groups (5). In cephalosporin studies (2), a double-bond migration was reported only when accompanied by dehydroacetoxylation; otherwise, the double bond remained at the C-3,4 position.

REFERENCES

(1) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **58**, 447(1969).

(2) J. M. T. Hamilton-Miller, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **116**, 371(1970).

(3) J. E. Dolfini, H. E. Applegate, G. Bach, H. Basch, J. Bernstein, J. Schwartz, and F. L. Weisenborn, *J. Med. Chem.*, **14**, 117 (1971).

(4) E. Brettmaier, G. Jung, and W. Voelter, *Angew. Chem. Int. Ed. Engl.*, **10**, 673(1971).

(5) J. M. Indelicato, T. T. Norvilas, and W. J. Wheeler, *J. Chem. Soc. Chem. Commun.*, **1972**, 1162.

ACKNOWLEDGMENTS AND ADDRESSES

Received December 26, 1972, from *The Squibb Institute for Medical Research, Princeton, NJ 08540*

Accepted for publication March 22, 1973.

The authors are indebted to Mr. John A. Hill for supplying the alkaline degradation product and to Dr. Joseph E. Dolfini for discussions of penam and cephem chemistry. They also greatly appreciate the ^{13}C pulse Fourier transform NMR spectrum provided by JEOL USA, Cranford, N. J.

▲ To whom inquiries should be directed.

Phytochemical Investigation of *Virola peruviana*, A New Hallucinogenic Plant

A. LAI, M. TIN-WA, E. S. MIKA, G. J. PERSINOS*, and N. R. FARNSWORTH▲

Abstract □ Although *Virola peruviana* has been described in ethnobotanical studies as being used as a hallucinogen, no proof of the presence of chemical agents explaining this activity existed previously. The present study resulted in the isolation of 5-methoxy-*N*,*N*-dimethyltryptamine as well as the identification of *N*,*N*-dimethyltryptamine and 5-methoxytryptamine, thus confirming the hallucinogenic aspect of *V. peruviana*. In addition, myoinositol and the lignans lirioresinol-A dimethyl ether and lirioresinol-B dimethyl ether were isolated and identified. A mixture of *n*-alkanols was isolated and shown to consist of octacosanol, triacontanol, and dotriacontanol, whereas a mixture of sterols was isolated and shown to consist of β-sitosterol, campesterol, and stigmasterol.

Keyphrases □ *Virola peruviana*—phytochemical investigation, hallucinogenic constituents isolated and identified □ Hallucinogens—isolation and identification of hallucinogenic constituents of *Virola peruviana* □ Phytochemistry—isolation and identification of hallucinogenic constituents of *Virola peruviana*

In the Rio Apaporis, Puinave Indians refer to *Virola peruviana* as yá-kee, suggesting its possible utilization in preparing the hallucinogenic snuff by the same name (1). Schultes and Holmstedt (1) suggested that this species may be used as a hallucinogen and determined that it contained alkaloids (1). Other virola species, *i.e.*, *V. theiodora*, *V. rufula*, *V. multinervia*, *V. venosa*, *V. calophylloidea*, *V. calophylla*, and *V. sebifera*, are well documented as hallucinogenic plants, with their active principles being determined as *N*,*N*-dimethyltryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, and/or *N*-methyltryptamine (2–5).

The purpose of this investigation was to determine whether *V. peruviana* contained chemical constituents that would explain its use as a hallucinogen.

Table I—Chromatographic Separation of Fraction A

Fraction Number	Eluent	Isolate	Yield, mg.
1–34	Benzene	—	—
35–39	Benzene	*n*-Alkanols	26
40–64	Benzene	—	—
65–81	Benzene–acetone (9:1)	Phytosterols	205
82–154	Benzene–acetone (9:1)	—	—
155–169	Benzene–acetone (6:1)	—	—
170	Benzene–acetone (6:1)	Lirioresinol-A dimethyl ether	185
171–174	Benzene–acetone (6:1)	Lirioresinol-B dimethyl ether	216
175–200	Benzene–acetone (6:1)	—	—
201–234	Benzene–acetone (3:1)	—	—
235–245	Acetone	—	—
246–280	Methanol	—	—

EXPERIMENTAL[1]

Plant Material—The plant material[2] used represented the bark of *Virola peruviana* (A.DC.) Warburg (Myristicaceae), collected near Leticia, Peru, during October 1968.

Extraction and Fractionation—Coarsely milled bark (4.7 kg.) of *V. peruviana* was defatted with 35 l. of petroleum ether (b.p. 30–60°) using a soxhlet apparatus for 24 hr. The combined extracts were filtered and evaporated to dryness to yield 4.0 g. of Fraction A. The defatted plant material was then exhaustively extracted in the soxh-

[1] All chemicals used were of reagent grade quality. Melting points were determined by means of a Thomas-Hoover apparatus or a Kofler hot plate and are uncorrected. Optical rotations were measured in a Carl Zeiss optical polarimeter. The UV spectra were recorded in methanol using a Beckman model DB-G spectrophotometer. IR spectra were taken using a Beckman model IR-18A instrument. Mass spectral analyses were made using the Hitachi Perkin-Elmer model RMD-6D single-focusing mass spectrometer. NMR spectra were taken using a Bruker model HFX-5 magnetic resonance spectrometer. GC analyses were carried out by means of a Perkin-Elmer model 881 linear programmed temperature gas chromatograph, equipped with a hydrogen flame-ionization detector and a Sargent model SR,S-72180-20 1-mv. recorder; a 1-sec. full-scale response was used. A borosilicate coiled glass column, 1.8 m. (6 ft.) × 2.0 mm. (i.d.), was packed with either 3% OV-1 or 5% OV-101 on 100–120-mesh Gas Chrom Q. Silica gel GF_{254} plates were used for monitoring the chromatographic separation of Fraction A; the solvent system used for development of plates was the same as the eluent used to elute the fractions. The same type plates were used for monitoring the chromatographic fractions from Fraction D, but development was with benzene–ethyl acetate–diethylamine (7:2:1). Spots were visualized after spraying the plates with sulfuric acid (70%) or Ehrlich reagent and heating at 110° for 5 min.

[2] A voucher specimen (2245) was authenticated by Dr. J. Wurdack, Smithsonian Institution, Washington, D. C. A specimen has been deposited at that address.

Table II—Chromatographic Separation of Fraction D

Fraction Number	Eluent	Isolate	Yield, mg.
1–20	Petroleum ether	—	—
21–44	Petroleum ether–benzene (9:1)	—	—
45–55	Petroleum ether–benzene (1:1)	5-Methoxy-*N,N*-dimethyltryptamine (as methiodide)	35
56–434	Petroleum ether–benzene (1:1)	5-Methoxy-*N,N*-dimethyltryptamine (as picrate)	259
435–633	Benzene	5-Methoxy-*N,N*-dimethyltryptamine (as picrate)	530
634–705	Benzene–ether (6:1)	—	—
706–936	Ether	—	—
937–1030	Chloroform	—	—
1031–1066	Chloroform–methanol (9:1)	—	—
1067–1087	Methanol	—	—

let apparatus with methanol until the final methanol extract no longer gave a positive alkaloid test with Mayer's reagent. Concentration of the methanol extract *in vacuo* gave 232 g. of a dark-brown syrupy liquid, which was redissolved in 2 l. of boiling methanol and filtered. A nonalkaloidal precipitate (Fraction B), which formed on standing, was removed by filtration (22.7 g.). The filtrate was poured into 6 l. of 2% acetic acid, and the methanol was removed by evaporation *in vacuo*. Suspended impurities were removed by filtration; the filtrate was extracted with chloroform (6 × 2 l.), and, after evaporation *in vacuo*, gave 11 g. of Fraction C.

The aqueous layer was made alkaline with 28% ammonium hydroxide and extracted with chloroform (9 × 2 l.). The combined chloroform extracts were evaporated to dryness *in vacuo* to yield 3.4 g. of Fraction D.

Chromatographic Separation of Fraction A—Fraction A (4.0 g.) was chromatographed over a column (5.5 × 62 cm.) of silica gel PF_{254} (300 g.) and eluted with solvents having increasing polarity (Table I). Each fraction (50 ml.) was monitored by means of TLC, and fractions were combined on the basis of TLC patterns.

Isolation and Identification of *n*-Alkanols—Combined fractions 35–39 from the column, which were eluted with benzene, formed a white precipitate when concentrated *in vacuo*. The precipitate was removed by filtration and crystallized from acetone several times. The IR spectrum of this isolate was typical of that seen with *n*-alkanols. GLC analysis (3% OV-1, 280° isothermal) of this isolate showed it to be a mixture of octacosanol (55%), triacontanol (35%), and dotriacontanol (15%).

Isolation and Identification of Phytosterols—Fractions 65–72 from the column, which were eluted with benzene–acetone (9:1), were combined and evaporated to dryness *in vacuo*. The residue (340 mg.) was crystallized from a benzene hexane mixture to give white plates. GLC analysis (5% OV-101, 250° isothermal) of this material showed it to be a mixture of campesterol (9.9%), stigmasterol (33.9%), and β-sitosterol (57.3%).

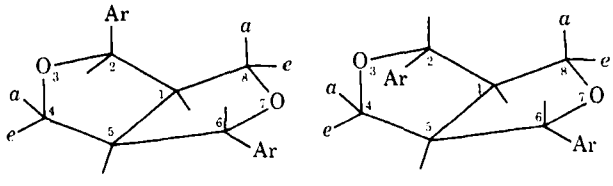
Isolation and Identification of Lirioresinol-A Dimethyl Ether—Concentration of fraction 170 from the column, which was eluted with benzene acetone (6:1), resulted in the formation of crystals. Recrystallization from acetone–hexane yielded 185 mg. of crystals having a melting point of 116° [lit. (6) m.p. 118–120°). The UV spectrum of the isolate showed absorptions at λ_{max} 208 (log ε 4.88) and 271 nm. (log ε 3.48), with a shoulder at 230 nm. (log ε 4.19). IR absorptions were found at 2900, 2800, 1590, 1510, and 810 $cm.^{-1}$. A molecular ion was observed in the mass spectrum at *m/e* 446, in agreement with a formula of $C_{24}H_{30}O_8$. Other prominent peaks were noted at *m/e* 416, 250, 249, 224, 207, 196, and 181, the fragmentation pattern of which is similar with that of lirioresinol-B dimethyl ether (7–9).

Since the presence of six methoxy groups was observed in the NMR spectrum at δ 3.85, the remaining two oxygen atoms were assigned as ether linkages in a lignan of the 3,7-dioxabicyclo[3.3.0]-octane type, considering the lack of hydroxy, phenolic, and carbonyl groups in the IR spectrum. However, the compound was not symmetrical as is the case with lirioresinol-B dimethyl ether. One benzylic hydrogen appeared as a doublet centered at δ 4.89 (J = 3 Hz.), while the other benzylic proton was at δ 4.47 (d, J = 3 Hz.). One methylene proton was seen upfield in the δ 3.38–3.47 region as a multiplet, which corresponded with the axial proton due to the shielding effect of the aryl group; another methylene proton was observed downfield in the δ 4.13–4.24 region, corresponding with the other axial proton affected by the equatorial aryl group. Two additional methylene protons appeared between δ 3.8 and 3.97. Furthermore, two nonequivalent methine protons appeared at δ 2.94 and 3.30, indicating that its NMR spectrum was similar to that reported for lirioresinol-A (10). The identity of this isolate as lirioresinol-A dimethyl ether was confirmed by measurement of the specific rotation $[\alpha]_D^{26}$ + 116 (c 0.65 in $chloroform_3$) [lit. (6) + 119] and preparation of the dibromo derivative (11), m.p. 122° [lit. (6) m.p. 124–126°].

Isolation and Identification of Lirioresinol-B Dimethyl Ether—When the pooled fractions 171–174 were eluted from the column with benzene–acetone (6:1) and concentrated, a crystalline material formed which was removed by filtration. This material was recrystallized from acetone containing a trace of hexane to give 216 mg. of a pure sample, m.p. 118–119° [lit. (6) m.p. 121–123°]. The UV spectrum showed absorption maxima at λ_{max} 208 (log ε 4.88) and 271 nm. (log ε 3.48), with a shoulder at 230 nm. (log ε 4.19). The IR spectrum was very similar to that of the lirioresinol-A dimethyl ether but not identical. The mass spectrum indicated a molecular ion at *m/e* 446, in agreement with a formula of $C_{24}H_{30}O_8$, suggesting that it might be an isomer of lirioresinol-A dimethyl ether. Its fragmentation pattern was in agreement with previously published data for lirioresinol-B dimethyl ether (7–9). This was further supported by the NMR spectrum, which indicated that the isolate was symmetrical; *i.e.*, it gave a proton count of 30 only if the apparent integrals were doubled. The assignments of the signals as indicated below is in agreement with those reported for lirioresinol-B dimethyl ether (9, 10), *i.e*, methine protons at C_1 and C_5 at δ 3.11 (m, 2H), benzylic protons at C_2 and C_6 at δ 4.78 (d, J = 3 Hz., 2H), axial methylene protons at δ 4.24 (m, 2H) and equatorial methylene protons at δ 4.00 (m, 2H), six methoxy protons at δ 3.87 (18H), and four aromatic protons at δ 6.60 (s, 4H).

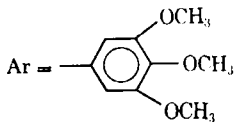
The identity of this compound as lirioresinol-B dimethyl ether was confirmed by measurement of the specific rotation $[\alpha]_D^{26}$ + 49 (0.65 in chloroform) [lit. (6) + 46.2] and preparation of the dibromo derivative (11), m.p. 153° [lit. (6) m.p. 152–155°].

Isolation and Identification of Myoinositol—Fraction B (22.7 g.) was dissolved in 70% ethanol and, on standing, formed crystals which were removed by filtration. Recrystallization from 70% ethanol afforded an analytical sample having a melting point of 220°, which was not depressed on admixture with a reference sample



lirioresinol-A dimethyl ether

lirioresinol-B dimethyl ether



of myoinositol. The IR spectrum of the isolate was identical with that of a reference sample of myoinositol, with $[\alpha]_D^{26} \pm 0$; paper chromatographic comparison of the isolate and myoinositol showed them to be identical (12). The identity of the isolate was further established by preparation and comparison of the acetates, m.p. 216° [lit. (13) m.p. 216°] and the benzoates (14), m.p. 260° [lit. (15) m.p. 258°] of the isolate and reference myoinositol.

Chromatographic Separation of Fraction D—Fraction D (3.0 g.) was chromatographed over a column (2.2 × 27 cm.) of neutral alumina[3] (100 g.), activity grade III. Fractions consisting of 15 ml. each were collected. Initially the column was eluted with petroleum ether (b.p. 30–60°), followed by solvents of increasing polarity (Table II).

Isolation and Identification of 5-Methoxy-*N*,*N*-dimethyltryptamine—Fractions 45–55 from the column were combined and taken to dryness *in vacuo* to yield 50 mg. of residue. This residue was shown by TLC to contain one major component, which gave a pale-yellow fluorescence under UV light and a positive reaction with the Ehrlich reagent. Since the material was resistant to crystallization as the free base, preparative TLC was employed to obtain the base in pure form. The zone at R_f 0.38 was removed from the plates and extracted with chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. A mass spectrum of this residue indicated a molecular ion at *m/e* 218, which corresponded with a molecular formula of $C_{13}H_{18}N_2O$. Other prominent peaks at *m/e* 174, 173, 160, 159, 145, and 58 were all in agreement with published data for 5-methoxy-*N*,*N*-dimethyltryptamine (16). The UV spectrum of the isolate showed absorptions at λ_{max} 224, 277, and 295 nm., which compares favorably with that found in the literature for 5-methoxy-*N*,*N*-dimethyltryptamine (16).

Reaction of the alkaloid residue (40 mg.) with ethanol (10 ml.), 80% sodium hydroxide (2 drops), and 8 ml. of methyl iodide at room temperature for 8 hr. gave the methiodide (17), m.p. 182–183° [lit. (16) m.p. 181–182°]. The IR spectrum was superimposable with that of a synthetic sample of 5-methoxy-*N*,*N*-dimethyltryptamine methiodide, prepared from 5-methoxy-*N*,*N*-dimethyltryptamine as described previously.

Pooled fractions 56–434 (304 mg.) and 435–633 (550 mg.) from the column were processed by the preparation of picrate derivatives. The concentrated residues were dissolved in methanol and mixed with an equimolar solution of picric acid dissolved in methanol. Orange-yellow picrates were obtained and recrystallized from methanol–acetone to give pure samples having melting points of 172° [lit. (16) m.p. 172°]. The IR spectra of these isolates were superimposable with those of a reference sample of 5-methoxy-*N*,*N*-dimethyltryptamine picrate.

Detection of 5-Methoxytryptamine and *N*,*N*-Dimethyltryptamine—GLC (5% OV-101, with a temperature of 150° initially for 2 min. followed by a 10° rise/min. from 150 to 300°) of Fractions C and D indicated the presence of small amounts of 5-methoxytryptamine in Fraction C, while *N*,*N*-dimethyltryptamine, 5-methoxytryptamine, and 5-methoxy-*N*,*N*-dimethyltryptamine were identified in Fraction D.

[3] Brockmann.

REFERENCES

(1) R. E. Schultes and B. Holmstedt, *Lloydia*, **34**, 61(1971).

(2) B. Holmstedt, W. J. A. Vandenheuvel, W. L. Gardiner, and E. C. Horning, *Anal. Biochem.*, **8**, 151(1964).

(3) S. Agurell, B. Holmstedt, J. E. Lindgren, and R. E. Schultes, *Acta Chem. Scand.*, **23**, 903(1969).

(4) J. M. Cassady, V. E. Tyler, M. Williams, and G. E. Blair, *4. Intern. Symp. Biokhim. Physiol. Alkal. Halle* (*Saale*), **1969**, 25.

(5) E. Corothie and T. Nakano, *Planta Med.*, **17**, 184(1969).

(6) E. E. Dickey, *J. Org. Chem.*, **23**, 179(1958).

(7) A. M. Duffield, *J. Heterocycl. Chem.*, **4**, 16(1967).

(8) A. Pelter, *J. Chem. Soc. C*, **1967**, 1376.

(9) H. Kakisawa, Y. P. Chen, and H. Y. Hsu, *Phytochemistry*, **11**, 2289(1972).

(10) L. H. Briggs, R. C. Cambie, and R. A. F. Crouch, *J. Chem. Soc. C*, **1968**, 3042.

(11) P. R. Jefferies, J. R. Knox, and D. E. White, *Aust. J. Chem.*, **14**, 175(1961).

(12) T. Pasternak, "The Cyclitols," Holden-Day, San Francisco, Calif., 1965, p. 53.

(13) R. J. Beveridge, R. F. H. Dekker, G. N. Richards, and M. Towsey, *Aust. J. Chem.*, **25**, 677(1972).

(14) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 5th ed., Wiley, New York, N. Y., 1965, p. 247.

(15) G. Harris, "Dictionary of Organic Compounds," vol. III, Oxford University Press, New York, N. Y., 1965.

(16) S. Ghosal and B. Mukherjee, *J. Org. Chem.*, **31**, 2284(1966).

(17) S. Wilkinson, *J. Chem. Soc.*, **1958**, 2079.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 19, 1973, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication March 22, 1973.

The authors are grateful to the Amazon Natural Drug Co. for supplying the plant material used in this investigation. They express their appreciation to Dr. Thomas Glonek for his assistance with the NMR spectral determinations and to Mr. Richard Dvorak for mass spectral analyses. They also thank Dr. S. Ghosal, Department of Chemistry, University of Kalyani, Kalyani, Nadia, West Bengal, India, for an authentic sample of 5-methoxy-*N*,*N*-dimethyltryptamine picrate.

• Present address: Bergstrom Toxicology Laboratory, Viers Mill Road, Rockville, Md.

▲ To whom inquiries should be directed.