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BRUNFELSAMIDINE: A NOVEL CONVULSANT FROM THE MEDICINAL PLANT BRUNFELSIA GRANDIFLORA

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Abstract: A convulsant isolated from Brunfelsia grandiflora is identified as pyrrole-3-carboxamidine.

Natives of the upper Amazon region use water extracts of the roots or bark of Brunfelsia grandiflora D. Don (Solanaceae) as a medicine, narcotic and in higher doses as a fish poison[1a-d]. This species is widely employed as one of the most important native remedies against rheumatism and arthritis; it is also used for fevers and snakebite[2]. Ingestion of these extracts is reported to cause sensations of chills (chiricaspi "cold tree"; chiric sanango "cold medicine") and hallucinations (borrachera "intoxicant")[2]. The bark of B. grandiflora and B. chiricaspi are added to the hallucinogenic drink made from Banisteriopsis caapi (Spruce) Morton (ayahuasca, yagé) to enhance its effects.

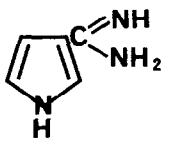
There are no accounts of previous chemical or pharmacological investigations of this species of Brunfelsia. We now report the isolation, purification and identification of the title compound from B. grandiflora D. Don subsp. Schultesii Plowman, collected near Pebas, Peru, on the banks of the Amazon[3].

Root bark (225g) was extracted with 2 l cold water according to typical native usage. The extract was found to induce convulsions in mice[4] and still produced the same effect after extraction with chloroform which removed a small amount of scopoletin (m.p. and m.m.p. 204-205°C; m.s: m/z 192 (M^+ 100%), 177 (61), 164 (25), 149 (41), 121 (15), 79 (11), 69 (24)). Part of the extract (200 ml) was chromatographed on a CM-Sephadex column using in turn water, dilute acetic and hydrochloric acids and the fractions were monitored biologically[4]. The active ingredient (0.2g) was obtained as a hydrochloride, m.p. 127-128°C, which showed ultraviolet absorption peak at λmax (MeOH) 236 (logε 4.65) and 263 (logε 4.45), suggesting aromatic character. Two dyes resulted when it was treated with diazotized p-anisidine (Rf 0.72 (red) and 0.2(yellow) in 1:1 benzene-ethyl acetate on cellulose). Its mass spectrum showed a molecular ion at m/z 109 (100%) and fragment ions at m/z 93 (M-16, 64%), 94 (15%) and 66 (M-16-27, 5%) with metastables for all three events. The nmr spectrum in CD_3OD exhibited three protons centered at δ 6.70 (q), 6.93 (q) and 7.69 (t). The ^{13}C spectrum showed four olefinic carbon

atoms: three were attached to hydrogen (δ 108, 122 and 124) while one was fully substituted (δ 112). A fifth carbon appeared in the carbonyl region at δ 162.

Treatment with acetic anhydride-pyridine converted the unknown to a diacetate (m.p.140-142°C) turning red in the air partly reverting to a monoacetate. The mass spectrum of the diacetate showed a molecular ion at m/z 194 ($C_9H_{10}N_2O_3$, formula deduced from other fragments) with loss of one ketene at m/z 152.0562 ($C_7H_8N_2O_2$), two ketenes at m/z 110.0438 ($C_5H_6N_2O$), ketene and CH_3CONH- at m/z 94.0297 (C_5H_4NO). The shift in parity of the molecular ion on acetylation suggested the formula of the original substance to be $C_5H_7N_3$; apparently an acetoxy displaced an NH group during the acetylation.

All the above data virtually exclude any other structure but that of pyrrole-2 or 3-carboxamidine. Both amidines were synthesized by established procedures[5] from the corresponding nitriles[6] and the 3-isomer (1) was identical in all respects (chemical and pharmacological) with the natural product. The di and monacetates are then assigned structures (2) and (3) and the diazo coupling reaction follows from the well-known property of pyrroles mimicking phenols.



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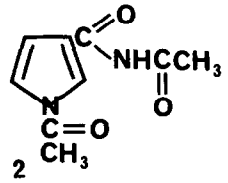

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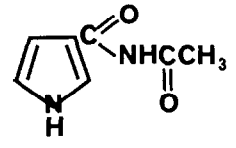

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It is not clear why such a structure should be physiologically active. The amidine function is not widespread in nature in contrast to the guanidino system. We are investigating the presence of other compounds in the extract which might shed some light on the biosynthesis of pyrrole-3-carboxamidine. Studies on the pharmacology of this substance and obvious analogs (indole-3-carboxamidine, pyridine-3-carboxamidine, etc.) are in progress.

References and Footnotes

1a. R. E. Schultes, Lloydia 29, 293 (1966).
b. R. E. Schultes, Science 163, 245 (1969).
c. R. E. Schultes, in "Flesh of the Gods," P. T. Furst, ed. (1972), Praeger Press Pub., New York, pp. 38, 52.
d. R. E. Schultes, "Hallucinogenic Plants" (1976), Golden Press, New York, pp. 140-141.
2. T. Plowman, Botanical Museum Leaflets (Harvard University) 25, pp. 289, 305 (1977).
3. Plant material was collected during phase VII of the Alpha Helix Amazon Expedition, 1977, and supported by NSF grant no. 76-81874 (R.E. Schultes & B. Holmstedt, co-principal investigators). Voucher specimens of the plant material, T. Plowman, R.E. Schultes & O. Tovar 6455, were collected on March 28, 1977, and are deposited in the following herbaria: ECON, F, GH, K, MO, US, USM.
4. Aqueous solutions of the natural product or of synthetic pyrrole-3-carboxamidine (or aliquots of chromatographic fractions) were administered intraperitoneally to mice and behavioral changes were observed according to the method of Irwin.[7] Within 40 min of injection the mice (NIH strain, 25-30 g) displayed a behavior characterized as "running excitement" and, depending on the dose given, they subsequently developed clonic convulsions and ultimately tonic convulsions which usually resulted in death. Spontaneous tonic convulsions were induced at a minimum dose (natural product or synthetic amidine) of 60 mg/kg.
5. S. Gronowitz and S. Liljefors, Acta Chem. Scand. B 31, 771 (1977).
6. C. E. Loader and H. J. Anderson, Can. J. Chem. 59, 2673 (1981).
7. S. Irwin, Psychopharm. 13, 22 (1968).

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