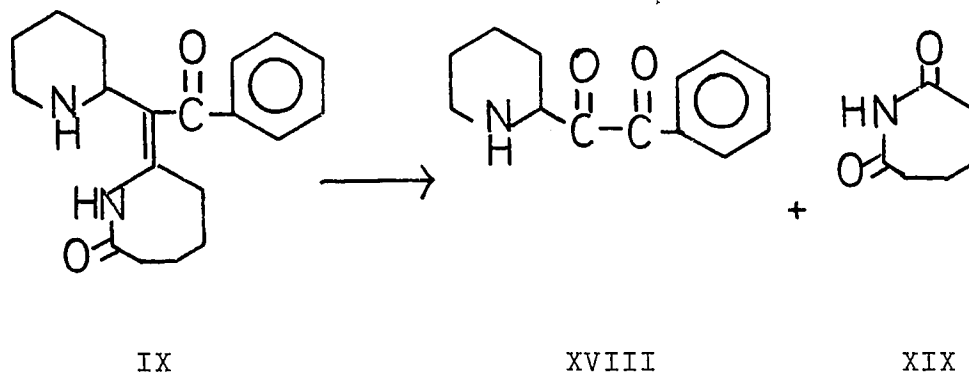
This reduced the number of possibilities to a six, seven, or eight-membered ring with two, one or zero carbon atoms remaining unassigned. The NMR spectrum of the free base of A-2 revealed the absence of C-methyl, N-methyl, O-methyl, and N-acetyl groups. The presence of a terminal methylene group was also considered possible. If this group were present, then hydrogenation of A-2 would result in a C-methyl group; no methyl peak was observed in the NMR spectrum of the hydrogenated product. This evidence proved that the remaining carbon atoms must be present as a chain connecting the piperidine ring and the lactam.

Evidence will be presented below under "e" which shows that the carbon-carbon double bond is tetra-substituted. This feature demands that the link between the piperidine and lactam rings consists of but one carbon. The lactam ring, therefore, must contain six carbon atoms and must be seven membered.

e. A Carbon-Carbon Double Bond

An examination of the molecular formula and of the known structural features of A-2 indicated the presence of one carbon-carbon double bond. Alkaloid A-2 was tested for unsaturation using Baeyer's reagent³⁸ whereupon a positive test resulted after 1 min. thus confirming the presence of a carbon-carbon double bond. The double bond

cannot be present in the piperidine ring because of a peak at m/e 84 in the mass spectrum of A-2, which would not be present if the piperidine ring were unsaturated. The double bond could either be placed in the lactam ring or in the side chain. If the double bond occurs in the lactam ring, then ozonolysis of A-2 would result in only one product; but if the double bond were present in the side chain, two products would result. The expected reaction for ozonolysis of A-2 is shown below. The ozonide was decomposed using hydrogen peroxide and evaporated to dryness. An



aqueous solution of the residue was extracted with chloroform resulting in a chloroform-soluble fraction and an aqueous fraction containing approximately equal quantities of material. Both fractions were found to be impure.

The residue from the aqueous solution gave positive tests with Mayer's, Dragendorff's and ninhydrin reagents and the UV spectrum showed a maximum at 255 μ . This data appeared consistent with a structure such as XVIII.

Weak but positive tests were also observed in testing for the presence of carbonyl groups.

The chloroform-soluble fraction differed from the aqueous fraction in that it gave negative tests with Mayer's ninhydrin reagents and positive tests with Dragendorff's and triphenyltetrazonium chloride (test for aldehydes and ketones). The UV spectrum of the chloroform-soluble fraction showed a shoulder at 272 $m\mu$ and strong end absorption, and the IR spectrum showed a band of medium intensity at 3390 cm^{-1} and strong broad absorption in the carbonyl region. The 3390 cm^{-1} band could be attributed to N-H stretching vibrations.^{47,53}

The separation of the ozonolysis reaction mixture on the basis of solubility into two fractions containing almost equal quantities of material; the different UV spectra of these fractions; and their behavior to various spot tests strongly suggested the presence of two major products. This places the double bond in the chain connecting the two rings.

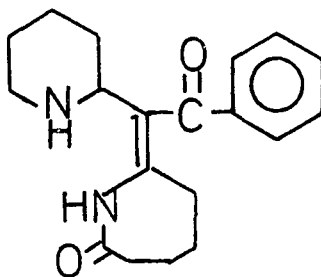
f. Placement of the Phenylcarbonyl Group

Although the existence of a double bond has been established, no olefinic protons could be assigned in the NMR spectrum of the free base of A-2. This implied that the double bond must be tetrasubstituted. The requirement of a tetrasubstituted double bond resulted in turn, in the necessary attachment of a phenylcarbonyl group directly to

the double bond. This places the carbonyl group on the alpha-carbon of the piperidine side chain, which is the same position that the carbonyl group occupies in all of the Lobelia alkaloids containing this structural moiety.⁸

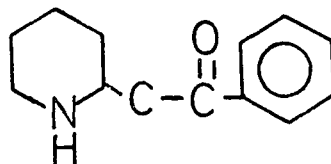
g. The Complete Structure

The above discussion shows that structure IX accommodates the bulk of the experimental evidence. It is



IX

a structure, part of which (XX) resembles structural features of other Lobelia alkaloids. But it also contains a seven-membered lactam, which is of uncommon occurrence.



XX

A seven-membered lactam ring has, however, recently been found to comprise part of the structure of a new Euphorbia-ceae alkaloid.⁵⁷ Cyclic seven-membered ring systems are

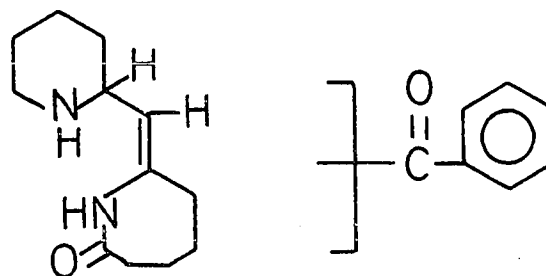
common and many are found in the Galanthamine and Lycorine alkaloids which occur in the plant family Amaryllidaceae.⁵⁸

Although evidence for many of the individual structural features in A-2 has been well established, the linkage of these structural moieties to form A-2 has resulted largely from indirect evidence.

The molecular formula ($C_{19}H_{25}N_2O_2Cl$), the presence of a phenylcarbonyl group, of an alpha substituted piperidine ring, and of a lactam ring have all been confirmed conclusively from the experimental evidence.

The size of the lactam ring, the position of the double bond were not proven conclusively and were based largely on indirect evidence.

The peaks in the NMR spectrum of the free base of A-2 in the region of 4-5 are anomolous. The two sharp peaks in this region might be explained by partial structure XXI where the alpha piperidine hydrogen causes the



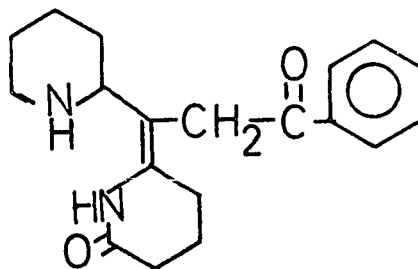
XXI

olefinic proton to be split into a doublet.

The attachment of the phenylcarbonyl group to

the carbon-carbon double bond results as a consequence of a tetrasubstituted double bond. This position is very favorable biogenetically. Nakanishi⁵⁹ states that diaryl ketones and α,β - α',β' unsaturated ketones absorb in the range of 1665 cm^{-1} while the phenylcarbonyl band in A-2 absorbs at 1684 cm^{-1} .

Table VII shows that the extinction coefficient of the carbonyl band in a phenylvinyl ketone system increases with branching at the beta carbon atom. The extinction coefficient of A-2 does not appear to be consistent with this observation. Both of these arguments are in conflict with other pieces of evidence which favor attachment of the phenylcarbonyl group to the double bond. The infrared interpretations cited above may be expressed in an alternate structure XXII.



XXII

Part Structure XXI and alternate structure XXII succeed in eliminating a weak point in structure IX, which was based on inconclusive NMR evidence. But both structures

are biogenetically unprecedented and fail to eliminate all inconsistencies in structure IX.

IV. SUMMARY AND CONCLUSION

An investigation of an alkaloidal extract of the root and stem bark of Lobelia yuccoides Hillebr. using two-dimensional TLC with four different systems of chloroform and methanol revealed the presence of 10-14 alkaloids. As a result of this investigation two alkaloids were isolated; one of these had been previously isolated from L. inflata and characterized, and the other was a new compound of unknown structure.

The first of the alkaloids was characterized as 8-phenylnorlobelol and was isolated in a quantity corresponding to ca. 0.0006% of dry bark. Isolation of the alkaloid was achieved exclusively through the use of alumina columns. A best-fitting molecular formula of $C_{13}H_{19}NO$, calculated from combustion data, was identical with the molecular formula of 8-phenylnorlobelol.⁴⁶ Alkaloid A-1 was characterized by comparison of its melting point, optical rotation, and chloroaurate derivative with the corresponding data of 8-phenylnorlobelol. Final proof of structure was made by comparison of alkaloid A-1 with a synthetic sample of 8-phenylnorlobelol by parallel infrared spectroscopy, which showed both materials to be identical.

Alkaloid A-2, a new Lobelia alkaloid, was isolated by a procedure similar to the one developed by Wieland¹⁴ and used by Schöpf.⁴⁶ Extraction of an aqueous solution of the alkaloid hydrochlorides with chloroform in

a liquid-liquid extractor resulted in dividing the crude alkaloids into two fractions: a chloroform-soluble fraction, and a fraction containing the alkaloids which remained in the aqueous phase. Trituration of the residue from the chloroform extract with chloroform, followed by cooling, resulted in the precipitation of alkaloid A-2 while all of the other alkaloids remained in solution. This fact by itself was an indication that the structure of A-2 might differ considerably from those of the other alkaloids in this fraction. Alkaloid A-2 was isolated in yields ranging from 0.003-0.004% of dry bark.

The results of chemical degradation and spectral evidence have led to a postulated structure (IX) for alkaloid A-2.

The analysis, mass spectrum, and potentiometric titration of A-2 indicated that the correct molecular formula was $C_{19}H_{26}N_2O_2Cl$.

The ultraviolet (λ_{max} 248 $m\mu$, ϵ 11, 136; λ_{max} 281 $m\mu$, ϵ , 1265), infrared (1684 cm^{-1}), and nuclear magnetic resonance (7.68-7.78 δ and 8.04-8.18 δ) spectra confirmed the presence of a phenylcarbonyl group.

A peak at 1637 cm^{-1} in the infrared spectrum was assigned to a lactam ring. This was supported by the presence of a neutral nitrogen atom in A-2, and by evidence that only one product resulted from the hydrolysis of A-2.

The presence of an alpha-substituted piperidine

ring, a common structural feature in Lobelia alkaloids, was established by the appearance of a peak at m/e 84 in the mass spectrum and was supported by a positive test for secondary amines.

The mass spectrum, the combustion data, and known structural features of A-2 indicated the presence of a carbon-carbon double bond. This was confirmed by a positive Baeyer's test.

The results of ozonolysis of A-2 placed the double bond in the carbon chain connecting the piperidine ring and the lactam. The absence of any olefinic protons in the NMR spectrum indicated that the double bond was tetra-substituted and this required in turn that the phenyl-carbonyl group be attached directly to the double bond.

Chemical work on A-2 was very troublesome because of the sensitivity of the alkaloid to base. This was indicated from the poor yields which resulted when the free base of A-2 was prepared. All attempts to hydrogenate A-2 resulted in more than one product. This might be attributed to fragmentation or to partial hydrogenation. The free base of A-2 and of the hydrolysis product were difficult to work with because they started to undergo decomposition after remaining in solution for a short time. This became apparent from the results of TLC.

Relatively little can be inferred from this research concerning botanical correlations. 8-Phenylnorlobelol

has been isolated from only one other plant, Lobelia inflata, and has been described in papers by Wieland²⁴ and Schöpf.⁴⁶ An unnamed alkaloid ($C_{19}H_{26}N_2O_3$) isolated from Lobelia inflata²⁴ in 1939, but whose structure is still unknown, has a molecular formula similar to A-2. By subtracting a molecule of water from this alkaloid, a formula identical with that of the free base of A-2 is obtained. The similarity in formulas of these two compounds suggests the possibility of a biogenetic relationship.

Finally, the structure postulated for A-2 differs from all Lobelia alkaloids previously characterized and may therefore constitute a new structural type.

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