PHYTOCHEMICAL INVESTIGATION OF
BOCCONIA ARBOREA AND *BOCCONIA FRUTESCENS*
FAMILY (PAPAVERACEAE)

by

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Professor of Medicinal Chemistry

Jan. 15, 1972
Date

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ABSTRACT

Anti-tumor tests conducted by the National Institute of Health, Cancer Chemotherapy National Service Center, showed that the anti-tumor activity resided in the defatted methanol fraction of the leaves and stems of Bocconia arborea and Bocconia frutescens family Papaveraceae.

Alumina column chromatography and thin-layer chromatography resulted in the isolation of nine alkaloids: dihydrosanguinarine, dihydrochelerythrine, oxysanguinarine, sanguinarine, oxychelerythrine, chelerythrine, 11-methoxychelerythrine, protopine and allocryptopine. Identification of each compound was determined by instrumental analysis, comparative thin-layer chromatography, mixed melting points and other analytical procedures.

Testing of the isolated alkaloids indicated that sanguinarine and protopine showed activity in the *in vitro* 9KB tissue culture, while chelerythrine showed activity in a live tumor system, 5WM (Walker Intramuscular).

A new derivative of dihydrochelerythrine was synthesized and identified as 11-N-propoxychelerythrine based on mass spectrometry and nuclear magnetic resonance.

CHAPTER 1

INTRODUCTION

In the continuing search for potential anti-tumor agents from plant sources, extracts of *Bocconia arborea* S. Wats. and *Bocconia frutescens* L. family Papaveraceae demonstrated tumor inhibitory properties in two biological screening systems of the Cancer Chemotherapy National Service Center. Both plants showed activity in the *in vitro* 9KB (Adenocarcinoma of the Nasopharynx) and the *in vivo* 5WM (Walker Intramuscular 256) test systems. The purpose of this investigation was to isolate and identify those agents responsible for the anti-tumor activity.

Occurrence and Description of the Plants

The genus *Bocconia* consists of shrubs or small trees with yellow or reddish juice. The stems are simple or sparsely branched, and the leaves are often very large. The small flowers are in large panicles. The small capsules are stipitate, dehiscent to the base, and contain one or more seeds (Standley 1922, p. 299).

Bocconia arborea is found in the Mexican states of Durango and Sinaloa south to Puebla and Oaxaca and also occurs naturally in Guatemala (Standley 1922,

p. 300). The material used in this investigation was collected 40 miles south of Tepic, Nayarit at the edge of the lava flow which crosses Mexican Highway 15. Confirmation of the plant's identity was made by Dr. Charles Mason, Jr., Director of The University of Arizona Herbarium, and a voucher specimen (#163199) was deposited in the Herbarium (Fig. 1).

Bocconia arborea is a tree 15-25 feet high and up to 2 feet in diameter. The leaves are ovate to oblong-lanceolate and deeply pinnatifid. They are glabrous above and either pale and tomentose or glabrous below. The bark is deeply cracked and corky (Standley 1922, p. 300).

Bocconia frutescens is found in the Mexican states of Tamaulipas, San Luis Potosi and Veracruz. It is distributed throughout Central America to Peru and also found in the West Indies (Standley 1922, p. 300). The material used in this study was collected at El Salto, 35 miles south of Morelia in the State of Michoacan. Confirmation was made by Dr. Mason, and a voucher specimen (#166630) was deposited in the Herbarium (Fig. 2).

Bocconia frutescens is usually a shrub or tree, 1 to 7.5 meters high. The bark is smooth and pale and the leaves range from 12 to over 40 cm in length. The leaves are petiolate and usually with numerous lobes.

Fig. 1. Herbarium Specimen of Bocconia arborea

Fig. 2. Herbarium Specimen of Bocconia frutescens

They are tomentose or sometimes glaucous beneath. The flowers are purplish and the fruit is usually glaucous (Standley 1922, p. 300).

CHAPTER 2

ISOLATION OF PLANT CONSTITUENTS

A review of the literature concerning the two species of the genus *Bocconia* indicated the presence of numerous alkaloids. Prior to this investigation *Bocconia arborea* was reported to contain the alkaloids chelerythrine, protopine, allocryptopine and several unidentified bases (Manske 1943). In work published after the completion of this study, these bases were identified (MacLean et al. 1969) and agree with this investigator's findings.

Bocconia frutescens has been reported to contain the alkaloids chelerythrine, protopine, allocryptopine and sanguinarine (Tani and Takao 1967).

Preliminary Biological Tests and Evaluation

The preliminary biological testing (Tables 1 and 2) showed that the defatted methanol extract of the dried leaves and stems was the most active fraction in both species.

On the basis of the preliminary test results, large samples of the defatted methanol extract were prepared for isolation and identification of the active components.

Table 1

Activity of Bocconia arborea in 9KB

Activity is defined as ED_{50} = 10 mcg/ml for plant extracts. Results are expressed as the dose that inhibits growth to 50% of control growth 3 days after drug addition (Cancer Chemotherapy Reports 1962)

Extract	mcg/ml Dilution
Petroleum Ether	2.2×10^1 mcg/ml
Petroleum Ether, Methanol	8.4×10^0 mcg/ml

Table 2

Activity of Bocconia frutescens in 5WM

Activity is defined as a percent T/C (Test/Control) of less than 60 in a satisfactory dose response test (Cancer Chemotherapy Reports 1962)

Extract	%(Test/Control)	Dose mg/kg
Petroleum Ether	102%	225
	92%	150
	89%	100
	97%	50
Petroleum Ether, Methanol	17%	225
	44%	150
	57%	100
	73%	50

Extraction of the Plants

Each species was processed separately in the following manner. Ten kilograms of dried leaves and stems were ground in a No. 2 Wiley Mill through a 3 mm mesh screen and placed in a large canvas bag. The bag and its contents were placed in a one gallon capacity Lloyd type extractor (Brighton Copper Works). The plant material was extracted continuously with 24 liters of petroleum ether until evaporation of a small amount which had passed through the plant material left no residue. This extraction was carried out for 38 hours. The petroleum ether extract was removed from the Lloyd extractor and evaporated. The marc was removed and dried. The marc was repacked in the Lloyd extractor and extracted for 46 hours with purified methanol. The exhausted marc was then discarded and the methanol extract removed from the Lloyd extractor and evaporated in an air stream. The yield of *Bocconia arborea* was 156 g, and the yield of *Bocconia frutescens* was 149 g.

Thin-Layer Chromatography of the Methanol Fraction

The methanol extract of each species was spotted on a 5 x 20 cm thin-layer chromatography plate 0.25 mm thick. The absorbant used was Silica Gel G (Merck). Ascending chromatography was employed using solvent system I (benzene, chloroform, methanol, 75:25:0.2). The plate was first examined under ultraviolet light and then developed with Dragendorff's reagent for the detection of alkaloids and other nitrogen-containing compounds. Nine possible alkaloids were detected in each species. A mixture of the methanol extracts of each species was compared on thin-layer chromatography using solvent system I. Inspection under ultraviolet light and development with Dragendorff's reagent showed that both species contained the same materials (Fig. 3).

The defatted methanol extract of Bocconia frutescens was used for further fractionation.

Alumina Neutral Column

In order to obtain sufficient quantity of each possible alkaloid for biological testing, a procedure involving a column chromatography separation was employed. A 100 mm glass column 1.5 m in length was packed with alumina neutral activity III (6% water). Four kilograms of alumina neutral activity I were shaken for six hours with 240 ml of distilled water. A small cotton plug was inserted in the bottom of the column and held in place with a long metal rod. Enough alumina was added to hold the plug in place so that the rod could be removed. Approximately 2 liters of benzene were added and the teflon stopcock was opened to drip at a moderate rate. The remainder of the alumina was added slowly until the 4 kg

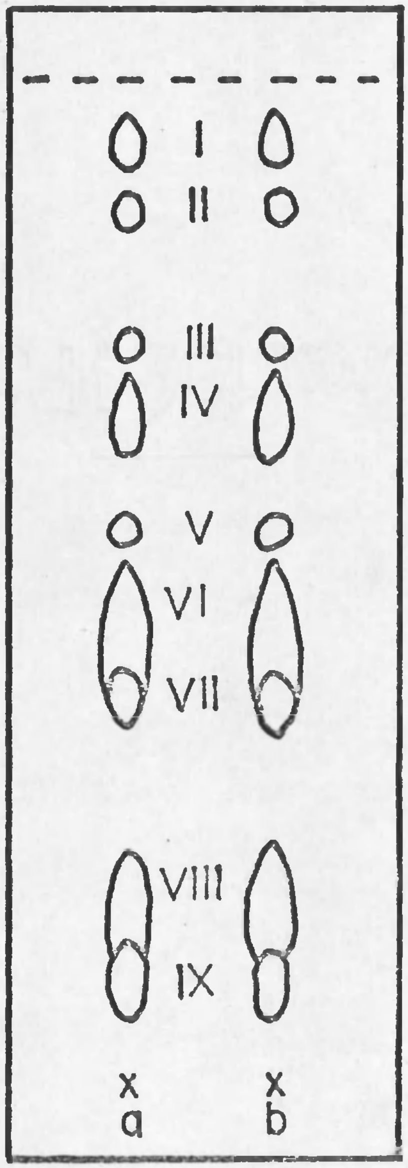

Fig. 3. Thin-Layer Chromatography of the Defatted Methanol Extract

(a) Bocconia arborea, (b) Bocconia frutescens were packed in the column. Several pieces of filter paper were placed on top of the alumina to provide a flat surface between the top of the alumina and extract. A small head of solvent still remained above the alumina.

Eighty grams of the defatted methanol extract of *Bocconia frutescens* was dissolved in a mixture of methanol and chloroform. This solution was slowly added to 250 g of alumina and evaporated *in vacuo*. The combination of extract and alumina was carefully placed on the column with the stopcock open to aid in packing the column evenly. Before more solvent was added, several more pieces of filter paper were placed in the column to prevent disturbance of the alumina absorbed extract mixture. The column was then filled with benzene in preparation for the collection of fractions (Fig. 4).

Fractions of 750 ml were collected and subjected to thin-layer chromatography for comparison with the original extracts. Sixteen benzene fractions were collected and applied to thin-layer chromatography using solvent system I and were revealed with Dragendorff's reagent. The solvent was then changed to chloroform and 18 more fractions were collected and subjected to thin-layer chromatography as indicated above. The fractions were combined in groups according to their thin-layer chromatography picture (Fig. 5).

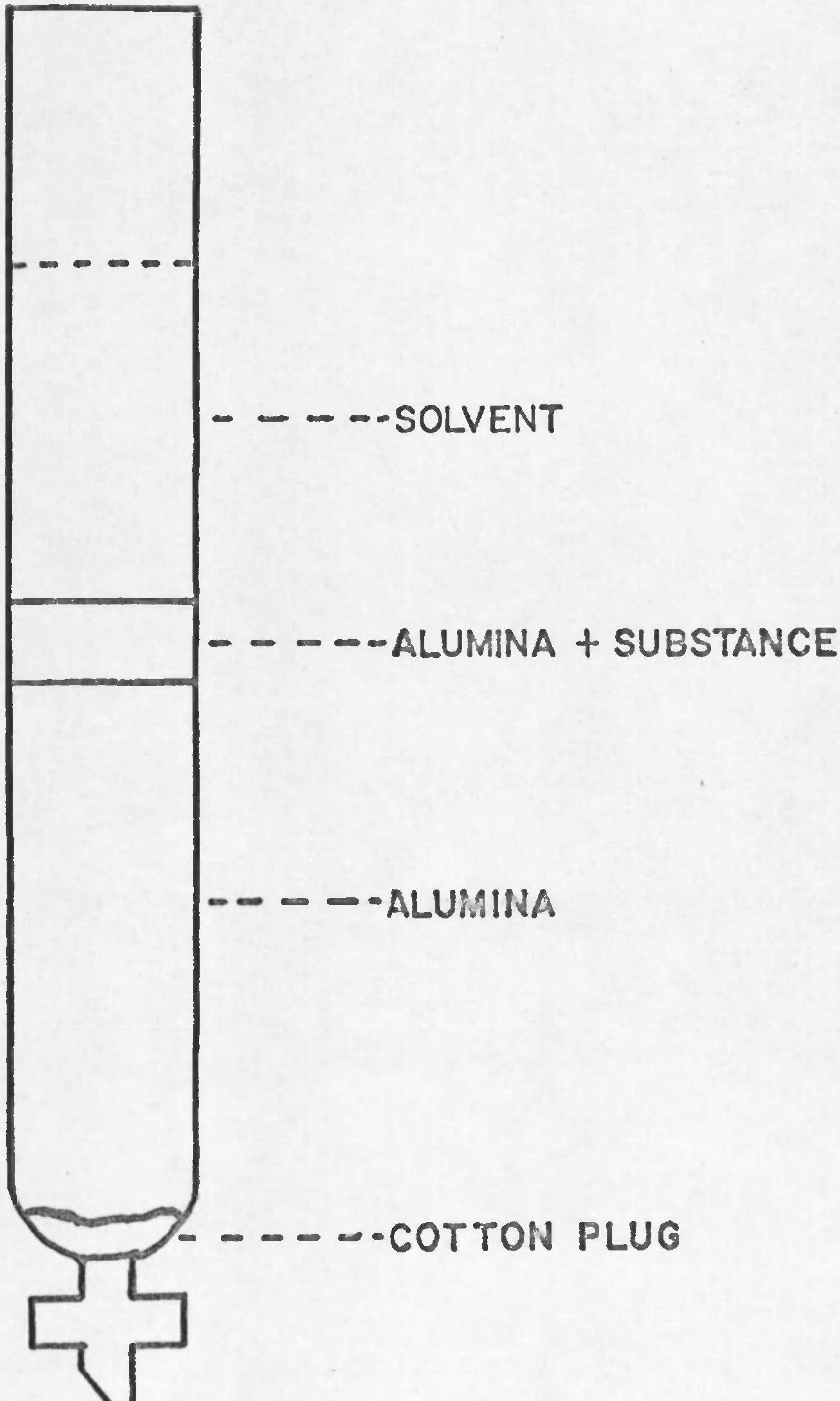

Fig. 4. Diagram of Packed Alumina Column

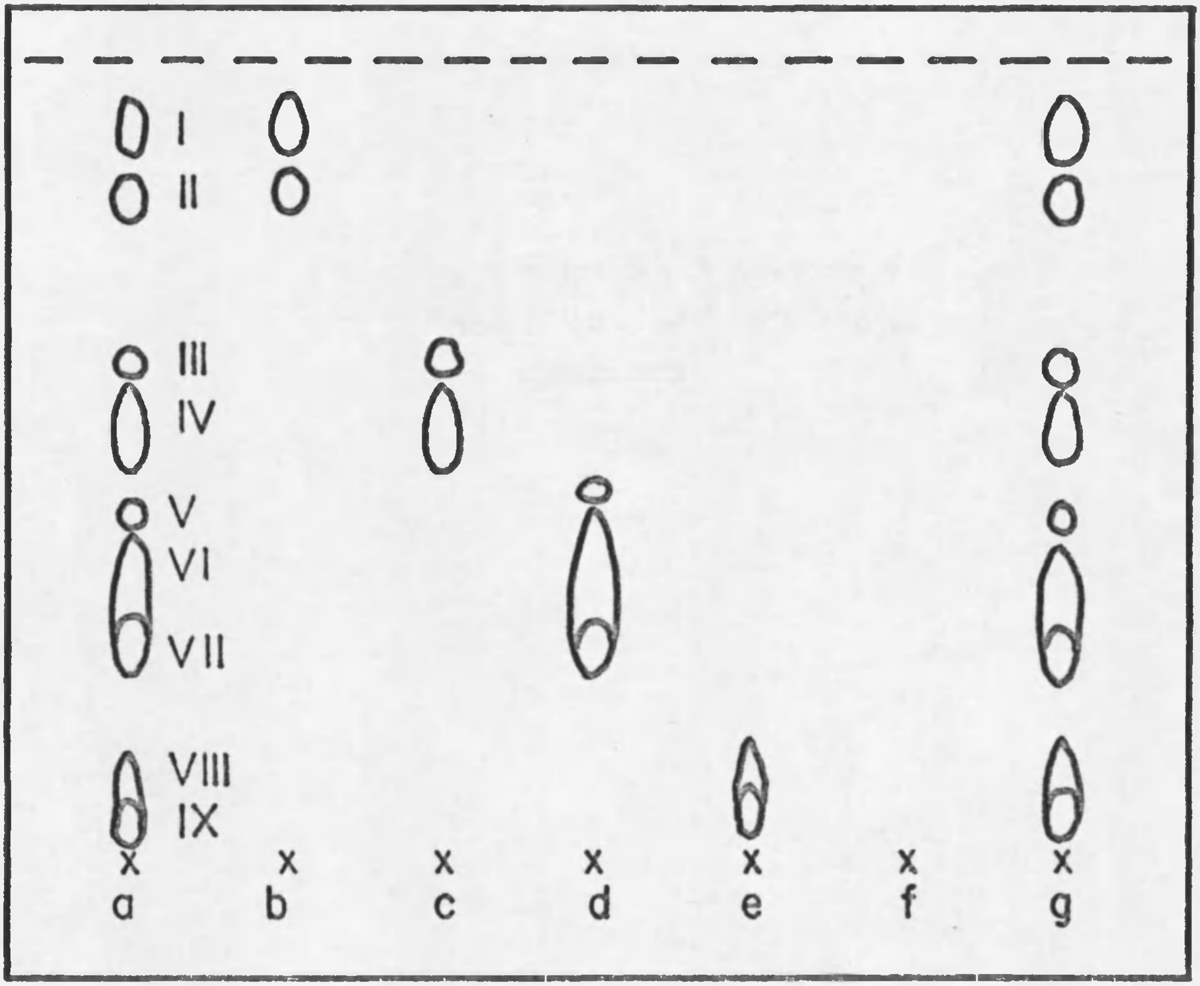

Fig. 5. Thin-Layer Chromatography of Alumina Column Fractionation of Bocconia frutescens

(a and g) Defatted Methanol, (b) Group A, (c) Group B, (d) Group C, (e) Group D, (f) Group E

A dried sample of each group was dissolved in 2% hydrochloric acid and 1 to 2 drops of Mayer's reagent was added. A positive test was indicated when precipitation occurred.

The results of the column chromatography and alkaloids test are shown in Table 3.

Table 3

Alumina Column Chromatography of *Bocconia frutescens*

Group	Solvent	Fractions	Amount	Mayer's	Dragendorff's
A	Benzene	1-6	2.78 g	Positive	Positive
B	Benzene	7-10	1.98 g	Positive	Positive
C	Benzene	11-18	3.08 g	Positive	Positive
D	Chloroform	19-23	10.51 g	Positive	Positive
E	Chloroform	24-34	2.02 g	Negative	Negative

Alkaloids of Group A

Thin-layer chromatography of Group A indicated the presence of two alkaloids. Ultraviolet examination of the plate revealed two violet spots. After spraying with Dragendorff's reagent, the spots showed red orange (Alkaloid I) and orange (Alkaloid II).

Separation of Alkaloids I and II

One gram of Group A was dissolved in chloroform and applied to 20 thin-layer chromatography plates (20 x 20 cm and .250 mm thick) coated with Silica Gel G. A solvent system of pure benzene (solvent system II) was used and the plates were allowed to run about 15 mm. The plates were then air dried and rechromatographed again in solvent system II to a point about 2.5 cm from top edge of the plate. The plates were removed from the solvent and air dried. Ultraviolet inspection indicated that the two alkaloids had separated and each one was carefully scratched off the plates into an Erlenmeyer flask. Acetone was added and the mixture was mechanically stirred for 24 hours. The mixtures were filtered through a fritted glass funnel and washed several times with acetone. The acetone filtrate was evaporated *in vacuo*. Each sample was chromatographed on thin-layer and compared with the original Group A (Fig. 6). Alkaloids I and II were relatively pure. They were recrystallized from a mixture of hot methanol and chloroform. The yield of Alkaloid I was 286 mg, and Alkaloid II was 194 mg. The melting point for Alkaloid I was 193-194°C, and Alkaloid II was 159-161°C.

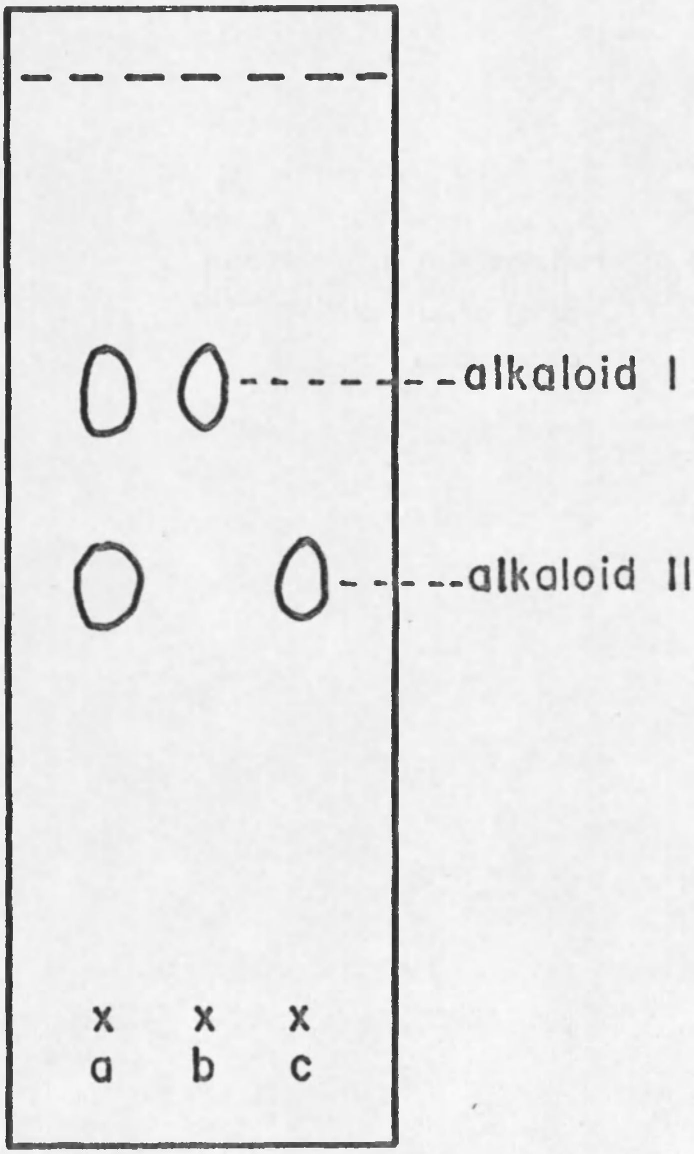

Fig. 6. Thin-Layer Chromatography of Group A
(a) Group A, (b) Alkaloid I, (c) Alkaloid II

Alkaloids of Group B

Thin-layer chromatography of this group showed the presence of two alkaloids upon spraying with Dragendorff's reagent. Alkaloid III gave the typical orange-red reaction to Dragendorff's reagent, while Alkaloid IV was bright red. Ultraviolet observation of III and IV indicated colors of violet and red respectively.

Separation of Alkaloids III and IV

Although the separation of Alkaloids III and IV on thin-layer chromatography was not as distinct as Alkaloids I and II, an attempt was made to use thin-layer chromatography as the method of obtaining them in pure form. While attempting to dissolve the mixture in chloroform and methanol, precipitation occurred. The material was filtered and all attempts to redissolve it in the usual solvents failed. The precipitate was not soluble in diluted hydrochloric acid or sulfuric acid. Hot dioxane was successfully used to solubilize the precipitate and a compound crystallized readily from the dioxane on cooling. The compound has a melting point of 356-358°C. A comparison on thin-layer chromatography using solvent system I of the dioxane soluble compound and the Group B mixture showed that the crystalline material was in fact Alkaloid III.

Hydrogen chloride gas was generated by dripping concentrated sulfuric acid into concentrated hydrochloric acid and drying in a sulfuric acid column. The gas was then introduced into the chloroform-methanol filtrate from above and a red precipitate appeared. The material was filtered, dissolved in water and ammonium hydroxide, placed in a separatory funnel and extracted with several portions of chloroform. The chloroform solutions were combined and evaporated *in vacuo*. The material was recrystallized from hot methanol:chloroform 1:1. It had a melting point of 242-244°C. The sample was compared with the original Group B on thin-layer chromatography using solvent system I and was shown to be identical with Alkaloid IV (Fig. 7).

Alkaloids of Group C

Thin-layer chromatography of this group, after spraying with Dragendorff's reagent, showed the presence of two alkaloids. Ultraviolet inspection prior to spraying indicated the possibility that a third component might be overlapping. Alkaloid V gave a slight orange color with Dragendorff's, and a violet color under ultraviolet light. Alkaloid VI gave a vivid yellow color on both spraying and ultraviolet observation. The tailing portion of Alkaloid VI gave a different yellow coloration. This was determined to be Alkaloid VII.

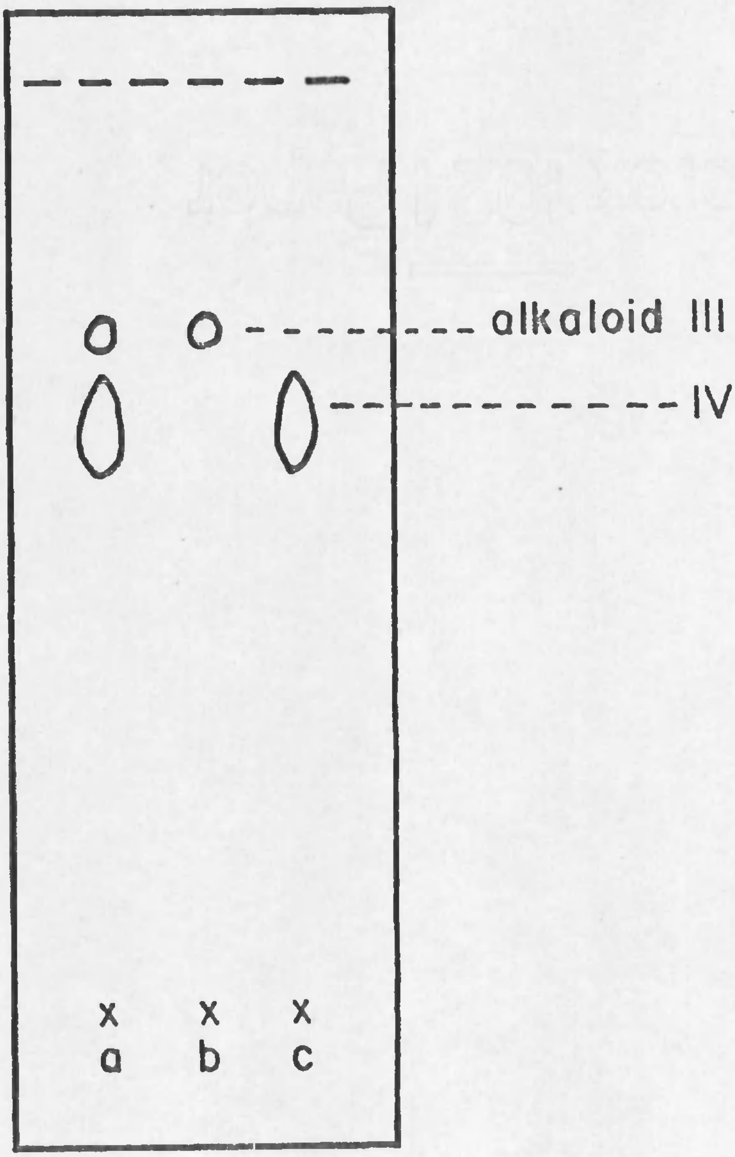

Fig. 7. Thin-Layer Chromatography of Group B

(a) Group B, (b) Alkaloid III, (c) Alkaloid IV

Separation of Alkaloids V, VI, and VII

In an attempt to redissolve Group C in chloroform and methanol, precipitation occurred. Again, this precipitation was filtered and was insoluble in the usual solvents. Hot dioxane was again utilized. The compound slowly crystallized as the dioxane cooled. It has a melting point of 197-198°C. Thin-layer chromatography showed this to be Alkaloid V.

The filtrate was left standing exposed to the air for several days and a crystalline precipitate occurred. This material was chromatographed and was found to be identical with Alkaloid VI. The mother liquid was evaporated in an attempt to crystallize more of Alkaloid VI, using hot methanol and chloroform as recrystallization solvents, but another compound was obtained. Comparison on thin-layer chromatography indicated that the compound was Alkaloid VII. Alkaloids V, VI and VII were then compared on thin-layer chromatography with the original Group C, using solvent system III (dichloromethane-benzene-methanol-ethyl acetate, 12:24:1:1.5) (Fig. 8). This resulted in separation of Alkaloids VI and VII.

Alkaloids of Group D

Thin-layer chromatography of this group showed the presence of two major alkaloids. Under ultraviolet

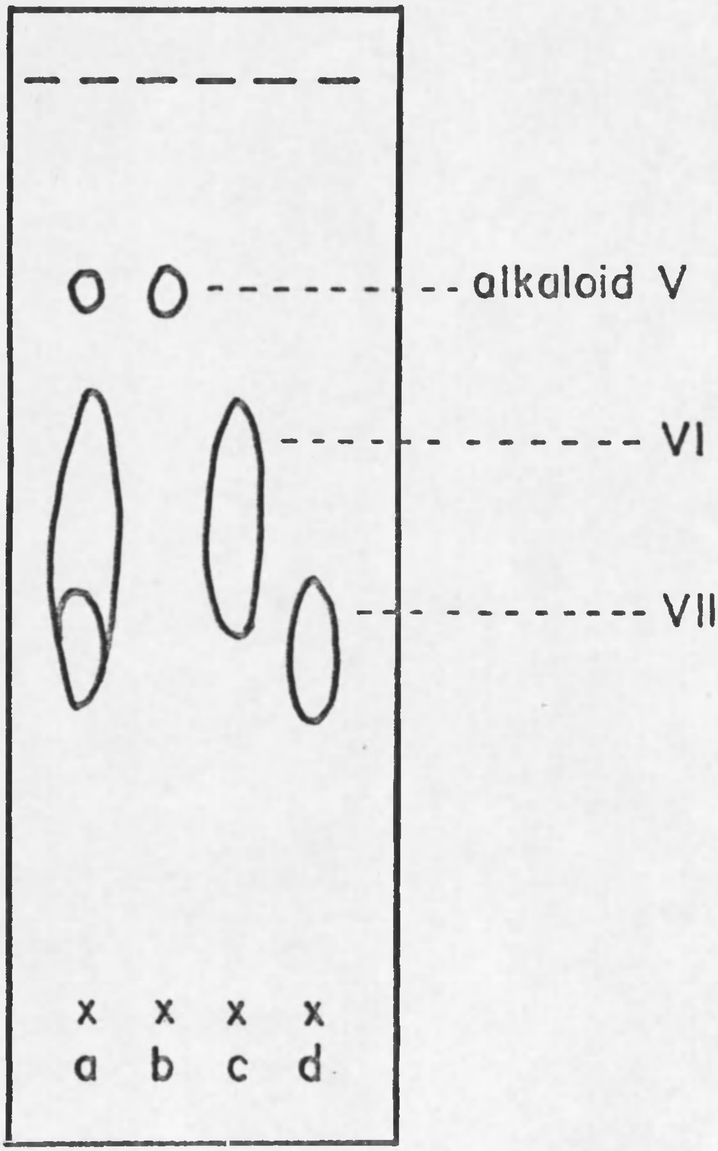

Fig. 8. Thin-Layer Chromatography of Group C

(a) Group C, (b) Alkaloid V, (c) Alkaloid VI, (d) Alkaloid VII light they were both observed as violet spots and after spraying, they gave the typical orange-red color for Dragendorff's positive reaction. The upper spot of this group was designated as Alkaloid VIII, and the lower spot as Alkaloid IX.

Separation of Alkaloids VIII and IX

A small column was packed with 300 g of alumina (Activity III). Two grams of the material from Group D were dissolved in chloroform and the solution carefully added to the top of the column. When all the solution was absorbed by the alumina, a piece of filter paper was introduced at the top of the alumina so that as more solvent was added, it would not disturb the column. Chloroform was then added to the column and collected at the bottom using an automatic fraction collector. Samples of about 10 ml were collected and each one was submitted for thin-layer chromatographic examination. As more solvent was needed, chloroform was introduced at the top of the column. Two-hundred sixty-eight samples were collected. Samples numbered 187-204 gave Alkaloid VIII in almost pure form. The samples were combined, evaporated _in vacuo_ and then recrystallized from methanol-chloroform. A compound was obtained with a melting point of 207-208°C. Samples 205-242 contained a mixture of Alkaloids VIII and IX. Samples 243-253 yielded relatively pure Alkaloid IX. It was crystallized as indicated above. The compound had a melting point of 160-161°C. Samples previous to 187 and after 253 were contaminated with other materials. Alkaloids VIII and IX were compared on thin-layer chromatography using solvent system III with a sample of Group D. They were identical (Fig. 9).

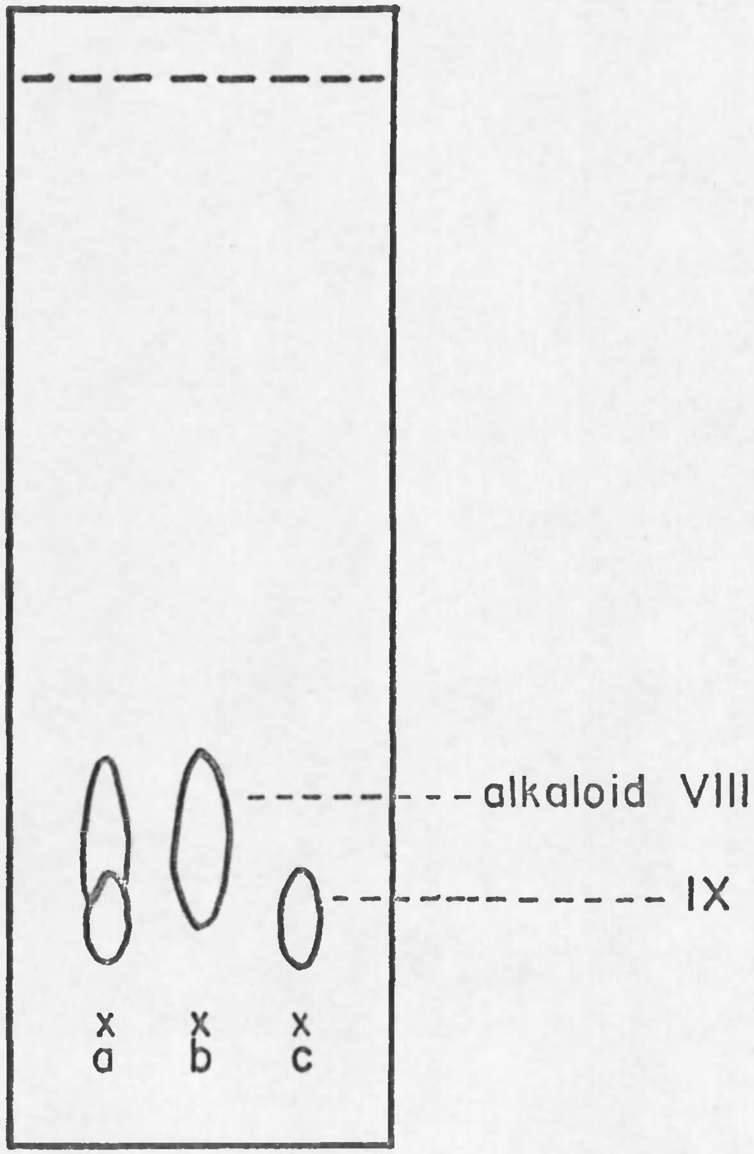

Fig. 9. Thin-Layer Chromatography of Group D

(a) Group D, (b) Alkaloid VIII, (c) Alkaloid IX

CHAPTER 3

IDENTIFICATION OF THE ALKALOIDS

Identification of the nine alkaloids was accomplished by instrumental analysis, thin-layer chromatographic comparisons, mixed melting points, synthetic and analytical procedure.

Alkaloid I

The mass spectrum of this alkaloid showed a m/e 333 corresponding to a molecular formula of $C_{20}H_{15}NO_4$. The nuclear magnetic resonance (N.M.R.) showed peaks corresponding to $N\text{-}CH_3$, benzylic methylene, and two methylene-dioxy groups. The aromatic region of the spectra integrated for 6 protons. The presence of the two methylenedioxy groups indicated a relationship to sanguinarine. The benzylic methylene protons established the identity of this alkaloid as dihydrosanguinarine (Figs. 10, 11, 12). Using standard reference ultraviolet (UV), infrared (IR) spectrum and melting point, this identification was confirmed.

As final proof, a small sample of dihydrosanguinarine was converted to sanguinarine according to the method of MacLean et al. (1969).

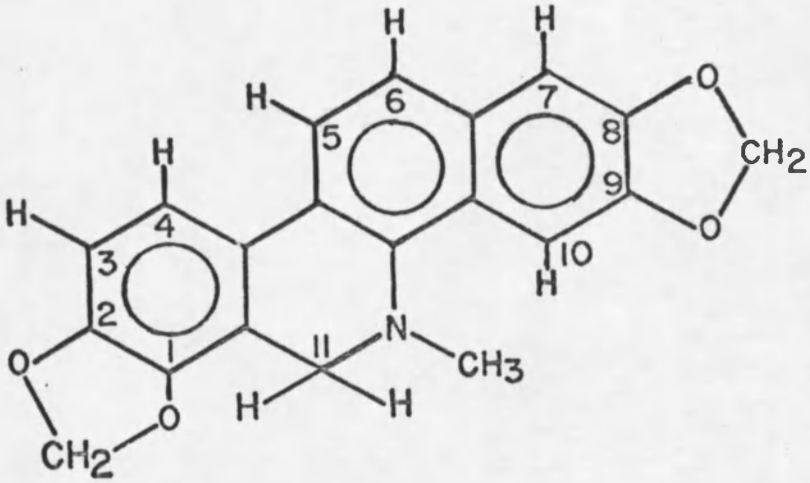

Fig. 10. Structure of Dihydrosanguinarine

Alkaloid II

The mass spectrum of this alkaloid showed m/e 349, corresponding to a molecular formula of $C_{21}H_{19}NO_4$. The N.M.R. indicated a benzylic methylene, N-CH_3, two methoxy groups and integrated for 6 aromatic protons. This information was sufficient to indicate this alkaloid as dihydrochelerythrine (Fig. 13).

A sample of chelerythrine nitrate was converted to dihydrochelerythrine by treatment with zinc dust and dilute hydrochloric acid in the usual manner (Scheuer, Chang, and Swanholm 1962). It was recrystallized from ethanol (melting point 162°C) and chromatographed with the isolated sample. In three different solvent systems (I, II, III) there was no difference in Rf values.

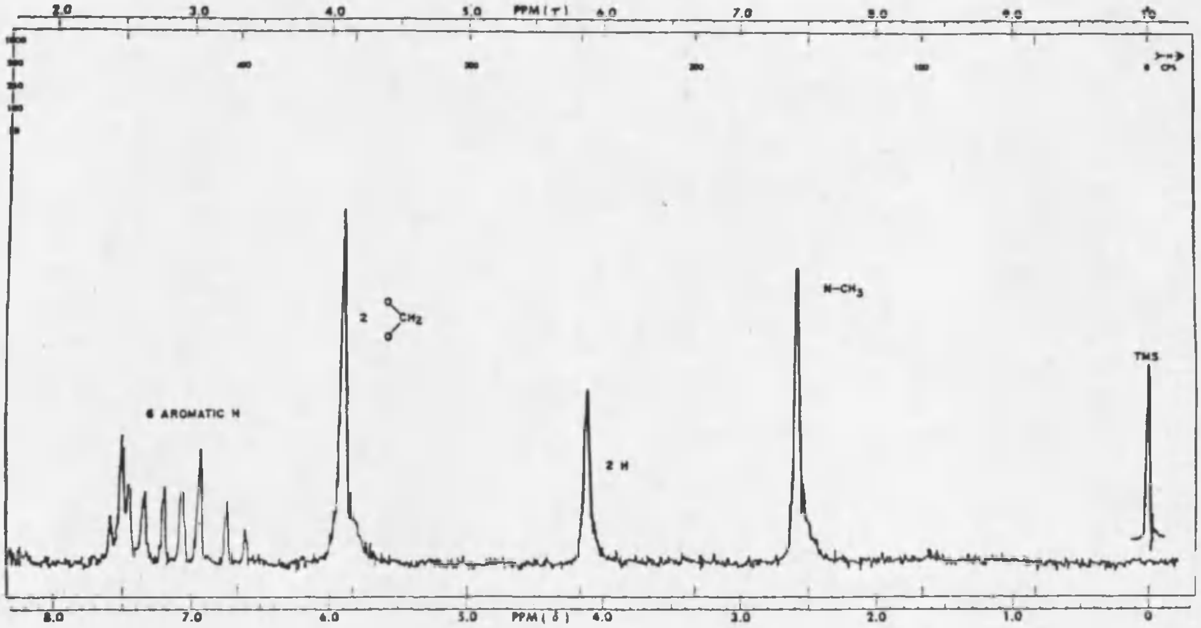

Fig. 11. N.M.R. Spectrum of Dihydrosanguinarine

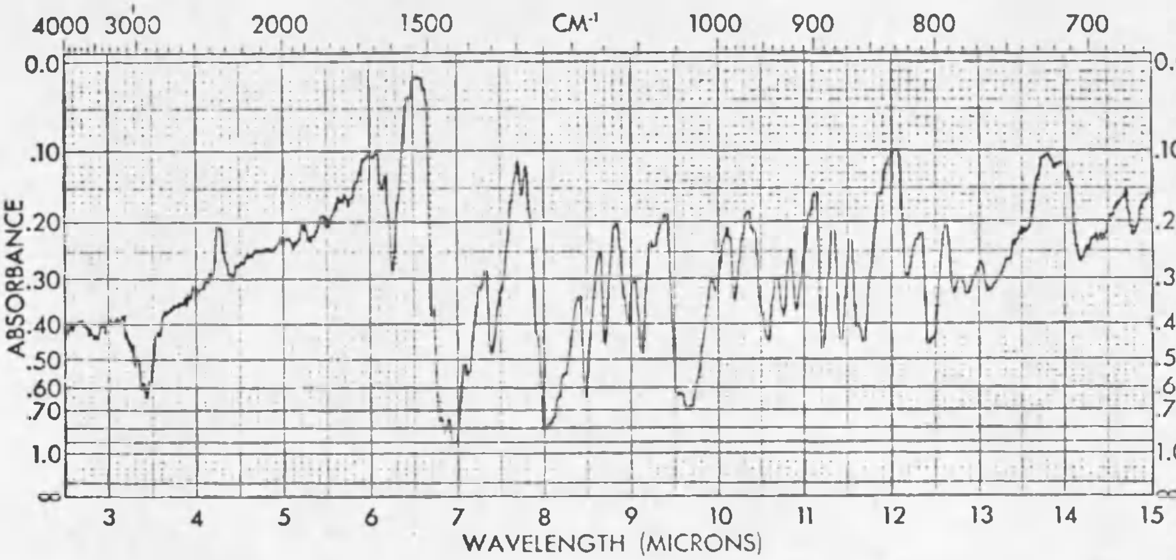

Fig. 12. Infrared Spectrum of Dihydrosanguinarine

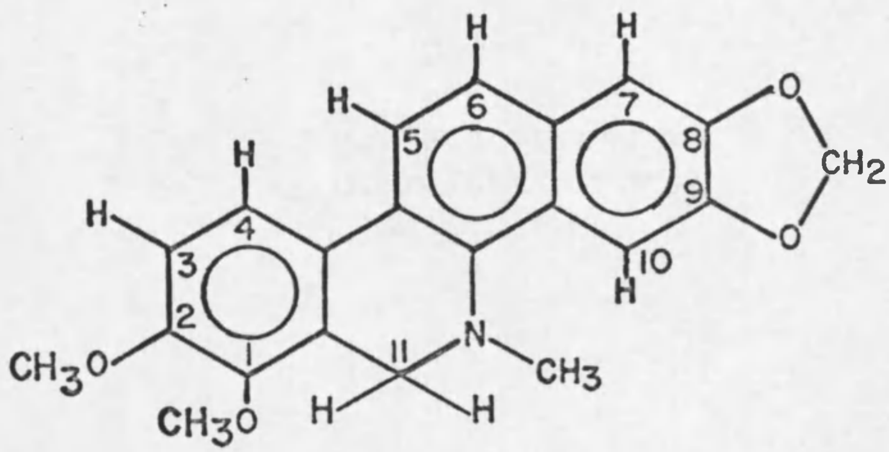

Fig. 13. Structure of Dihydrochelerythrine

Alkaloid III

The mass spectrum of this alkaloid showed a m/e 347 corresponding to a molecular formula of $C_{20}H_{13}NO_5$. The IR spectra showed a carbonyl absorption at 1649 cm^{-1} characteristic of a six membered aromatic lactam. The N.M.R. spectra run in trifluroacetic acid indicated two methylene-dioxy groups, an $N\text{-}CH_3$ and 6 aromatic protons. On the basis of these properties Alkaloid III was determined to be oxysanguinarine (Figs. 14, 15, 16).

To further substantiate this, a sample of oxysanguinarine was prepared from sanguinarine nitrate (Spath et al. 1937). The thin-layer chromatography comparison and superimposable IR spectra indicated that the compounds were the same.

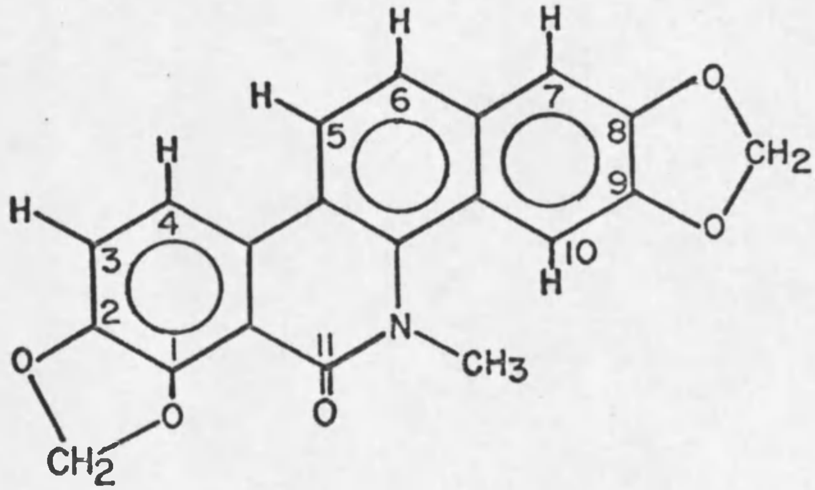

Fig. 14. Structure of Oxysanguinarine

Alkaloid IV

Previous workers have identified sanguarine, a bright red alkaloid in the salt form, from <u>Bocconia frutescens</u> (Tani and Takao 1967). An authentic sample of sanguinarine (courtesy of the Cancer Chemotherapy National Service Center) was compared on thin-layer chromatography to the isolated alkaloid and gave the same Rf as Alkaloid IV. Thin-layer chromatography of Alkaloid IV, the authentic sample and a mixture of the two showed the same Rf values in three different solvent systems used previously (I, II, III). A mixed melting point, N.M.R. and IR comparison and a molecular weight determination demonstrated that Alkaloid IV was indeed sanguinarine (Figs. 17, 18).

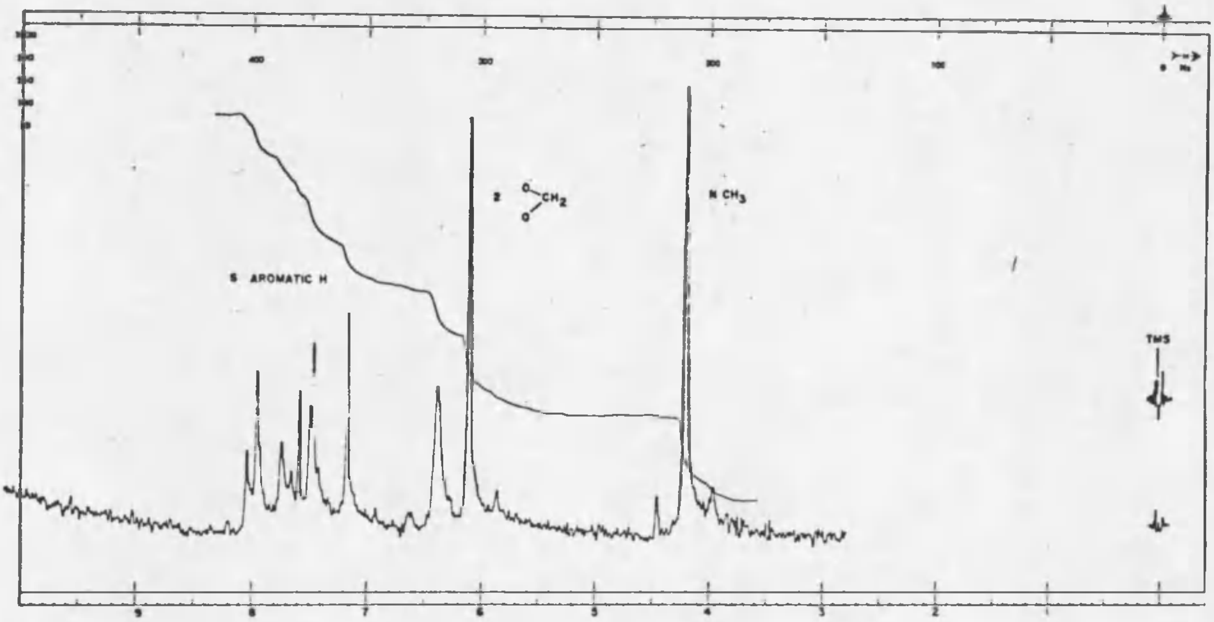

Fig. 15. N.M.R. Spectrum of Oxysanguinarine

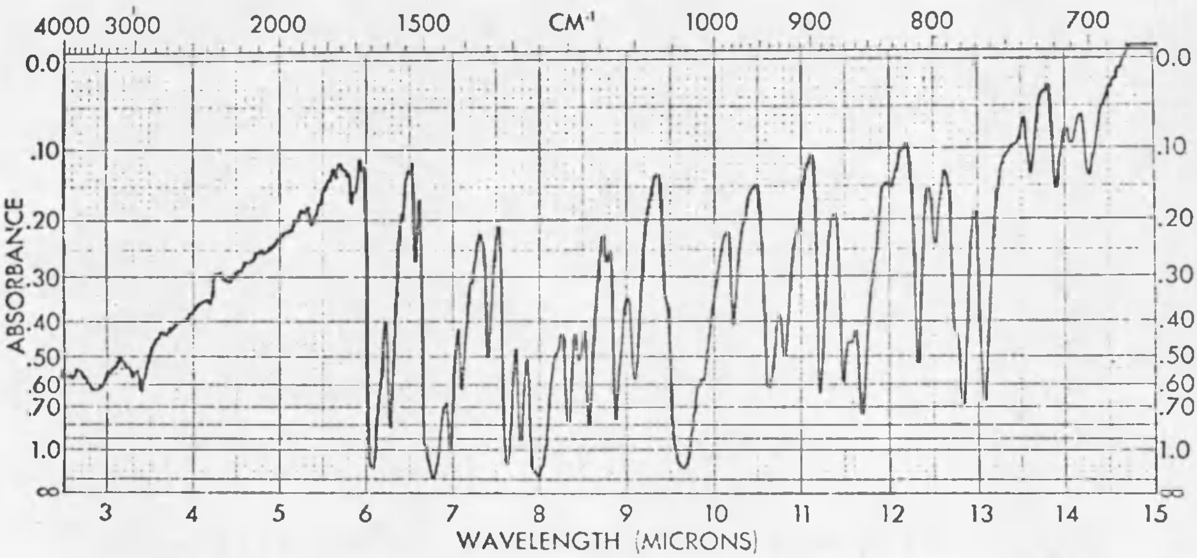

Fig. 16. Infrared Spectrum of Oxysanguinarine

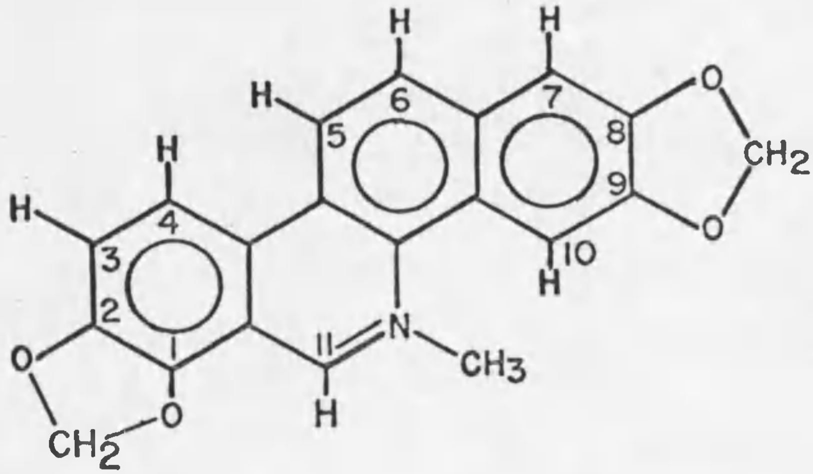

Fig. 17. Structure of Sanguinarine

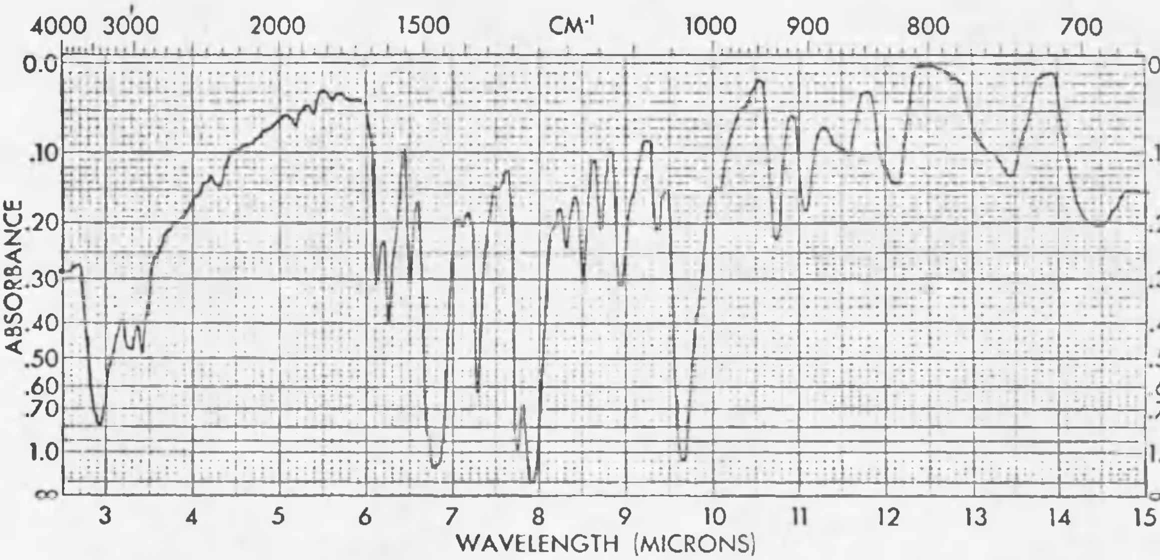

Fig. 18. Infrared Spectrum of Sanguinarine

Alkaloid V

The mass spectrum of Alkaloid V showed a m/e 363 corresponding to a molecular formula of $C_{21}H_{17}NO_5$. The IR spectra showed carbonyl absorption a 1649 cm^{-1} characteristic of a six membered aromatic lactam. The N.M.R. spectra in chloroform indicated the presence of two methoxy groups, a methylene-dioxy, a $N-CH_3$ and 6 aromatic protons. By comparison to other isolated alkaloids of this group and the foregoing information, it was determined that this alkaloid was oxychelerythrine (Figs. 19, 20).

To further substantiate this identification, a sample of oxychelerythrine was prepared from chelerythrine chloride in a similar way to the preparation of oxysanguinarine. Thin-layer chromatography proved conclusively that it was oxychelerythrine.

Alkaloid VI, VIII and IX

Based on reports in the literature (Manske 1943), authentic samples of chelerythrine (courtesy of Cancer Chemotherapy National Service Center), protopine (Pierce Chemical Co.), allocryptopine (Pierce Chemical Co.), were obtained for comparison with the isolated alkaloids. These samples were compared on thin-layer chromatography. The results indicated that Alkaloid VI was chelerythrine (Figs. 21, 22, 23). Alkaloid VIII was protopine

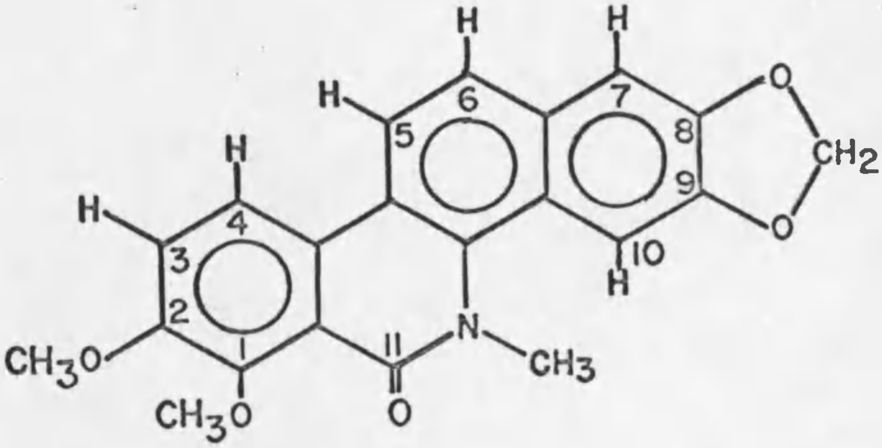

Fig. 19. Structure of Oxychelerythrine

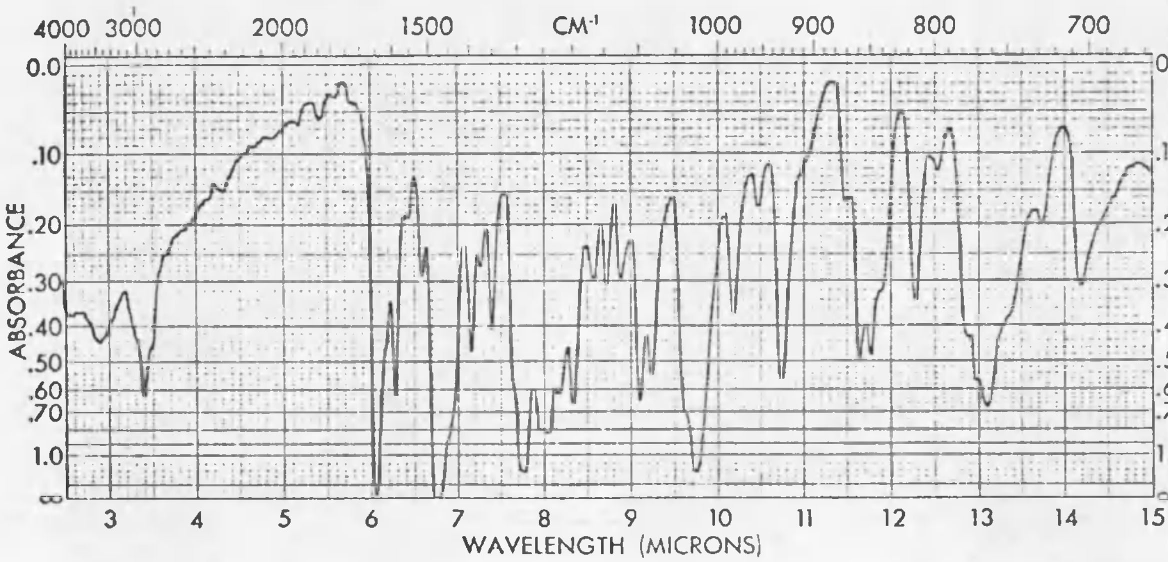

Fig. 20. Infrared Spectrum of Oxychelerythrine

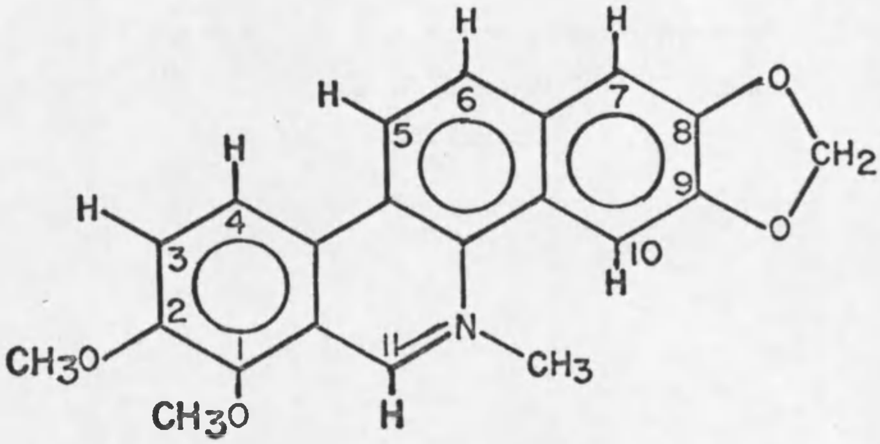

Fig. 21. Structure of Chelerythrine

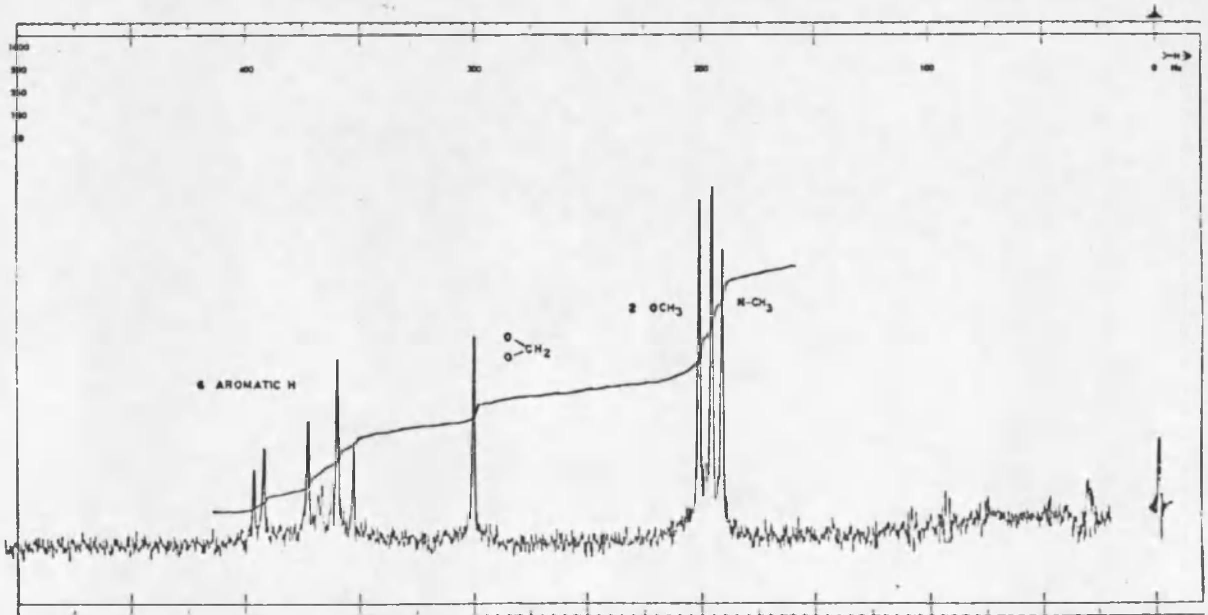

Fig. 22. N.M.R. Spectrum of Chelerythrine

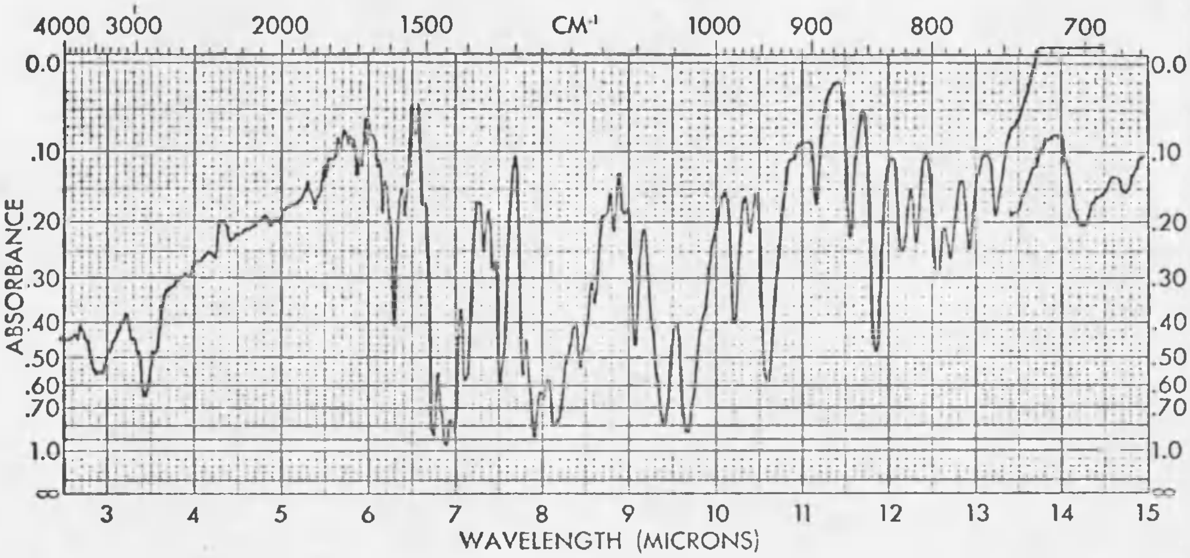

Fig. 23. Infrared Spectrum of Chelerythrine

(Figs. 24, 25), and Alkaloid IX was allocryptopine (Figs. 26, 27). Each of these three alkaloids were spotted separately with the authentic sample and a mixture of the authentic sample and the isolated alkaloid. They were run in three different solvent systems (I, II, III), and each showed identical Rf values. Mixed melting points for these three alkaloids with the authentic samples showed no depression. Comparison of N.M.R. and IR spectra were identical and the mass spectra gave the appropriate molecular weights.

Alkaloid VII

The mass spectrum of this alkaloid gave an m/e 379 corresponding to $C_{22}H_{21}NO_5$. The mass spectrum gave

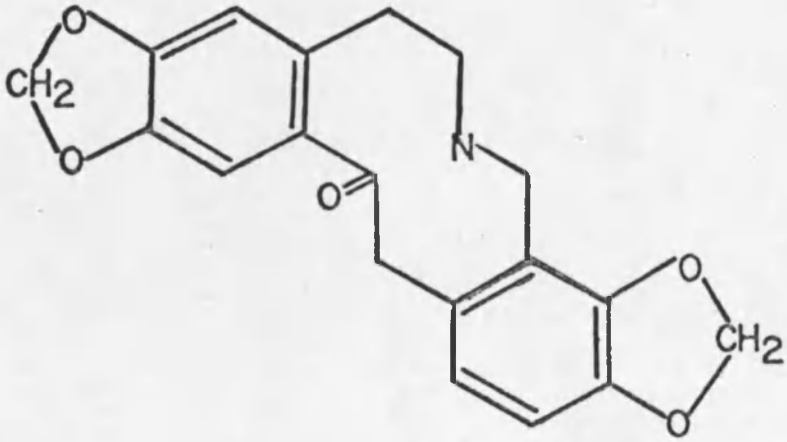

Fig. 24. Structure of Protopine

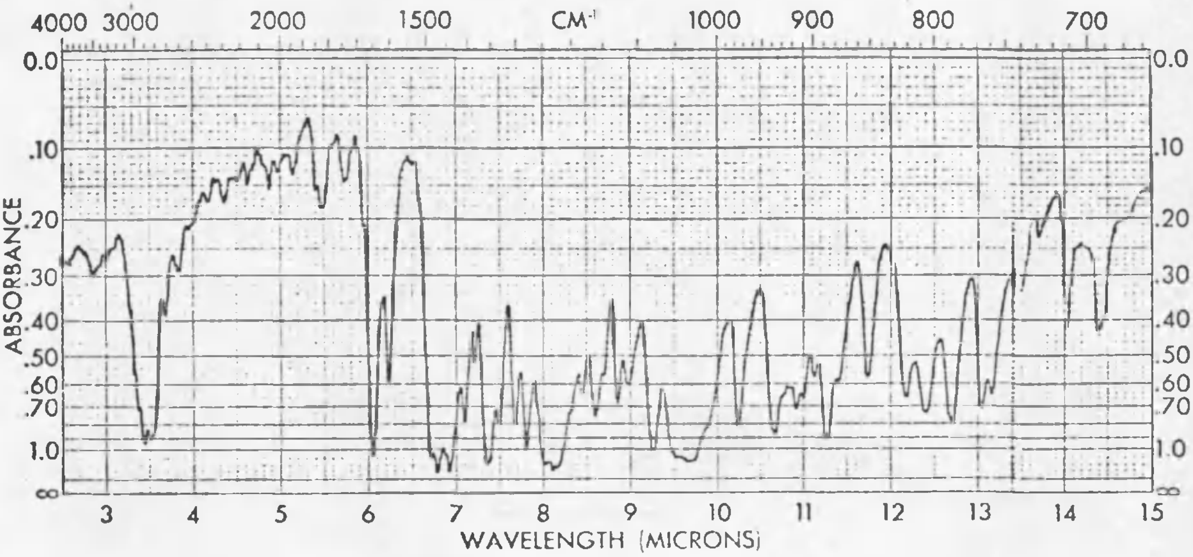

Fig. 25. Infrared Spectrum of Protopine

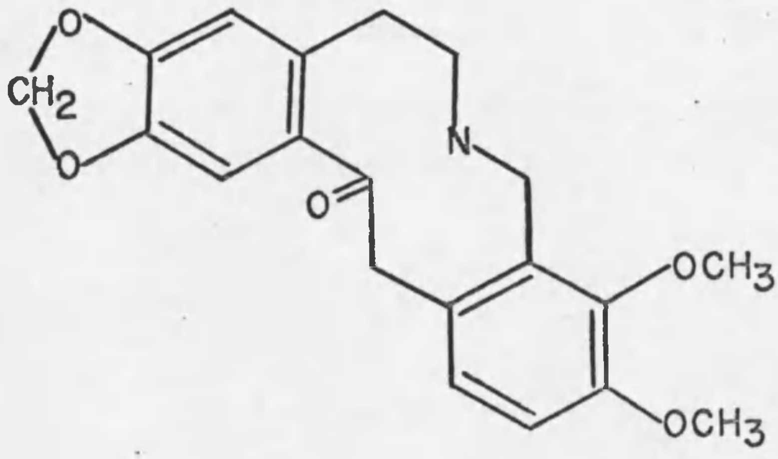

Fig. 26. Structure of Allocryptopine

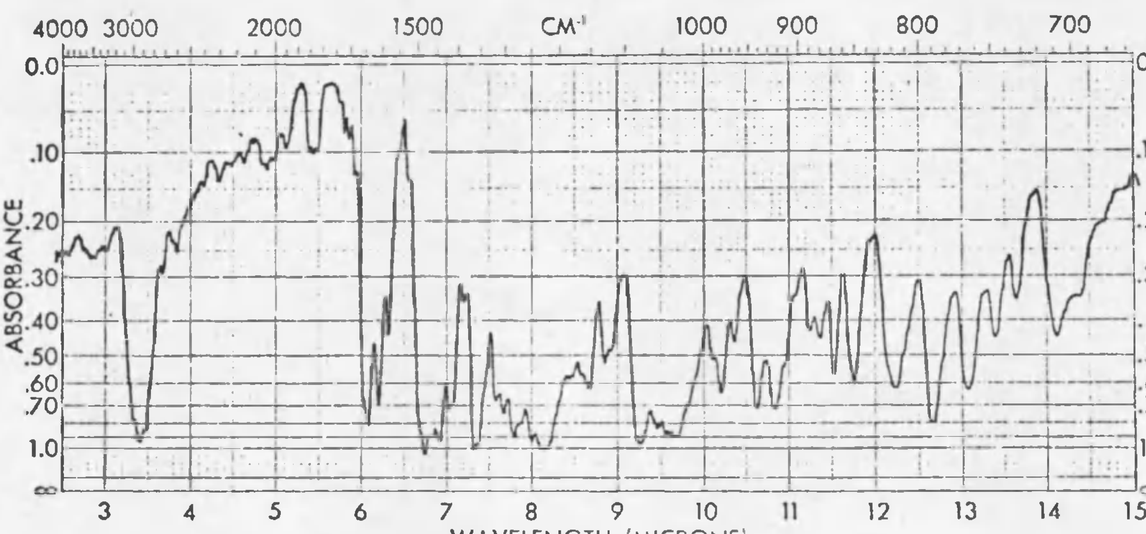

Fig. 27. Infrared Spectrum of Allocryptopine

a fragmentation pattern similar to the other 11-dihydro derivatives. The N.M.R. integrated for 3-methoxy groups, a methylene-dioxy substituent, N-CH_3, one aliphatic proton and six protons in the aromatic region. This indicated the compound was an eleven methoxy substituted chelerythrine (Figs. 28, 29, 30). Recent work (MacLean et al. 1969) substantiates these conclusions.

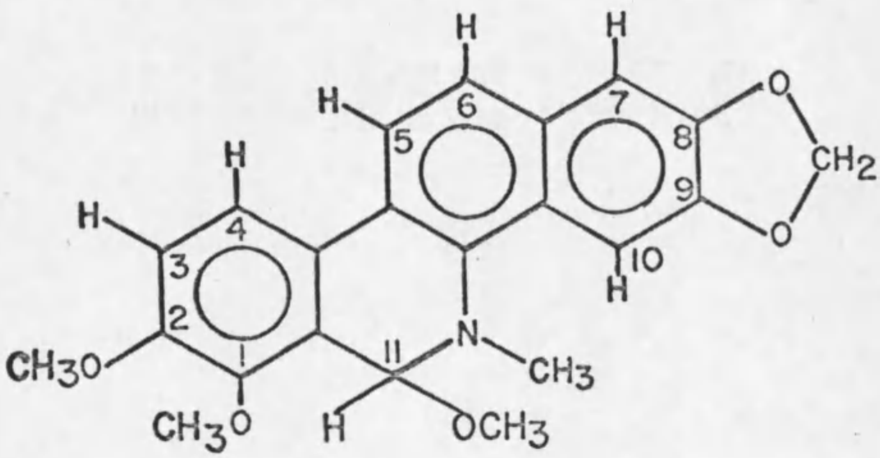

Fig. 28. Structure of 11-Methoxychelerythrine

Methoxychelerythrine was converted to ethoxychelerythrine (Fig. 31) by dissolution in hot ethanol and recrystallization from absolute ethanol (melting point 238-242°C). This compares with the literature value of 239-242°C (Scheuer et al. 1962). The N.M.R. showed the loss of the third methoxy group, but a doublet and triplet corresponding to an ethyl substitution was evident (Fig. 32).

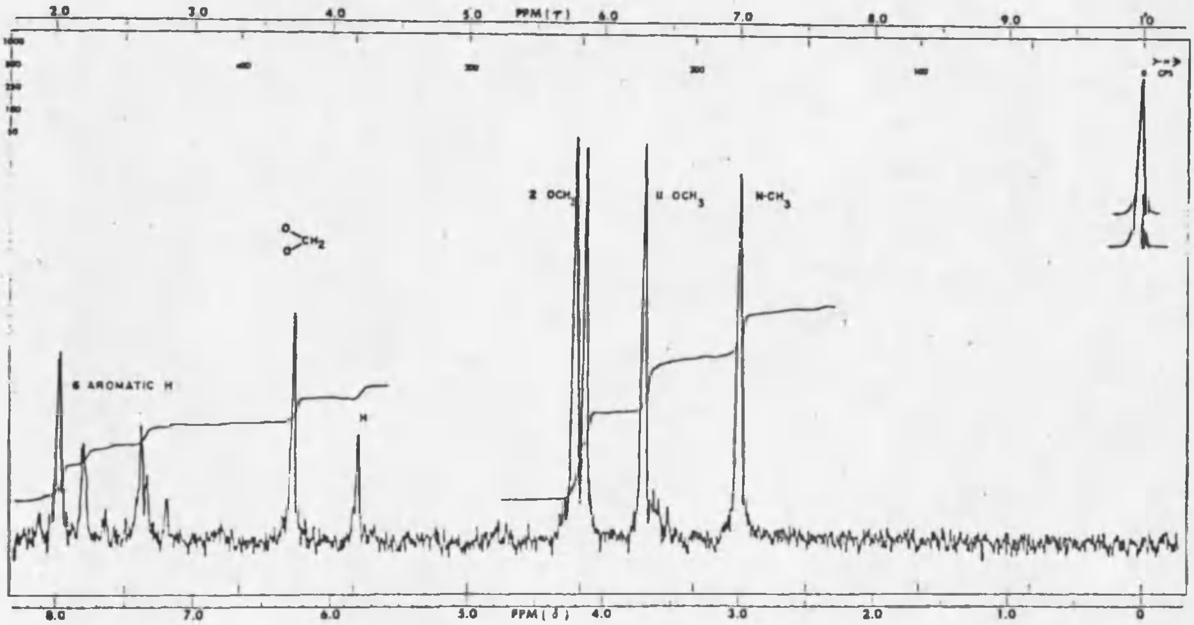

Fig. 29. N.M.R. Spectrum of 11-Methoxychelerythrine

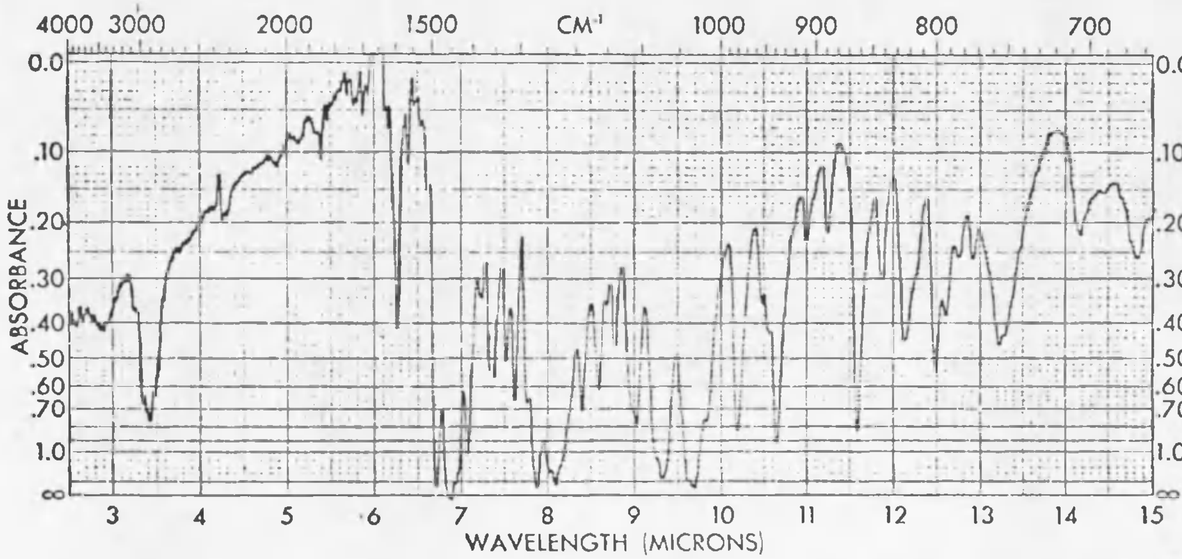

Fig. 30. Infrared Spectrum of 11-Methoxychelerythrine

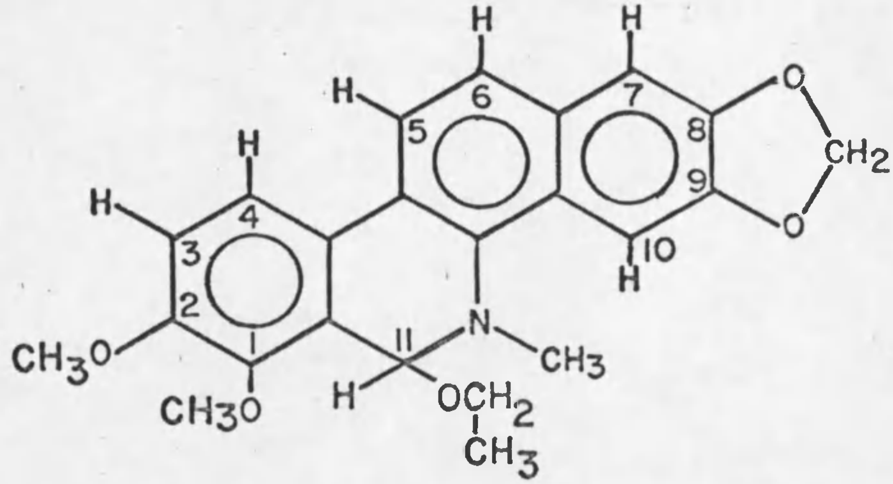

Fig. 31. Structure of 11-Ethoxychelerythrine

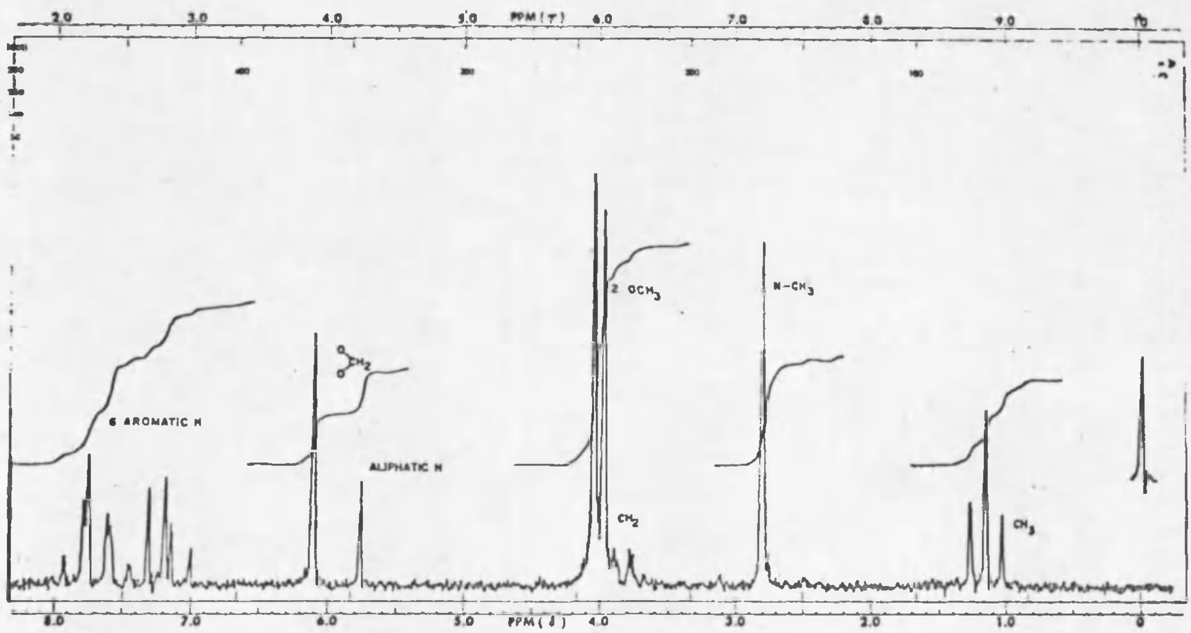

Fig. 32. N.M.R. Spectrum of 11-Ethoxychelerythrine

A conversion of the ethoxy derivative to an N-propoxy derivative (Fig. 33) was carried out in a similar manner using N-propanol. The melting point of this compound was 155-156°C. The N.M.R. showed the corresponding protons, triplet, quartet, doublet for the N-propyl derivative (Fig. 34).

An isopropyl derivative was attempted, but it did not form. The inability to prepare this derivative could be explained by steric hindrance.

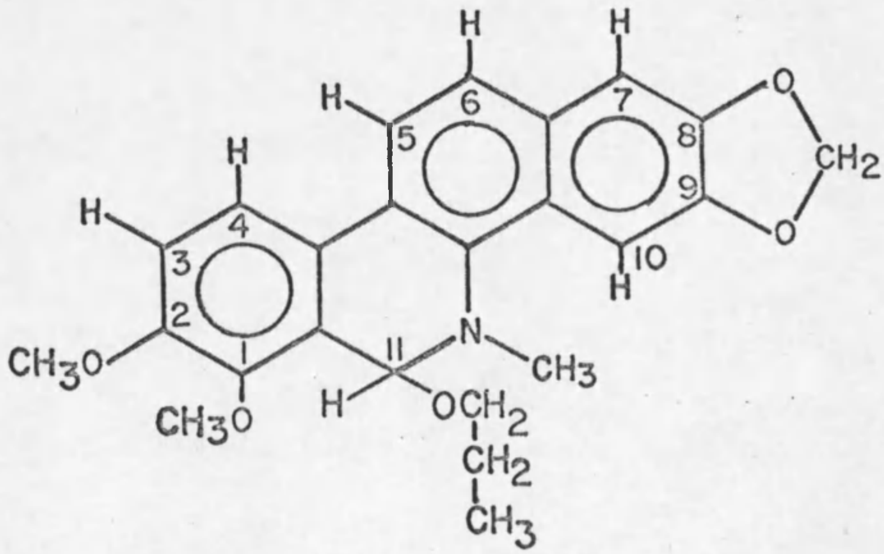

Fig. 33. Structure of 11-N-Propoxychelerythrine

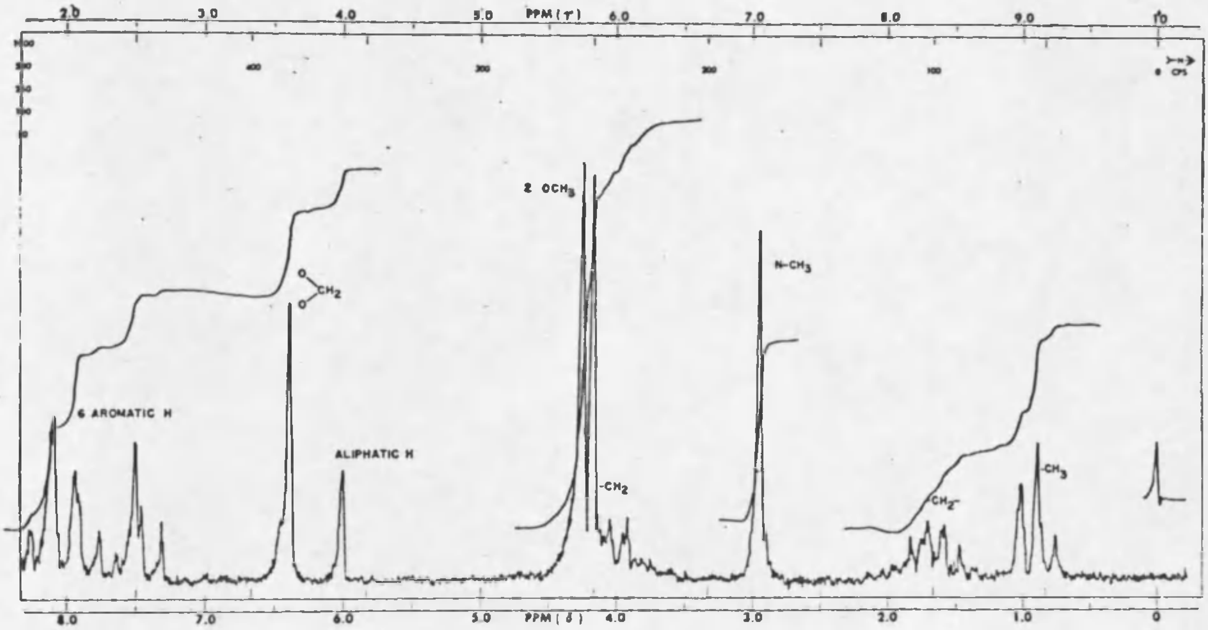

Fig. 34. N.M.R. Spectrum of 11-N-Propoxy-chelerythrine

CHAPTER 4

EXPERIMENTAL

This section deals with the types of instrumentation used in the identification of the isolated alkaloids. Also included is a description of the materials involved in the separation and qualitative determination of the alkaloids.

Thin-Layer Chromatography

Thin-layer plates were prepared with silica gel G made by Merck and distributed by Brinkman Instruments, Inc., Westbury, New York. All plates were prepared with an automatic plate maker so that the dried thickness was .25 mm and were activated at 110°C before use.

Alumina III for Column Chromatography

Alumina III was prepared from alumina I obtained from Fisher Scientific Co. by adding 6% water by weight and shaking in automatic shaker for six hours.

Dragendorff's Reagent

It was prepared by mixing equal volumes of a solution containing .85 g of basic bismuth nitrate and 10 g of acetic acid in 40 ml of water with a solution of 8 g of potassium iodide in 20 ml of water. One milliliter of this mixture is mixed with 2 ml of acetic acid and 10 ml of water. This final solution is sprayed on the developed dry plates (Stahl 1969).

Mayer's Reagent

It was prepared by dissolving 1.358 g of mercuric chloride in 60 ml of water and pouring it into a solution of 5 g of potassium iodide in 10 ml of water. A sufficient amount of water was then added to make 100 ml. One or two drops of reagent are sufficient to produce a positive test in one milliliter of test solution (Weast, Selly, and Hodgman 1966).

Infrared Spectrometry

All infrared spectra were performed on a Perkin Elmer Infracord model 137B. The spectra were obtained using potassium bromide pellets (5 mg of substance to 50 mg of potassium bromide and pressed into a micro pellet using 10,000 PSI pressure).

Nuclear Magnetic Resonance

All spectra were performed by the Department of Chemistry, University of Arizona, using a Varian HA 100 or an A60 instrument. Deutero chloroform or trifluro-acetic acid were used as solvents and tetramethylsilane as the standard. The concentration of the solutions used was ten percent.

Mass Spectrometry

All mas spectra were performed by the Department of Chemistry, University of Arizona, on a RMU6E Hitachi-Perkin Elmer spectrometer.

Melting Point Determinations

Melting points were taken of a Kofler hot stage apparatus made by Arthur H. Thomas Co., Philadelphia, Pennsylvania, and are uncorrected.

Ultraviolet Spectrometry

Ultraviolet spectral comparisons were performed on a Beckman DB-G spectrophotometer.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The active anti-tumor fraction of *Bocconia arborea* and *Bocconia frutescens* was found to contain a number of alkaloids. Nine of the alkaloids were isolated, identified and tested for anti-tumor activity. Protopine (3.5×10^{0} mcg/ml) and sanguinarine (6.8×10^{-1} mcg/ml) showed activity against 9KB tissue culture. Chelerythrine showed in vivo activity in the 5WM system (16% T/C at 10 mg/kg).

Of the nine alkaloids that were isolated and identified from each species, five were not previously reported from *Bocconia frutescens*, and two were not previously reported from *Bocconia arborea*. These unreported alkaloids are dihydrochelerythrine, dihydrosanguinarine, oxysanguinarine, oxychelerythrine and 11-methoxychelerythrine from *Bocconia frutescens*, and dihydrochelerythrine from *Bocconia arborea*. In addition, a new derivative of dihydrochelerythrine was synthesized and identified as 11-N-propoxychelerythrine.

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