Arch. Invest. Méd. (Méx.), 1990; 21: 175 - 7

Recibido: 27-II-1990
Received: 27-II-1990

Aceptado: 15-III-1990
Accepted: 15-III-1990

n, n-dimethyltryptamine alkaloid in *mimosa tenuiflora* bark (tepescohuite)

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n, n-dimetiltriptamina alcaloide de la corteza de *mimosa tenuiflora* (tepescohuite)

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Introduction

In Mexico, the plant product "tepescohuite" (toasted and powdered bark of *Mimosa tenuiflora* (Willd.) Poir., Leguminosae) has received considerable attention as an empirically applied remedy for skin burns. Following unpublished clinical trials, "tepescohuite" was claimed to relieve pain and itching, reduce infection and healing of second degree burns. More recently, "tepescohuite" has been promoted as a treatment of stomach ulcers and other miscellaneous ailments. However, little is known about its pharmacological properties and chemical constituents. We have recently undertaken phytochemical and pharmacological studies on *M. tenuiflora* bark extracts. Antimicrobial, hemolytic, hemagglutination, and smooth muscle activity effects were observed *in vitro*.[1,2] An alkaloidal fraction strongly inhibited the peristaltic reflex in the isolated guinea pig ileum.[3] The present communication reports the identification of the principal alkaloids of this fraction.

The well known central nervous system stimulant, N_b,N_b-dimethyltryptamine (DMT), was obtained in a yield of approximately 0.03 % from the powdered bark. Subsequent HPLC investigation of the alkaloid fraction additionally confirmed the presence of serotonin, in approximately 0.001 % yield. Other related tryptamines, including bufotenine, 5-methoxytryptamine, N_b-methyltryptamine, and 5-methoxy-N_b,N_b-dimethyltryptamine, were not found to be present at the trace levels detectable by our HPLC method.

Occurrence of tryptamine derivates in other species of *Mimosa* has been previously reported.[4,5] DMT was detected in the roots of *Mimosa hostilis* Benth.[6] and in the bark of *Mimosa verrucosa.*[7] DMT alone is apparently inactive orally, probably because it is metabolized by visceral monoamine oxidase (MAO). However, the co-occurrence of beta-carboline alkaloids in the same plant product, a circumstance common since tryptamines are also the precursors for these reversible inhibitors of MAO, can protect the DMT from deamination and render it orally active.[8] In the case of *M. tenuiflora,* which is used for the treatment of burns, the powdered bark is sprinkled on the damaged skin surface. This raises the question of possible direct absortion of DMT into the blood stream, suggesting that practical use of the crude "tepescohuite" remedy may involve some health risk.

Further studies will be required to determine if beta-carbolines are present as minor constituents, and what is the significance of the alkaloid content to patient health.

Experimental

General experimental procedures. Solvents for column chromatography were glass distilled (BDH Inc., Toronto). Solvents for HPLC were HPLC grade (BDH). Tryptamine standars: 5-hydroxytryptamine (serotonin), 5-hydroxy-N_b,N_b-dimethyltryptamine (bufotenine), 5-methoxytryptamine, N_b-methyltryptamine, 5-methoxy-N_b,N_b-dimethyltryptamine, and N_b,N_b-dimethyl-tryptamine were obtained from Sigma Company, St. Louis, Mo., U.S.A. Bufotenine and DMT were obtained and used under special permission from the Bureau of Dangerous Drugs, Health and Welfare Canada. Proton and ^{13}C nmr spectra were obtained at 300 and 75.4 MHz; respectively, on a varian XL-300 spectrometer. HPLC was performed on a 4.6 x 250 mm, 5 um C18 reverse-phase column (ODS-Altex Ultrasphere), using programmable Perkin-Elmer LC-480 autoscan diode array UV detector with detection at 280 nm, and a Perkin Elmer Nelson 900 integrator for quantitation.

Plant material. Trunk bark of *Mimosa tenuiflora* (Willdenow) Poiret was collected by personnel of the National Union of Tepescohuite Producers Company, in Chiapas, Mexico, in the summer of 1986. Reference vouchers were deposited at the IMSSM Medical Herbarium after their taxonomic verification.[9] The bark samples had been toasted (150°, 2 hours), sliced finely and packed in plastic bags according to the usual procedure applied by the Company. The same product is distributed for medicinal purposes.

Isolation. The alkaloidal fraction was obtained by conventional partition procedures alredy described.[3] Preliminary purification was accomplished by step-gradient flash column chromatography (silica gel 60, 230-400 mesh) using a solvent mixture of hexane:acetone:methanol (100:0:0 - 0:100:0 - 0:0:100 in steps of 20 %, aliquots of one liter). The alkaloid-positive fractions, detected by Ehrlich's reagent, were combined and subjected to centrifugal preparative thin-layer chromatography, using a Harrison Research Chromatotron. The sample was applied to a 2 mm layer of silica gel PF-254 (Merck) and eluted with a solvent mixture of ethyl ether and methanol (2:1, then 1:1, 200 ml each) at a pump flow rate of 6 ml/min. Concentric bands were detected by UV light (254 nm) and collected as they eluted. Dowex-50 ion-exchange column chromatography was used to further purify the principal alkaloid, followed by preparative TLC on silica crystallized from methanol and had a melting point of 45.5-46.8°.

The combined fractions containing minor alkaloids were purified by ion-exchange column chromatography and preparative TLC. The mixture was then subjected to HPLC (20 ul, 10 ug/ml in MeOH), eluted at a flow rate of 1.0 ml/min using a gradient of acetonitrile/ammonium carbonate 0.1 M (30 min: 20-30 % A in 3 min; 30-40 % A in 10 min; 40-100 % A in 12 min; 100-20 % A in 2 min, and finally, equilibration at 20 % A for 3 min).

Identification. Spectroscopic analysis of the major alkaloid (eims, cims, ^1H nmr, and ^{13}C nmr) provided results in close agreement with published values for DMT (10-16). The HPLC capacity factors for the two main peaks of the minor alkaloid fraction corresponded with 5-hydroxytryptamine ($k' = 0.44$) and DMT ($k' = 7.28$). Cochromatography of the sample with a pure reference sample of 5-hydroxytryptamine gave a single peak at the same k' with an enhanced integration.

Acknowledgements

Funding for the project was provided by the Canadian natural Sciences and Engineering Research Council through an International Collaborative Grant, and by the Mexican Social Security Institute, and by the National Council of Science and Technology of Mexico. We thank Health and Welfare Canada for their asistance in obtaining the tryptamine standards.

References

1. X. LOZOYA, V. NAVARRO, J.T. ARNASON and E. KOURANY. Archiv. Invest. Med. (Mex). 1989; 20: 87.
2. M. MECKES-LOZOYA, X. LOZOYA and J.L. GONZALEZ. Archiv. Invest Med (Mex) 1990; 21:
3. M. MECKES-LOZOYA, X. LOZOYA, J.L. GONZALEZ and M. MARTINEZ. Archiv. Invest. Med. (Mex.) 1990; 21:
4. M-P. GUPTA and T.D. ARIAS. Jour. Nat. Prod. 1979; 42: 234.
5. M.F. BALANDRIN, A.D., KINGHORN, S.J. SMOLENSKI and R.H. DOBBERSTEIN, J. Chromatog. 1978; 157: 365.
6. T.A. SMITH. Phytochemistry 1977; 16: 171.
7. I.J. PATCHER, D.E. ZACHARIUS and O. RIBEIRO. J. Org. Chem. 1959; 24: 1285.
8. D.J. MCKENNA, G.H.N. TOWERS and F. ABBOTT. J. Ethnopharmacol 1984; 10: 195.
9. R. GRETHER. Bol. Soc. Bot. Mexico 1988; 48: 151.
10. S. AGURELL, B. HOLMSTEDT, J.-E. LINDGREN and R.E. SCHULTES. Acta Chem. Scand 1969; 23: 13.
11. A.C. THOMPSON, G.F. NICOLLIER and D.F. POPE. J. Agric. Food Chem. 1987; 35: 361.

12. C. POUPAT, A. AHOND and T. SEVENET. Phytochemistry 1976; 15: 2019.
13. M.S. MORALES-RIOS, J. ESPINEIRA and P. JOSEPH-NATHAN. Magn. Reson. in Chem. 1987; 25: 377.
14. E. WENKERT, J.S. BINDRA, C.-J. CHANG, D.W. COCHRAN and F.M. SCHELL. Accounts of Chem. Res. 1974; 7: 46.
15. L.A. COHEN, J.W. DALY, H. KNY and B. WITKOP. J. Am. Chem. Soc. 1960; 82: 2184.
16. G. CIMINO and S. DE STEFANO. Comp. Biochem. Physiol. 1978; 61c: 361.