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ARTAVENUSTINE, A CATECHOLIC BERBINE FROM *ARTABOTRYS VENUSTUS*[1,2]

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ABSTRACT.—The stem bark of *Artabotrys venustus* yielded 0.34% of crude bases, mostly known aporphinoids and berbinoids. One of the minor constituents, (−)-artavenustine (**12**), is a new member of the rare class of catecholic isoquinoline alkaloids which are of interest as biogenetic precursors of novel alkaloid types. Its structure was elucidated by spectroscopic methods, in particular nOeds and 2-D nmr, and by chemical correlation with xylopinine. Other substances isolated are nornuciferine, asimilobine, anonaine, norstephalagine, norushinsunine, nuciferine, lirinidine, (*S*)-reticuline, norcorydine, discretamine, and 10-*O*-demethyldiscretine.

The genus *Artabotrys* (Annonaceae, tribe Unoneae) comprises over a hundred species of climbers and scandent shrubs distributed in tropical Africa and East Asia (1), of which only the widespread Asian *Artabotrys suaveolens* and the Gabonese *Artabotrys lastourvillensis* have been analyzed for alkaloids. In studies dating back to 1939 (2,3), *A. suaveolens* afforded isocorydine and suaveoline, a purportedly C-10, C-11 diphenolic aporphine whose structure is in doubt. Quite recently, *A. lastourvillensis* was shown to contain glaucine and the C-1, C-2 catecholic aporphine lastourvilline (4).

Artabotrys venustus King is a climber attaining a length of 25 m, which grows throughout the Malay peninsula (1). In the recent study, stem bark of this species collected in Pahang, Malaysia, was extracted by conventional methods to yield 0.34% of crude alkaloids. This mixture consisted mainly of noraporphines and aporphines with the 6a(*R*) configuration and no ring D substituents: (−)-nornuciferine (**1**) (30% isolated yield based on crude alkaloids), (−)-asimilobine (**2**) (5%), (−)-anonaine (**3**) (1%), the C-3-oxygenated (−)-norstephalagine (**4**) (1.5%), the C-7-oxygenated (−)-norushinsunine (**5**) (0.4%), and the aporphines (−)-nuciferine (**6**) (1%) and (−)-lirinidine (**7**) (1%). (+)-(*S*)-Reticuline (**8**) (2%) was also isolated, along with its biogenetic derivatives (+)-norcorydine (**9**) (0.3%) and the berbines (−)-discretamine (**10**) (15%), (−)-10-*O*-demethyldiscretine (**11**) (7%), and the new catecholic (−)-artavenustine (**12**) (3%), described here for the first time.

The previously known alkaloids were identified by spectroscopic methods and by comparison with reference samples. The ms of the new compound (**12**) showed a molecular ion peak at *m/z* 313, a base peak at *m/z* 178, and a fragmentation pattern generally indicative of a berbine skeleton bearing two hydroxyl groups on ring D and one

[1]Part LXIII in the series "Alcaloïdes des Annonacées." For Part LXII, see D. Cortes, J. Saez, R. Hocquemiller, A. Cavé, and Ad. Cavé, *Can. J. Chem.*, in press.

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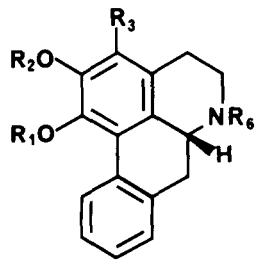
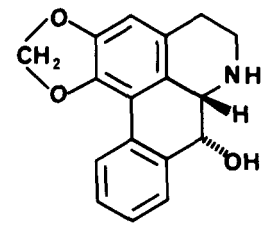


	R_1	R_2	R_3	R_6
1	CH_3	CH_3	H	H
2	CH_3	H	H	H
3	—CH_2—		H	H
4	—CH_2—		OCH_3	H
6	CH_3	CH_3	H	CH_3
7	H	CH_3	H	CH_3



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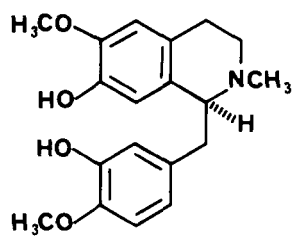

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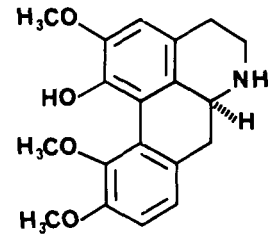

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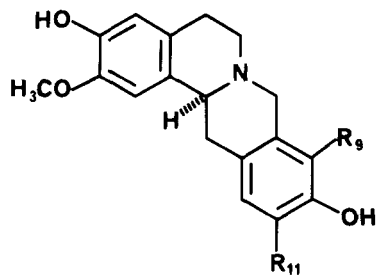

	R_9	R_{11}
10	OCH_3	H
11	H	OCH_3
12	H	OH

hydroxyl and one methoxyl groups on ring A (5). This unusually low degree of methylation was confirmed by the ^{1}H-nmr spectrum, which exhibited a single methoxyl signal at 3.75 ppm, four aromatic proton singlets at 6.44, 6.48, 6.54, and 6.74 ppm, and the significant absence of any C-8 methylene resonance near 4.35 ppm, showing that the oxygen substituents must be located at C-2, -3, -10, and -11 (6). Chemical proof of this partial structure was obtained by methylation with CH_2N_2, which afforded xylopinine (2,3,10,11-tetramethoxyberbine). Acetylation yielded a tri-*O*-acetyl derivative.

A homonuclear (^{1}H-^{1}H) *J*-correlated 2-D nmr spectrum (COSY) (Figure 1) established the existence of three spin systems in the aliphatic region: an isolated AB system (signals at 3.75 and 3.94 ppm) attributable to the C-8 protons; an AMX system which could be assigned to the C-13 protons (2.69 and 3.34 ppm) and H-14[3] (3.82 ppm); and

[3]Numbering of the berbine skeleton as in Pelletier (7), C-14 is often referred to in publications as C-13a.

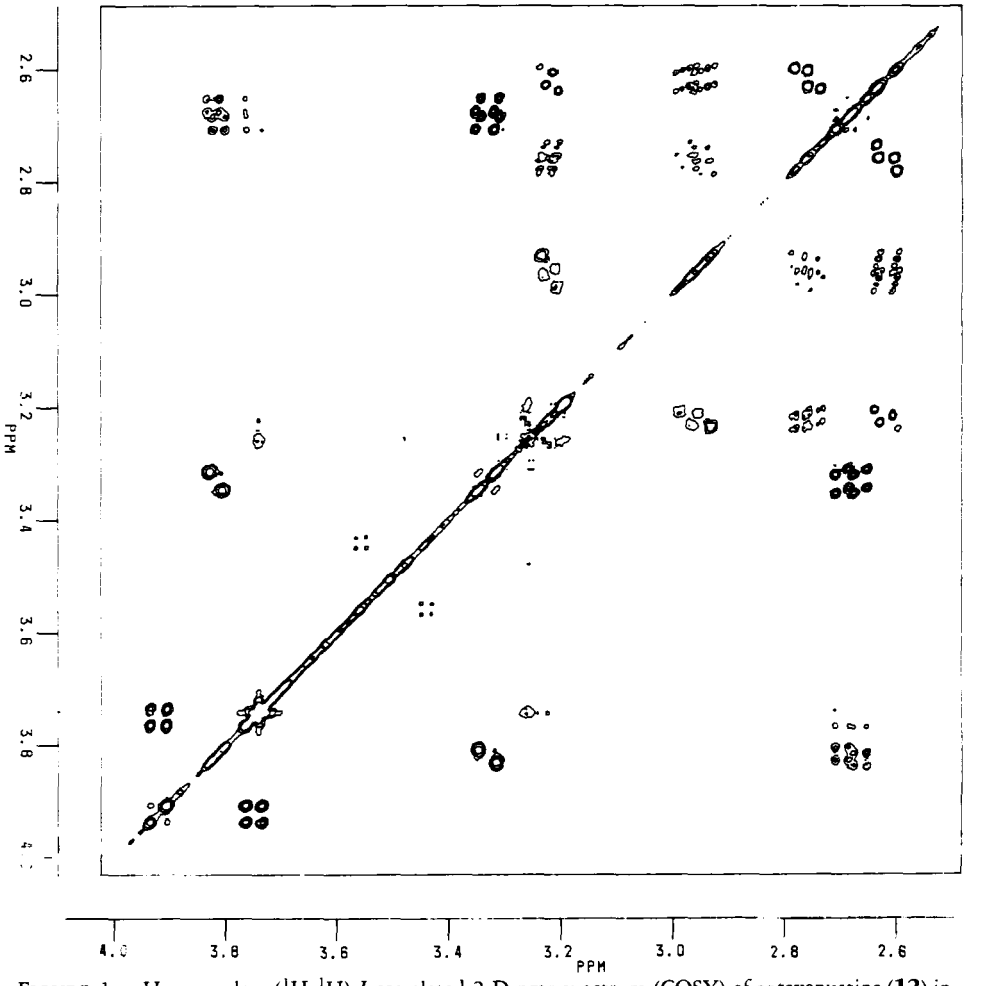

FIGURE 1. Homonuclear (^{1}H-^{1}H) *J*-correlated 2-D nmr spectrum (COSY) of artavenustine (**12**) in CD_3OD at 500 MHz.

a set of four multiplets (2.62, 2.74, 2.96, and 3.23 ppm) for the C-5 and C-6 methylene groups. These results, in combination with the coupling constants measured at 500 MHz, permitted all the aliphatic proton resonances to be assigned as indicated in Figure 2.

Selective irradiation at the resonance frequencies of the aromatic protons showed the existence of long-range couplings (^4J and ^5J) between aromatic and aliphatic protons, consistent with an attribution of the signals at 6.74 and 6.48 ppm to H-1 and -4, and 6.44 and 6.54 to H-9 and -12. To complete the spectral assignments and, in particular, to clarify the position of the methoxyl group, a nOeds study was carried out. By saturating the methoxy protons, only the signal at 6.74 ppm was enhanced appreciably. Irradiation at the latter frequency led to a marked increase in the intensity of the methoxyl signal and a smaller but still meaningful intensification of the doublet of doublets centered at 3.82 ppm, already shown to correspond to H-14, thus proving that the methoxyl group is located at C-2. Other nOe's which served to demonstrate the relative positions of the aromatic ring protons and the neighboring methylene groups are represented in Figure 2.

Catecholic isoquinoline alkaloids appear to be rare in nature, although the fact that only very few are known may well be a reflection of their ease of oxidation and of their

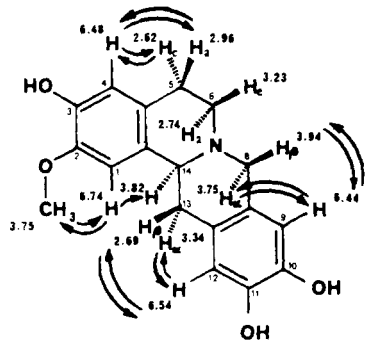

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FIGURE 2. ^{1}H-nmr chemical shifts and nOe's of artavenustine (**12**) in CD_3OD at 500 MHz.

high polarity. Higenamine (demethylcoclaurine) has been isolated from *Nelumbo nucifera* (Nelumbonaceae) (8), *Aconitum japonicum* (Ranunculaceae) (9), *Asiasarum heterotropoides* (Aristolochiaceae) (10), and *Annona squamosa* (Annonaceae) (11), a distribution which suggests that it may be present, albeit in low concentrations, in a broad range of "primitive" Angiosperms. As the synthesis of this alkaloid from dopamine and *p*-hydroxyphenylacetaldehyde is catalyzed very efficiently by (*S*)-norlaudanosoline synthase (12), it can be concluded that demethylcoclaurine is very probably an intermediate in all plants accumulating coclaurine metabolites, i.e., ring D-monosubstituted or unsubstituted aporphinoids, or bisbenzylisoquinolines. Demethyleneberberine has been reported as a constituent of *Thalictrum javanicum* (Ranunculaceae) (13). *Polyalthia oligosperma* (Annonaceae) contains an incompletely characterized alkaloid which would seem to be 10,11-dihydroxy-2,3-dimethoxyberbine, the C-3-*O*-methyl derivative of the new artavenustine (14). Finally, as mentioned in the introduction, the two other *Artabotrys* species studied to date have yielded catecholic aporphines (2-4). As partial *O*-methylation seems to precede further biosynthetic elaboration of the 1-benzyl-1,2,3,4-tetrahydroisoquinoline skeleton (15), all these compounds, with the exception of the biosynthetically early metabolite demethylcoclaurine, can most reasonably be envisioned as *O*-demethylation products of commonplace aporphinoids and berbinoids. The question arises, therefore, as to whether *O*-demethylation occurs frequently as the initial step in the catabolism of isoquinoline alkaloids. Indirect evidence that this is so is provided by the growing number of substances whose formation can be explained as passing through the cleavage of unknown catechol intermediates. Thus, the erythroidines and erymelanthines may be derived from erythrinans (16), and eupolauramine (17), aconcaguine (18), and the biogenetically related eupolauridine, cleistopholine, and onychine (19) probably arise by oxidation of ring A of appropriate aporphinoids. Chiloenamine and chiloenine (20), santiagonamine (21), and andesine (22) are presumably ring C-oxidized dimethylaminoethylphenanthrenes. By analogy, modified berbines should eventually be found in which ring A or ring D has been opened and reclosed to give a γ- or δ-lactone, a lactam, or a pyridine ring.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler block and are uncorrected. Eims were obtained on a VG Micromass 70 instrument. Uv spectra were recorded on a Pye Unicam SP 1800 spectrophotometer. ^{1}H-nmr spectra were recorded at 90 MHz on a Varian EM-390 instrument, and at 500 MHz on a Bruker WM-500 spectrometer. Optical rotations were determined using a Schmidt-Haensch Polartronic I electronic polarimeter.

The COSY spectrum was recorded at 500 MHz using the pulse sequence $(90°\text{-}t_1\text{-}45°)_n$; 256 spectra were acquired with 1K data points and a sweep width of 1000 Hz. The first dimension was zero-filled twice

before 2-D processing. The window used in the processing was a pure sine bell. The matrix was symmetrized before plotting.

PLANT MATERIAL.—Aerial parts of *A. venustus* were collected in flower, in January 1984, at an altitude of about 1300 m, along Robinson Walk, Cameron Highlands, Pahang, Malaysia, in disturbed primary forest. Voucher specimens are deposited at the Department of Chemistry, University of Malaya, and in the Herbaria of the Forest Department, Kepong, Selangor, Malaysia, and of the Laboratoire de Phanérogamie, Muséum d'Histoire Naturelle, Paris (Deverre 92).

EXTRACTION AND ISOLATION.—Stem bark (1.5 kg) of *A. venustus* was defatted with petroleum ether and extracted with MeOH. The MeOH extract was concentrated to dryness, and the residue was extracted with 5% H_2SO_4. The aqueous acid solution was basified with concentrated aqueous NH_3 and extracted with CH_2Cl_2, and the organic layer was concentrated to give 5.16 g (0.34%) crude alkaloids which were separated by column chromatography on silica gel, eluting with CH_2Cl_2-MeOH (9:1).

All previously reported alkaloids were characterized spectroscopically and by direct comparison with authentic samples.

ARTAVENUSTINE (**12**).—Amorphous, $[\alpha]_D$ −127° (c 0.26-EtOH); λ max (MeOH) nm (log ε) 228 (4.09), 288 (3.86); eims *m/z* (rel. int.) 314 (13), 313 (73, M^+), 312 (37), 179 (8), 178 (100), 177 (28), 176 (57), 136 (33); 500 MHz ^{1}H nmr (CD_3OD) δ 2.62 (1H, ddd, J=16.8, 3.6, 2.7 Hz; H-5ψ e), 2.69 (1H, dd, J=16.3, 11.6 Hz; H-13β), 2.74 (1H, ddd, J=11.7, 11.5, 3.6 Hz; H-6ψ a), 2.96 (1H, ddd, J=16.8, 11.5, 5.1 Hz; H-5ψ a), 3.23 (1H, ddd, J=11.7, 5.1, 2.7 Hz; H-6ψ e), 3.34 (1H, dd, J=16.3, 4.5 Hz; H-13α), 3.75 (3H, s; OMe), 3.75 (1H, d, J=14.5 Hz; H-8), 6.44 (1H, s; H-9), 6.48 (1H, s; H-4), 6.54 (1H, s; H-12), 6.74 (1H, s; H-1).

O-METHYLATION OF ARTAVENUSTINE.—Artavenustine, dissolved in MeOH, was methylated with CH_2N_2 in Et_2O, yielding xylopinine: 90 MHz ^{1}H nmr ($CDCl_3$) δ 3.88 (9H, s; 3 OMe), 3.90 (3H, s; OMe), 6.56 (1H, s; H-12), 6.60 (1H, s; H-4), 6.63 (1H, s; H-9), 6.73 (1H, s; H-1); identical with an authentic sample by tlc.

TRI-*O*-ACETYLARTAVENUSTINE.—Artavenustine was acetylated with 1:1 Ac_2O-pyridine, affording the tri-*O*-acetyl derivative: 90 MHz ^{1}H nmr ($CDCl_3$+CD_3OD) δ 2.22 (9H, s; 3 OAc), 3.72 (3H, s; OMe), 6.71, 6.79, 6.86, and 6.94 (1H each, 4 s; H-1, -4, -9, and -12); eims *m/z* (rel. int.) 439 (87, M^+), 438 (65), 397 (28), 396 (32), 354 (12), 220 (4), 218 (21), 179 (21), 178 (43), 177 (16), 176 (77), 149 (19), 137 (22), 136 (100), 44 (37).

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