The Isolation of 6-Methoxyharmalan and 6-Methoxyharman from *Virola cuspidata*[1]

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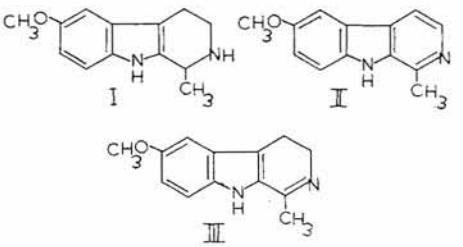
Various species of the genus *Virola* (Myristicaceae) have been used in the preparation of the intoxicating snuffs yakée, epéna, and nyakẃana by certain South American Indian tribes (1–4). Several reports have now appeared in the literature describing the chemical constituents of these plants. The psychoactivity of these snuffs is apparently due to the presence of various tryptamines and β-carbolines. Holmstedt and Lindgren (5) reported the presence of *N,N*-dimethyltryptamine, *N*-monomethyltryptamine, and 5-methoxy-*N,N*-dimethyltryptamine in *Virola calophylla* Warb. Agurell and co-workers investigated several Virola species (6,7) and found 5-methoxy-*N,N*-dimethyltryptamine to be the main base component of *Virola multineria* Ducke, *Virola rufula* (A.D.C.) Warb., and *Virola venosa* (Benth.) Warb. In addition, *Virola rufula* was shown to contain a carboline, 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline. *Virola theiodora* (Benth.) Warb. was reported to contain *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine, 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline, and 2-methyl-1,2,3,4-tetrahydro-β-carboline (6,7). We have also investigated *Virola theiodora* and found *N*-monomethyltryptamine to be the major component of the base fraction of the bark along with minor amounts of *N,N*-dimethyltryptamine and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (8).

Our investigation of *Virola cuspidata* (Benth.) Warb. sensu Ducke led to the isolation of the compounds otobaene, hydroxyotobain, and 3,4',5-trimethoxy-*trans*-stilbene (9). The alkaloid fraction of this plant contained one major and three minor constituents and the structure of the major component has been reported as 6-methoxytetrahydroharman (8) (I in figure 1). We have now identified two of the minor components as 6-methoxyharman (II) and 6-methoxyharmalan (III).



EXPERIMENTAL AND DISCUSSION

The dried and powdered leaves and stems of *Virola cuspidata* were extracted with chloroform–tetrahydrofuran–ammonium hydroxide (12:8:2) at room temperature (10); the mixture filtered, and the organic layer evaporated to dryness. The residue was partitioned between chloroform and dilute hydrochloric acid. After washing with chloroform, the acid layer was made basic with ammonium hydroxide and extracted with chloroform. The chloroform layer was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated to give the crude bases. Chromatography of the crude bases on Woelm neutral alumina

[1]Dedicated to Prof. Dr. K. Mothes on the occasion of his seventieth birthday.

gave three major fractions. One fraction contained the major component I. Two of the fractions were enriched in compounds II and III. These compounds were further purified by sublimation followed by preparative thin-layer chromatography on Brinkmann pre-coated silica gel plates developed with isopropyl alcohol–ethyl acetate–ammonium hydroxide (40:10:1). Compounds II and III were readily detected on the plates by viewing with a short wavelength ultraviolet light source. Compound II gave a characteristic purple fluorescence, while compound III fluoresced yellow.

The ultraviolet spectrum of 6-methoxyharman (II) showed absorption at λ_{max}^{EtOH} 232 (33,800), 246 (sh, 29,400), 257 (sh, 21,900), 285 (sh, 15,000), 290 (15,600), 296 (25,000) and 354 nm (7500). Its spectrum was identical with that of an authentic reference sample obtained by refluxing 6-methoxytetrahydroharman (I) with 5% Pd/C in xylene. The compound melted with decomposition at 273°. Its mass spectrum showed major peaks at m/e 212 (M^+), 198, 197 (base peak), 169 and 168 in agreement with a spectrum of reference 6-methoxyharman.

The ultraviolet spectrum of 6-methoxyharmalan (III) showed absorption at λ_{max}^{MeOH} 212, 230 (sh), and 325 nm. Its spectrum was identical with that of an authentic reference sample of 6-methoxyharmalan obtained from Regis Chemical Company, Chicago, Illinois. The mass spectrum showed a parent ion at m/e 214, as expected. Although the mass spectrum does not distinguish between III and II and isomers such as harmine (7-methoxyharman) and harmaline (7-methoxyharmalan), these compounds can be distinguished by ultraviolet spectral differences which arise as a consequence of the position of attachments of the methoxy group (11). No separation of the isolated compounds from reference material was obtained when they were co-chromatographed on silica gel (Brinkmann) plates with isopropyl alcohol–ethyl acetate–ammonium hydroxide (40:10:1).

In preparing the *Virola* snuffs one step involves the concentration of the resinous exudate to a thick syrup which is subsequently dried and powdered (1, 2). Since compound I was the major component in *Virola cuspidata*, it was of interest to determine what effect this type of treatment might have on it. Thus I was refluxed in water for eight hours and concentrated. Thin-layer chromatographic examination of the residue indicated that I had been partially converted to II and III by this treatment (figure 1). The presence of the fully aromatic compound (II) in the plant extract and the fact that its concentration is probably increased by the treatment is important, since as pointed out by Agurell (6) this compound with its known monoamine oxidase inhibitory activity may potentiate the hallucinogenic effects of such compounds as I and III.

ACKNOWLEDGMENT

The authors thank the National Institutes of Health for financial support (RR05586) and Dr. R. E. Schultes for identifications of the plant material used in this study. Voucher specimens have been deposited in the Economic Herbarium of Oakes Ames Botanical Museum, Harvard University.

Received 16 October 1970.

LITERATURE CITED

1. SCHULTES, R. E. 1954. A new narcotic snuff from the Northwest Amazon. *Botan. Museum Leaf., Harvard University* **16**: 241.
2. SCHULTES, R. E. 1967. In *Ethnopharmacologic Search for Psychoactive Drugs*. Public Health Service Publication no. 1645, U. S. Government Printing Office, Washington, D.C. pp. 291–306.
3. SCHULTES, R. E. and B. HOLMSTEDT. 1968. The vegetal ingredients of the myristicaceous snuffs of the Northwest Amazon. *Rhodora*. **70**: 113.
4. WASSÉN, S. H. and B. HOLMSTEDT. 1963. The use of paricá, an ethnological and pharmacological review. *Ethnos* **1**: 5.
5. HOLMSTEDT, B. and J. E. LINDGREN. 1967. In *Ethnopharmacologic Search for Psychoactive Drugs*. Public Health Publication no. 1645, U. S. Government Printing Office, Washington, D.C. pp. 339–373.
6. AGURELL, S., B. HOLMSTEDT, J. E. LINDGREN and R. E. SCHULTES. 1968. Identification of two new β-carboline alkaloids in South American hallucinogenic plants. *Biochemical Pharmacology* **17**: 2487.
7. AGURELL, S., B. HOLMSTEDT, J. E. LINDGREN and R. E. SCHULTES. 1969. Alkaloids in certain species of *Virola* and other South American plants of ethnopharmacologic interest. *Acta. Chem. Scand.* **23**: 903.
8. CASSADY, J. M., V. E. TYLER, M. WILLIAMS and G. E. BLAIR. 1970. The isolation of 6-methoxytetrahydroharman from *Virola cuspidata* and *N*-monomethyltryptamine from *Virola theiodora*. Proceedings of the Symposium on the Biochemistry and Physiology of Alkaloids, Halle (Saale).
9. BLAIR, G. E., J. M. CASSADY, J. E. ROBBERS, V. E. TYLER and R. F. RAFFAUF. 1969. Isolation of 3,4',5-trimethoxy-*trans*-stilbene, otobaene and hydroxyotobain from *Virola cuspidata*. *Phytochemistry* **8**: 497.
10. FISH, M., N. JOHNSON and E. HORNING. 1955. Piptadenia alkaloids—indole bases of *P. peregrina* (L.) Benth. and related species. *J. Am. Chem. Soc.* **77**: 5892.
11. NEUSS, N., Ed. 1964. *Physical data of indole and dihydroindole alkaloids, vol. I*, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana. pp. 48 and 50.