PART I

THE ISOLATION AND STRUCTURE OF TWO ALKALOIDS FROM TABERNAEMONTANA RUPICOLA

PART II

THE STRUCTURE OF NECROSAMINE

Thesis by

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ABSTRACT

Part I

Two alkaloids have been isolated from the leaves and twigs of Tabernaemontana rupicola, a South American shrub. Physical and chemical data indicate that one of these alkaloids, named rupicoline, is 18-carbomethoxyiboluteine, a pseudoindoxyl compound closely related to the alkaloid voacangine. The second alkaloid, named montanine, is believed to be 18-carbomethoxy-20-hydroxyiboluteine. However, attempts to prove this structure for montanine by converting some of this alkaloid to rupicoline were unsuccessful. Experiments were also conducted which suggest that these alkaloids are not artifacts of the isolation procedure.

Part II

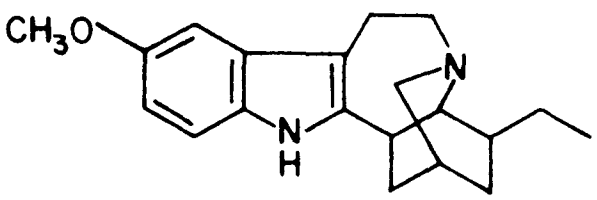
Necrosamine, a base isolated from an Escherichia coli lipopolysaccharide causing the regression of an experimental mouse tumor, yields, when oxidized with periodate and permanganate, n-butyric acid. Necrosamine is therefore an isomer of 4, 5-diaminoeicosane. Several attempts were made to resolve synthetic 4, 5-diaminoeicosane. These were unsuccessful as were attempts to prepare a derivative of necrosamine with a detectable optical rotation.

PART I

THE ISOLATION AND STRUCTURE OF TWO ALKALOIDS FROM TABERNAEMONTANA RUPICOLA

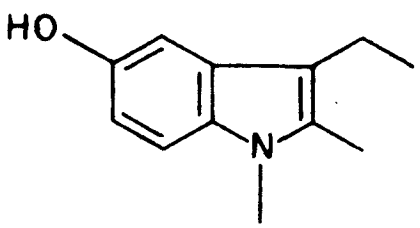
INTRODUCTION

The root bark of the African hardwood Tabernanthe iboga Baillon (Apocynaceae) has yielded a number of alkaloids. The principal member of these is ibogaine (I), first reported (1) in 1901. Ibogaine has long been thought an indole on the basis of

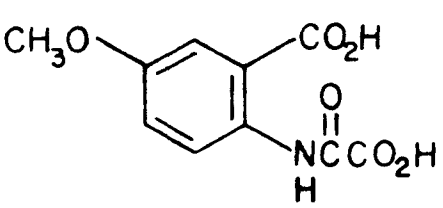


I

color tests (2) and, in 1953, 3-ethyl-5-hydroxy-1, 2-dimethylindole (II) was isolated after a potash fusion of ibogaine (3). Earlier, 5-methoxy-N-oxalylanthranilic acid (III) had been obtained by

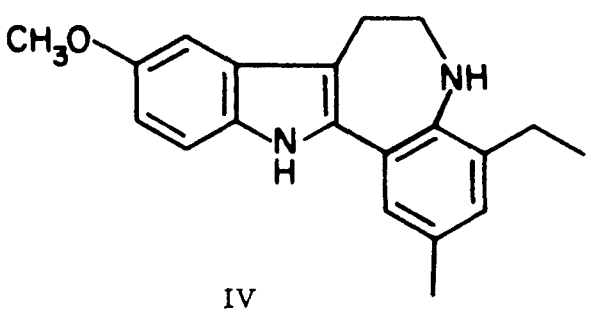


II

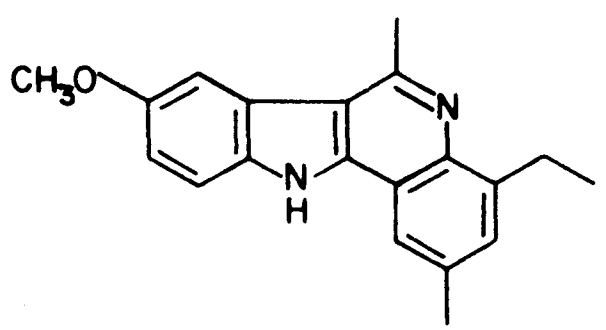


III

permanganate oxidation (4). A clue to the remainder of the molecule was provided by the isolation of 3-ethyl-5-methylpyridine from the potash fusion mixture (5). Taylor et al. (6) confirmed the potash fusion results and carried out a selenium dehydrogenation of ibogaine from which they obtained 4-ethyl-5, 6, 7, 12-tetrahydro-9-methoxy-2-methylindolo [3, 2-d] [1] benzazepine (IV) and 4-ethyl-8-methoxy-2, 6-dimethyl-11H-indolo [3, 2-c] quinoline (V). These



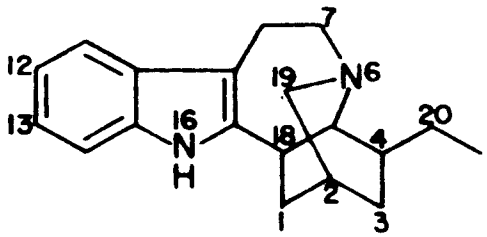
IV



V

experiments led Taylor et al. to deduce the structure (I) for ibogaine. This structure has subsequently been confirmed by X-ray analysis (7).

Ibogamine (VI) is another alkaloid isolated (8) from



VI

Tabernanthe iboga. The structure of this compound was also determined by Taylor et al. (6b). Ibogamine is the least substituted member of a group of compounds possessing the skeleton* shown (VI). These are now generally referred to as iboga alkaloids although they are isolated from a number of different plant species.

The alkaloids of this group isolated to date include substitution of this ring system at the 12 and 13 positions by methoxyl groups, at the 18 position by a carbomethoxyl group, at the 3-4 position by a double bond, and at the 20 position by a hydroxyl or keto-group. Table 1 is a collection of the twelve iboga alkaloids which may be classified in this manner.

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*The numbering system is that used by Taylor et al. (6b) extended to the ethyl group.

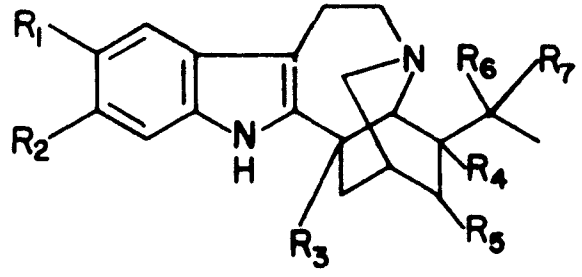


TABLE 1. The Iboga Alkaloids

Name	R_1	R_2	R_3	R_4R_5	R_6R_7	First Isolated	Structure
ibogamine	H	H	H	H H	H_2	(8)	(6, 9)
ibogaine	CH_3O	H	H	H H	H_2	(1)	(6)
tabernanthine	H	CH_3O	H	H H	H_2	(10)*	(6, 11)

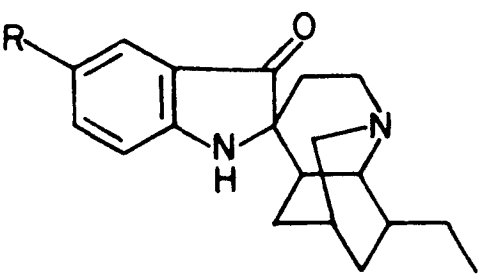
TABLE 1. (Continued)

ibogaline	CH_3O	CH_3O	H	H H	H_2	(12)	(12)
coronaridine	H	H	CO_2CH_3	H H	H_2	(13)	(13)
voacangine	CH_3O	H	CO_2CH_3	H H	H_2	(14)	(6, 15, 16)
isovoacangine	H	CH_3O	CO_2CH_3	H H	H_2	(17)	(17)
conopharyngine	CH_3O	CH_3O	CO_2CH_3	H H	H_2	(18)	(18)
catharanthine	H	H	CO_2CH_3	——	H_2	(19)	(19, 20, 21)
iboxygaine	CH_3O	H	H	H H	H, OH	(22)	(16, 23, 24)
voacangarine	CH_3O	H	CO_2CH_3	H H	H, OH	(25, 26)**	(25, 23)
voacryptine	CH_3O	H	CO_2CH_3	H H	O=	(27)	(28)

*Tabernanthine may have been discovered by Haller and Heckel (1b) who determined only the melting point. It was isolated and named by Delourme-Houdè (10).
**Renner named this compound voacristine (26).

In addition to those tabulated in Table 1 there are several other alkaloids which appear to be closely related to the iboga group.

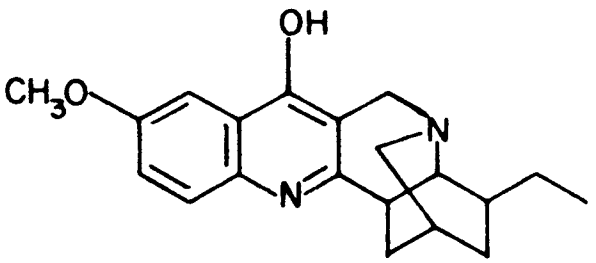
Iboluteine (VIIa), isolated (11) from a plant extract, has been shown to be a 5-methoxypseudoindoxyl related to ibogaine (29). Similarly, desmethoxyiboluteine (VIIb) has been isolated and



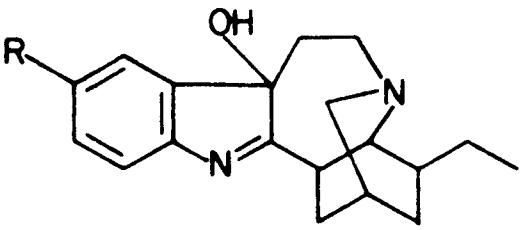
VIIa iboluteine R = CH_3O

VIIb desmethoxyiboluteine R = H

identified by Taylor et al. (30) as one of the minor alkaloids of Tabernanthe iboga. Other minor alkaloids isolated at that time include iboquine (VIII) and the hydroxyindolenine derivatives of ibogamine (IXa) and ibogaine (IXb). These compounds may be



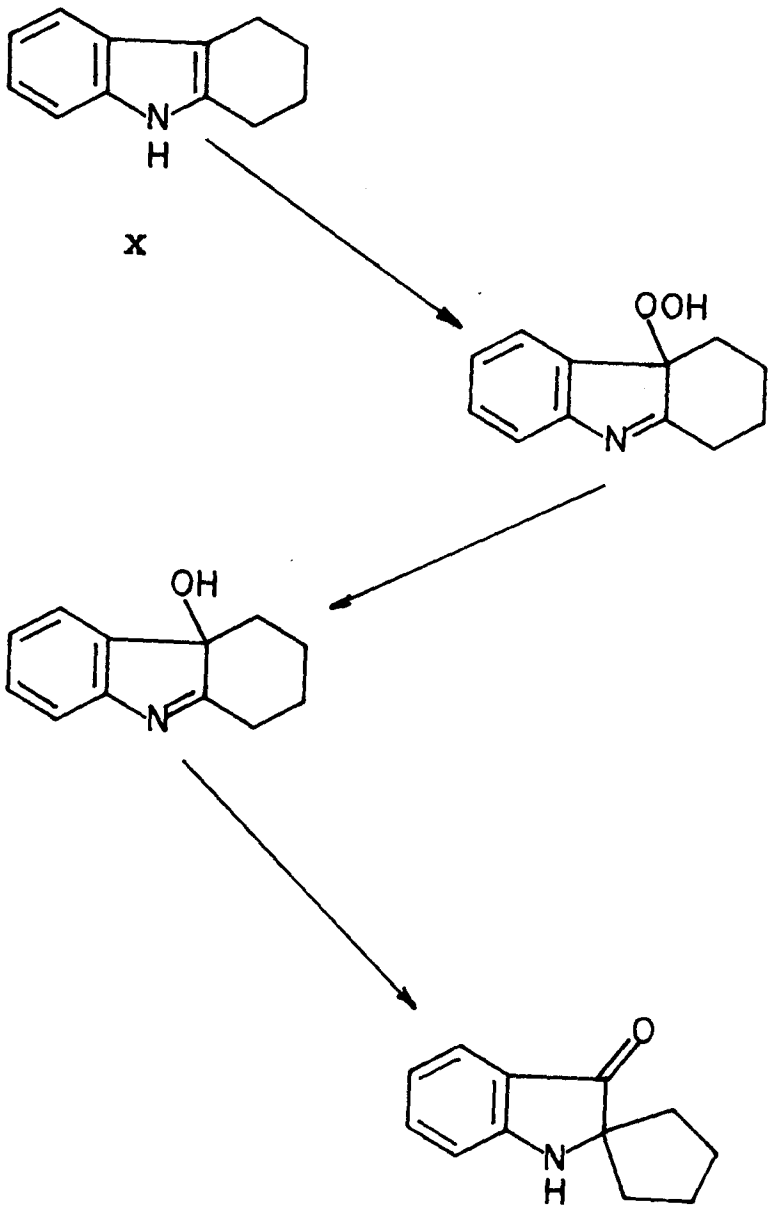
VIII



IXa ibogamine hydroxyindolenine R = H

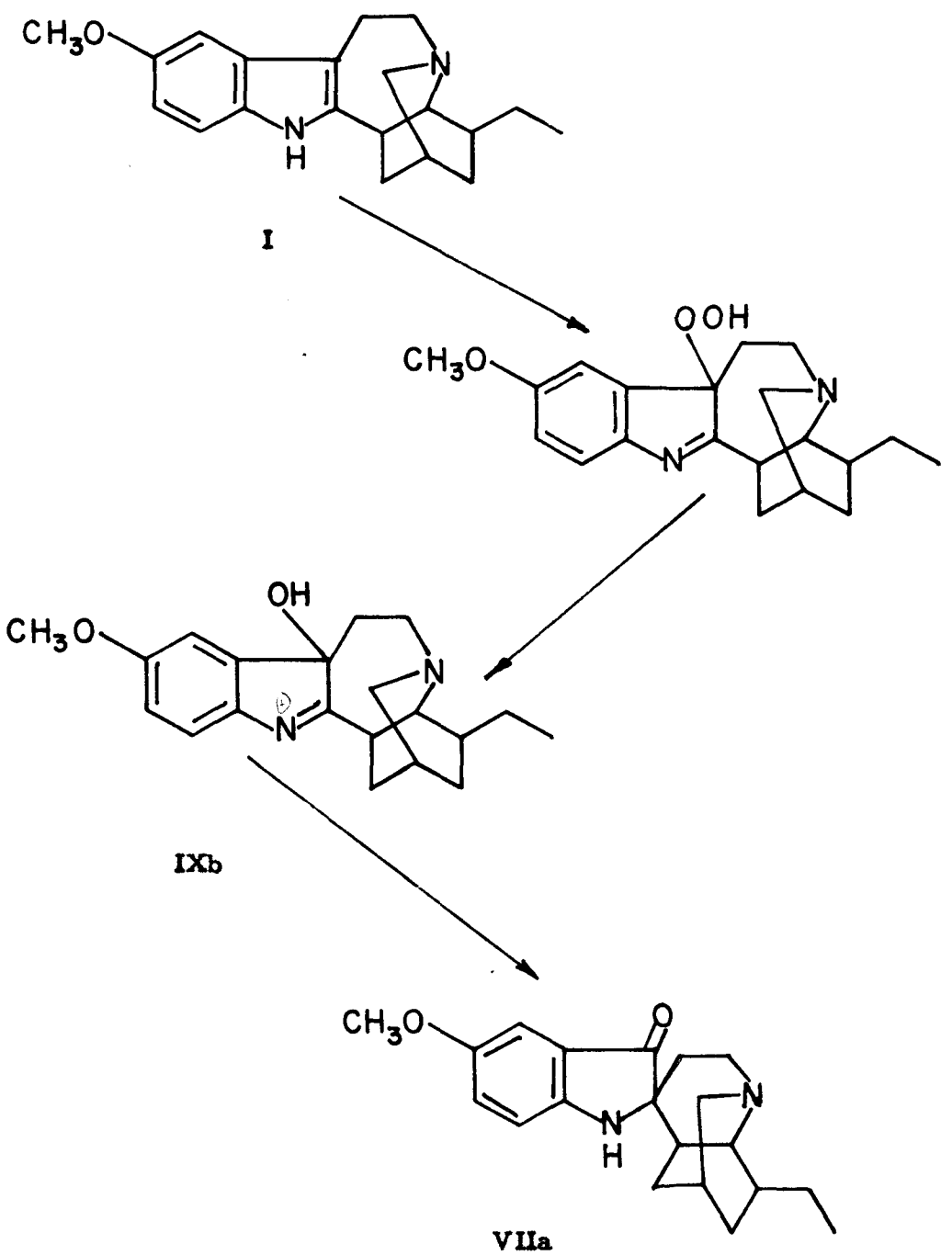
IXb ibogaine hydroxyindolenine R = CH_3O

prepared by oxidation of ibogaine and ibogamine (6b, 11, 29). The oxidation and rearrangement of tetrahydrocarbazole (X) to 2, 2-spirocyclopentanopseudoindoxyl (XI) has been studied by Witkop and Patrick (31) and, in a similar fashion, Goutarel et al. (29) have prepared iboluteine from ibogamine.



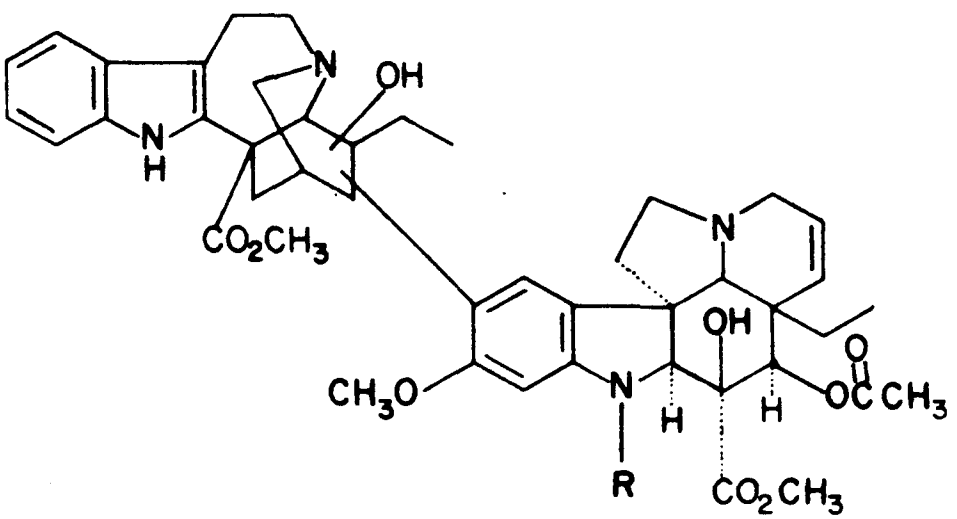
x

xi


CH3O
N
H
I
OOH
CH3O
N
N
OH
CH3O
N
N
IXb
CH3O
O
N
H
N
VIIa

It has been suggested (30) that, while they may occur naturally, these compounds (VII-IX) might also be attributed to a facile autoxidation of the parent alkaloids.

The alkaloids leurocristine (XIIa) and vincaleukoblastine (XIIb), isolated from the white-flowered periwinkle Vinca rosea Linn. (32-34), have been shown by Neuss et al. (35, 36) to be

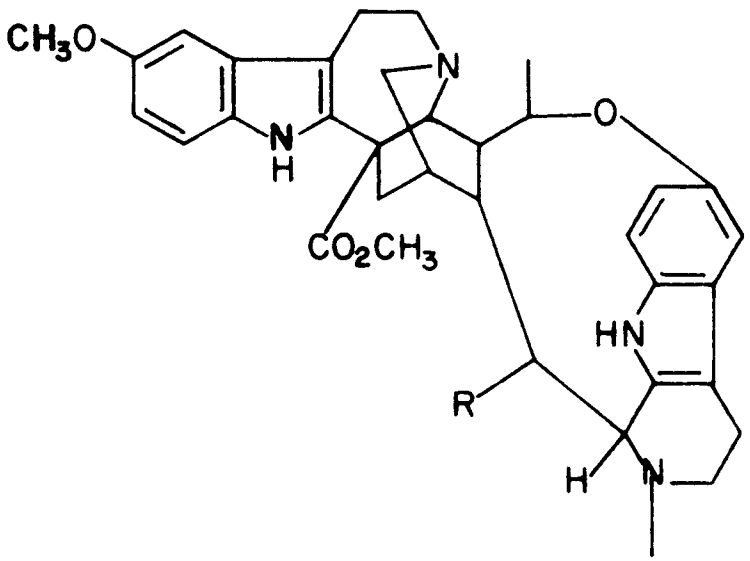


XIIa R = CHO

XIIb R = CH_3

composed of indole and dihydroindole moieties with the partial structures shown in Figure XII. Leurosine, another alkaloid isolated (37) from this plant, is quite similar to vincaleukoblastine (20, 35) and it seems likely that it also contains the iboga skeleton.

Voacamine, isolated by Janot and Goutarel (38) from Voacanga africana, apparently also has the iboga skeleton attached to a second indole nucleus. Figure XIII has been suggested for the partial structure of this compound (39).



XIII R = $C_{10}H_{17}O_2$

Thus, the iboga skeleton occurs in a number of alkaloids found in plants within the plant family Apocynaceae. Alkaloids of this type apparently occur widely in species of the tribe tabernaemontaneae (13, 40).

The genus tabernaemontana is one of twenty* of this tribe (41). It is closely related to the genus Ervatamia and in some species Ervatamia and Tabernaemontana are synonymous. These two genera are by far the largest of the tribe with 232-236 species between them (13).

Alkaloids have been noted in Tabernaemontana crispa (42) and isolated from T. spharocarpa (43), T. dichatoma (44), Ervatamia caronaria syn. Tabernaemontana caronaria (45, 46, 13), T. undulata (13), T. psychotrifolia (13), T. oppositifolia (13), and T. australis (13).

Tabernaemontana rupicola, first described by Bentham (47) in 1841, is a woody shrub four to five feet in height bearing white flowers. It is found in Brazil in the area of the Amazon and its subsidiaries.

The present investigation was initiated as a result of interest in the chemistry of alkaloids and other natural products and because a quantity of T. rupicola was available. This work describes the isolation of alkaloids from T. rupicola and the separation and characterization of two of these compounds. The structure of the first of these has been elucidated and a similar structure proposed for the second.

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*Monachino (48) also places the genus Macoubea in this tribe. Raffauf and Flagler (40) mention three additional genera, not listed by Pichon (41), as belonging in this tribe.

EXPERIMENTAL

Elemental analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Michigan.

All melting points are corrected. Melting points were determined with a capillary apparatus. The apparatus was calibrated using melting point standards from a Kofler hot stage (A. D. Thomas Company).

Measurements of specific rotations were carried out using a Schmidt and Haensch polarimeter with a Lippich double field.

Infrared absorption spectra were recorded using a Beckman Model IR-7 infrared spectrophotometer.

Ultraviolet absorption spectra were obtained using Cary Model 11M and Model 14 recording spectrophotometers.

Nuclear magnetic resonance spectra were taken at 60 megacycles with a Varian A-60 High Resolution spectrometer.

The first sample of Tabernaemontana rupicola, 5.1 kg. of leaves and twigs, was collected by Dr. W. A. Rodriguez of the Instituto Nacional do Pesquisas d' Amazonia, Manaus, Brazil on the Rio Negro in October, 1959. This was examined at Smith, Kline and French Laboratories in Philadelphia and an ethanol extract of the material was made. Preliminary examination of

the extract revealed that it contained a considerable quantity of organic bases. This extract, concentrated to about 2 liters in volume, was submitted to us for our study in January 1961.

A second collection of the plant was made by Dr. R. F. Raffauf with the assistance of Sr. N. T. da Silva of the Instituto Agronomico do Norte, Belem, Brazil. This material consisting of upwards of 50 kg. of leaves and twigs, was collected on the grounds of the Instituto Agronomico do Norte in Belem, Brazil in October, 1961.

Alkaloid Reagents Several reagents were used to detect alkaloids in the various phases of isolation and purification. Wagner's, Mayer's, and Dragendorff's reagents were prepared and used as described by Cromwell (49) to determine completeness of extraction or the possible presence of alkaloids in a chromatographic fraction. For the detection of colorless or nonfluorescent spots on paper or thin layer chromatograms a modified Dragendorff's reagent (50) or the iodoplatinate reagent (51) was used.

Paper Chromatographic Methods The paper chromatographic method of Waldi (52) was used with codeine as a reference. Whatman #1 paper was dipped in a 20% solution of formamide in

acetone and the excess solution removed by pressing the paper between sheets of filter paper. The acetone was then allowed to evaporate for 2-3 minutes. The alkaloid solution was spotted carefully and this solvent evaporated with a stream of nitrogen. The sheet was developed, ascending, with 9:1 cyclohexane-diethylamine.

A second method used in some cases was based on a system used for natural products by Smith, Kline, and French Laboratories (53). A 1:1 by volume mixture (100 ml.) of isoamyl alcohol and tertiary amyl alcohol was shaken with 100 ml. of a 1:5 by volume mixture of formic acid (88%) and water. Half of each layer was transferred to the bottom of the chromatographic tank. The remainder of the upper layer was used as the moving phase. Whatman 3 mm paper was used. The sheet was spotted with the alkaloid and placed in the chromatographic tank for five hours. The moving phase was then added and the chromatogram run, decending, for 8 hours (or as desired). Since no nitrogenous compounds were used in this solvent system the chromatogram could be air dried after development and then sprayed with the iodoplatinate reagent to develop the spots. With the formamide/cyclohexane-diethylamine method it is necessary to dry the chromatogram for some time at 80-100° before development.

Thin Layer Chromatography Thin layer chromatography, as described by Stahl (54), was used in the following manner. A slurry of 20 g. of silica gel G (Merck AG) in 50 ml. of water was shaken for two minutes and then spread onto the glass plates in a layer 275 μ thick. The plates were dried at 110-120° for one hour. During the first fifteen minutes the oven was opened frequently. After drying, the plates were stored over Drierite until used. In order to assure uniform rise of solvent on the plate the walls of the chromatography tank were covered with paper saturated with the solvent system used.

Separation of Organic Bases A portion (500 ml.) of the ethanol extract of the first collection of Tabernaemontana rupicola was evaporated to a thick sirup under vacuum. This material was then extracted with 500 ml. of 1N HCl and the solution filtered. The residue was extracted with sixteen successive 250 ml. portions of 1N HCl. The combined extracts were made basic by the addition of 1.2 equivalents of NaOH. The solution was then shaken, in thirds, with 100 ml. portions of chloroform until the extract no longer gave a precipitate with Wagner's or Mayer's reagents. The combined chloroform extracts were evaporated to dryness with vacuum. The aqueous phase was then tested (see page 18) for

the presence of quaternary amines. The residue from the evaporation of the chloroform extract was taken up in dilute acid, the pH brought to below 2, and the solution diluted to 500 ml. The solution was then extracted exhaustively with ether in a continuous liquid-liquid extractor. This was continued until extraction for 24 hours followed by evaporation of the solvent yielded less than 0.3 mg. of material. The aqueous phase was then brought to pH 11.2 with dilute NaOH. It was then again extracted exhaustively with ether. The ether extract was concentrated and treated with ferrous sulfate to reduce any peroxides formed. It was then filtered. Evaporation of the ether yielded 1.28 g. of organic bases. Further extraction of the aqueous phase with dichloromethane yielded 0.98 g. of bases.

In all, a volume of the ethanol extract equivalent to 3700 g. of the dried plant was treated in the manner described above yielding 6.44 g. of bases extracted by ether (0.17% yield from the dried plant). Further extraction with dichloromethane gave a total of 3.92 g. of bases (0.11% yield from the dried plant).

Test for Quaternary Amines The first aqueous extract above, after extraction with chloroform, was tested for quaternary amines

by bringing a portion of the solution to pH 2, filtering, and adding an equal volume of 5% $HgCl_2$. No precipitate occurred and this was taken as a negative test (55).

Chromatography of the Ether Extractable Bases A sample of the ether extractable bases (0.73 g.) was chromatographed on 250 g. of acid washed alumina (activity grade V) (56) in a column having an inner diameter of 2.1 cm., making a column of alumina 55.5 cm. long. The column was developed with the solvents shown in Table 2. A total of 653 fractions, having volumes of 80-110 ml. each, were taken. Each of the fractions was tested with Wagner's reagent. The fractions shown in Table 3 gave positive tests.

Fractions 66-98 were combined and treated with methanolic HCl. The methanol and excess HCl were then removed under vacuum. The residue crystallized slowly from methanol and could be recrystallized from isopropanol giving yellow needles possessing a yellow-green fluorescence.

Paper Chromatography of the Chromatographic Fractions The formamide/cyclohexane-diethylamine system was used with codeine as a reference. A sample of the material of chromatographic

TABLE 2. Solvents Used in the Initial Chromatographic Study

Volume		Solvent	Fractions
3.2 liters		benzene	1 - 75
6.4	3:1	benzene - chloroform	76 - 135
7.1	1:1	benzene - chloroform	136 - 210
7.0	1:3	benzene - chloroform	211 - 305
6.6		chloroform	306 - 395
3.6	3:1	chloroform - acetone	396 - 440
2.3	1:1	chloroform - acetone	441 - 468
1.5	10:1	chloroform - methanol	469 - 490
1.4	5:1	chloroform - methanol	491 - 510
6.0	3:1	chloroform - methanol	511 - 620
1.1	1:10	chloroform - methanol	621 - 653

TABLE 3. Fractions Giving Positive Tests with Wagner's Reagent

Fractions	Weight on Evaporation
20 - 39	0.009 g.
66 - 98	0.102
136 - 163	0.132
474 - 486	0.031
547 - 603	0.104
619 - 650	0.029

fractions 66-98 was chromatographed by this method. In addition to the main component, a yellow spot fluorescing a bright yellow-green, there were several other spots indicating that the material was not very pure. The Rf value of 0.41 for the main component does not correspond to any of the known alkaloids listed by Waldi (52).

Paper Chromatography of the Ether Extractable Bases A sample of the ether extractable bases of the first collection of T. rupicola was paper chromatographed using the formamide/cyclohexane-diethylamine method. A total of eleven fluorescent spots could be detected on the chromatogram under ultraviolet light. Two of these were yellow and were easily seen in visible light. These had Rf values (not corrected to codeine) of 0.59 and 0.06. Both of these compounds had a bright yellow-green fluorescence on the chromatogram. The compound of Rf value 0.59 was the same as that isolated above from fractions 66-98 of the column chromatographic experiment. These two compounds were designated alkaloids A (Rf value 0.59) and C (Rf value 0.06).

Separation of Organic Bases - Second Collection The second collection of T. rupicola, made in October 1961, was assayed

at Smith, Kline, and French Laboratories as having alkaloids to the extent of 0.007%, a value much lower than that of the first collection. The dried leaves and twigs (560 g.) of the second collection were extracted with ethanol and the organic bases of the extract separated in the manner described for the first collection. A sample of the ether extractable bases derived in this manner was paper chromatographed by the formamide/cyclohexane-diethylamine method. This chromatogram revealed only two fluorescent spots under the ultraviolet lamp. These had Rf values of 0.00 and 0.97. The later spot had a blue fluorescence.

Separation of Bases by Means of Ion Exchange Resin A second sample (552 g.) of twigs of the second collection of T. rupicola was stirred with 5 liters of methanol for three days. The solvent was then filtered off. A 3.3 by 15 cm. column of the weakly acid ion exchange resin, Amberlite IR120, was prepared in the hydrogen form. The methanol extract was then passed slowly over the column and the column rinsed by passing one liter of methanol over it. This removed most of the coloring matter adhering to the column. It was then washed with 0.1N methanolic hydrochloric acid. The first 500 ml. of the effluent of the column was collected

and evaporated to dryness with nitrogen at reduced pressure. The residue was then taken up in a small volume of methanol.

Paper chromatography of this material by the formamide/cyclohexane-diethylamine method gave a chromatogram with three fluorescent spots. None of these, however, were either of the alkaloids designated A and C.

Due to the great qualitative and quantitative differences between the two alkaloid collections, the balance of the material of the second collection was not used.

Isolation of Alkaloids A and C The balance of the ether extractable bases of the first collection was chromatographed in batches of 0.5 - 1 g. on columns of acid washed alumina (activity II - III) 2 by 25 cm. in size. The columns were washed with benzene, and then with chloroform. A solvent gradient (57) was then established by adding, with stirring, 500 ml. of 1:1 methanol-pyridine to 2 liters of chloroform. The rate of this addition was one fourth the rate at which the mixed solution was run onto the chromatographic column. The rate of flow of the chromatographic

column was limited to approximately four ml. per minute. In this way a bright yellow band containing alkaloids A and C was developed and eluted.

This fraction was evaporated to dryness under nitrogen. It was then taken up in benzene and chromatographed on a column of 200 - 300 mesh florosil. The column was washed with chloroform followed by chloroform containing a few percent methanol. The alkaloids A and C were developed in a single narrow band and eluted. This fraction was evaporated under nitrogen.

The residue was taken up in benzene and chromatographed using the preparative paper chromatographic method of von Arx and Neher (58) with the formamide/cyclohexane-diethylamine system. In this method a number of sheets of paper were clamped together between steel plates. Solvent was poured into a depression cut into the top of the sheets and they were developed descending. Alternately, the alkaloids were chromatographed on 11 1/2 x 12 1/2 inch sheets of Whatman #3 mm paper. In this case the sheets were developed ascending. After development (by either method) the sheets were allowed to dry somewhat and the spots corresponding to alkaloids A and C were cut from the

paper and separated. The alkaloids were then eluted from the paper with 9:1 chloroform-diethylamine. The alkaloidal solutions were diluted with ether and washed with several portions of dilute base and then with water. They were dried over potassium carbonate, filtered, and evaporated to dryness under vacuum. The residues were treated with methanolic hydrochloric acid and again evaporated, under vacuum, to dryness. The alkaloid A hydrochloride was then recrystallized once from isopropyl alcohol to give a total of approximately 60 mg. of fine yellow needles. Alkaloid C hydrochloride crystallized, after standing for some time, from isopropyl alcohol, giving a total of approximately 55 mg. of yellow needles in clusters.

Properties of Alkaloid A Alkaloid A has been given the name rupicoline. The free base may be crystallized from cold methanol giving A·MeOH. After three recrystallizations from methanol this material had a melting point of 205.4 - 206.9° with decomposition. Rupicoline hydrochloride, recrystallized several times from isopropyl alcohol, had a melting point, in vacuum, of 264.7 - 264.9° with decomposition. The specific rotation of rupicoline hydrochloride is $[\alpha]_D^{25.5} = -228°$ (c = 1.21 in water).

Rupicoline hydrochloride gave, on analysis, C,62.44, 62.30; H,7.11, 7.20; N,6.21, 6.35; Cl,8.08. An earlier sample gave: C,63.16; H,6.67; N,7.06. $C_{22}H_{29}N_2O_4Cl$ requires: C,62.77; H,6.94; N,6.66; Cl,8.42.

Properties of Alkaloid C Alkaloid C has been given the name montanine. The free base of this alkaloid was not obtained in crystalline form. The amorphous base melts at 137.1 - 137.7° in vacuum. The alkaloid forms a hydrochloride salt which crystallizes from isopropanol very slowly and melts, in vacuum, at 257.7 - 258.7° with decomposition. Montanine forms a hydrobromide salt which may be recrystallized from isopropyl alcohol containing 2-3 percent water. The recrystallized compound melts, in vacuum, at 272.8 - 273.6° with decomposition. For montanine hydrobromide $[\alpha]_D^{26} = -206°$ (c = 1.21 in water). Montanine hydrobromide gave, on analysis, C,55.02, 54.80; H,6.19, 6.06; N,5.84, 5.76; Br,16.72, 16.80. $C_{22}H_{29}O_5N_2Br$ requires C,54.89, H,6.07, N,5.82, and Br, 16.60.

Sublimation of Rupicoline A small sample of impure rupicoline was sublimed at 100° and 0.05 mm. pressure for several hours.

Paper chromatography of the product by the formamide/cyclohexane-diethylamine method revealed that some of the alkaloid had sublimed. However, a prominent spot was noticed in the sublimate at Rf 0.07 which had not been in the material before sublimation. This indicated that the material had decomposed extensively.

Mass Spectrometric Examination of Rupicoline and Montanine

Samples of rupicoline and montanine which had been vacuum dried for several days were examined by mass spectrometry using a heated inlet system at 180°. The spectra of these compounds lacked peaks of mass greater than 250. This indicates that the compounds were completely decomposed and the spectra are consequently valueless.

Molecular Weight of Rupicoline The molecular weight of rupicoline was studied by two methods. The isothermal distillation method of Childs (59) was used with primary standard benzoic acid as the reference compound. Reagent acetone was used as the solvent. The apparatus were equilibrated in a bath at 40.0° for 233 hours. By this method molecular weight values of 377 and 590 were obtained for rupicoline.

The difference between these results far exceeds the error expected for this method and suggests that a manipulative error was involved in one of these measurements. For this reason the measurement of the molecular weight was repeated, using a thermistor bridge device based on the instrument of J. J. Newmayer (60). By this method molecular weight values of 380, 382, 383, and 375 were obtained for rupicoline. The mean of the five best values obtained by these methods is 379.

Ultraviolet and Visible Spectra of Rupicoline and Montanine

Rupicoline and montanine had practically identical ultraviolet and visible spectra. These consisted of a peak with λ_{max} at 228 mμ (log ϵ = 4.34 for rupicoline, 4.48 for montanine) and a shoulder at approximately 260 mμ (log ϵ = 3.42 for rupicoline, 3.75 for montanine). In addition there was a peak in the visible region, λ_{max} 410 mμ (log ϵ = 3.45 for rupicoline, 3.53 for montanine). These spectra were unchanged by the addition of acid.

Infrared Spectra of the Alkaloids The infrared spectra of rupicoline hydrochloride and montanine hydrobromide were taken in potassium bromide pellets. Each of these spectra had strong bands in the carbonyl region at 1684 cm^{-1} and 1737 cm^{-1}. These spectra are reproduced in Figures 1 and 2.

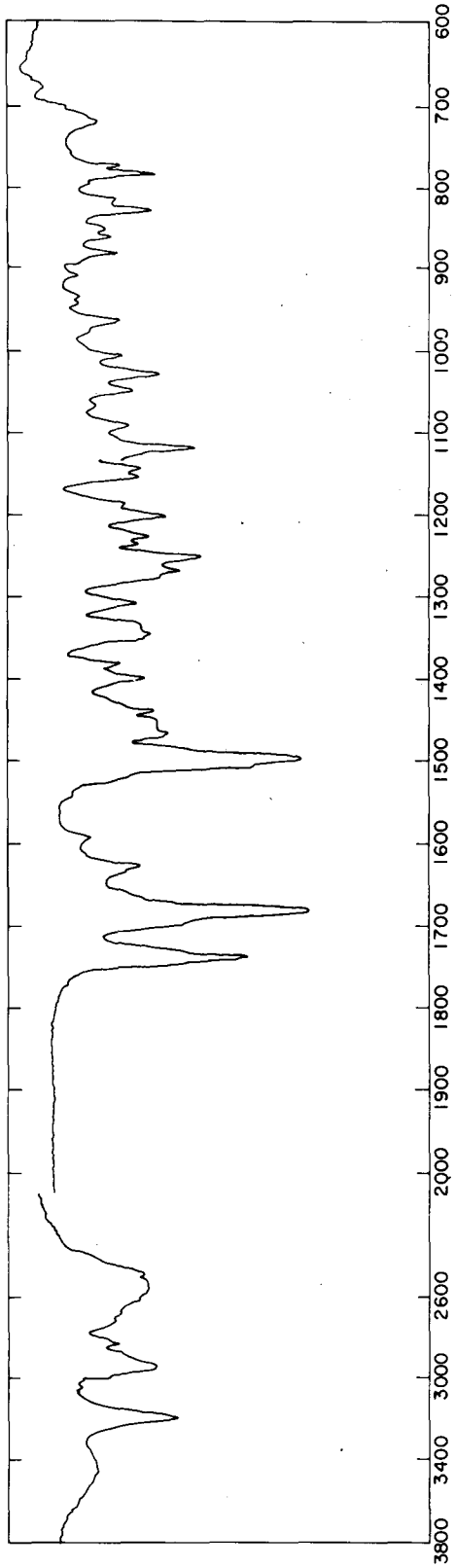


Figure 1. The infrared spectrum of rupicoline hydrochloride.

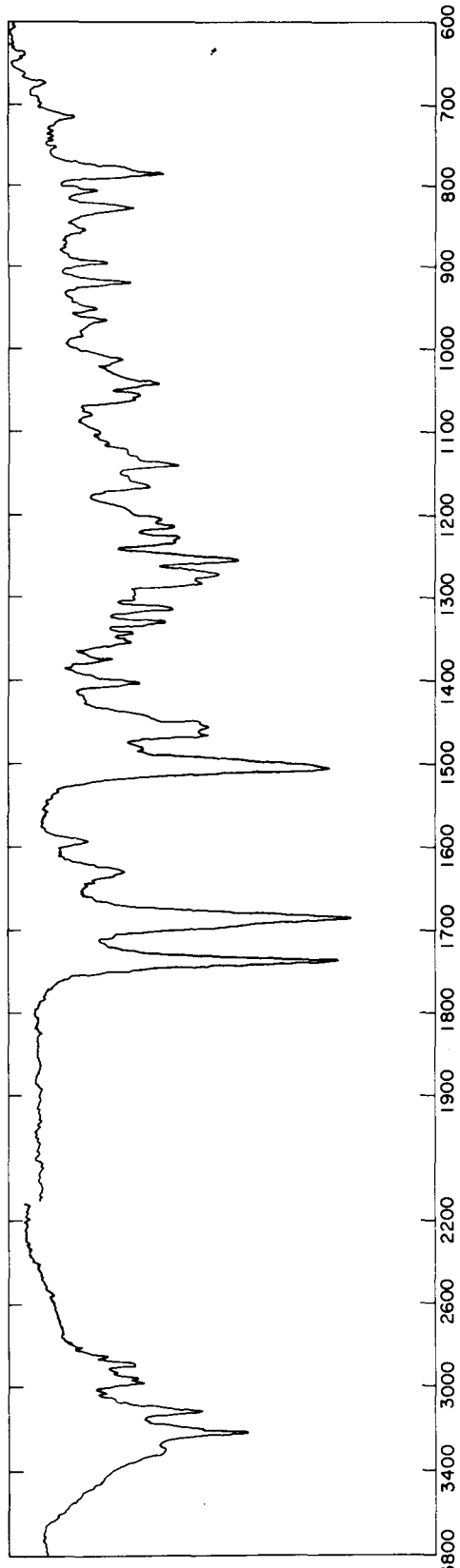


Figure 2. The infrared spectrum of montanine hydrobromide.

Nuclear Magnetic Resonance Spectra of the Alkaloids The nuclear magnetic resonance spectra of rupicoline and montanine were taken in deuteriochloroform using a nylon microcell to limit the quantity of material required for the spectra. These spectra are reproduced in Figures 3 and 4. Chemical shifts are given in cycles per second downfield from tetramethylsilane (=0 cps.). A trace of chloroform present in the deuteriochloroform causes the peaks at 440. 5 cps. in the rupicoline spectrum and at 436.5 cps. in the montanine spectrum (Tetramethylsilane was added for the calibration of the spectrum of montanine. The spectrum of rupicoline, Figure 3, may be calibrated by subtracting 4 cps. from the observed positions of the peaks.).

Figure 3 is the spectrum of rupicoline methanolate. The group of peaks at 390-434 cps. had an integral of 3/32 the total hydrogen. The three major peaks at 195-230 cps. each had integrals of 3/32 the total hydrogen. Of these the peak at 207.5 cps. was believed to be the methyl hydrogen of methanol. This peak, as well as the peak at 263.5 cps. (which had an integral of 1/32 the total hydrogen), was attenuated to 1/2 height when the crystalline rupicoline methanolate was vacuum dried at room temperature for 48 hours before use. The peak at 232.5 cps.

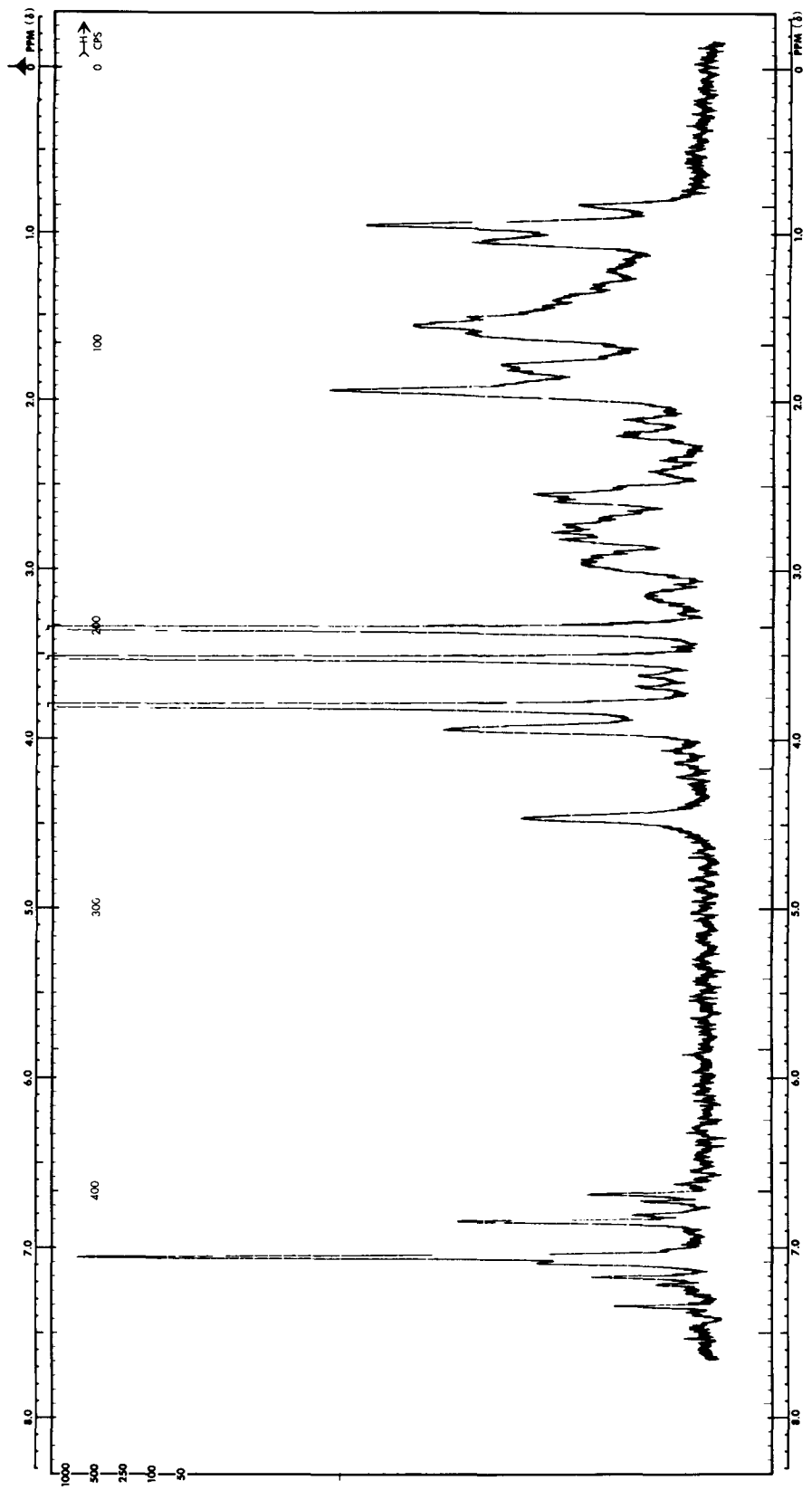


Figure 3. The nuclear magnetic resonance spectrum of rupicoline methanolate.

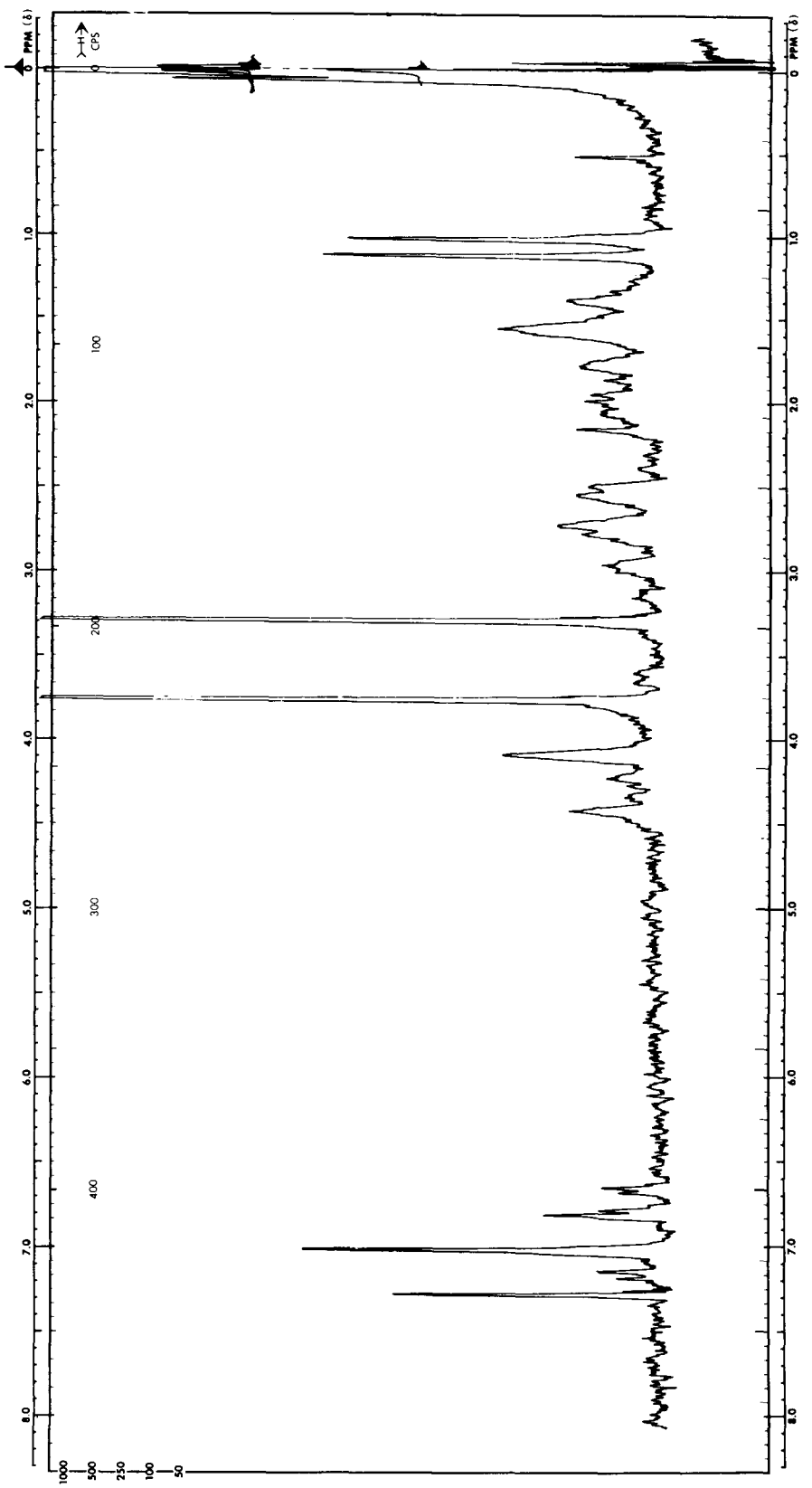


Figure 4. The nuclear magnetic resonance spectrum of montanine.

had an integral of 1/32 the total hydrogen while the triplet centered at 53 cps. had an integral 3/32 the total hydrogen of rupicoline methanolate.

Figure 4 is the spectrum of montanine. Montanine hydrobromide was treated with aqueous base and extracted with ether. The ether extract was evaporated and the residue dried in vacuum. This residue was then dissolved in deuteriochloroform and the spectrum taken. The group of peaks at 390-434 cps. had an integral of 3/28 the total hydrogen. The peaks at 197.5 cps. and 225.5 cps. and the doublet centered at 64 cps. each had integrals of 3/28 the total hydrogen. The peaks at 245.5 cps. and 265.5 cps. each had integrals of 1/28 the total hydrogen.

Air Oxidation of Voacangine Voacangine (0.010 g.) was dissolved in 10 ml. of chloroform and the solution refluxed on the steam bath in sunlight during the day for four days. The solution was then evaporated and thin layer chromatographed with rupicoline and voacangine as standards. A faint yellow spot could be detected at the Rf value corresponding to rupicoline. Unreacted voacangine was also observed as were other spots corresponding to decomposition products.

This experiment was repeated with the difference being that a small portion of the solution was withdrawn every 4 hours during the day. This sample was evaporated and chromatographed. In this case, and in a subsequent attempt to repeat the first experiment, no spot corresponding to rupicoline could be detected.

Catalytic Oxidation of Voacangine Voacangine was oxidized in a manner similar to that described by Goutarel et al. (29) for the conversion of ibogaine (I) to iboluteine (VIIa). The voacangine (0.040 g.) was placed in a platinum boat in a microhydrogenation apparatus (61). Platinum oxide (0.011 g.) was then placed in the apparatus and 5 ml. of ethyl acetate added. The apparatus was then evacuated and filled with hydrogen. After the platinum oxide had been reduced the system was again evacuated and filled with oxygen. When the catalyst no longer absorbed oxygen, the voacangine was introduced into the solution. After approximately thirty hours at 25°, 0.97 equivalents of oxygen had been taken up. The catalyst was then filtered off and the solvent evaporated. The residue was an amorphous solid and all attempts to crystallize it were unsuccessful. The residue was placed in a platinum boat in the microhydrogenation apparatus. Platinum oxide (0.020 g.)

and 5 ml. of ethanol were then added and the air in the system was displaced by hydrogen. After the catalyst had been reduced the material in the platinum boat was added and 0.75 equivalents of hydrogen added. At 25° the reaction was complete in 5 - 10 minutes. The catalyst was then removed by centrifugation and decantation. The solvent was evaporated and the residue divided into two portions. To one portion 5 ml. of 2N methanolic NaOH was added. After 30 hours at room temperature, chromatography revealed that no spot corresponding to rupicoline was present in the solution. The solution was then refluxed for two hours and again tested with thin layer chromatography with the same results. The second portion of the reduced material was treated with 5 ml. of 2N methanolic HCl and the solution allowed to stand for 30 hours at room temperature. Thin layer chromatography of this solution revealed a spot having an Rf value similar to that of rupicoline. The methanolic solution was then evaporated and the residue chromatographed preparatively on thin layer chromatographic plates using 9: 1 cyclohexane-diethylamine as the solvent.

This method of oxidation and rearrangement of voacangine was repeated with an additional 0.040 g. of voacangine. The bands on the thin layer chromatograms corresponding in Rf value to

rupicoline were removed from the plates and eluted with methanol. The base derived in this manner was converted to a hydrochloride salt and recrystallized from isopropyl alcohol. The total yield was approximately 2 mg. of fine yellow needles.

After a second recrystallization the infrared spectrum of this material was taken in a potassium bromide pellet. This spectrum is reproduced in Figure 5, superimposed on the spectrum of rupicoline. It is identical in every respect with the spectrum of rupicoline.

X-Ray Powder Data The X-ray powder method was used to prove the identity of the compound prepared by oxidation and rearrangement of voacangine with rupicoline. A sample of each of these compounds was powdered and placed in a capillary tube. X-ray exposures of 20 and 122 hours were then made using the Straumanis technique (62). Chromium radiation with a vanadic acid filter was used (the λ_{max} of CrK α = 2.2909Å). The camera used had a diameter of 99.812 mm. Arcs were measured on a comparator to ±0.02 mm. Relative intensities of the lines were estimated visually as very strong (vs), strong (s), medium (m), weak (w), or very weak (vw). Table 4 is a comparison of the spacings and

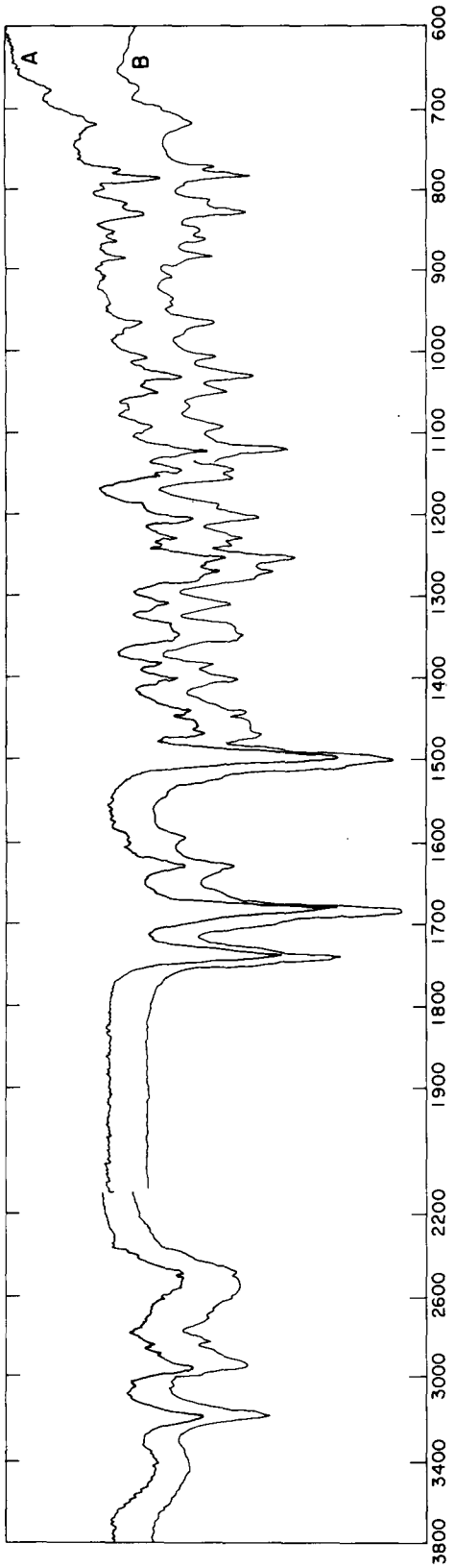


Figure 5. The infrared spectrum of the hydrochloride salt of the pseudoindoxyl compound prepared by oxidation and rearrangement of voacangine (A); rupicoline hydrochloride (B).

Table 4. Spacings and Intensities of Rupicoline and the Material Prepared From Voacangine.

Rupicoline		Material Prepared From Voacangine	
d	I	d	I
8.23	w	8.23	w
7.85	vs	7.84	vs
7.16	w	7.15	w
6.49	vs	6.49	vs
6.13	w	6.12	w
5.67	m	5.67	m
5.41	w	5.42	w
5.10	w	5.10	w
4.69	w	4.69	w
4.54	w	4.53	w
4.30	w	4.31	w
4.01	m	4.01	m
3.86	m	3.86	m
3.78	w	3.78	w
3.58	w	3.59	w
3.48	w	3.48	w
3.36	w	3.36	w
3.20	w	3.20	w
2.96	w	2.96	w
2.86	vw	2.86	vw
2.65	vw	2.64	vw
2.44	vw	2.44	vw
2.41	vw	2.41	vw
2.31	vw	2.31	vw
2.24	vw	2.24	vw
2.12	vw	2.12	vw
1.94	vw	1.94	vw
1.92	vw	1.92	vw
1.71	vw	1.70	vw

intensities obtained for rupicoline and the compound prepared by oxidation and rearrangement of voacangine.

Further Oxidations of Voacangine A small sample of voacangine (0.010 g.) was treated in the exact manner described earlier for the isolation of the ether extractable organic bases from the plant. The resulting fraction was thin layer chromatographed with rupicoline as a comparison. No spot corresponding to rupicoline could be detected in the material treated in this way.

A sample of voacangine (0.010 g.) was refluxed in ether for approximately one month and the ether evaporated to dryness. Thin layer chromatography of a sample of the residue did not reveal a spot of Rf value corresponding to rupicoline. The residue was then allowed to stand for two weeks in the sunlight and another sample taken and chromatographed. A faint spot corresponding to rupicoline in Rf value and color could then be detected. Most of the material was unreacted voacangine.

Preparation of the p-Toluenesulfonate of Montanine Montanine (0.017 g.) was dissolved in 10 ml. of pyridine (reagent grade, dried over BaO for several days). p-Tolenesulfonyl chloride (0.051 g.), washed with 5% NaOH, dried, and recrystallized from

ligroin, was then added and the solution stirred at room temperature for 24 hours. The solvent was then evaporated and the residue washed with several portions of ether. The residue was then thin layer chromatographed preparatively. Examination of the fractions with ultraviolet spectroscopy disclosed that the fraction having Rf value 0.0 was the p-toluenesulfonate of montanine. This fraction was evaporated and attempts were made to crystallize it from several solvents. However, these were unsuccessful.

Reduction of Montanine p-Toluenesulfonate Absolute ethanol (20 ml.) was refluxed with 1 g. of ammonium chloride for 24 hours and then allowed to cool. A portion of this solution (5 ml.) was added to a solution of the crude montanine p-toluenesulfonate in 2 ml. of methanol. Ethyl orthoformate (0.5 ml.) was then added and the solution refluxed for two hours. The pale yellow solution was then evaporated to dryness and the residue extracted with a few ml. of ethanol. This solution was treated with 200 mg. of sodium borohydride in small portions and the solution allowed to warm to reflux temperature. On cooling, water was added and the solution extracted with ether. The extract was then evaporated to dryness and treated with methanolic HCl containing 5% water.

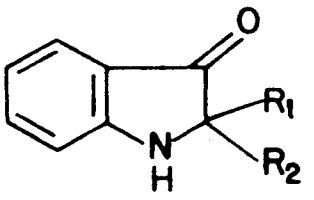
This solution was evaporated to dryness and the residue examined with thin layer chromatography. Rupicoline was used as a comparison. The Rf values of the material prepared above and rupicoline were not the same.

DISCUSSION

It is often possible to demonstrate a close structural relationship between alkaloids from plants within a given taxonomic group such as a plant family (40).

The structural correlations found among the alkaloids of the tribe Tabernaemontaneae suggest that biogenetically related alkaloids may be found in other species of the tribe. This is apparently true for Tabernaemontana rupicola.

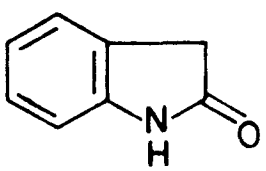
The ultraviolet and visible spectra of iboluteine (VIIa) have maxima at 230 mμ (log ε = 4.3) and 420 mμ (log ε = 3.5) and a shoulder at 260 mμ (log ε = 3.7) (29). Similarly, the spectra of desmethoxyiboluteine (VIIb) have maxima at 230 mμ (log ε = 4.44) and 400 mμ (log ε = 3.39) and a shoulder at 256 mμ (log ε = 3.77) (29). The spectra of rupicoline and montanine are very similar to these with peaks at 223 mμ (log ε = 4.34, 4.48 respectively) and 410 mμ (log ε = 3.45, 3.53) and a shoulder at 260 mμ (log ε =3.42, 3.75). Like iboluteine and desmethoxyiboluteine, these alkaloids have bright yellow-green fluorescences. These facts suggest that rupicoline and montanine are pseudoindoxyls (XIV). This view is supported by the fact



XIV

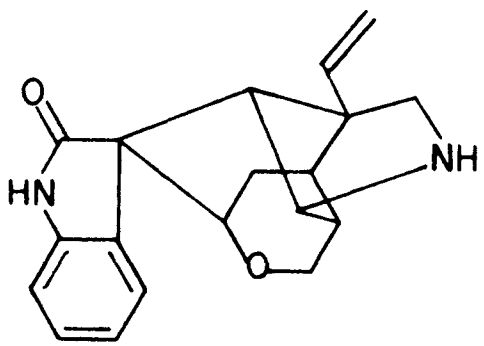
that carbonyl bands occur in the infrared spectra of rupicoline and montanine at 1685 cm^{-1}. The spectra of iboluteine and desmethoxyiboluteine have bands at approximately the same wavelength (1675 cm^{-1} and 1680 cm^{-1} respectively).

A structural element somewhat similar to the indoxyl group, also prepared from indoles by oxidation (63), is the oxindole group (XV). Compounds with this group may be distinguished

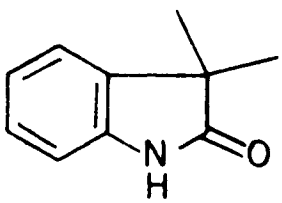


XV

from indoxyl compounds on the basis of spectra. The oxindole gelsemine (XVI), for example, has an ultraviolet spectrum with maxima at 252 mμ (log ε = 3.3) and 285 mμ (log ε = 3.9) (64, 65). 3, 3-Dimethyloxindole (XVII) has an ultraviolet spectrum with



XVI



XVII

maxima at 250 mμ (log ε = 3.2) and 285 mμ (log ε = 3.9) (65). In their infrared spectra gelsemine and 3, 3-dimethyloxindole also differ from indoxyl compounds. Gelsemine has peaks at 1720 cm^{-1} and 1619 cm^{-1}. 3, 3-Dimethyloxindole has peaks at 1718 cm^{-1}

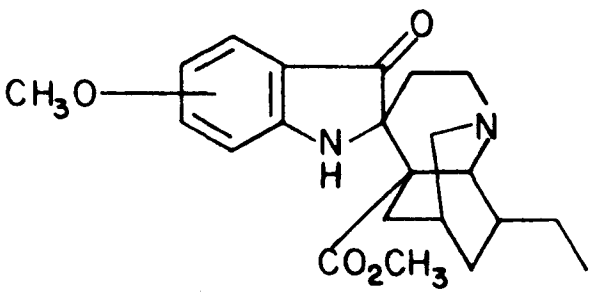
and 1618 cm^{-1}. Oxindole has similar peaks at 1714 cm^{-1} and 162: cm^{-1} (65). These differences in spectral properties are sufficient to eliminate the oxindole group from further consideration with respect to the structure of rupicoline and montanine.

The elemental analyses of the alkaloids are consistant with the formulae $C_{22}H_{28}O_4N_2$ for rupicoline and $C_{22}H_{28}O_5N_2$ for montanine. The molecular weight of rupicoline was found to be 379 and this value is in agreement with the formula $C_{22}H_{28}O_4N_2$ (molecular weight 384). Although both alkaloids contain two nitrogen atoms the analyses indicate that they each form salts with only one equivalent of acid.

The nuclear magnetic resonance spectrum of rupicoline provides insight into the structure of the molecule. The group of peaks at 390-434 cps. is in the region associated with aromatic protons and they have an integral equal to 3/28 the total hydrogen or three protons. The absence of peaks in the region 270-390 cps. suggests that olefinic protons are not present in rupicoline. The two peaks at 224 cps. and 197 cps. fall in the region associated with O-methyl groups. Each of these peaks has an integral equivalent to three hydrogens. In addition, the infrared spectrum of rupicoline has a band at 1737 cm^{-1}. A number of saturated esters have

carbonyl stretching frequencies in the region 1735-1750 cm^{-1}. This data suggests that rupicoline contains an aromatic methoxyl group and a nonconjugated carbomethoxyl group. The triplet centered at 53 cps. (J = 6.5 cps.) is due to the methyl hydrogens of an ethyl group.

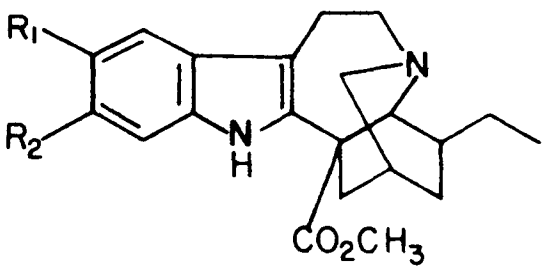
In view of the fact that rupicoline contains the pseudoindoxyl partial structure and that pseudoindoxyl alkaloids of the iboga group are present in plants closely related to T. rupicola it seems likely that rupicoline is closely related to the iboga group and on this basis a structure (XVIII) may be proposed for the alkaloid. The peak in



XVIII

the nuclear magnetic resonance spectrum of rupicoline at 197 cps. is approximately 30 cps. upfield from the position at which carbomethoxyl or methoxyl protons would be expected. However, ring currents associated with the benzene ring would be expected to shield the methyl protons of the carbomethoxyl group in structure XVIII and shift them upfield.

Structure XVIII bears the same relationship to either voacangine (XIXa) or isovoacangine (XIXb) that iboluteine (VIIa) has

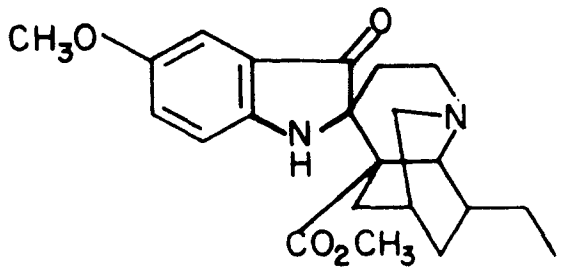


XIXa $R_1 = OCH_3$, $R_2 = H$

XIXb $R_1 = H$, $R_2 = OCH_3$

with respect to ibogaine (I). The conversion of ibogaine to iboluteine by Goutarel et al. (29) suggests a method of confirming this structure for rupicoline since oxidation and rearrangement of either voacangine or isovoacangine to the corresponding pseudoindoxyl should give a compound of this structure.

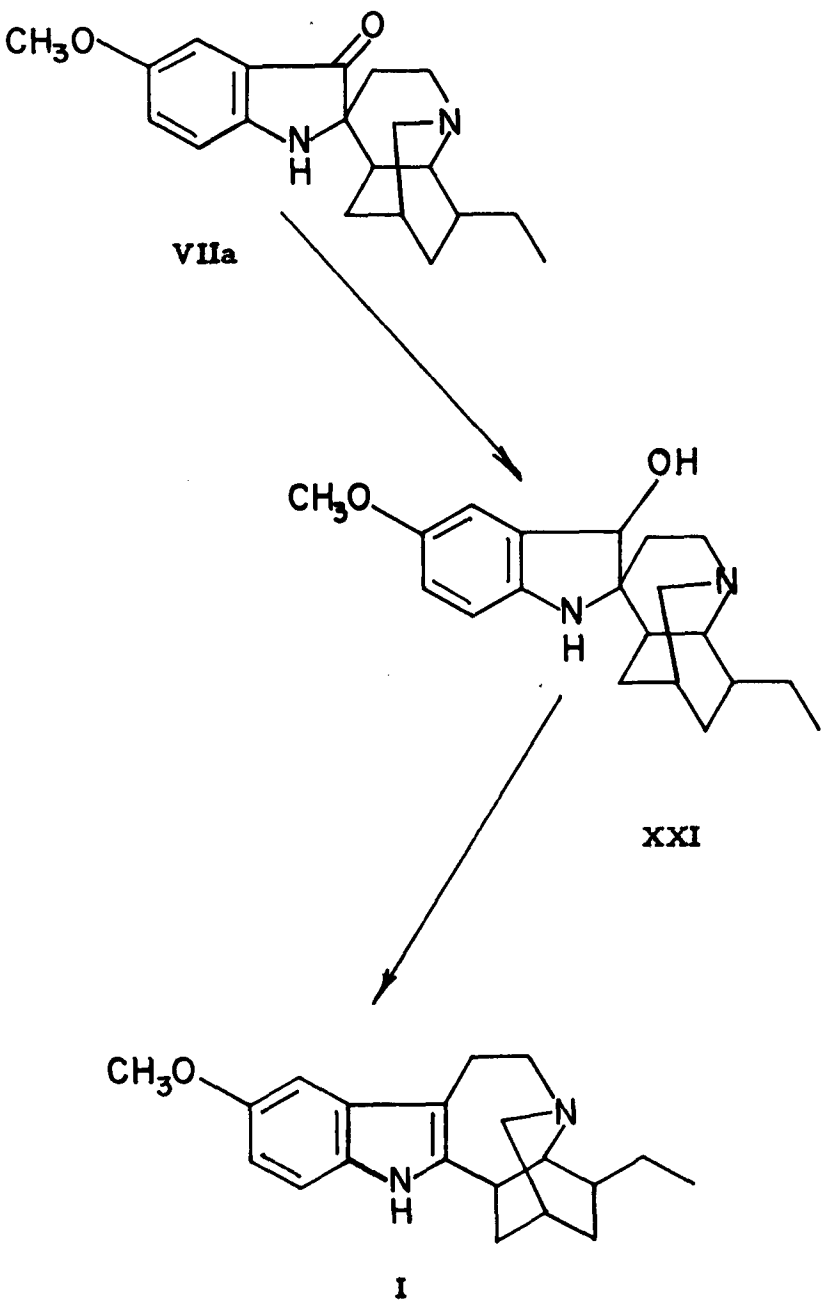
Goutarel et al. (4) state that Goutarel (66) carried out the conversion of ibogaine to iboluteine in a single step by refluxing ibogaine in chloroform in the presence of air and light. Treating voacangine in approximately the same manner did not produce any rupicoline, however. Catalytic oxidation, in the manner described by Goutarel et al. (29), of voacangine and rearrangement of the product with dilute acid did yield a compound having an infrared spectrum and X-ray powder pattern identical with those of rupicoline. This data confirms compound XX as the structure of



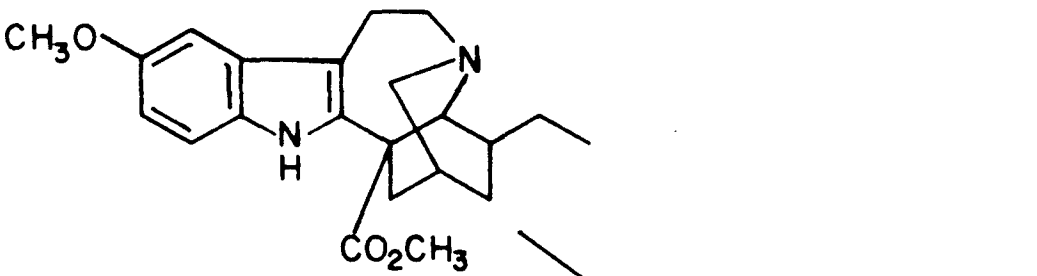
XX

rupicoline. Both the infrared spectra and X-ray powder data leave absolute stereochemistry undertain although differences in spectra would be expected for differences in relative stereochemistry. The fact that these spectra are identical does prove that the compounds are either the same or enantiomers.

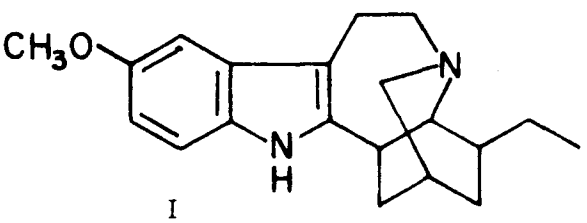
It is interesting to note that the conversion of voacangine to rupicoline proceeds with the introduction of an asymmetric center. Since a single product with the characteristic yellow-green indoxyl fluorescence was observed (in this case or by Goutarel et al. (29)), the rearrangement is highly stereoselective. It is also unlikely that the rearrangement is accompanied by any change in the adjacent asymmetric center (C-20) since iboluteine may be reduced to dihydroiboluteine (XXI) which is rearranged in acid to ibogaine (67).


CH3O
O
N
H
N
VIIa
OH
CH3O
N
H
N
XXI
CH3O
N
H
N
I

The close relationship between the iboga alkaloids is demonstrated by the conversion of voacangine to ibogaine by saponification and decarboxylation (68).



XIXa



I

In this way the sterochemistry of voacangine has been related to that of ibogaine. The relative stereochemistry of ibogaine has been established by the study of the structure by means of X-ray diffraction (7).

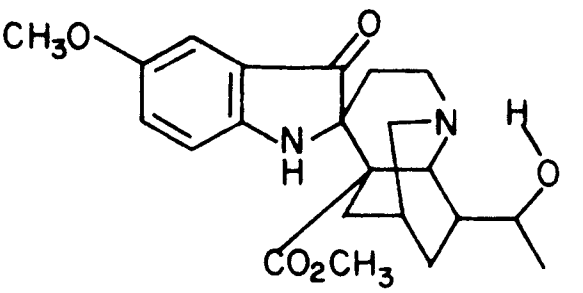
These experiments suggest that rupicoline has the same relative stereochemistry as ibogaine.

Montanine, as mentioned earlier, has ultraviolet and visible spectra identical with those of rupicoline. This suggests that the chromophore of montanine is the same or very similar to that of rupicoline. The infrared spectrum of montanine has two

strong bands in the carbonyl region at 1685 cm^{-1} and 1737 cm^{-1} as did the infrared spectrum of rupicoline. In addition to the peak at 3200 cm^{-1} common to the spectra of both alkaloids (This peak is probably due to N-H stretching vibration. This hydrogen is intramolecularly hydrogen bonded to the carbomethoxyl carbonyl.), the spectrum of montanine has a broad peak centered at 3300 cm^{-1} which may be due to an intramolecularly hydrogen bonded hydroxyl group.

The nuclear magnetic resonance spectrum of montanine is also very similar to that of rupicoline. The group of peaks at 390-434 cps., which have an integral of 3 hydrogens, appear to have the same pattern as those in the rupicoline spectrum suggesting the same pattern of substitution on the aromatic ring. The absence of peaks in the region 270-390 cps. appears to suggest the absence of olefinic protons. The peaks at 197.5 cps. and 225.5 cps., each having an integral of 3 hydrogens, have almost identical chemical shifts as those in the spectrum of rupicoline. The triplet at 53 cps. in the spectrum of rupicoline is missing and is replaced by a doublet (J = 6.5 cps.) centered at 64 cps. which also has an integral of 3 hydrogens. In addition a peak at 266 cps., having an integral of one hydrogen, appears in the spectrum of montanine.

The similarities between the spectra of rupicoline and montanine suggest very similar structures for these compounds. It is interesting, as well, that the molecular formulae differ only by one oxygen atom. The replacement of the triplet at 53 cps. in the nuclear magnetic resonance spectrum of rupicoline by a doublet at 64 cps. in the spectrum of montanine reveals the location of this oxygen atom. A structure accommodating this change is XXII. This structure is consistent with the spectra of montanine.

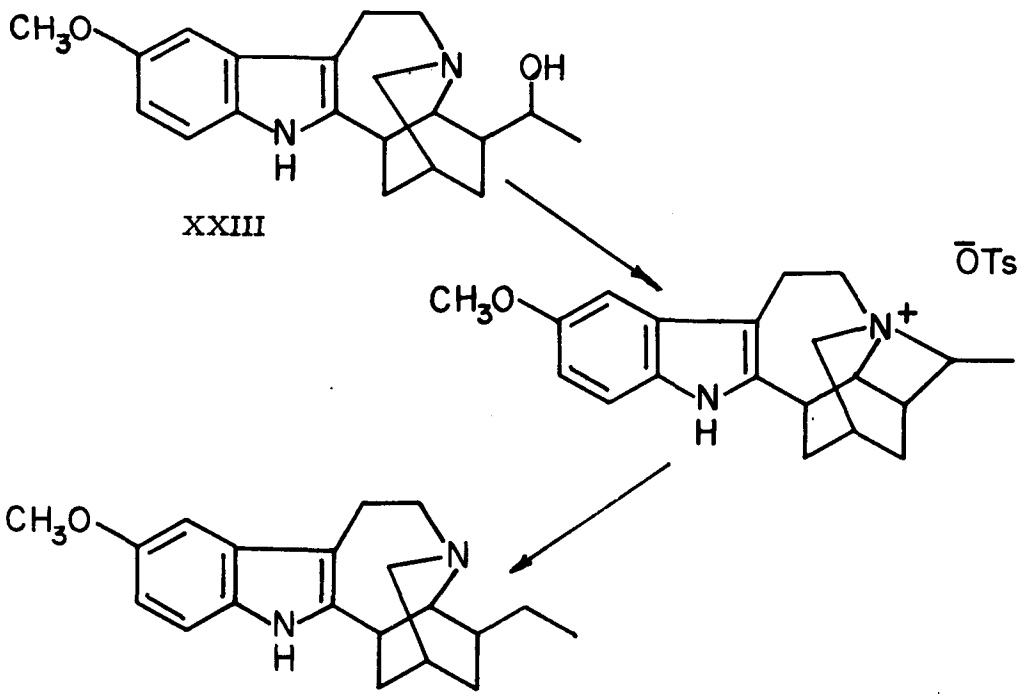


XXII

The peak in the nuclear magnetic resonance spectrum at 266 cps. may be attributed to the hydroxyl hydrogen. This hydroxyl hydrogen may hydrogen bond with the adjacent nitrogen as the infrared spectrum suggests.

This structure is the pseudoindoxyl corresponding to the alkaloid voacangarine (see Table 1, page 4). A sample of this alkaloid was not available, however, for possible correlation with montanine.

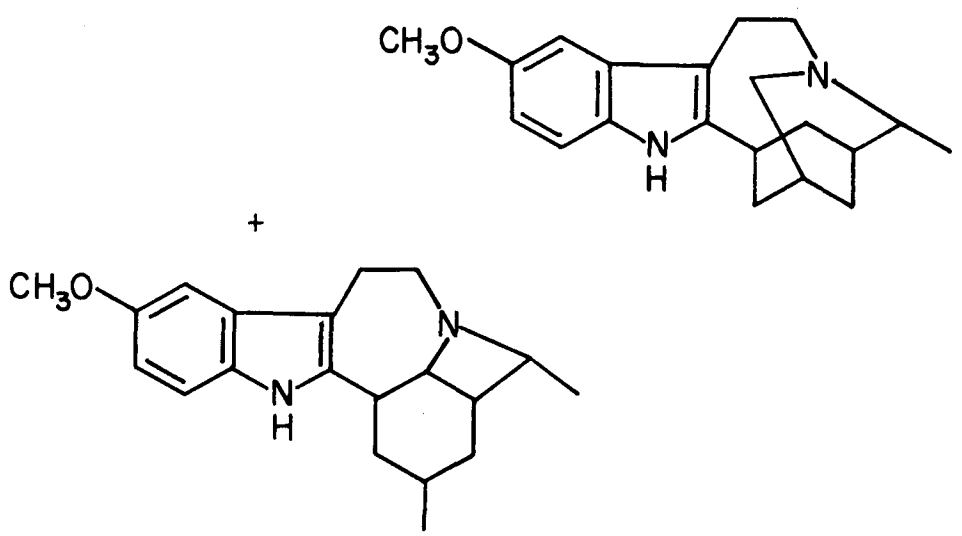
Renner and Prins (23) prepared the tosylate of iboxygaine (XXIII) which they were able to reduce with sodium to a mixture of ibogaine and several isomeric compounds.



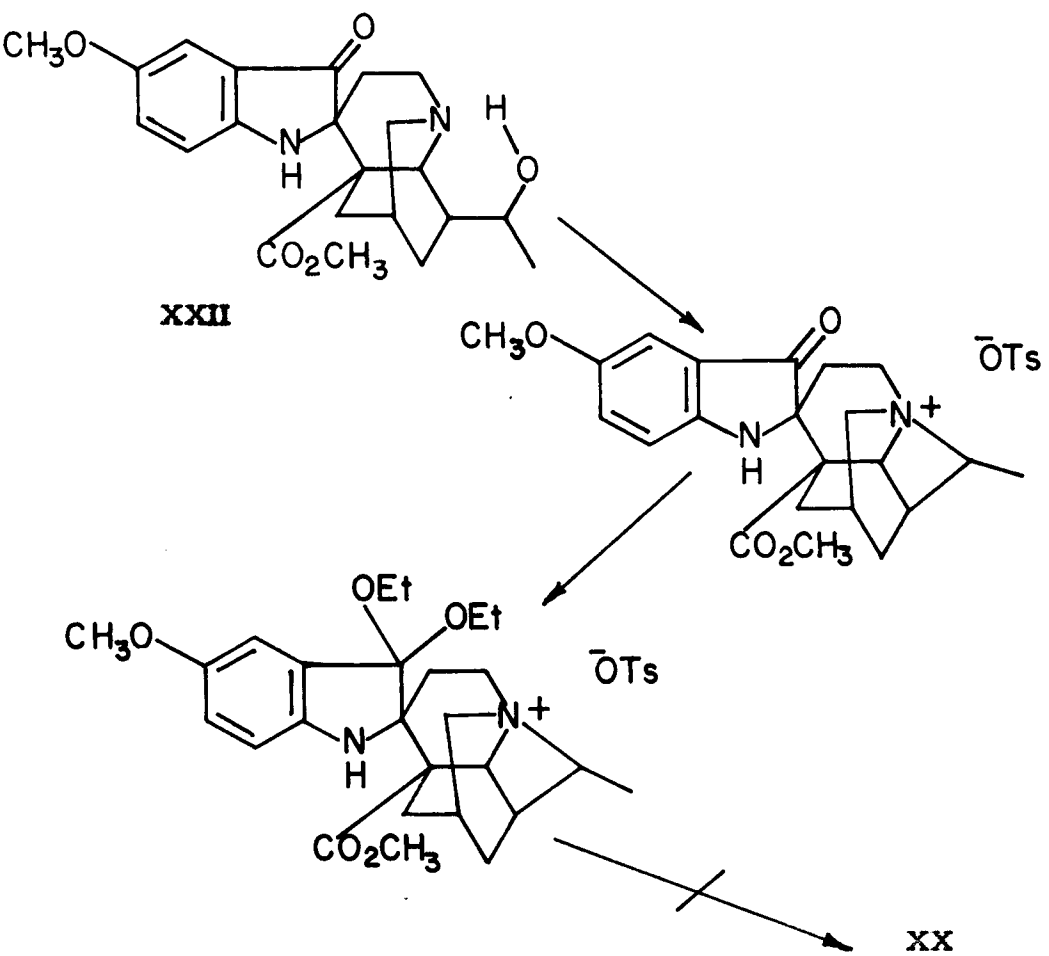
XXIII

I

+ isomeric compounds such as:



The tosylate of montanine was prepared in a manner similar to that used by Renner and Prins. This compound was hydroscopic and could not be obtained crystalline. It was treated with ethyl orthoformate to convert the ketone to an acetal and the product reduced with sodium borohydride. This material was then treated with acid to regenerate the ketone and the product chromatographed. Only one spot on the chromatogram had the bright yellow-green indoxyl fluorescence. The Rf value of this spot was distinctly different from that of rupicoline with which it was compared.



Earlier workers (29, 30) have suggested that the pseudoindoxyl and hydroxyindolenine iboga alkaloids might be attributed to a facile autoxidation of the parent alkaloids. This view cannot be completely excluded in the present investigation. If this were the case, the parent indole alkaloids might also be expected to be present in the plant. The initial chromatographic experiment revealed that the early fractions, in which the indole alkaloids would appear if present, contained only a trace of material reacting with alkaloidal reagents. This fraction was not investigated in this study.

Some experiments were carried out with small samples of voacangine to determine whether or not the conditions of isolation were such that the alkaloid would be oxidized to rupicoline. In these experiments, no rupicoline could be detected. However, these experiments do not exclude the possibility of such an oxidation occurring in the presence of other plant materials, either in the plant or during the isolation.

The material of the second collection of the plant was apparently subject to environmental conditions of growth differing significantly from that of the first collection. Examination of the

alkaloids of the material from the second collection revealed the absence of voacangine (and probably voacangarine) as well as rupicoline and montanine.

PART II

THE STRUCTURE OF NECROSAMINE

INTRODUCTION

In 1952, Ikawa et al. (69) reported an agent isolated from cultures of Escherichia coli which caused hemorrhage and spontaneous regression of a mouse tumor (Crocker mouse sarcoma 180). Experiments indicated that the substance was a complex polysaccharide which had both peptide and phospholipid components. On treatment with aqueous trichloroacetic acid most of the peptide fraction was removed leaving a lipopolysaccharide which had approximately the same biological activity as the original material. Reagents which attacked the lipopolysaccharide fraction of the agent decreased its activity (70).

Hydrolysis of the agent has yielded several component monosaccharides and fatty acids (71, 72). In addition, ethanolamine and an aliphatic diamine were isolated from the hydrolysate (73). The latter compound, which has been given the name necrosamine, was shown to be a saturated acyclic diamine with the molecular formula $C_{20}H_{44}N_2$. Necrosamine reacted with carbon disulfide forming a dithiocarbamate salt which was converted to a cyclic thiourea by heating. This suggested that the amino groups were vicinal and this view was confirmed by the fact that necrosamine reacted with lead tetraacetate. This oxidation reaction yielded

palmitaldehyde, isolated as the 2, 4-dinitrophenylhydrazone. Oxidation with permanganate gave palmitic acid, while periodate followed by permanganate gave palmitic and either butyric or isobutyric acids. On this basis the structure $CH_3(CH_2)_{14}CHNH_2CHNH_2C_3H_7$ was proposed for necrosamine. The terminal C_3H_7 group was not elucidated. Although the proposed structure for necrosamine has two asymmetric carbon atoms, no detectable optical activity was observed for the base or its dihydrochloride salt. Since necrosamine is one of the isomers of either 4, 5-diaminoeicosane or 2-methyl-3, 4-diaminononadecane, the synthesis of these compounds was carried out (74). These compounds were each easily separated into two pairs of enantiomers on the basis of the solubility of the dihydrochloride salts in ether. Necrosamine dihydrochloride was observed to be insoluble in ether.

The purposes of the present research were to investigate the configuration of the groups about the two asymmetric carbon atoms and to determine the structure of the terminal propyl group.

EXPERIMENTAL

Elemental analyses were performed by Dr. A. Elek, Los Angeles, California or by Spang Microanalytical Laboratories, Ann Arbor, Michigan.

All melting points are corrected. Melting points were determined with a capillary apparatus. The apparatus was calibrated using melting point standards from a Kofler hot state (A. D. Thomas Company).

Infrared absorption spectra were recorded using a Perkin Elmer Model 21 infrared spectrophotometer.

Nuclear magnetic resonance spectra were taken at 60 megacycles on Varian Model A-60 and HR-60 Spectrometers.

Measurement of specific rotations were carried out using a Schmidt and Haensch polarimeter with a Lippich double field.

Gas chromatographic experiments were carried out using a Wilkins Instrument Company Aerograph Model A-100C Gas Chromatograph and a Model A-600B "Hy-Fi" Gas Chromatograph with a flame ionization detector.

4, 5-Diaminoeicosane and 2-methyl-3, 4-diaminononadecane

4, 5-Diaminoeicosane and 2-methyl-3, 4-diaminononadecane were

prepared in the manner described by Ikawa and Niemann (74). The ether insoluble fraction of the 4, 5-diaminoeicosane dihydrochloride was recrystallized from water and from ethanol giving colorless plates. On analysis this material was shown to have the composition: C,62.27, 62.16; H,11.97, 11.97; N,7.22, 7.34; Cl,18.58, 18.37. $C_{20}H_{46}N_2Cl_2$ requires: C,62.31; H,12.03; N,7.27; Cl,18.39.

The ether insoluble fraction of the 2-methyl-3, 4-diaminononadecane dihydrochloride was recrystallized from ethanol giving colorless crystals which, on analysis, were found to have the composition: C,62.22; H,11.95; N,7.33; Cl,18.51. $C_{20}H_{46}N_2Cl_2$ requires: C,62.31; H,12.03; N,7.27; Cl,18.39.

Necrosamine A small sample (ca. 40 mg.) of necrosamine dihydrochloride isolated by Ikawa et al. (batch I 185 IX) was available. The infrared spectrum of this material was taken in a potassium bromide pellet. Since it consisted of only a few broad, weak peaks the material was presumed to be impure.

Isolation of Necrosamine Mouse tumor hemorrhagic agent (fraction A, 10.10 g.) isolated from E. coli by Ikawa et al. (batches 48, 49, 54, 56, 61, 63, 70, and 90) was fractionated with ethanol as described by Ikawa et al. (69) giving 4.16 g. of fraction B_1. This material

and 13.24 g. of fraction B_1 isolated by Ikawa et al. (batches II 82 XI, II 88 XI, II 91 XI, II 98 XI, II 111 XI, II 121 XI, II 147 XI, and II 149 XI) were hydrolyzed with dilute sulfuric acid by the method of Ikawa et al. (72) giving 2.739 g. of acetone insoluble lipids.

This lipid fraction was then hydrolyzed with 5N hydrochloric acid at 100° for 16 hours in sealed tubes. The necrosamine dihydrochloride isolated from this hydrolysate was recrystallized twice from ethanol giving 0.242 g. of colorless crystals. After this material was recrystallized once more from ethanol and once from water the melting point, in vacuum, was 285° with decomposition. Analysis revealed that the compound had the composition: C, 62.18; H, 12.13; N, 7.35; Cl, 18.58. $C_{20}H_{46}N_2Cl_2$ requires: C, 62.31; H, 12.03; N, 7.27; Cl, 18.39.

Oxidation of the Amines Several methods of oxidation were studied using the synthetic amines 4, 5-diaminoeicosane and 2-methyl-3, 4-diaminononadecane. The method used successfully was based on the method of Ikawa et al. (73). The amine dihydrochloride (4.5 mg.) was dissolved in 2 ml. of water and 25.5 mg. of sodium periodate added. Potassium permanganate (45.4 mg.) was then added, the solution heated under reflux for 2 minutes, and 0.45 ml. of

1N NaOH added. The solution was then refluxed for 3 minutes. After cooling the solution was made acidic with sulfuric acid and then decolorized with a minimum of sodium bisulfite. A boiling stone was then added, the flask attached to a micro still, and 0.5 ml. of the solution distilled.

The distillate was then examined by means of gas chromatography. The A-600B chromatograph was equipped with a 1/8" x 5' column of 15% carbowax 20M and 5% phosphoric acid on 20/30 firebrick. Carbon dioxide, at 15 psi, was used as the carrier gas. The column temperature was 119 ±1° while the injector temperature was approximately 300°. Samples of 0.010 ml. of the distillate were injected and the retention times of the peaks noted. These samples were immediately followed by samples of dilute aqueous solutions of n-butyric, isobutyric, propionic, and acetic acids. The retention times of the fatty acids of the distillate and the known solutions were compared several times in this way. The retention times of the lower fatty acids under these conditions were: n-butyric acid 15.1 min., isobutyric acid 11.3 min., propionic acid 10.5 min., and acetic acid 7.5 min. Samples of the distillates were also chromatographed simultaneously with each of these fatty acids. The added acid either increased the size of a peak or added a peak to the chromatogram.

4, 5-Diaminoeicosane, when oxidized and examined in this manner, gave n-butyric acid and, in much smaller amounts, propionic and acetic acids. 2-Methyl-3, 4-diaminononadecane yielded isobutyric acid and much smaller peaks attributed to n-butyric and acetic acids. Necrosamine gave n-butyric acid and, in much smaller quantities, propionic and acetic acids.

In each case before chromatographing the distillate 0.010 ml. of water was injected to demonstrate that the column was free of spurious peaks and that the syringe was clean. Samples of each of the synthetic amine hydrochlorides and necrosamine hydrochloride were also treated with the oxidation procedure described with the difference being that the oxidizing agents were not added. In these cases the distillates did not yield any fatty acid on chromatography. Similarly when the oxidation experiment was repeated leaving out only the amine hydrochloride, the distillate was free of fatty acids.

Solubility of Necrosamine The ether insoluble fraction of the synthetic 4, 5-diaminoeicosane dihydrochloride was readily soluble in dimethyl sulfoxide. At room temperature a 25% by weight solution could be prepared. Necrosamine dihydrochloride was dissolved in dimethyl sulfoxide by warming and stirring the solution. When

a 10% solution was allowed to cool to room temperature a precipitate of necrosamine dihydrochloride formed.

The free base 4, 5-diaminoeicosane (from the ether insoluble dihydrochloride fraction) was very soluble in benzene. At room temperature a 20.8% solution could be prepared. A 5.2% solution of necrosamine, prepared by warming the base in benzene, formed a precipitate when cooled to room temperature.

Cyclic Thiourea of Necrosamine The cyclic thiourea of necrosamine was prepared using the method of Ikawa et al. (73). It was recrystallized from ethanol giving a white powder which melted at 71.0-72.8°. The specific rotation of this compound was $[\alpha]_D^{24} = 0 \pm 0.7°$ (c = 1.47 in chloroform).

Complex of 4, 5-Diaminoeiscosane with Cupric Chloride Anhydrous cupric chloride (1/2 mol. equiv.) was added to a chloroform solution of 4, 5-diaminoeicosane forming a deep blue complex. This compound was recrystallized from chloroform and the visible spectrum taken. The complex has a broad absorption maximum centered at 678 mμ (log ε = 2.2). The broad absorption band of this complex prevents its being used at useful concentrations in a polarimeter employing the sodium D line.

Complex of 4, 5-Diaminoeicosane with Potassium Tetrachloroplatinate

Potassium tetrachloroplatinate (0.47 g.) was dissolved in 50 ml. of water and 0.40 g. of 4, 5-diaminoeicosane dihydrochloride (1 mol. equiv.), in 50 ml. of water, added with stirring. A white precipitate formed immediately. Water (100 ml.) was then added and the solution warmed to 60°. On cooling the precipitate was collected and washed with several small portions of water. It was then suspended in 200 ml. of 0.05 N hydrochloric acid and the mixture heated to boiling. After 1/2 hour a bright yellow precipitate was filtered from the hot solution. This precipitate was washed with water and then with several portions of acetone. It was then dried in vacuum and stored over phosphorus pentoxide overnight. The compound was insoluble in water and most organic solvents but it was soluble in and forms a gel with chloroform or carbon tetrachloride. The material could not be crystallized. Samples of the impure compound were analyzed for platinum by heating them in a platinum crucible at a bright yellow heat for five minutes (At this temperature potassium chloride vaporizes and is driven off). On cooling the platinum residue was weighed. When analyzed in this way the material was found to contain Pt, 34.60, 34.76. $C_{20}H_{44}N_2PtCl_2$ requires 33.74% platinum.

Attempted Resolution of 4, 5-Diaminoeicosane Several attempts were made to resolve the 4, 5-diaminoeicosane from the ether insoluble dihydrochloride. (-)-Mandelic acid was prepared as per Wood et al. (75). (-)-α-Methylbenzyl alcohol and its phthalate half ester were prepared as per Houssa and Kenyon (76). Salts of 4, 5-diaminoeicosane with these acids and with (+)-tartaric, (+)-lactic, and (-)-malic acids were prepared and studied in several solvent systems. None of these experiments yielded a crystalline salt of the amine.

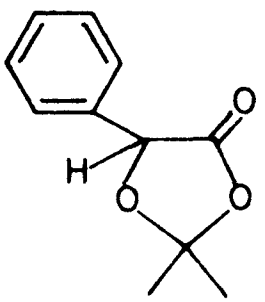
(-)-2, 2-Dimethyl-5-phenyl-1, 3-dioxolan-4-one was prepared by the reactions of (-)-mandelic acid with acetone (77). The product was recrystallized from absolute ethanol giving fine, colorless crystals. $[\alpha]_D^{25} = -95.3°$ (c = 2.2 in methanol). Freudenberg et al. (78) state that $[\alpha]_{578}^{18} = -99°$ (in methanol) for this compound. 4, 5-Diaminoeicosane (1.0 g.) was dissolved in 10 ml. of absolute methanol and 2.46 g. (4 mol. equiv.) of (-)-2, 2-dimethyl-5-phenyl-1, 3-dioxolan-4-one added. The solution was then stirred at 40° for 24 hours. The solvent was then evaporated and the residue chromatographed on silicic acid. The nuclear magnetic resonance spectra of the products were then examined. The products consisted of unreacted starting materials and polymeric material. None of the mandelamides of 4, 5-diaminoeicosane were observed.

DISCUSSION

The amount of necrosamine available without a very extensive isolation program was rather limited and for this reason the method used to determine the nature of the terminal propyl group had to be one which would not require more than approximately 20 mg. of the amine. The method used successfully involved oxidation of the necrosamine to butyric and palmitic acids. The aqueous solution of these acids was then distilled and a small volume of distillate collected. Butyric acid (and isobutyric acid) forms a low boiling azeotrope with water and therefore the initial distillate contained any of this acid present in the solution. This solution was studied using a gas chromatograph equipped with a flame ionization detector. The flame ionization detector is almost completely insensitive to water while extremely sensitive to most organic compounds. The oxidation of necrosamine gave n-butyric acid proving that the carbon skeleton of this amine is not branched.

Necrosamine is one of the isomers of the 4, 5-diaminoeicosane forming an ether insoluble dihydrochloride and for this reason several attempts were made to resolve this synthetic base by means of the formation of salts with optically active acids and

separation of the salts by crystallization. In addition an attempt was made to prepare the diamide of 4, 5-diaminoeicosane and (-)-mandelic acid by reaction of (-)-2, 2-dimethyl-5-phenyl-1, 3-dioxolan-4-one (XXIV) with the amine.



XXIV

Freudenberg et al. (78) found that this method of preparing optically active amides of mandelic acid was easily carried out and resulted in less racemization of the acid than previous methods. None of the attempted resolutions of 4, 5-diaminoeicosane were successful.

The formation of complexes of 4, 5-diaminoeicosane with metal ions was studied since such a complex with necrosamine might enhance a very low rotation present but unobserved in this compound. Complexes of 4, 5-diaminoeicosane with cupric salts were made but these absorb strongly in the region of the sodium D line and could not be used. A compound formed by reacting 4, 5-diaminoeicosane with potassium tetrachloroplatinate was prepared but the low yield and the difficulties of purification prevented its being used for this purpose.

The thiourea of necrosamine was prepared and its optical rotation taken in chloroform. It was thought that the thiocarbonyl group would enhance the rotation of the diamine but this was not the case.

It seems likely that the groups about the two asymmetric carbon atoms in necrosamine are arranged in an erythro configuration (73) and that the contributions of the n-propyl and the n-pentadecyl groups are very nearly equal. This quasi-meso configuration could be disturbed by reaction at one of the amine groups. This presents an additional problem. Since the reactivities of the two amino groups are probably very nearly the same any such reaction should give two products representing substitution at each of these sites. For example benzoylation of necrosamine with one mole of benzoyl chloride should give two benzoylnecrosamines. Both of these should have significant specific rotations. The small amount of necrosamine available at this time is not sufficient for the completion of this experiment.

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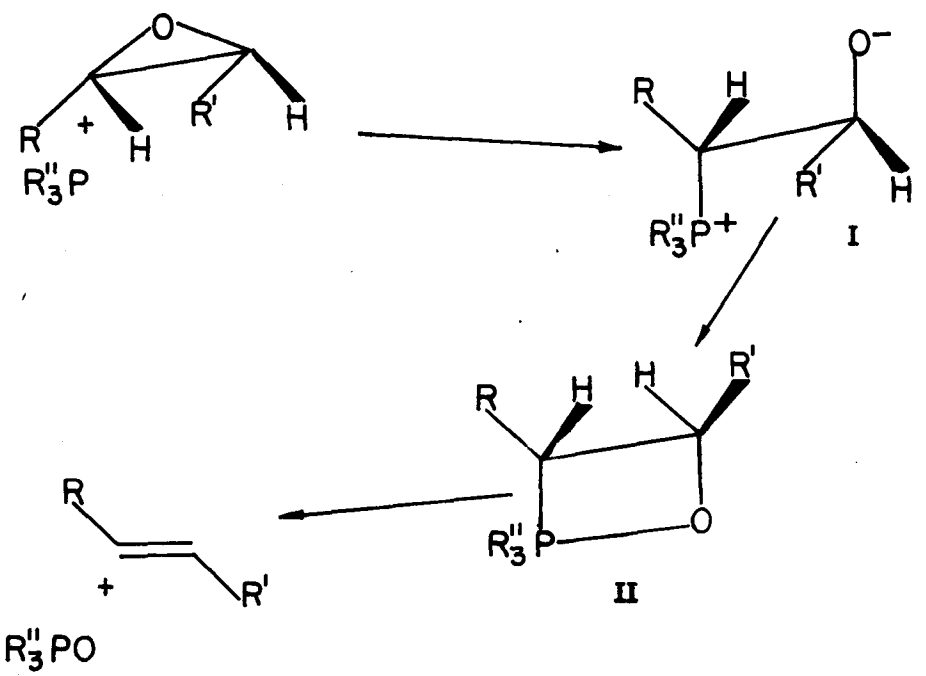
PROPOSITIONS

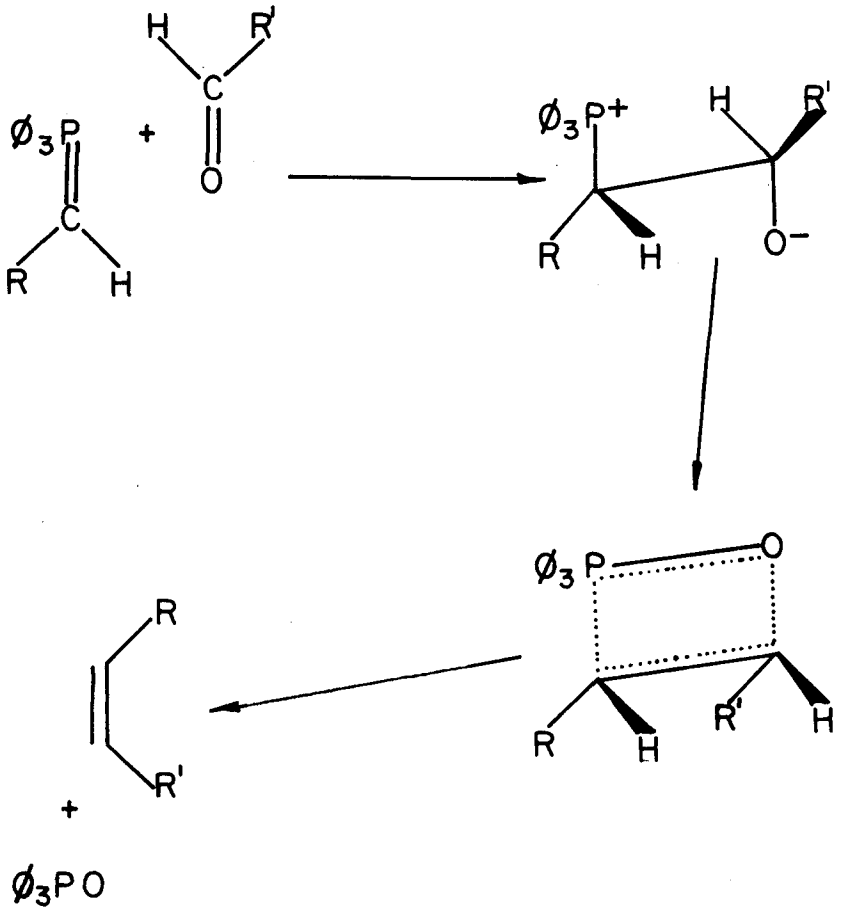
PROPOSITION I

Epoxides are smoothly reduced to olefins by triphenyl- or tributylphosphine (1, 2) or by triethylphosphite (3). Boskin and Denney (2), studying the deoxygenation of the 2-butene epoxides at 150° with tributylphosphine, found that trans-2-butene epoxide yielded 72% cis-2-butene and 28% trans-2-butene while cis-2-butene epoxide gave 81% trans-2-butene and 19% cis-2-butene. The mechanism proposed (1, 3) for this reaction involves nucleophilic displacement of the oxygen by the phosphine to give the betaine I, followed by rotation and cyclization to give II. Cleavage of II



gives the olefin. This mechanism accounts for the major product of the reaction but not for the minor component.

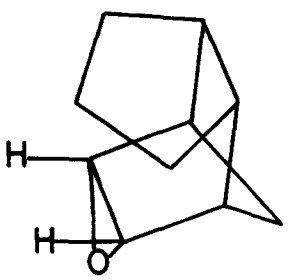
The betaine (I) is identical with one of the intermediates proposed for the Wittig reaction (4). However, in the Wittig



reaction additional factors, such as the orientation of the starting materials, are involved in determining the stereochemistry of the products.

The following two part study of the reduction of epoxides by trialkylphosphines is proposed:

1. The reaction of a trialkylphosphine with exo-2, 3-epoxy-endo-5, 6-cyclopentanonorbornane (III). Cadogan (5) has

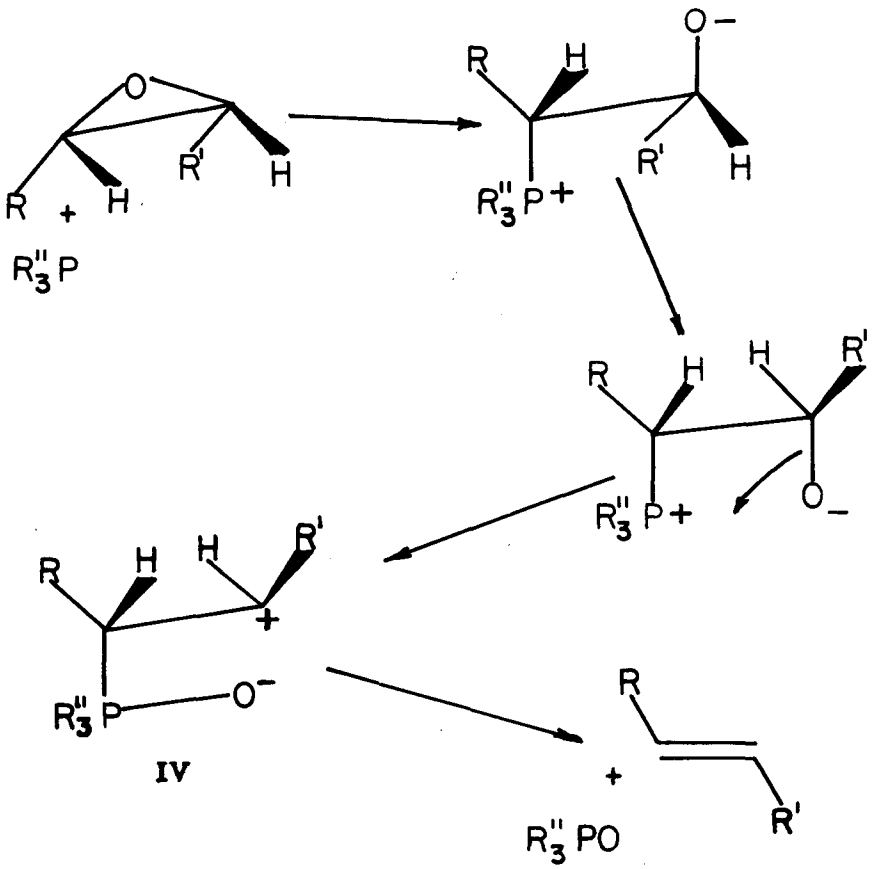


III

stated that, in order to account for the minor product observed in the reaction of epoxides with trialkylphosphines, attack of phosphorus directly on oxygen is required. Nucleophilic displacement of the oxygen by the phosphine would be prevented in III by steric hindrance while direct attack at the oxygen could occur. If both products of the reaction are derived from reactions of the betaine (I), no olefin would be expected from III. exo-2, 3-Epoxy-endo-5, 6-cyclopentanonorbornane (III) would be the expected product of the reaction of endo-1, 2-dihydrodicyclopentadiene (6) with perbenzoic acid.

2. The reaction of a trialkylphosphine with cis- and trans-1, 2-dideuterioethylene epoxides. Previous discussions of the

mechanism of this reaction have implied a concerted elimination of the trialkylphosphine oxide from the betaine. However, a two step elimination involving an intermediate such as IV may be



considered. If this intermediate persists long enough, rotation about the central carbon-carbon bond may occur giving the minor product. The presence of an intermediate such as IV could be tested by the reaction of a trialkylphosphine with <u>cis</u>- and <u>trans</u>-1, 2-dideuterioethylene epoxide. In this case the intermediate ion IV

would be a primary carbonium ion and would be less stable than the corresponding intermediate in the reduction of the 2-butene epoxides. The resulting olefins should thus favor the major product to a greater degree than is observed in the case of the 2-butene epoxides.

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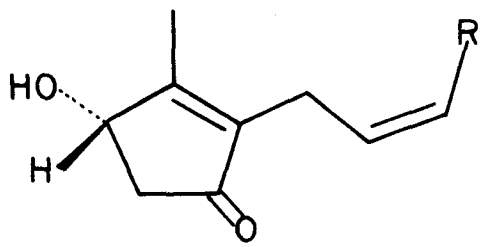
PROPOSITION II

Hydrolysis of the natural pyrethrins, four esters isolated from Chrysanthemum cinerariaefolium Vis., has yielded the keto alcohols pyrethrolone (Ia) and cinerolone (Ib) (1). One isoprene



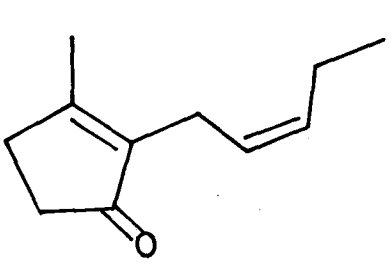
Ia R = $CH{=}CH_2$

Ib R = CH_3

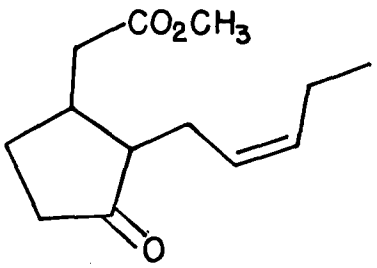
unit may be formally dissected from these compounds. However, Crowley et al. (2, 3), investigating the biosynthesis of the pyrethrins, found that 2-^{14}C-mevalonic acid was not incorporated into pyrethrolone. Feeding experiments were also carried out with 2-^{14}C-acetate and, as might be expected, this unit was incorporated into pyrethrolone. Degradation experiments to determine the pattern of labeling by the acetate have not been performed.

The pyrethrin keto alcohols are closely related to jasmone (IIa) (4), a constituent of the oil of Jasminum grandiflorum L.

Methyl jasmonate (IIb) (5) is another constituent recently isolated from the same source.

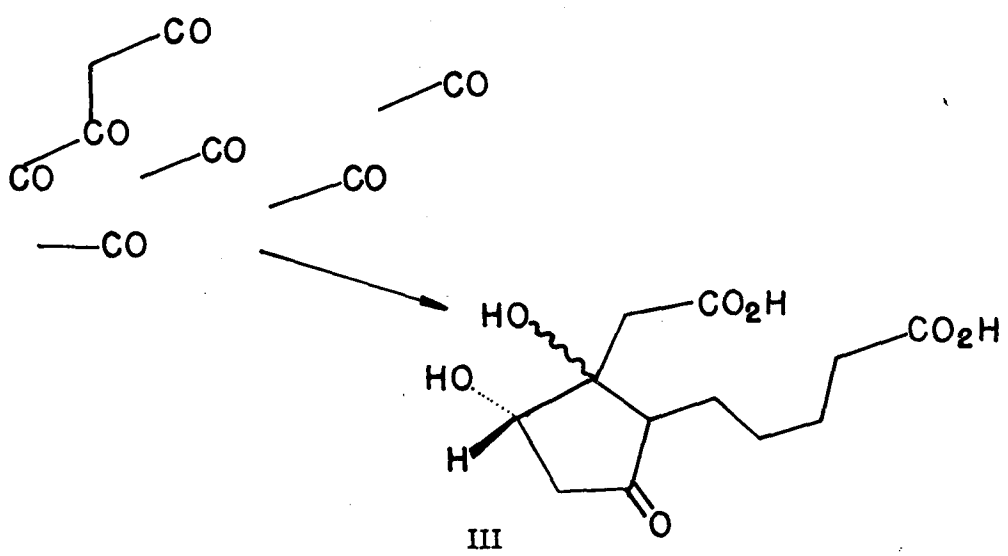


IIa



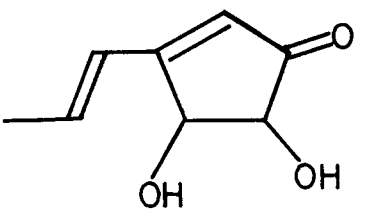
IIb

It is proposed that the biogenesis of these compounds be studied by means of feeding experiments with labeled 2-^{14}C-acetic and 2-^{14}C-oxaloacetic acids and by degradation studies to determine the position of the labeling. The condensation of oxaloacetic acid with acetate units would provide an intermediate (III) from which these ketones could be easily derived.

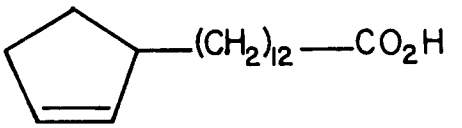


III

It should be noted that the cyclopentane derivatives terrein (IV) and chaulmoogric acid (V) do not fit into this scheme



IV



V

since they would necessitate oxidative loss of the acetic acid side chain followed by several steps of oxidation or reduction.

Richards and Hendrickson (6) have suggested that the biosynthesis of these cyclopentane compounds involves intramolecular oxidative coupling of a polyacetate chain. This view has the advantage that it involves only the well known biosynthetic precursor, acetate. The experiments proposed would distinguish between these possible biosynthetic methods.

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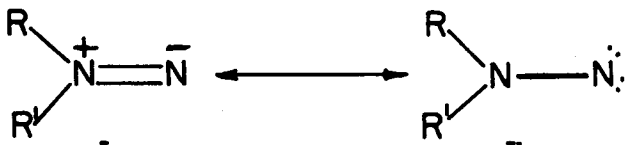
PROPOSITION III

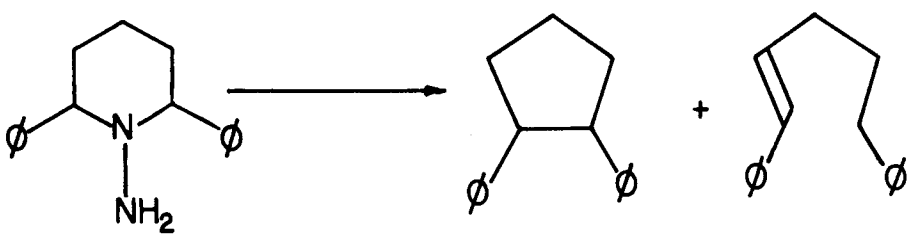
Azamines (I) occur as intermediates in the oxidation of



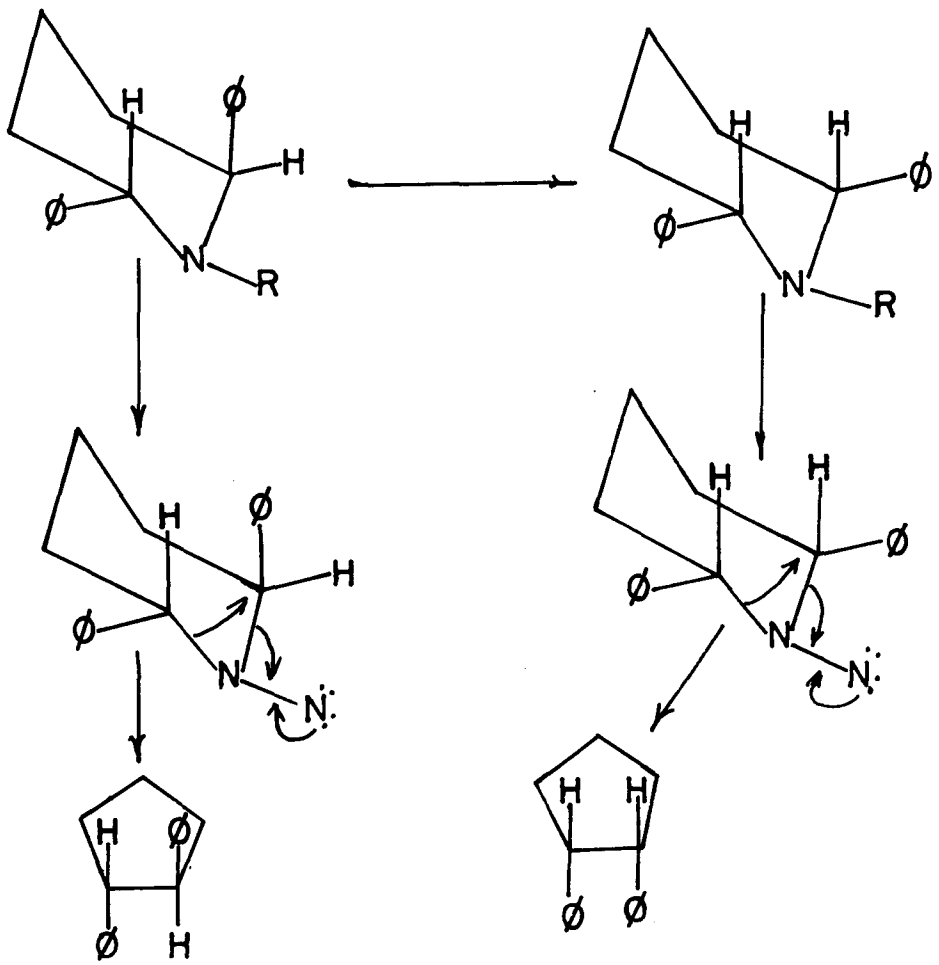
Ia Ib

1, 1-disubstituted hydrazines (1), the reduction of nitrosoamines in base (2), the alkaline degradation of sulfonhydrazides (1b, 3, 4), and the reaction of difluoramine (HNF_2) with secondary amines (5). An important reaction of this intermediate is the elimination of nitrogen with coupling of the substituants. It is proposed that the mechanism of the decomposition of azamines be studied.

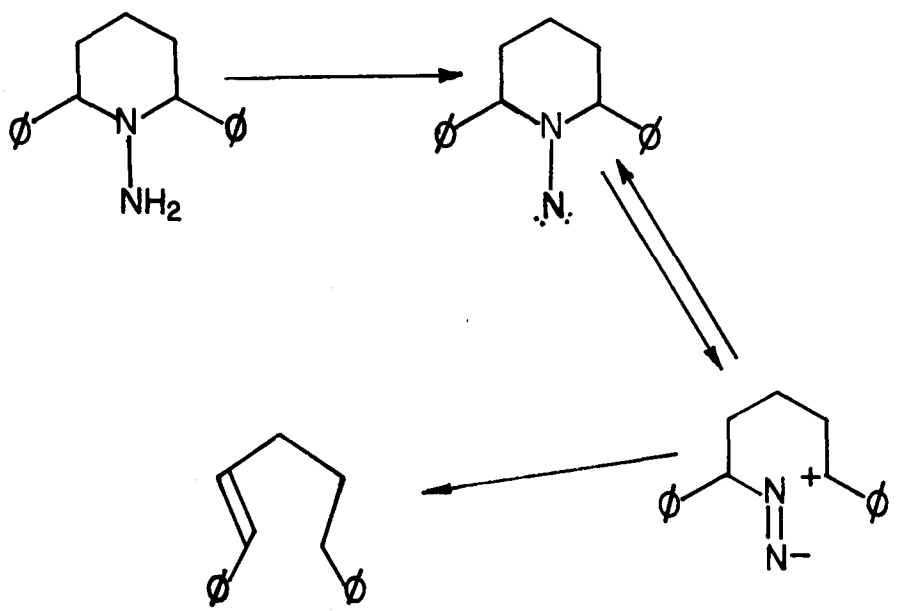
Overberger et al. (2) investigated the mercuric oxide oxidation of cis- and trans-N-amino-2, 6-diphenylpiperidine. The cis- isomer lead to cis-1, 2-diphenylcyclopentane while the



trans-n-amino-2, 6-diphenylpiperidine gave a mixture of cis- and trans-1, 2-diphenylcyclopentane with the trans-isomer predominating. Similar results were obtained from the reduction of cis- and trans-N-nitroso-2, 6-diphenylpiperidine with sodium hydrosulfite in base or with lithium in ammonia. A by-product in each of these reactions was 1, 5-diphenyl-1-pentene (2, 6). It has been proposed (2, 7) that the reaction of the azamine intermediate to give diphenylcyclopentane is a concerted process.

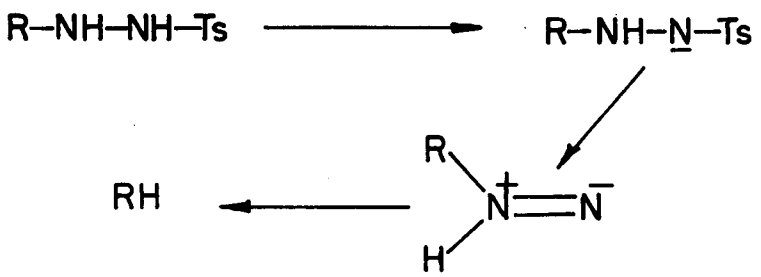


The small amount of cis-1, 2-diphenylcyclopentane produced in the reaction of the trans- compounds is thought to be due to isomerization of the reactant under the conditions of the reaction to the more stable cis- isomer. An ionic decomposition was suggested to account for the acyclic olefin.

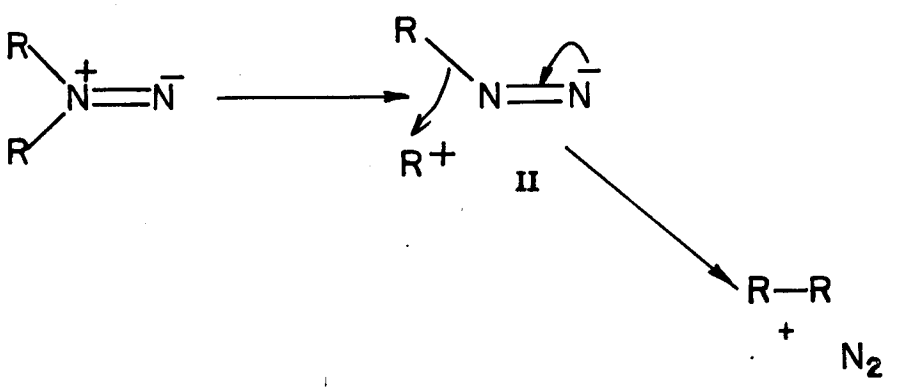


This proposed mechanism may be studied by the reaction of cis- and trans-2, 6-diphenylpiperidine with difluoramine. The conditions of this reaction are such that isomerization of the amine would be very unlikely. However, unreacted amine could be isolated and examined to determine whether or not racemization had occurred.

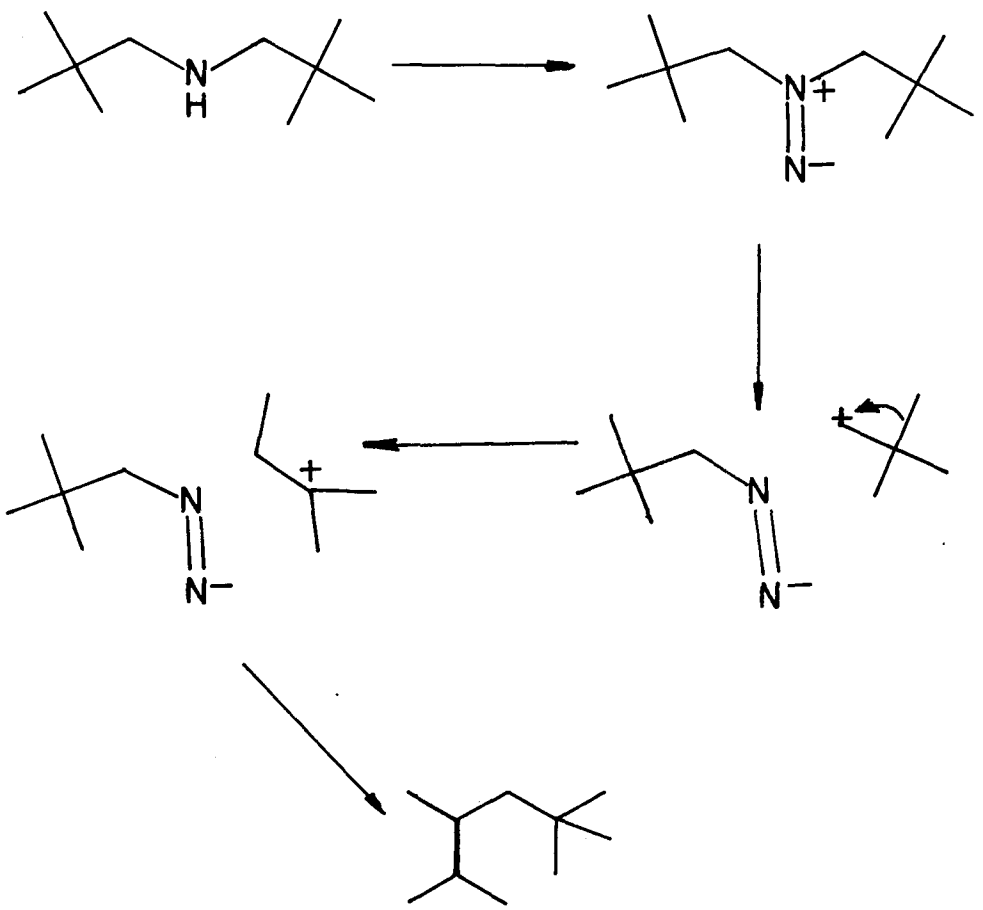
The observed lack of complete retention of configuration in the N-amino- or N-nitroso-2, 6-diphenylpiperidines may also be explained by an ionic decomposition of the azamine. This is supported by the results of Cram and Bradshaw (4) who suggest an azamine intermediate in the cleavage of (+)-2-phenyl-2-butylhydrazine-p-toluenesulfonamide in concentrated aqueous base. The reaction proceeds with 70-77% net retention of configuration rather than the complete retention of configuration a concerted mechanism would suggest.



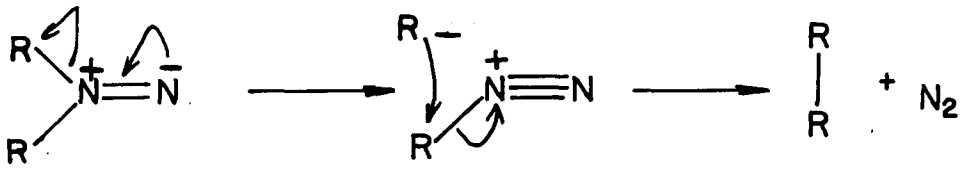
The ionic decomposition of azamines by way of an intermediate such as II could be confirmed by the reaction of



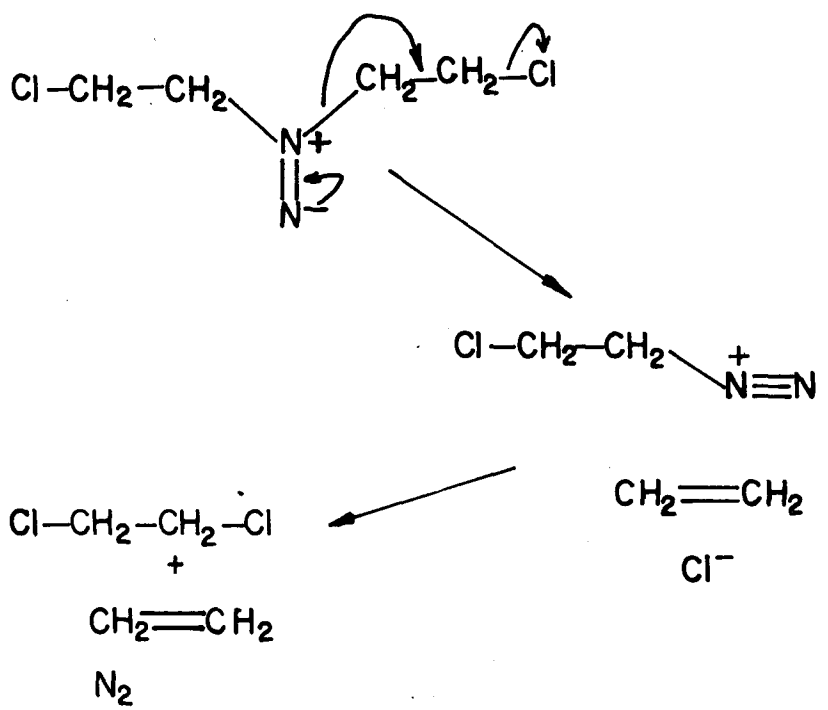
dineopentylamine with difluoramine. A carbonium ion formed in the reaction of this compound would rearrange with the migration of a methyl group to give 2, 2, 4, 5-tetramethylhexane rather than the 2, 2, 5, 5-tetramethylhexane expected in a concerted or radical process.



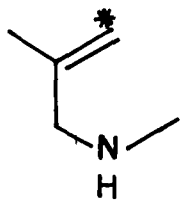
If the azamine intermediate in this reaction decomposed to give a carbanion and diazonium cation, rearrangement might also occur providing the rate of decomposition of the neopentyl



diazonium cation were greater than the rate of attack on this ion by the carbanion. However, decomposition of the azamine to give a carbanion and a diazonium cation could be considered unlikely if the reaction of 2, 2′-dichlorodiethylamine with difluoramine did not yield ethylene.



The elimination of nitrogen from azamines with the formation of radicals has been suggested (3, 7). Providing the reactions suggested above do not indicate that ionic intermediates are involved, this possibility could be tested by the reaction of 1-^{14}C-4-aza-2-methyl-1-pentene (III) with difluoramine.



III

Randomization of the label between carbons 1 and 3 of the resulting 2-methyl-1-butene would suggest a radical process.

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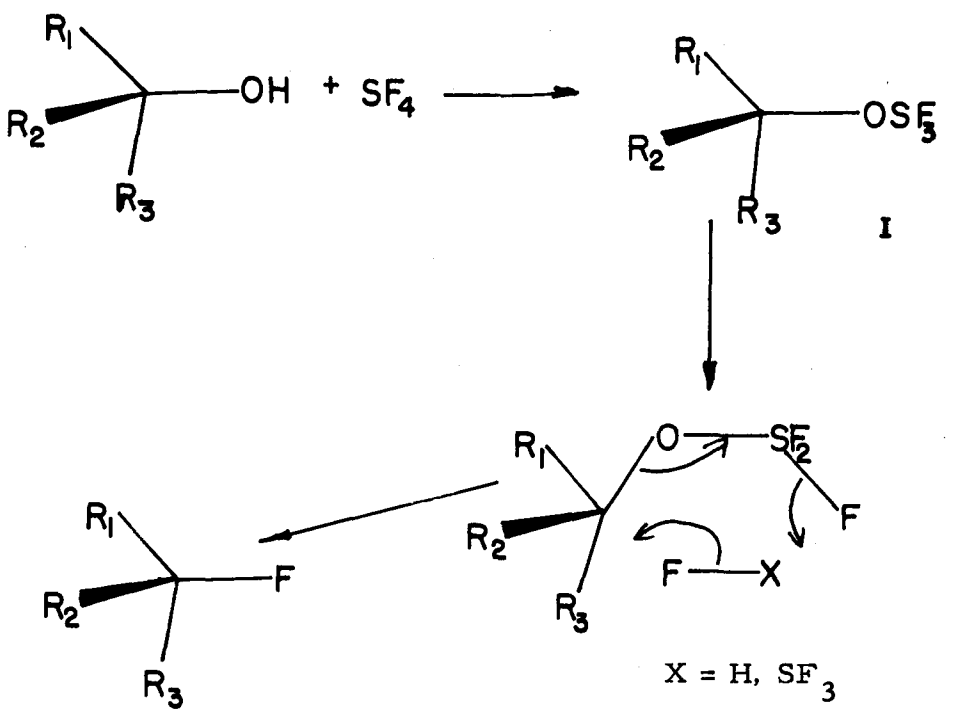
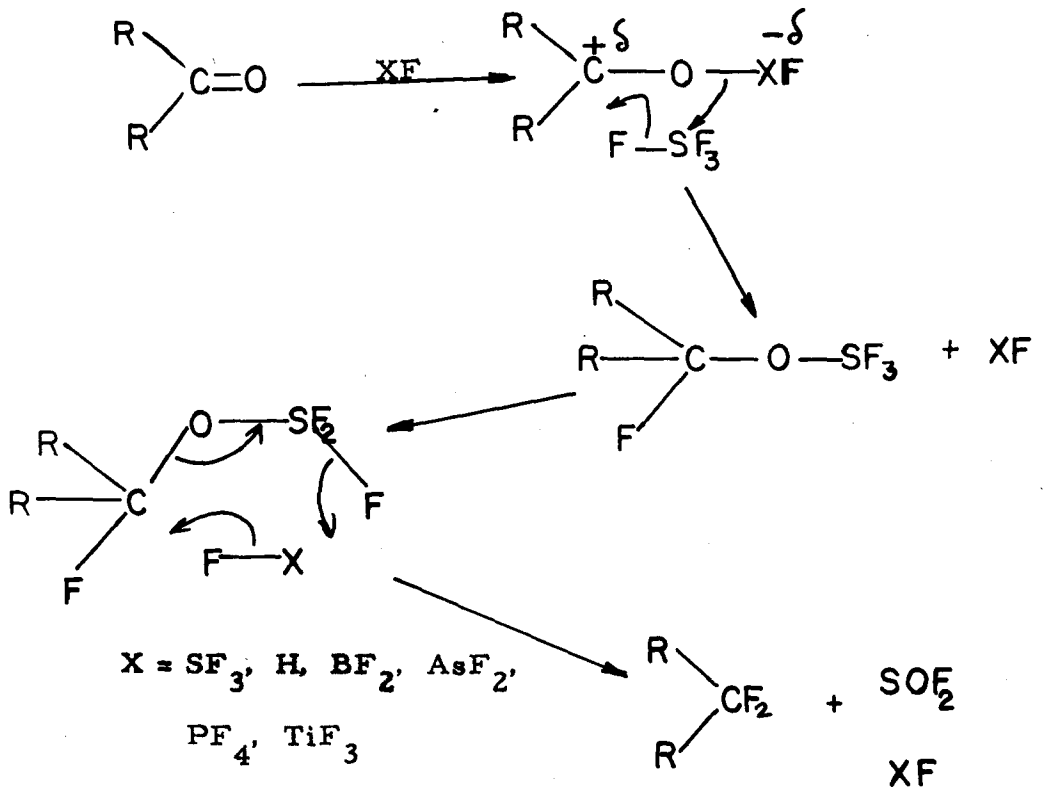
PROPOSITION IV

Sulfur tetrafluoride reacts with alcohols to yield the corresponding fluorides (1). It is proposed that the stereochemical course of this reaction and the effect of added hydrogen fluoride on the rate and stereochemistry of the reaction be studied.

Smith (1) has proposed that this reaction takes place with the elimination of hydrogen fluoride to give the intermediate $ROSF_3$. This compound is thought to react with sulfur tetrafluoride or hydrogen fluoride to give the product. Retention of configuration is implied by this mechanism.



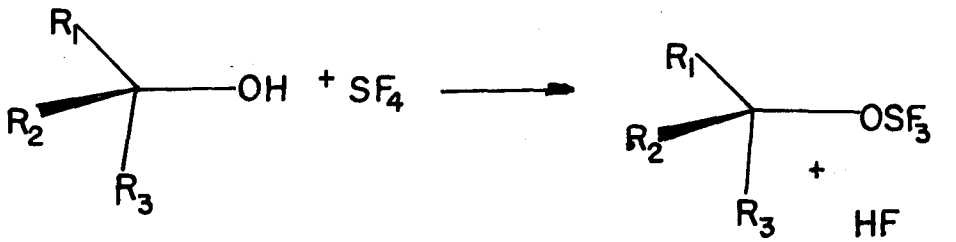
This reaction is analogous to the reaction of sulfur tetrafluoride with ketones for which the following mechanism has been proposed (2):

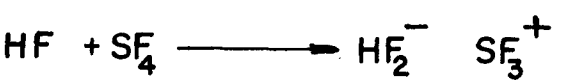


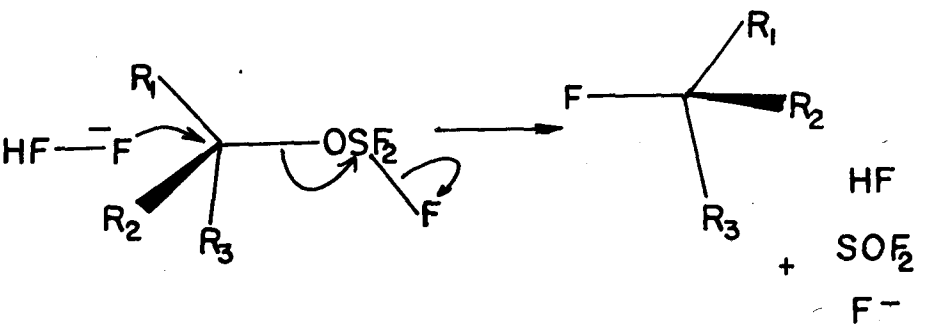
As noted this reaction is catalyzed by HF, BF_3, and other fluorides.

Sulfur tetrafluoride forms adducts with a number of Lewis acids (3). These were initially suggested (4) to be simple donor-acceptor complexes: $SF_4 \rightarrow XF_n$. More recently evidence has been presented (5) in favor of the view that they are ionic compounds of the type $SF_3^+XF_{n+1}^-$.

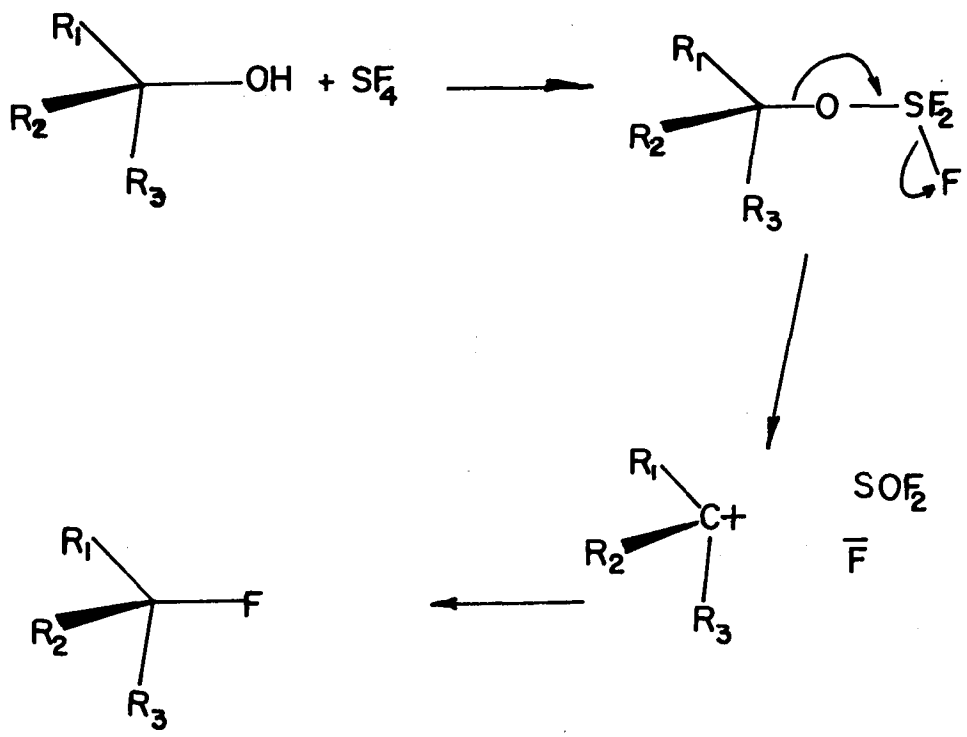
Since this is the case the possibility that fluorine is donated by HF^-_2 must be considered in the reaction of alcohols with sulfur tetrafluoride. If this were to take place the resulting fluoride should be produced with inversion of configuration.







The mechanism initially proposed by Smith (1) for the reaction of alcohols with sulfur tetrafluoride is based on the related reaction of ketones. The catalysis suggested has not been demonstrated. For this reason the decomposition of I to give an ion pair should also be considered.



In this case retention of configuration would be expected and hydrogen fluoride added to the system should not effect the rate.

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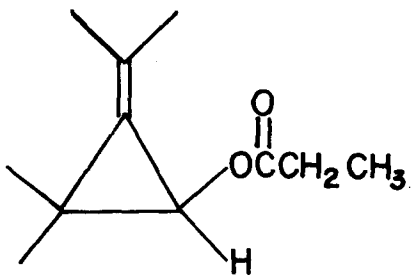
PROPOSITION V

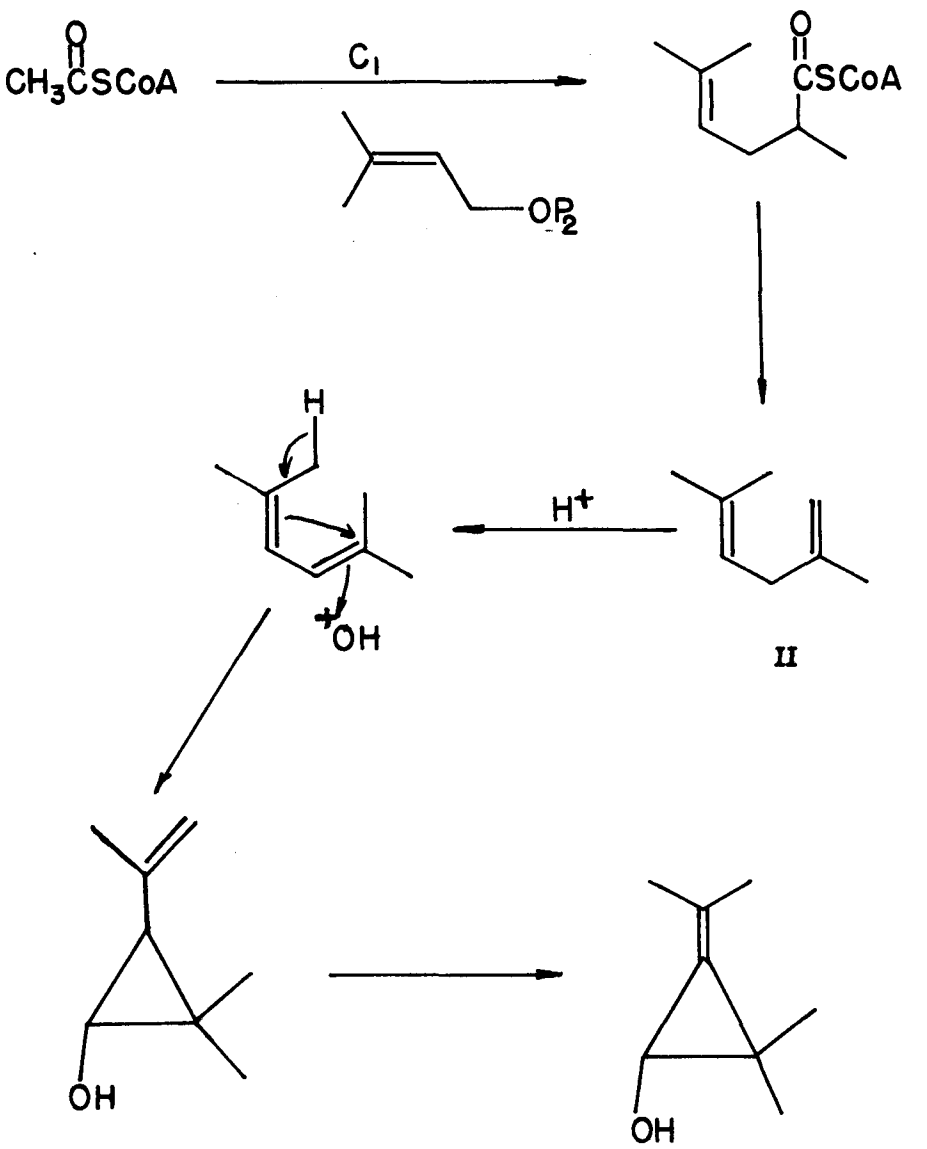
The virgin female of the American cockroach, Periplaneta americana (L.), emits a powerful attractant (for males of that species) which has been shown to have the structure I (1).



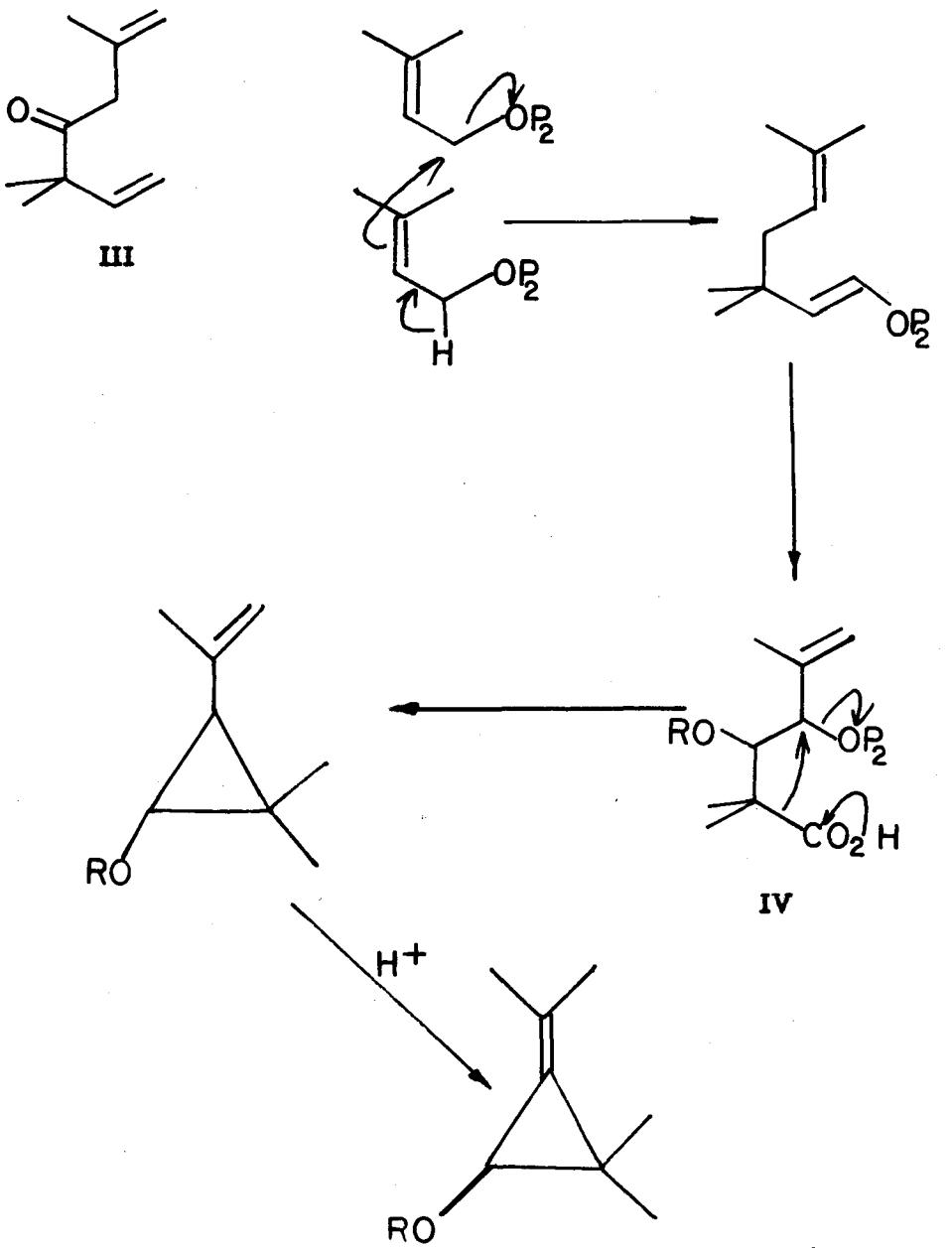
I

It is proposed that the biogenesis of this compound be studied by means of feeding experiments with 2-^{14}C-mevalonic acid and 2-^{14}C-acetate.

The attractant may result by substitution on a single acetate unit by a methyl and a 3, 3-dimethylallyl group. Reduction of the resulting acid and elimination of the elements of water would yield the diene II. Cyclization of this compound would lead to the ring system of the attractant.



Inspection of the structure of the attractant suggests that it could be derived from two isoprene units with the loss of two carbons. Condensation of the isoprene units in the manner observed in the artemisia ketone (III) (2) and oxidation of the product would lead to IV. Decarboxylation and cyclization of this intermediate would lead to the ring system of the attractant.

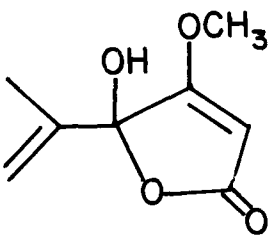


III

IV

The fact that an isoprene unit may be formally dissected from the molecule need not indicate that the compound is derived by way of mevalonic acid. Birch et al. (3) have noted that

penicillic acid (V) incorporates labeled mevalonic acid only very slightly and in this material the label is completely randomized.



V

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