# MASS SPECTRA OF ALKALOIDS FROM CISSAMPELOS PAREIRA L.

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## RESUMEN

Se presenta una discusión de los espectros de masas de warifteina y metil-warifteina, dos alcaloides bis-benzil- isoquinolínicos aislados de *Cissampelos pareira,* una especie de importancia etnobotánica. Se da información sobre las posiciones relativas de dos grupos hidroxilo y dos metilos en warifteina, así como sobre la presencia de una parte p-xilílica en la molécula de ambos compuestos.

Key word index: Cissampelos pareira, mass spectra, alkaloids, menispermaceae.

## SUMMARY

The mass spectra of the tertiary bis-benzyl-isoquinoline alkaloids, warifteine and methyl-warifteine, extracted from the ethnobotanically important species, *Cissampelos pareira* L., are discussed. Information about the reltive positions of two hydroxyl and two methoxyl groups in warifteine and the presence of a p-xylyl moiety in both compounds in provided.

## INTRODUCTION

Kupchan et al. (1960), reviewed the work on *Cissampelos pareira* L. and listed four principal alkaloids in the species: hayatine, hayatinine, (+)-curi-

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ne and (+)- isochondodendrine. The first compounds were claimed to possess curare-like actions comparable to that of (+)-tobocurarine (Pradham, 1953). The most recent work on *C. pareira* yielded a novel tetiary bis-benzyl-isoquinoline base, cissamperine, with no other alkaloid reported (Kupchan, et al., 1966).

Since *C. pareira* L. might be the origin of, at least, some of the calabash curare preparations used by the Macushi tribe of South America, the species has interested chemists and botanists since the early days of commercial preparations of drugs such as Radix Pareirae Bravae, curare, and the various arrow poisons used by South American indians.

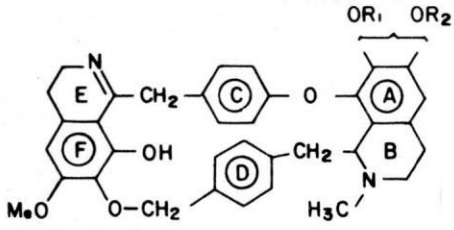
Furthermore, the isolation of warifteine from leaves of *C. pareira* is of interest since that compound has never been found in this species by the research workers who investigated it, although none of them appear to have had access to samples of *C. pareira* from Colombia or from the north-western Andean part of the South American continent. Kupchan, et al. (1966), studied Peruvian samples of *C. pareira* from the Department of Huanaco in 1965 and reported one alkaloid called by him cissamperine which appears to be, spectrally and chromatographically, very similar to methyl-warifteine. The application of Kupchan's method to Colombian material of *C. pareira* resulted in the isolation of small amounts of methyl- warifteine and warifteine.

It seems important to consider if those two 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 methyl-warifteine are of pharmacological potencial. Cissamperine, itself, was evaluated for activity against human carcinoma of the nasopharinx and was found to have significant and reproducible inhibitory activity (Kupchan, 1965), as did 1-curine, d-isochondodendrine and tetrandrine (Kupchan et al., 1960). Thus, apart from its uses in popular medicine in Asia, Africa and America (Chopra, 1958), one might reasonably expect methyl-warifteine to possess some, or all, of the medical properties which seem to be common to the other four bis-benzyl-isoquinoline alkaloids tested by Kupchan and coworkers.

## EXPERIMENTAL

Samples of *C. pareira* were collected in the Andean region of Colombia (Departamento de Cundinamarca, Salto de Tequendama 2.000 m; Departamento de Risaralda, La Virginia 1.070 m), botanically identified and voucher specimens deposited in the Herbario Nacional Colombiano (Gorinsky and Idrobo, SN, 1980).

MS measurements were carried out in a VG micromass ZAB-IF apparatus, fitted with solid probe (70 ev) and ionizing current 300 A. The atomic compositions of the more intense ions were obtained by measurement of ion mass to a precision of ± 10 ppm. The theoretical masses of the assigned compositions differed from the measured masses by less than 10 ppm im each case, while the measured masses of the metastable ions agreed with the theoretical to within 0.1 mass units.




I $R_1 \equiv H$ ; $R_2 \equiv Me$

II $R_1 \equiv R_2 \equiv Me$

The spectra were taken at two vaporization temperatures (150°C and 200°C) to allow the observation of effects on the mass spectrum resulting from the changes caused by the excess of vibrational and rotational energy gained by the molecule before it is ionized and for comparison, so as to decide the exact mass-to-change ratio (m/e), of the molecular ion M + 1 peaks.

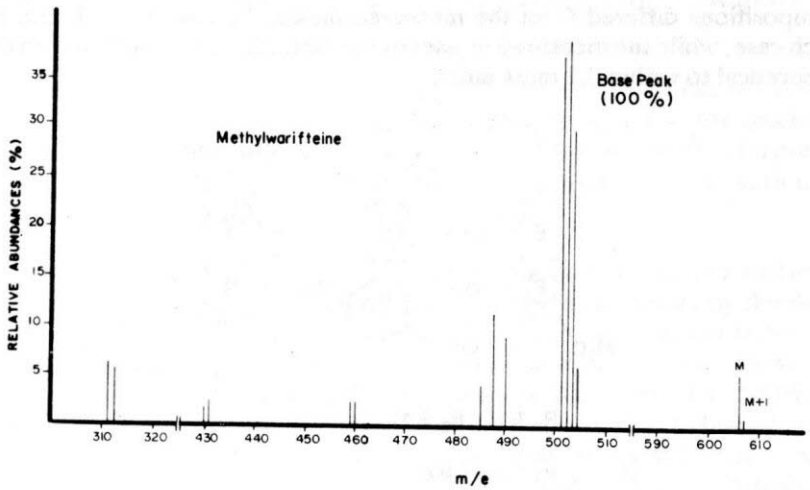
After the spectra were obtained, all the peak heights were related to the base peak and expressed as the porcentage of the base peak height, thus obtaining the normalized ion abundances of the partial mass spectra. Peaks below m/e 300 are of less structural significance and were omitted for clarity.

## RESULTS AND DISCUSSION

The partial mass spectra of warifteine (I) and methyl- warifteine (II) are shown in figure 1.

The presence of two hydroxyl and two methoxyl groups in warifteine was corroborated by infrared and NMR studies. Thus, two hydroxyl bands 2.87 nm and 2.72 nm are observed in the IR spectrum and two methoxyl proton bands at $\tau = 6.10$ in the NMR spectrum (90 MHz).

In both compounds the predominant mode of cleavage occurs at positions a and b with elimination of the p-xylyl residue. The ion so formed (m/e 502 and 488 for methyl-warifteine and warifteine respectively) contained an umpaired electron which appears to induce fragmentation of ring F. with successive loss of $CH_3^{\cdot}$, CO and CHO as shown in figure 2.



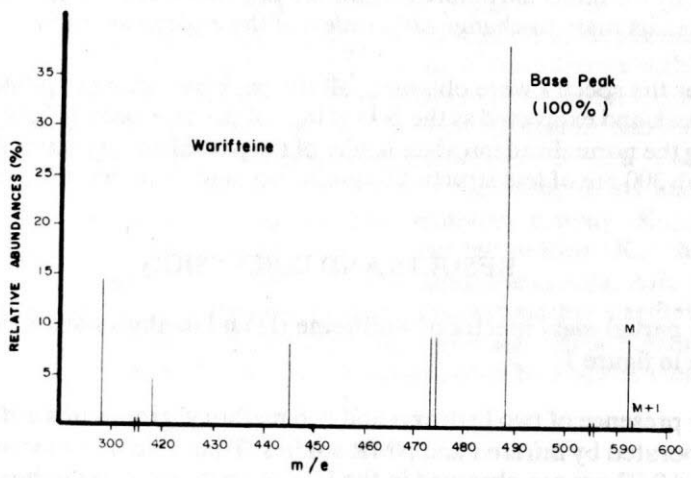




Figure 1 - Mass Spectra of Methylwarifteine and Warifteine from Cissampelos spp.

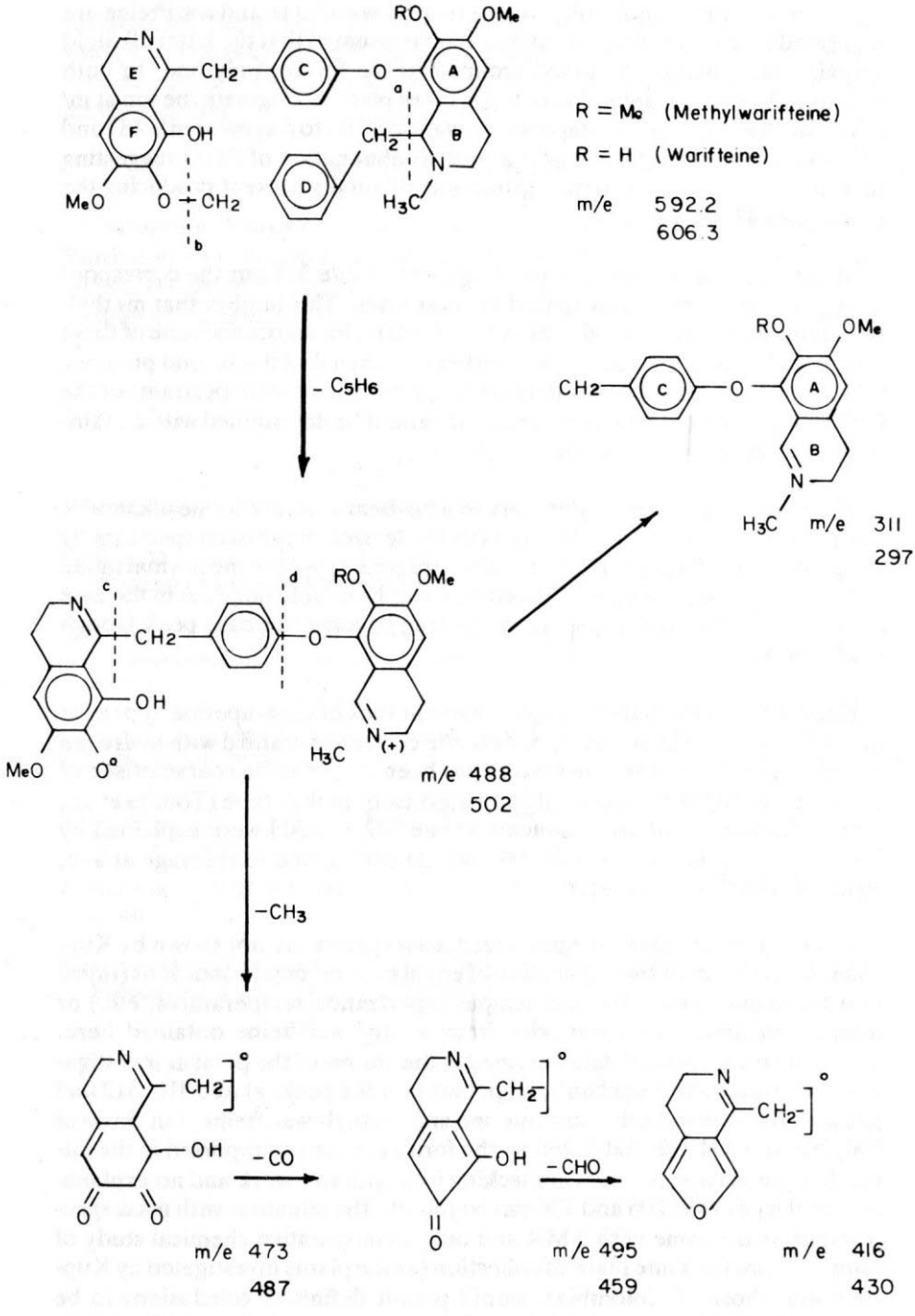



Figure 2 - Fragmentation patterns in the Mass Spectrum of Warifteine and Methylwarifteine

The masses of the molecular ions of methyl-warifteine and warifteine are separated by 14 units, from which it could be assumed that the latter alkaloid contains two phenolic hydroxyl groups and the former only one. In both compounds, ready elimination of $C_8H_8$ takes place to originate the ion at m/e 502 and 488, followed by stepwise cleavage of $CH_3$ to fragment m/e 487 and 473; CO to ions m/e 450 and 445; as well as elimination of CHO suggesting that, in ring F, only one methyl group was originally present producing the ions of m/e 430 and 416.

Methyl-warifteine gives rise to a fragment of m/e 311 but the corresponding ion from warifteine is shifted 14 mass units. This implies that methyl-warifteine has two methyl groups in ring A while, for warifteine, one of them is replaced by an OH group. This further confirms that the second phenolic OH of warifteine must be localetd on ring F but the relative positions of the hydroxyl and methyl groups on each ring cannot be determined with certainty by mass spectrometry alone.

The ocurrence of a p-xylyl moiety in a bis-benzyl-isoquinoline alkaloid is comparativaly rare. Its presence is easly recognized in the mass spectrum by the production of a prominent M- $C_8H_8^+$ fragment. Where the normal mode of fragmentation at (a-c) is inhibited by a vinylic double bond, as in the case of warifteine, the M-$C_8H_8$+ fragment may become the base peak (Jongh et al., 1966).

Kupchan reports that the major fragment ions of cissamperine 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 alkaloids of the symmetrical bis-benzyl-isoquinoline 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.

It is unfortunate that the normalized mass spectra are not shown by Kupchan, so as to permit the application of equal experimental conditions (injection technique, ionization and sample vaporization temperatures, etc.) or direct comparison with the ones from methyl-warifteine obtained here. When the mass spectral data obtained in the course of the present investigation is compared to Kupchan's, it appears that the peaks at m/e 310, 312 and 502 are common to both, cissamperine and methyl- warifteine, 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 with mass spectrometry is the same with NMR and only a comparative chemical study of samples from the same place of collection (as the plants investigated by Kupchan and those of Colombia), would permit definitive conclusions to be drawn. The impression gained throughout this work is that probably cissamperine and methyl- warifteine 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. Since the botanical identity of the plant material used in the investigación leading to the isolation of cissamperine appears to be well documented, there is every reason to assume that this kind of study should be most rewarding.

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