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ISOLATION OF A NEW ALKALOID FROM *ARTABOTRYS LASTOURVILLENSIS*

JACQUELINE ELOUMI-ROPIVIA,

Institut de Pharmacopee et des Medicines Traditionnelles, Libreville, Gabon

JACQUES BELIVEAU,

Faculty of Pharmacy, University of Montreal, Montreal, Quebec, H3C 3J7, Canada

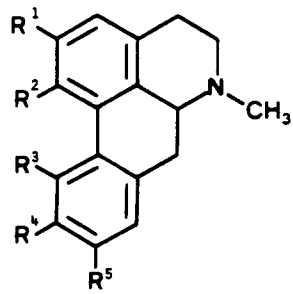
and DAVID ZVI SIMON

Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada

The genus *Artabotrys* (1) of the family Annonaceae is a paleotropical genus composed of about one hundred species. *Artabotrys lastourvillensis* was first described by Pellegrin (2) and is found only in Gabon in a region close to Lastourville. The bark of this plant has long been used in folk medicine in Gabon. It is a climbing plant with smooth branches, and its twigs are covered with long, rust-colored hairs. The flowers are red, and the fruit has not yet been studied. This species has not yet been studied phytochemically, but a study done on a closely related species. *Artabotrys suaveolens*, native to the Philippines, suggested the presence of a catecholic aporphine (3-5), a class of alkaloids reported for the first time in the family Annonaceae. The structure of suaveoline (**2**) (6), however, has been contested, for it was thought that catecholic aporphines would not be stable enough to isolate and characterize. The extraction and identification, in our laboratories, of lastourvilline (**1**), a new alkaloid which is 1,2-dihydroxy-9,10-dimethoxyaporphine, lends credibility to the structure of suaveoline (**2**) and opens to the phytochemist a new field of study.

Preliminary tests showed the presence of alkaloids in the bark of *A. lastourvillensis*. These alkaloids were extracted by conventional methods and the tertiary alkaloids obtained were separated into phenolic and nonphenolic fractions. The nonphenolic portion was found, by tlc, to contain three alkaloids which were separated by flash chromatography (7), which is a time-saving inexpensive technique (30 min) permitting a fast and efficient separation of the alkaloids compared to the classical column system. One nonphenolic alkaloid was present in sufficient quantity for a clear elucidation of its structure. The physical and spectral characteristics permitted its identification as glaucine (**3**). Glaucine had already been isolated from the family Annonaceae but has been isolated here for the first time from the genus *Artabotrys* (8).

The phenolic fraction was shown, by tlc, to contain two alkaloids, only one of which was present in large enough quantity to be isolated and studied. The alkaloid, in the form of large pale-yellow crystals, was studied by ms, ^{1}H nmr, nOeds, and ^{13}C nmr. ^{1}H nmr showed 21 protons, including two protons at 3.47 ppm which were easily replaced on addition of D_2O. Two closely spaced singlets at 3.90 and 3.91 ppm indicated two methoxy groups in a similar chemical environment. ^{13}C-nmr spectra showed nineteen carbon atoms with methoxy groups at 56.2 ppm. Ms showed M^+ 327 and enabled us to set a molecular formula of $C_{19}H_{21}NO_4$ for the alkaloid. Methylation of the hydroxy groups with CH_2N_2 gave glaucine. Only four aporphines are possible containing two noncatecholic hydroxyl and methoxy groups, which will give glaucine upon methylation, and these are known.



Compound	R^1	R^2	R^3	R^4	R^5
1 lastourvilline	OH	OH	H	$O-CH_3$	$O-CH_3$
2 suaveoline	$O-CH_3$	$O-CH_3$	OH	OH	H
3 glaucine	$O-CH_3$	$O-CH_3$	H	$O-CH_3$	$O-CH_3$
4 boldine	OH	$O-CH_3$	H	$O-CH_3$	OH
5 isoboldine	$O-CH_3$	OH	H	$O-CH_3$	OH
6 bracteoline	$O-CH_3$	OH	H	OH	$O-CH_3$
7 liriotulipiferine	OH	$O-CH_3$	H	OH	$O-CH_3$

These are boldine (**4**) isoboldine (**5**), bracteoline (**6**), and liriotulipiferine (**7**).

The physical properties and spectral data (6,9) for these compounds all differ from those of the isolated alkaloid. This leaves only two possibilities. Either the two hydroxyl groups are on positions 1 and 2, or they are on positions 9 and 10. In the aporphines, the ^{13}C-nmr spectra indicate that a methoxy on position 1 gives a signal ca. 60 ppm (9). Also, the ^1H-nmr peak for methoxy protons on position 1 falls ca. 3.6 ppm rather than at 3.90 ppm as observed in our alkaloid. The methoxy groups in our alkaloid (^{13}C nmr) were observed at 56.2 ppm, placing them on positions 2, 9, or 10 but excluding position 1. The nOeds experiments clearly place an hydroxyl group on position 2, thus ruling out the possibility of the alkaloid being isoboldine, which has similar physical constants. The ir spectra of isoboldine and our alkaloid are greatly different, especially in the finger-print region between 1600 cm^{-1} to 1000 cm^{-1}. Their migration is different on a silica plate and mixed melting points show a depression. Thus, in this new alkaloid the two methoxy groups must be placed in positions 9 and 10 (nOeds and ^{13}C-nmr assertions), and with the same assertion, the two hydroxy groups are in positions 1 and 2. This new alkaloid has been named lastourvilline (**1**) and is 1,2-dihydroxy-9,10-dimethoxyaporphine.

EXPERIMENTAL

PLANT MATERIAL.—The plant material (the bark of *A. lastourvillensis*) used in this investigation was collected by the Institut de Recherche en Ecologie Tropicale, Makokou, Gabon, and was identified by Dr. Jean-Noel Gassita of L'Institut de Pharmacopee et de Medicines Traditionnelles, Libreville, Gabon, where a voucher specimen is deposited.

INSTRUMENTS.—Nmr spectra were recorded on a Bruker WH 400 MHz instrument. Ir spectra were recorded on a Beckman IR 18A and a Perkin-Elmer 257, while uv spectra were recorded on a Unicam SP 800 recording spectrophotometer. Melting points were obtained on a Buchi melting point apparatus and are uncorrected. ^{13}C nmr was performed in the chemistry department of the University of Montreal. Mass spectra were obtained by Mr. J.C. Ethier of the Drug Identification Division, Ottawa. An ir spectrum of isoboldine was furnished by Dr. Paul L. Schiff, Jr., University of Pittsburgh.

EXTRACTION.—The air-dried, milled bark (300 g) was extracted with 95% EtOH (52 liters) in the cold, for two months, under constant stirring. The solvent was changed weekly. The solvent was removed in vacuo to yield a viscous residue (43 g) which was dissolved in 5% HCl (1.5 liters). This acidic solution ws extracted with CH_2Cl_2 (5 liters). The CH_2Cl_2 portion, still positive to alkaloidal precipitating agents, was

washed, dried over anhydrous Na_2SO_4, and evaporated to dryness (2.5 g). The acidic aqueous portion was brought to pH 9 with 20% NH_4OH and extracted with CH_2Cl_2 (8 liters), dried and evaporated, yielding the tertiary alkaloids (6 g) which were redissolved in CH_2Cl_2 and extracted with 10% NaOH (100 ml). The CH_2Cl_2 yielded 500 mg of nonphenolic alkaloids. The darkly colored NaOH solution was brought to pH 2 with 5% HCl and then to pH 9 with 20% NH_4OH. Extraction with CH_2Cl_2 gave 5 g of total phenolic tertiary alkaloids. From the neutral and acidic solutions, a further 1.7 g of tertiary nonphenolic alkaloids was extracted.

SEPARATION OF THE TERTIARY PHENOLIC BASES.—The tertiary phenolic bases (1 g) were separated by flash chromatography (7). A 30-mm column, fitted with a pressure valve, was filled to a height of 20 cm with silica gel 60 (E. Merck, 230-400 mesh). The sample was deposited on the surface of the silica and the column filled with eluant. Sufficient nitrogen pressure was maintained through the pressure valve to allow the surface of the eluant to drop at a constant rate of 5 cm/min. The eluant used was that which gave the best separation on silica tlc plates. In this case, the eluant was CH_2Cl_2-MeOH-NH_4OH (90:10:0.4). Fifty fractions of 50 ml were collected, and lastourvilline was found in the pure state in fractions 8 to 10 (yield from 300 g of bark, 0.12%).

IDENTIFICATION OF LASTOURVILLINE.—The alkaloid (350 mg) was crystallized repeatedly from CH_2Cl_2/MeOH. Large pale-yellow crystals (200 mg) were obtained, mp 128-136° (dec). When exposed to air, the crystals turned violet and so were kept in the cold under nitrogen. $[\alpha]^{25}D = +120°$ (c=0.5 EtOH).

METHYLATION OF THE PHENOL GROUPS ON LASTOURVILLINE.—Lastourvilline (100 mg) was dissolved in EtOH (10 ml). A solution, in Et_2O, of CH_2N_2 was prepared from *N*-nitroso-*N*-methyl-urea (300 mg) and KOH 40% (15 ml). This latter was added to the solution of lastourvilline in EtOH, and the mixture was kept at room temperature for 48 h, after which the solvent was evaporated under a stream of N_2. The residue was purified on preparative plates of silica gel G 254 with $CHCl_3$-CH_3OH-NH_4OH (95:5:0.2). A product, Rf=0.71, identical with that of glaucine was obtained. The ir, uv, ^{1}H-nmr, and ^{13}C-nmr spectra were identical to those of a known sample of glaucine. The mp was that of glaucine (119° uncorrected), and mmp with the known sample gave no depression.

SPECTRA OF LASTOURVILLINE.—Uv λ max (log ε) in MeOH 224 (4.08), 279 (3.64), and 303 nm (3.69) (bathochromic shift in alkaline medium); ir (KBr) 3450, 3000, 2950, 2850, 2800, 1600, 1590, 1510, 1480, 1460, 1405, 1370, 1330, 1310, 1280, 1240, 1160, 1110, 1080, 1020, 985, 960, 865, 820, 770, 755, and 705 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) ppm 2.52 (s, N-CH_3), 2.69-3.13 (m, $(CH_2)_3$ and CH), 3.47 (s, $(OH)_2$), 3.90 (s, O-CH_3 on C-10), 3.91 (s, O-CH_3 on C-9), 6.53 (s, H aromatic on C-3), 6.80 (s, H aromatic on C-8), and 8.02, (s, H aromatic on C-11); ^{13}C nmr ($CDCl_3$) ppm 140.6 (C-1), 119.8 (C-1a), 124.3 (C-1b), 145.9 (C-2), 108.9 (C-3), 127.7 (C-3a), 29.1 (C-4), 53.6 (C-5), 62.7 (C-6a), 34.5 (C-7), 129.9 (C-7a), 114.0 (C-8), 144.7 (C-9), 145.1 (C-10), 112.1 (C-11), 124.1 (C-11a), 56.2 (O-CH_3 on C-9 and C-10), and 43.9 (N-CH_3); ms (% rel. abundance) M^+ 327 (80), *m/z* 326 (100), other fragments 312 (27), 296 (15), 284 (37), 269 (20), 253 (30) (all common to aporphines), 44 (47), and 42 (80).

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