PHYTOCHEMICAL NOTES ON CISSAMPELOS SPP

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RESUMEN

Se discuten aspectos fitoquímicos y farmacológicos de *Cissampelos ovalifolia D.C. y C. pareira L.* y se describen procedimientos de extracción, aislamiento, purificación, cromatografía (CC, CCF) y espectroscopia (IR, H^+-RMN, SM) de dos alcaloides BBI aislados de esas especies. Se hace una comparación entre material estudiado proveniente de la Guayana Británica, la India y varias partes de Colombia.

SUMMARY

Phytochemical and pharmacological aspects of *Cissampelos ovalifolia D.C. and C. pareira L.* from Guyana, India and several regions of Colombia are discussed. A description of extraction procedures, isolation, purification, chromatographic (CC, TLC) and spectroscopic techniques (IR, H^+-NMR, MS) as well as a comparison of the BBI alkaloids present in the material studied, are presented.

Key word index: Menispermaceae. *Cissampelos*. BBI-alkaloids. Spectroscopy.

INTRODUCTION

The varied pharmacological effects and diverse structures of bisbenzylisoquinoline (BBI) alkaloids have interested chemists, biologists and pharmacologists since the early days of commercial preparation of drugs such as Radix Pareirae Bravae, curare, and the various arrow poisons used by South American indians.

Shama (1972a, 1972b), has written an excellent review which covers different chemical aspects of BBI alkaloids. The sources of the BBI alkaloids are mainly, representatives of *Menispermaceae* along with various members of the *Aristolochiaceae, Anonnaceae, Berberidaceae, Buxaceae, Hernandiaceae, Lauraceae, Magnoliaceae, Monimiaceae, Nymphacaceae, Ranunculaceae, Rhamnaceae and Umbelliferae*. Among the former, *Chondodendron, Cissampelos* (Table 1), *Cocculus, Stephania* and *Menispermum* are probably the richest in BBI alkaloid content and, certainly, the most studied genera. *M. dauricum, for instance*, synthetizes some alkaloids with antitumoral activity, specially in its rhizome, yielding N-desmethyldauricine (Par, 1992; Zhao, 1989).

More recently a number of alkaloids have been isolated from *C. pareira*, namely laudasoline, nuciferine, balbocarpine, corituberine, magnoflorine (Ahmar, et al., 1992), and the novel antileukaemic tropoisoquinoline alkaloid perereirubrine (Morita, Matsumoto, et al., 1993). From the aereal parts of *C. fasciculata*, Galinis, Weiner et al. (1993), isolated the BBI alkaloid cissampentim which was shown, after bioassays, to possess a repellent activity to the leafcutter *Acromyrmex octopinosus* as well as a mild antifungal activity.

Regarding the other families, *Abuta. Anisocyclea, Cyclea, Epinetrum Limacia, Menispermum, Pachygone, Paracyclea, Picnarrhema, Sciadotenia, Tiliacora and Triclisia* are also known to be rich sources of these compounds.

Among BBI alkaloids, wrifteine (5) and cissamperine (4), not found in Colombian material so far, are thought to be ingredients of curare preparations and shown to be of pharmacological potential. Cissamperine was evaluated for activity against human carcinoma of the nasopharynx and found to possess significant inhibitory activity (Kupchan, 1965). This alkaloid might be isomeric to methylwarifteine, one of the compounds isolated in the course of this work.

The action of warifteine in blood pressure studies (Gorinsky, 1973), could be the result of the two isoquinoline moieties within the molecule. The N-methylated end of the compound might be expected to act

TABLE No. 1
BOTANICAL SOURCES AND CHEMICAL STRUCTURES OF BBI ALKALOIDS FROM *Cissampelos spp* (*)

PLANT SPECIES	PART STUDIED	BBI ALKALOID	FORMULA	REFERENCE
Cissampelos fasciculata	L	Cissampentine		Galinis (1993)
Cissampelos insularis	R	Cycleanine	1	Kondo (1937)
	R	Insularine	3	Kondo (1937)
C. mucronata	R	Isochondodendrine	2	Ferreira (1965)
C. ovalifolia	R	Dihydrowarifteine	7	Snedden (1970)
	R	Dimethylwarifteine	9	Snedden (1970)
	R	Methyldihydrowarifteine	8	Snedden (1970)
	R	Methylwarifteine	6	Snedden (1970)
	R	Warifteine	5	Snedden (1970)
C. pareira	P1	Cissamperine	4	Kupchan (1965)
	R	Insularine	3	Dwuna-Badu (1975)
	R	Isochondodendrine	2	Dwuna-Badu (1975)
	R	O-methylcurine	11	Haynes (1966)
	R	Cycleanine	1	Haynes (1966)
	L, R	(-) Curine	10	Bhattacharji (1956)
	L, R	Hayatidine	12	Bhattacharji (1956)
	L, R	Hayatine	13	Bhattacharji (1956)
	L, R	Hayatinine	14	Bhattacharji (1956)
	L	Laudasoline Nuciferine Balbocarpine Magnuflorine	(**)	Ahmar, et al. (1992)
	P1	Pereireirubrine		Morita, et al (1993)

(*) Abbreviations: L: leaf
R: root
P1: whole plant

(**) Formula not described in the original publication

on β-receptors and the pressor effect would be related to the dihydroisoquinoline end (Gorinsky, 1973). This interpretation assumes a sympathomimetic anime-like effect of warifteine to be operative in *in vivo* systems.

Regular contractions of ileum preparations were obtained with doses of 10 μg warifteine. These concentratios were equivalent in height to those produced in response to doses of 1 μg acethylcholine (París, 1967).

An oxytoxic effect on isolated rat uterus preparations was indicated by maximal concentrations being obtained at doses of 20-100 μg warifteine (Gorinsky, 1973).

When an anticonvulsant dose of phenobarbitone (20 mg/Kg), was given subcutaneously to mice and, after ten minutes, a convulsant dose of warifteine (100 mg/Kg), was given to the same mice and also to other mice used as controls, these which received warifteine alone convulsed and died. The animals which received phenobarbitone followed by warifteine did not convulse and no behavioral changes were observed (Tackie, 1968).

A test for local anaesthesic action was carried out with 4 mg warifteine injected into guinea pigs. Anaesthesia of 100% was observed for a period of about 10 minutes. An hour after injection, 50% anaesthesia was still apparent. Falls in blood pressure were obtained whit 1 μg acethylcholine and, 5 minutes later, with 100 μg warifteine. After another pause of about 5 minutes, 40 μg atropine was given. After atropine, 1 μg acethylcholine gave a rise in blood presure. Warifteine gave the usual response of a fall in blood pressure in the atropinized animal (Doskotch, 1971).

EXPERIMENTAL

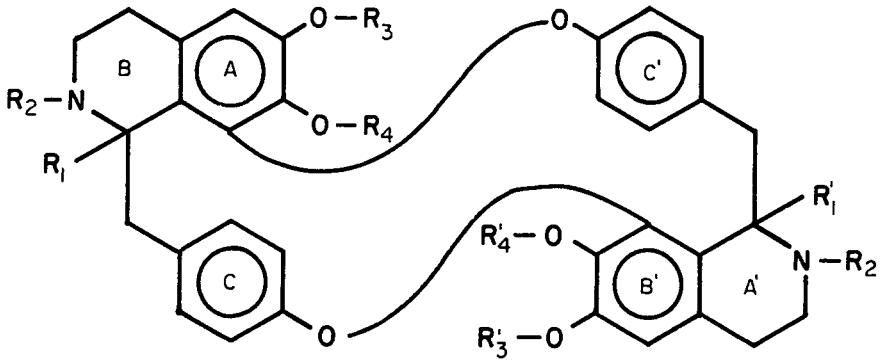
Samples of *C. ovalifolia* and *C. pareira* were collected in Colombia, India and Guyana (Table 2), botanically identified and voucher specimens deposited either in the Herbario Nacional Colombiano (COL) or in the Herbarium of the Royal Botanic Gardens, Kew, Surrey, England (KEW).

The extraction procedure using a soxhlet with methanol is summarized in (Fig. 1). Extractions by infusion, aqueous and methanolic, were also carried out in order to compare the different yields and percentages of alkaloids being extracted.

TABLE No. 2
PERCENTAGE OF WARIFTEINE IN SAMPLES OF *Cissampelos spp* (*)

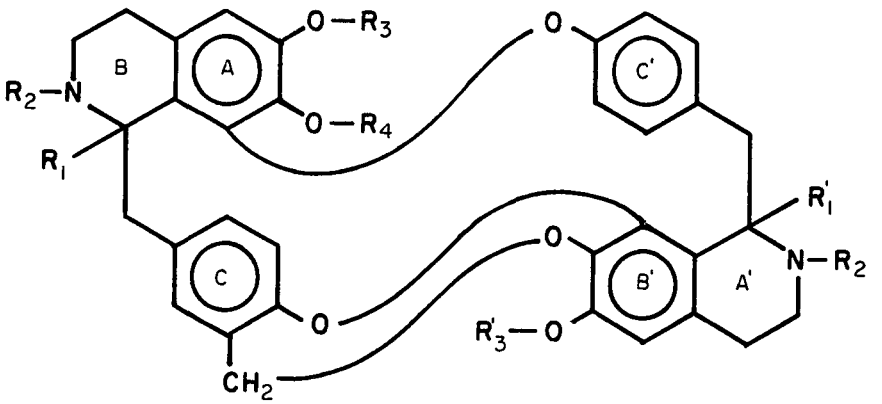
SPECIES HERBARIUM	ASPIRATOR	SOXHLET		PLACE OF COLLECTION	
	MeOH	0.1M HCl	EXTRACTION AND COLECTOR		
C. ovalifolia	0.012 (0.14)	0.20 (0.22)	0.11 (0.09)	Guyana, Rupunini. open savannahs. Gorinsky, S. N.	KEW
	0.013 (-)	0.016 (-)	0.004 (-)	India Vadlamudi, P., S. N.	KEW
	0.07 (0.05)	0.06 (0.004)	0.05 (0.03)	Colombia, Risaralda, La Virginia. Morales, G., S. N.	COL
	0.014 (-)	0.032 (-)	0.01 (-)	Colombia, Medina, Departamento Cundina-marca 900m. Morales, G., S. N.	COL
C. pareira	0.042 (-)	0.041 (-)	0.021 (-)	Colombia, Medina, Departamento Cundi-namarca, open savannah, 1000 m. Morales, G., S. N.	COL
	0.031 (-)	0.052 (-)	0.01 (-)	Colombia, Villavicencio, Meta, open savannahs, 2000 m. Gorinsky, S.N.	COL
	0.036 (-)	0.065 (-)	0.024 (-)	Colombia, Fusagasugá, Departamento Cundinamarca. Burned contry-side. 2000 m Morales, G., S. N	COL
	0.04 (-)	0.081 (-)	0.017 (-)	Colombia, Salto Tequendama, 2000 m. Gorinsky, S. N.	COL

(*) First figures correspond to methylwarifteine; figures between parenthesis correspond to warifteine.

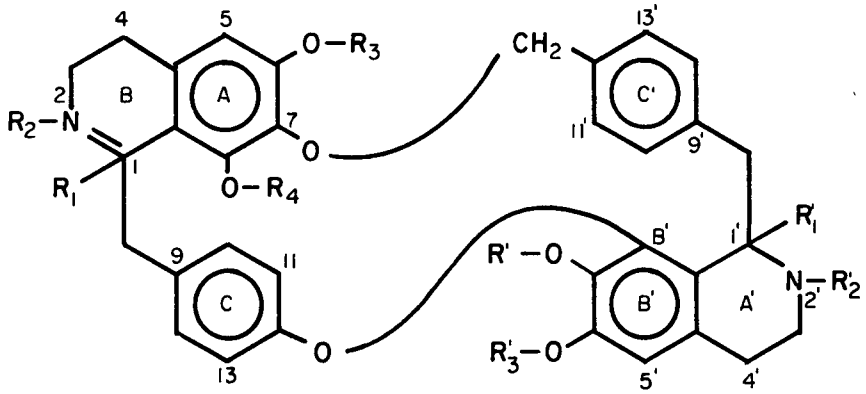


(1) : $R_2 = R_3 = R_4 = R'_2 = R'_3 = R'_4$ = Me ; $R_1 = R'_1$ =H = Cycleanine

(2) : $R_2 = R_3 = R'_2 = R'_3$ =Me ; R_4 ; $R'_4 = R_1 = R'_1$ =H = Isochondrodendrine



(3) : $R_2 = R_3 = R_4 = R'_2 = R'_3$= Me ; $R_1 = R'_1$ =H = Insularine



(4) : $R_1 = R'_1 = R_2 = R_4 = H$; $R'_2 = R'_3 = R'_4 = R_3 = Me$ = Cissamperine

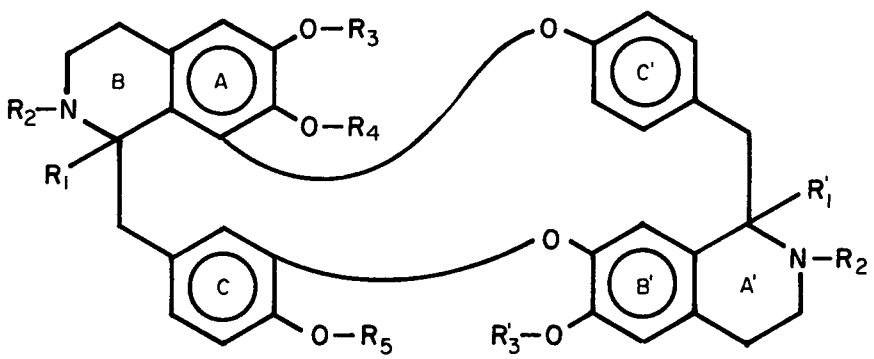
(5) : $R'_2 = R'_4 = R'_1 = H$; $R'_3 = R_3 = Me$ = Warifteine

(6) : $R_3 = R'_1 = H$; $R'_2 = R'_3 = R'_4 = R_2 = Me$ = Methylwarifteine

(7) : 1, 2 Dihydrowarifteine = Dihydrowarifteine

(8) : 1, 2 Dihydromethylwarifteine = Methyldihydrowarifteine

(9) : $R_1 = R'_1 = R_2 = H$; $R_4 = R'_2 = R'_3 = R'_4 = R_3 = Me$ = Dimethylwarifteine



(10) : $R_2 = R_3 = R'_2 = R'_3 = Me$; $R_4 = R_5 = R_1 = R'_1 = H$ = (–) Curine

(11) : $R_2 = R_3 = R_5 = R'_2 = R'_3 = Me$; $R_4 = R_1 = R'_1 = H$ = O- Methylcurine

(12) : $R_2 = R_3 = R_5 = R'_2 = R'_3 = Me$; $R_4 = R_1 = R'_1 = H$ = Hayatidine

(13) : $R_2 = R_3 = R'_2 = R'_3 = Me$; $R_4 = R_5 = R_1 = R'_1 = H$ = Hayatine

(14) : $R_2 = R_3 = R_5 = R'_2 = R'_3 = Me$; $R_4 = R_1 = R'_1 = H$ = Hayatinine

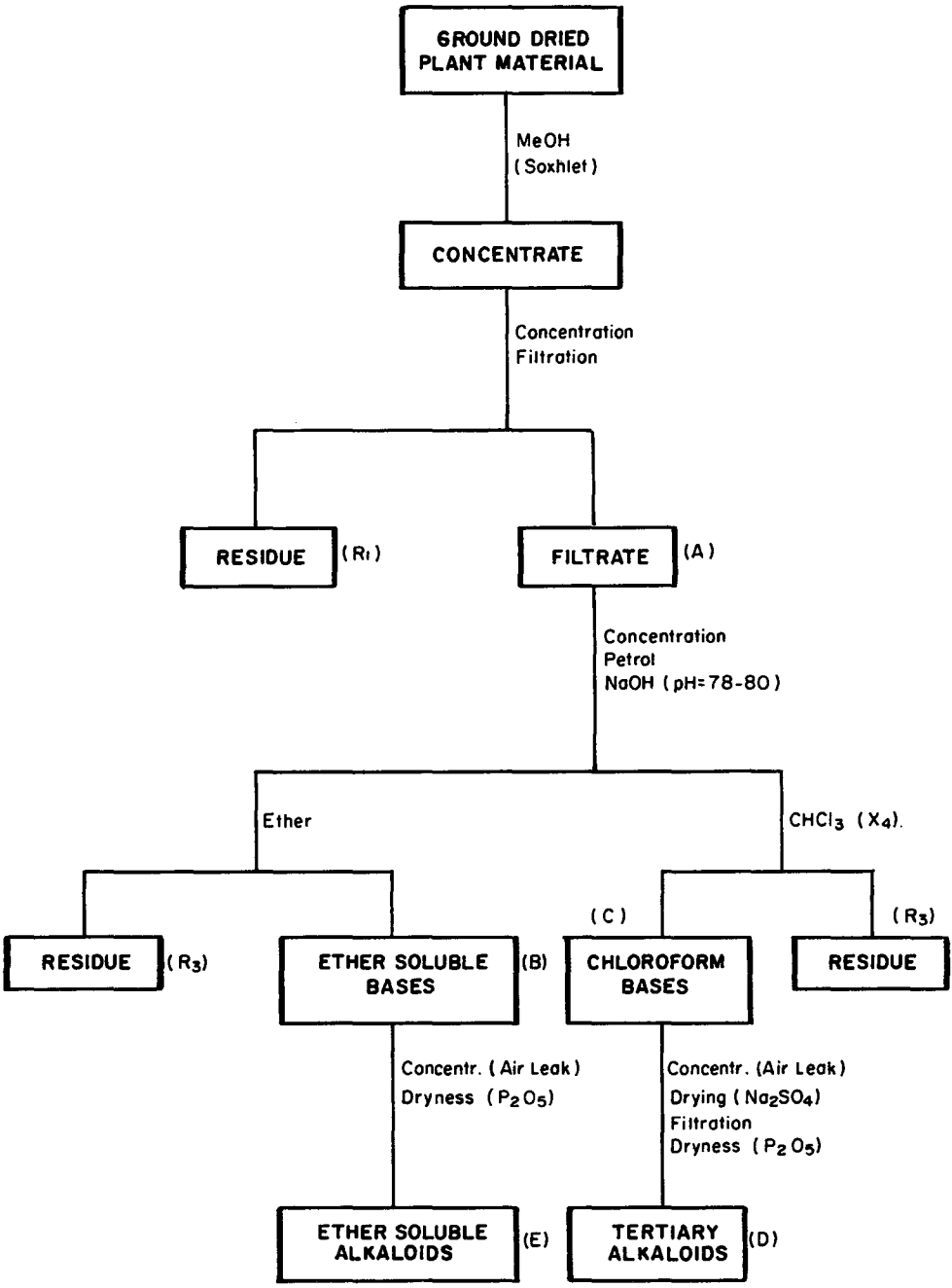


FIGURA No. 1 Methanolic extraction of Warifteine and Methylwarifteine from *Cissampelos spp.*

Preparative and analytical TLC was carried out using silica gel (Merk F 254) in distilled water, eluted with either ethanol, chloroform or a mixture of these. The solvent system was a mixture of chloroform and methanol 4:1 (V/V).

Infrared spectra were taken in a double-beam Perkin-Elmer 237 Grating Infrared spectrophotometer using KBr disks and Nujol-mull semi-solid cells. Liquid cell spectra were recorded in carbon disulphide using the latter solvent as reference.

Protonic Nuclear Magnetic Resonance spectroscopy was carried out in a Perkin-Elmer R-32 spectrophotometer (90 MHz) with a resolution better than 50:1 and a decoupling field strengh of up to 8 milligaus available on field and frequency sweep modes. The solvent used throughout was deuterochloroform and tetramethylsilane was added as internal reference.

Mass spectroscopy spectra were taken on a VG Micromass ZAB-IF spectrophotometer; the technics and procedures of which have been described elsewhere (Aguirre-Galviz, 1988).

RESULTS AND DISCUSSION

As can be seen in Table 2, the various extraction procedures show different yields, the most successful method being the aspirator (infusion) extraction by means of 0.1 N HCl (Fig. 1). These findings are in agreement with the ones reported by Gorinsky (1973), who found that in *C. ovalifolia*, warifteine was present in 0.24-0.27 per cent, that it was extracted by acidic aqueous infusions and that warifteine appears to be unstable upon long standing in methanol.

Ethanol, however, was found not to show detectable decomposing effects on the isolates provided it did not act on them for long periods of time, and if this condition was met, it could be used as an eluent to separate the compounds from silica gel powder after preparative TLC.

The infrared spectrum of warifteine (5) isolated from *C. ovalifolia* is identical to that obtained from *C. pareira* collected in Risaralda. They show a double broad band at 3.490 cm^{-1} and 3.350 cm^{-1}, respectively, corresponding to two phenolic hydroxyl substituents. A sharp band located at 1.610 cm^{-1} is assignable to a carbon to nitrogen double bond.

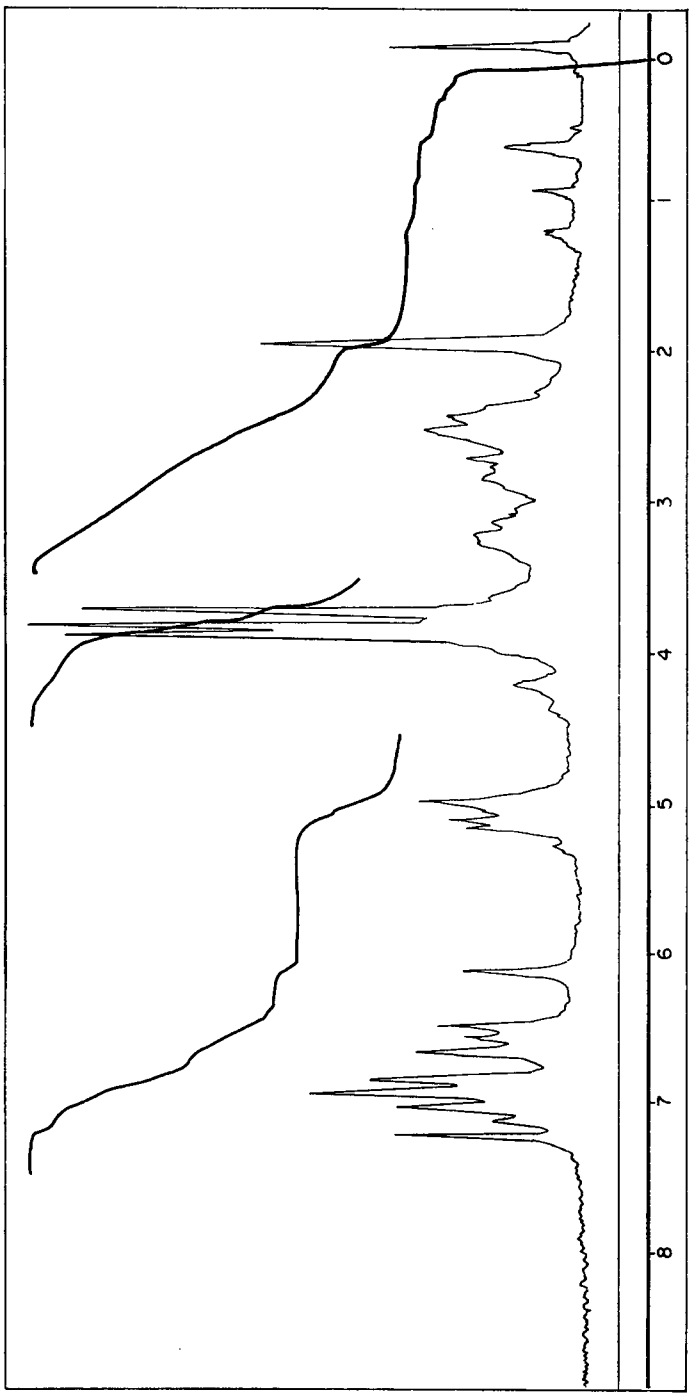


FIGURA No. 2 $^{+}$H-90 MHz NMR Spectrum of Methylwarifteine from *Cissampelos pareira* in $CDCl_3$

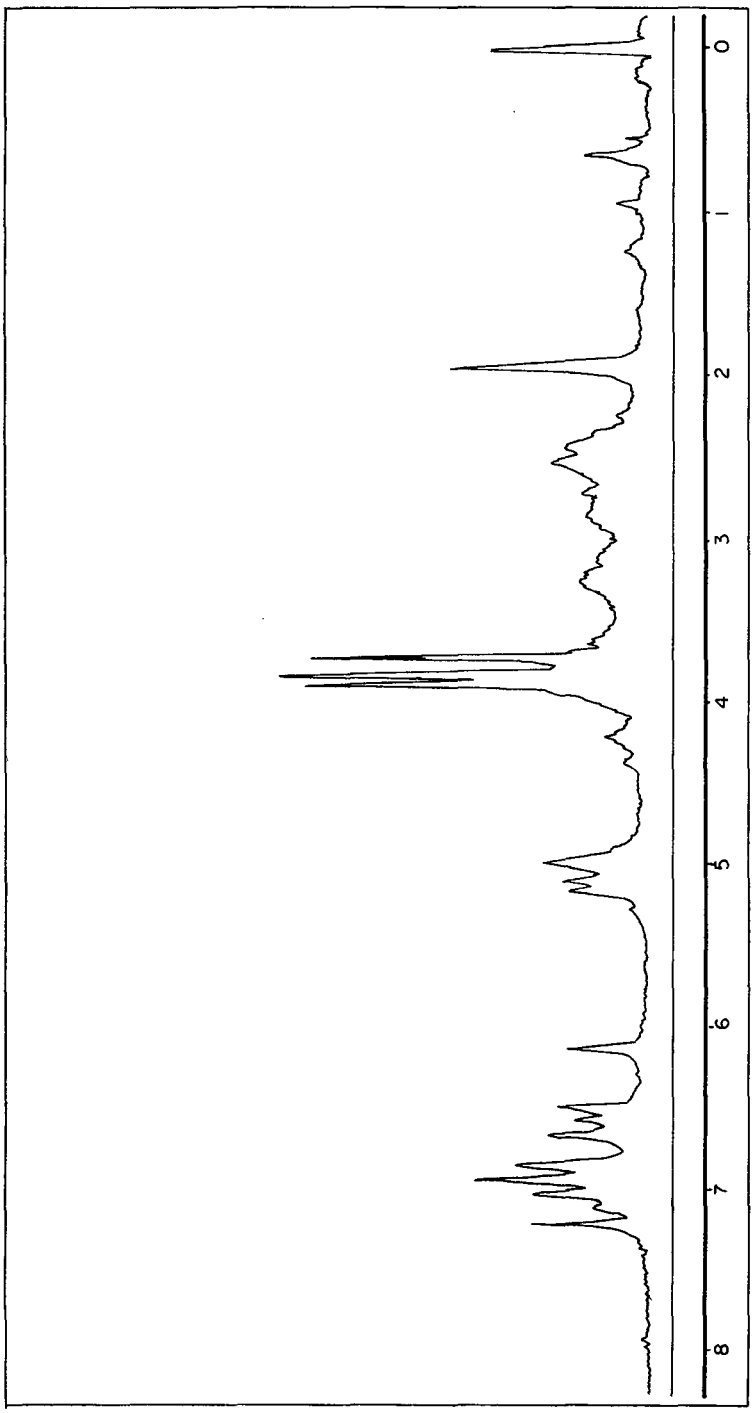


FIGURA No. 3 $^{+}$H-90 MHz NMR Spectrum of Methylwarifteine from *C. ovalfolia* in $CDCl_3$

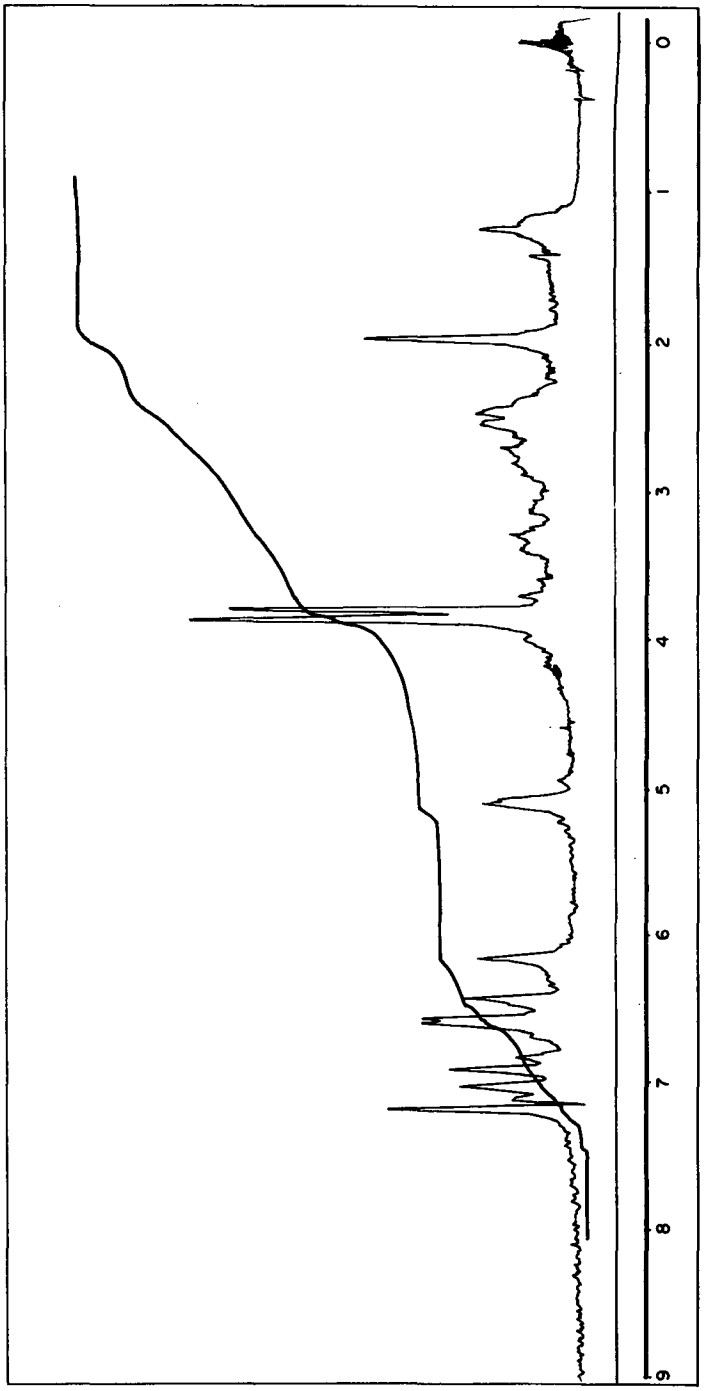


FIGURA No. 4 $^{+}$H-90 MHz NMR Spectrum of warifteine from *Cissampelos pareira* in $CDCl_3$

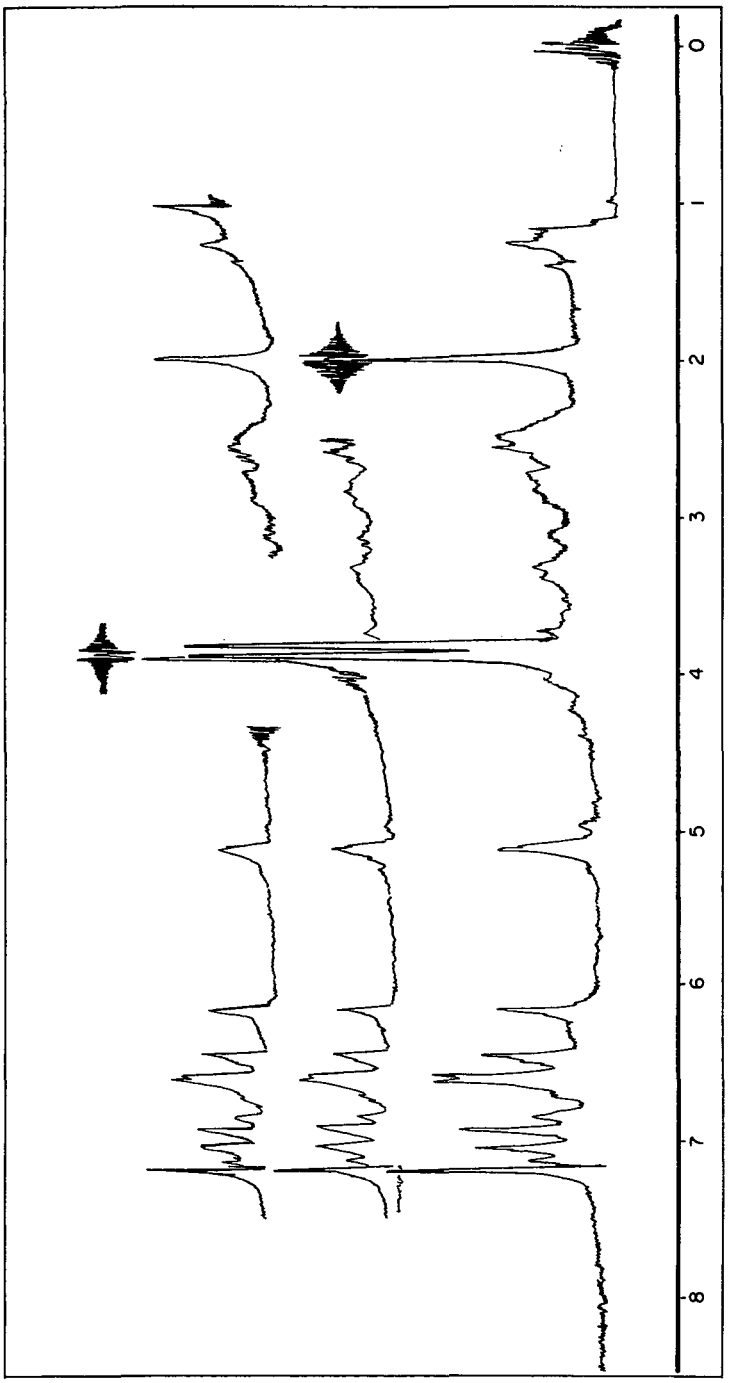


FIGURA No. 5 $^{+}$H-90 MHz NMR Spectrum of warifteine from *C. ovalfolia* in $CDCl_3$

All the IR spectra of the samples of methylwarifteine (6) isolated from the various species studied were superimposable and exhibited an identical intense band at 1.610 cm^{-1} due to C = N double bond, but, instead of two bands for hydroxyl substituents, one broad absorption peak at 3.490 cm^{-1} appears, which can be assigned to one phenolic hydroxyl group.

The mass spectra of these tertiary bisbenzylisoquinoline alkaloids have been discussed in an earlier work (Aguirre-Galviz, 1988).

The NMR spectra of warifteine and methylwarifteine (Figures 2-5) resemble far more those of symmetrical bisbenzylisoquinoline alkaloids than those of unsymmetrical type as, for instance, the spectra of the symmetrical molecules of curine (10). The 90 MHz H^+ - NMR assignments of warifteine and methylwarifteine in $CDCl_3$ are summarized in Table 3, which shows that the triplet (3.72 - 3.88 δ) is finely split into a doblet with a centre at 3.85 (δ) which must correspond to the methoxy groups at 6' and 7' respectively, probably rotating freely and non-equivalent, whereas the third peak at 3.88 δ can be assumed to be due to the methoxy substituent at position 8. The whole region respresents an integration of 23 protons, nine of which are represented by the triplet, while the remaining 14 protons are the eight heterocyclic ones, the protons at positions 6,1', and the two $-CH_2-$ groups of the isoquinolines, between positions 9,1 and 9',1' respectively.

The aromatic region of the NMR spectrum shows a series of peaks between 7.22 δ which could be interpreted as follows: the benzene ring with the ether linkage shows a A_zB_2 quartet at 6.82 δ, and 6.68 δ; the symmetrical ring exhibits a large singlet at 7.22 δ and the two single aromatic protons on the isoquinoline rings show signals at 6.50 and 6.22 δ, Irradiation of the peaks at 1.95 and 3.81 δ did not change the pattern in either the region 3.8 - 4.4 δ or the region 1.96 - 2.01 δ, indicating the equivalence of the methylene protons and the single character of the peak at 1.96 δ (Figure 5).

Warifteine exhibits a spectrum which is similar to methylwarifteine but, instead of triplet at 3.7 - 3.9 δ. the former compound exhibits a doublet centered at 3.86 δ, which integrates for six protons corresponding to the O-methyl groups at 8 and 6' (Figure 4). The sharp signal at 1.98 δ, equivalent to three protons, could be assigned to the 2' - N methyl group. Similarly to methylwarifteine, the aromatic paraxylyl group at position 12' occurs at 4.98 δ and is equivalent to two protons (Table 3).

TABLE No. 3
90-MHz $H^{\pm}$NMR SPECTRAL DATA AND ASSIGNMENTS OF WARIFTEINE ($C_{36}H_{36}O_6N_2$) AND METHYLWARIFTEINE ($C_{37}H_{38}O_6N_2$) IN DEUTEROCHLOROFORM

COMPOUND INTEGRATION (*)	SIGNAL (δ UNITS)	ASSIGNMENT	
W	3.86, 3.90	O-methyl	6
	1.98	2'-N-methyl	3
	4.98	Ar-CH_2-OR	2
	7.21 - 6.12	Aromatic protons	10
	2.2 - 3.4	-CH_2-; heterocyclic protons	15
MeW	1.95	2'-N-methyl	3
	3.72; 3.81; 3.88	O-methyl	9
	4.99	Ar-CH_2-OR	2
	7.21 - 6.12	Aromatic protons	10
	2.2 - 3.4	-CH_2-; heterocyclic protons	14
(*) Abbreviations:		W: Warifteine MeW: Methylwarifteine	

As it was pointed out before, the use of methanol, both in hot and cold, resulted in lower amounts of warifteine and methylwarifteine being extracted and in a sizable decomposition of these alkaloids if left for long in presence of this solvent. However, methanolic percolation at room temperatures has been used as a method of extracting related bisbenzylisoquinolines from *Cyclea peltata* (Kupchan, 1973) whereas Boissier (1965), used hot alcohol (soxhlet) extraction for *C. pareira* from Madagascar and Ahmed and Cava (1977), studied this species by extraction with aqueous ammonia-ether. The procedures, however appear to be aimed at a qualitative extraction of a representative number of alkaloids in the plant species investigated, rather than a quantitative search into a particular kind of compound, such as in an ethnobotanical approach.

The yields from the species examined must be considered in respect to their distribution, since ecologic factors are known to affect the nature, relative proportions and amounts of alkaloidal composition as pointed out by the various studies carried out on *C. pareira* (Tomita and Abe, 1952; Bhattacharji et al., 1956; Boissier et al., 1965).

This might be the case with the alkaloids found in the various samples of *C. pareira* examined here, where leaves of the plant collected in Risaralda (Colombia), showed the presence of warifteine and methylwarifteine but those plants found in other parts of the same country were devoid of warifteine and contained only methylwarifteine, as was the case with the material originated from India.

The isolation of warifteine from leaves of *C. pareiraa* is of particular interest since that compound has not been found in this species. Kupchan, studied peruvian samples of *C. pareira*. from the Department of Huanaco in 1966 and reports one alkaloid called by him, cissamperine (4), which appears to be, spectrally and chromatographically, very similar to methylwarifteine. The application of Kupchan's method to Colombian material of *C. pareira* (leaves) resulted in the isolation of small amounts of one compound which was identical to methylwarifteine with no traces of other compounds being observed.

It seems important to consider whether cissamperine and methylwarifteine, two very similar compounds, constitute the same alkaloid isolated from two different populations of *C. pareira*; whether the samples from Peru and Colombia contain different alkaloids or whether they possess a hydroxyl substituent at isomeric positions within the same structural skeleton. This distinction appears to be important not only from the ecological point of view but also because warifteine and methylwarifteine are of pharmacological potential. Cissanperine, itself, was evaluated for activity against human carcinoma (Kupchan, 1965), as did 1-curine, d-isochondodendrine and tetrandrine (Kupchan and Yodoyama, 1960).

Thus, apart from its uses in popular medicine in Asia, Africa and America (Chopra, 1958), one might reasonably expect methylwarifteine to possess some, or all, of those medical properties which seem to be common to the other four bisbenzylisoquinoline alkaloids tested by Kupchan and co-workers. Cissamperine possesses three O-methyl groups and one N-methyl group, and the NMR spectrum of this compound, as described by Kupchan and co-workers, confirms this substitution pattern by showing nine O-methyl protons (3.99 δ, and 3.75 δ) and three N-methyl protons (199 δ) with the high chemical shift corresponding to these protons probably attributable to a large anisotropic effect of a neighbouring aromatic ring, as in the case of warifteine. Cissamparine also shows a singlet at 5.15 δ (2 protons), assignable to Ar-CH_2-OR and a hindered phenolic hydroxyl group located in the benzyl 3'-4' dihydroisoquinoline moiety, as shown by sodium in liquid ammonia reduction of derivatives (Kupchan, 1966). Unfortunately, the author does not reports the full NMR spectrum of this compound, nor does he mention decoupling experiments, providing only spectral data of derivatives and decoupling experiments carried out in them. Methylwarifteine obtained from *C pareira* and *C. ovalifolia*, on the other hand, also exhibit very similar spectra to the one cited by Kupchan and it is difficult, solely from NMR data, to draw a conclusión as to whether the two compounds are the same or not.

Mass spectra support the assignment of a symmetrical bisbenzylisoqionoline structure for cissamperine (Kupchan, 1965) and also for warifteine and methylwarifteine isolated from *C. pareira* and *C. ovalifolia* (Aguirre-Galviz, 1988). Kupchan reports that the major fragment ions of the former compound appear at m/e 312 and 310 which corresponds to the cleavage a-c and d with hydrogen transfer. This type of fragmentation has been shown to be characteristic of the symmetrical bisbenzylisoquinoline type (Tomita, et. al., 1966). Further important fragments at m/e 502 and 500 were explained by fission at a-b, and those at m/e 206 and 204 correspond to cleavage at a-c, again with hydrogen transfer. When the mass spectral data obtained in the course of the present investigation are compared to Kupchan's, it appears that the peaks at m/e 310, 312 and 502 are common to both cissamperine and methylwarifteine but those at 500, 206 and 204 m/e, exhibited by the former, were not reported in the latter, Fragmentation patterns are lacking in Kupchan's work and no explanation of the peaks at 206 and 204 can be found. The situation of mass spectrometry is the same as with NMR and only and comparative chemical study of samples from the same places of collection (as the plant investigated by Kupchan and those of Colombia), would permit definitive conclusions to be drawn.

The impresion gained throughout this investigation is that probably cissamperine and methylwarifteine are isomeric compounds and that only a thorough study of their derivatives and sodium reduction products could indicate the exact location and rotational behaviour of their phenolic hydroxyl substituents.

In order to ascertain whether or not warifteine was present in more Colombian material, studies on plant samples of *C. pareira* from other parts of the country were carried out. These investigations showed the species to be devoid of warifteine and containing only methylwarifteine.

A closer consideration of the habitats of the various samples and parts of the plant studied shows several differences that could be summarized as follows.

1. *Cissampelos ovalifolia* is a small shrub characteristic of the Orinoco River, growing to a height of about 1 m. The habitat of the plant is usually on relatively high ground quite near, or under, the small trees of the open savannah.

   The plant survives continuous burning thanks to an extensive root system and it is able to use its xerophytic leaves in order to resist the dry season (Gorinsky, personal communication). The samples of this species were collected in the open savannahs of the Rupunini

District of Guyana and they consist of nodular roots which have been dried and stored without losing their content of warifteine and methylwarifteine. The species is one of the alleged constituents of macushi curare and it is also used as a therapeutic drug by the local peoples. Both warifteine and methylwarifteine have been shown to be the two main constituents of its bisbenzylisoquinoline fraction (Gorinsky, 1973).

2. *Cissampelos pareira* from India, was brought from that country by Dr. P. Vadlamudi in the form of stems obtained from local herbalists who claimed that the material was of therapeutic use, but no voucher specimens were gathered and the botanical identity was solely determinated by its vascular morphology with xyleme and phloem vases arranged in such a way that the stems possess a stellar appearance when viewed in cross-section. Besides, the presence of methylwarifteine, even if in the lowest concentration among the samples studied, was a further proof of the identity of this material. Since there is no voucher specimen, it is impossible to know the habitat of the plant, but it seems reasonable to assume that it comes from fairly dry areas around Hyderabad, the locality of collection which is, itself, situated in a dry region of India.

3. There were six samples of Colombian *C. pareira* available to this study, two of them from the same geographical region (Table 2) but collected in different habitats: one open burned-out savannah and the other in places located at lower altitudes, well watered by the River Gazaunta, a tributary of the Meta, and growing among several other species in the woods along the river banks. Both samples consist of leaves with no warifteine but only methylwarifteine.

   The material (leaves) collected near Fusagasugá comes from plants growing in areas which are heavily populated and subjected to cultivation. The stems obtained in Tequendama, on the other hand, grow in Andean mountain woodlands, which have been undisturbed for many years and are well watered by the Bogotá river. While these two samples contain methylwarifteine and no warifteine, the leaves collected at 1.070 m. above sea level in Risaralda (La Virginia) showed a very significant content of warifteine since this compound has not been isolated, as yet, from *Cissampelos pareira* and also yielded larger amounts of methylwarifteine (Table 2). Medina, on the other hand, is in the flat lands between 500 and 1.000 meters of altitude, consisting of mildly humid to mildly dry prairies.

The two samples of *C. pareira* gathered at Medina, one coming from the open savannahs and the other from the river banks, belong to different climatic regimes and could be expected to exhibit differences in their content of warifteine and methylwarifteine. Notwithstanding, the evidence obtainded (Tables 1 and 2), suggests a similar alkaloidal composition in the two samples and makes it difficult to apply the criteria of different habitats governing the synthesis of these compounds, unless one consideres that the soil factor is a special one.

No explanation has been found to account for the absence of warifteine in the samples collected in Tequendama and Fusagasugá, areas which are similar to Risaralda. The assumption could be made that the stems are organs where plants do not store alkaloids and only roots and leaves do so, when the correct climatic and edafologic conditions are available. However, without having examined several organs of each plant population, it is difficult to draw any conclusions.

Thus, from the evidence gathered, it appears that *C. pareira* accumulates both warifteine and methylwarifteine in leaves (or roots) when it grows at medium altitudes in the warm and well watered lands of the Andean valleys and plateaus. This hypothesis could only be tested when a wide range of samples from environments are available and consisting of as many plant organs as possible. Unfortunately, no samples from the Amazonian rain forest were available for investigation but it is evident that, when studying material from various parts of South America, the different habitats and hydric conditions in which those plants grow must be kept in mind.

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