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SHORT COMMUNICATION

ISOLATION OF 3,4',5-TRIMETHOXY-*TRANS*-STILBENE, OTOBAENE AND HYDROXYOTOBAIN FROM *VIROLA CUSPIDATA**

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Abstract—Investigation of the petroleum ether extract of *Virola cuspidata* (Benth.) Warb. led to the isolation of 3,4',5-trimethoxy-*trans*-stilbene and the lignans, otobaene and hydroxyotobain. The structure of 3,4',5-trimethoxy-*trans*-stilbene was determined on the basis of its u.v. and NMR spectra. Otobaene and hydroxyotobain were identified by comparison of physical data with literature values.

INTRODUCTION

ETHNOBOTANICAL investigations[4] have revealed that plant parts from species of the genus *Virola* have been employed by South American Indians in the preparation of narcotic snuffs. Holmstedt[5] has reported the presence of tryptamine derivatives in a snuff known to be prepared from *Virola calophylla* Warb. and *V. calophylloidea* Markgraf. Unfortunately, there was no assurance that the snuff did not contain parts of other plants unrelated to *Virola* which could account for the presence of the tryptamine derivatives. More recently, Holmstedt and Lindgren,[6] with the use of gas–liquid chromatography in combination with mass spectrometry, have demonstrated the occurrence of *N,N*-dimethyltryptamine, *N*-monomethyltryptamine, and 5-methoxy-*N,N*-dimethyltryptamine in the bark of *V. calophylla*.

On the other hand, because *Virola* is a member of the family Myristicaceae, Shulgin[7] has speculated that the narcotic principle in this genus could be myristicin, a psychotomimetic substance found in the essential oil of the seed kernels of *Myristica fragrans* Houttuyn. There have been no reports in the literature on the chemical investigation of the genus *Virola* for the occurrence of myristicin; however, because of the taxonomic relationship of *Virola* to *Myristica*, the narcotic properties attributed to species of *Virola* may, in part, be due to the presence of myristicin in these plants.

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[4] R. E. SCHULTES, *Botanical Museum Leaflets*, Harvard University **16**, 241 (1954).
[5] B. HOLMSTEDT, *Arch. Int. Pharmacodyn.* **156**, 285 (1965).
[6] B. HOLMSTEDT and J. E. LINDGREN in *Ethnopharmacologic Search for Psychoactive Drugs*, pp. 339–373, U.S. Government Printing Office, Washington, D.C. (1967).
[7] A. T. SHULGIN, *Nature* **210**, 380 (1966).

We recently obtained a sample of *V. cuspidata* (Benth.) Warb. *sensu* Ducke[8] which was collected in South America. Since no definitive work had appeared on the occurrence of myristicin in the volatile or ethereal oils of *Virola* species, our initial studies were directed to the investigation of these fractions for the presence of myristicin or related compounds. In the course of our search for myristicin, we have isolated three compounds, otobaene (I), hydroxyotobain (II), and 3,4′,5-trimethoxy-*trans*-stilbene (III).

RESULTS AND DISCUSSIONS

The petroleum ether extract and volatile oil fraction of *Virola cuspidata* bark were examined for the presence of myristicin.[9] Comparison of R_f values and color reactions of components of the above fractions of *V. cuspidata* with myristicin (silica gel G, benzene, anisaldehyde–sulfuric acid) indicated the absence of myristicin in both fractions.

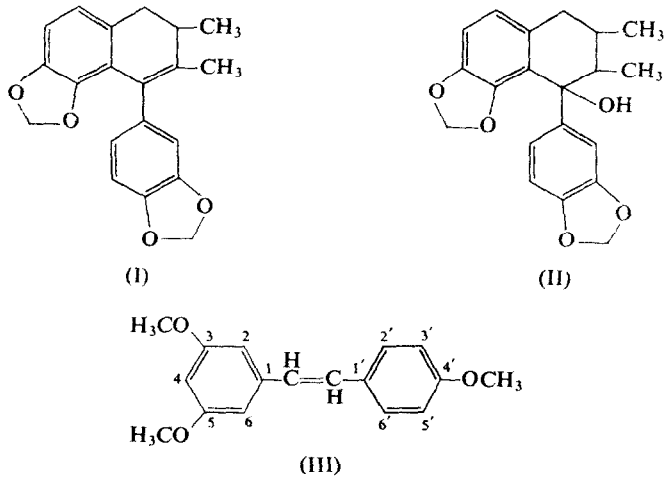

FIG. 1.

The petroleum ether extract of *V. cuspidata* upon TLC revealed two spots of similar R_f to myristicin. Therefore, in an attempt to determine if related compounds were present, the mixture was chromatographed on a column of silicic acid. Elution with petroleum ether–benzene (1 : 3) gave a compound which melted at 128–129° on recrystallization from petroleum ether. This compound was identified as otobaene (I) by comparison of u.v., i.r., and NMR spectral data with literature values.[10] Otobaene was previously isolated by Kohen and co-workers[10] from *Myristica otoba* Humb. and Bonpl. and shown to be an artifact arising from dehydration of hydroxyotobain[10,11] (II), during column chromatrogaphy. The subsequent isolation of II from later fractions during the chromatography of *V. cuspidata* extract, coupled with the absence of I in the petroleum ether extract prior to chromatography, indicated that I was probably an artifact in this case as well.

[8] A. J. DUCKE, *J. Washington Acad. Sci.* **26**, 253 (1936).
[9] An enriched fraction of myristicin was obtained through the courtesy of Dr. Alexander T. Shulgin, 1483 Shulgin Road, Lafayette, California 94549.
[10] F. KOHEN, L. MACLEAN and R. STEVENSON, *J. Chem. Soc.* (c) 1775 (1966).
[11] T. GILCHRIST, R. HODGES and A. L. PORTE, *J. Chem. Soc.* 1780 (1962).

Continued elution of the silicic acid column with benzene gave a third crystalline compound which was purified by recrystallization from ethanol, m.p. 56–57°. The molecular formula, $C_{17}H_{18}O_3$, was established on the basis of elemental analysis and molecular weight determination by mass spectrometry (M^+ 270). This compound has been identified as 3,4',5-trimethoxy-*trans*-stilbene (III). Takaoka[12] synthesized III (reported m.p. 56–57°) by reaction of resveratrol (3,4',5-trihydroxy-*trans*-stilbene) with diazomethane. This, however, is the first reported isolation of III from a natural source.

The u.v. spectrum of III showed absorption at 218 (22,000), 235 sh (15,600), 305 (30,300), 319 (30,000) and 335 sh (18,500) nm (ϵ values in parentheses), characteristic of a substituted *trans*-stilbene.[12–14]

Final structural assignment was based on an interpretation of the NMR spectrum of III which is consistent in every detail with the structure proposed. A sharp singlet at 3·78 δ (9H) was assigned to protons of the three methoxy groups. An AB system, 6·83δ (*d*, $J=9$ cps, 2H) and 7·41δ (*d*, $J=9$ cps, 2H) was assigned to the 2',6' and 3',5' aromatic protons, the coupling constant of 9 cps being typical for protons in an *ortho* relationship on the aromatic nucleus. The signal at 6·83δ was assigned to the 3',5' protons adjacent to methoxy at 4'. The 4-proton flanked by two methoxy groups, gave a signal at 6·35δ (*t*, $J=2$ cps, 1H). A signal for the 2,6 protons *meta* to position 4 appeared at 6·62δ (*d*, $J=2$ cps, 2H). The vinyl protons appeared at 6·95δ (*br s*, 2H) further confirming the *trans* relationship about the double bond.[15]

EXPERIMENTAL

M.ps were determined using a Thomas-Hoover Unimelt Apparatus. U.v. spectra were recorded on a Bausch and Lomb Spectronic 505 spectrophotometer. I.r. spectra were recorded in KBr disc on a Perkin-Elmer 21 Spectrophotometer. NMR spectra were determined in deuteriochloroform with tetramethylsilane as internal standard on a Varian Associates A-60-A instrument. The mass spectra were measured on a Hitachi Perkin-Elmer RMU-6D instrument, using a direct inlet to the source.

The air-dried bark was ground and 50 g of the powdered material was extracted in a soxhlet apparatus with petroleum ether (30–60°). The remaining marc was steam distilled to yield a second extract. Portions of the petroleum ether extract and the volatile oils were co-chromatographed with reference myristicin on silica gel, precoated glass plates (Merck). The plates were developed in benzene and sprayed with a mixture of anisaldehyde; sulfuric acid; glacial acetic acid; methanol (1:10:20:170). Upon heating at 100° for 10 min, aromatic compounds give dark colors. Myristicin gave a distinctive pink–orange spot with this reagent. Column chromatography on 100 mesh, silicic acid (Mallinckrodt) was used to separate the constituents in the petroleum ether extracts. Otobaene was eluted from the column with petroleum ether–benzene (1:3); elution with benzene first removed the stilbene and then hydroxyotobain.

Physical Data

3,4',5-Trimethoxy-*trans*-stilbene: m.p. 56–57°; i.r. bands at 3·5, 6·3, 6·65, 7·0, 8·0–8·5 (strong, broad), 9·5, 9·75 and 10·45 μ. Found: C, 75·46; H, 6·94. Calc. for $C_{17}H_{18}O_3$: C, 75·55; H, 6·67.

Otobaene: m.p. 128·5–129·5°; mass spectrum showed M^+ at *m/e* 322; u.v. absorption λ_{max}^{EtOH} 216 (24,200), 230 (22,700), 274 (12,800) and 284 nm (12,100); NMR signals δ 1·00 (*d*, $J=6$ cps), 1·75 (*s*), 5·52 and 5·57 (*dd*, $J=1$ cps), 5·93 (*s*), 6·60 (*m*). These data were identical with literature values reported by F. Kohen and coworkers.[10]

Hydroxyotobain: m.p. 113–114°; mass spectrum M^+ at *m/e* 340; u.v. absorption λ_{max}^{EtOH} 233 (8000), and

[12] M. TAKAOKA, *J. Faculty Sci.*, Hokkaido Imp. Univ. [III] **3**, 1 (1940); *Chem. Abs.* **34**, 7887 (1940).

[13] G. BILLEK, in *Fortschritte Der Chemie Naturstoffe* (edited by L. ZECHMEISTER), Vol. XXII, pp. 115–146, Springer-Verlag, Vienna, N.Y. (1964).

[14] A. I. SCOTT, *Interpretation of the Ultraviolet Spectra of Natural Products*, pp. 97–100, Macmillan, N.Y. (1964).

[15] D. Y. CURTIN, H. GRUEN and B. A. SHOULDERS, *Chem. & Ind.* 1205 (1958).

287 nm (6700); and NMR signals at δ 0·87 (*d*, *J* = 6 cps), 1·03 (*d*, *J* = 6 cps), 1·64 (*m*), 2·22 (*br s*), 2·62 (*m*), 5·52 and 5·64 (*dd*, *J* = 1 cps), 5·88 (*s*), 6·7 (*m*). These data were identical to literature values.[10, 11]

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[16] A voucher specimen has been deposited in the Economic Herbarium of Oakes Ames Botanical Museum, Harvard University.