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Aporphine Alkaloids from *Guatteria stenopetala* (Annonaceae)

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This paper is dedicated to Professor P. Joseph-Nathan for his 65th birthday.

Guatteria species (Annonaceae) are rich in isoquinoline alkaloids that contain a restricted β-phenethylamine moiety and thus have potential as monoamine oxidase (MAO) inhibitors for the treatment of Alzheimer's and Parkinson's diseases. Three aporphine alkaloids have been isolated from *G. stenopetala*, the known oxoporphine dicentrinone (**1**), and two novel compounds, *N*-methyldicentrinone (**2**) and 4,5-dioxodehydrodicentrine (**3**). Their inhibitory effects on MAO-B were evaluated, but none of them was significantly active.

Keywords: *Guatteria stenopetala*, dicentrinone, *N*-methyldicentrinone, 4,5-dioxoaporphine, aporphine alkaloids, Annonaceae, MAO-B.

Guatteria is the largest Annonaceous genus, comprising approximately 265 species, and is one of the largest genera of neotropical trees, along with *Inga* and *Ocotea*. The genus is exclusively neotropical, and widely distributed throughout Mesoamerica (ca. 30 species), the Caribbean (3 species), and South America (ca. 230 species) [1]. In Venezuela, 35 species are registered, of which 21 are encountered in Venezuelan Guayana. The fibrous bark of many species, commonly called majagua, is used as cordage throughout this region [2]. About 140 alkaloids, mostly containing the aporphine skeleton, have been isolated and identified from ca. 30 phytochemically studied species of this genus [3].

G. stenopetala R. E. Fr. is classified as VU D2 (taxon that is vulnerable, although not critically endangered, but is facing a high risk of extinction in the wild in the medium-term future), according to the IUCN (International Union for Conservation of Nature and Natural Resources) red list of threatened species (1994) Categories & Criteria [4]. To our knowledge there is no report of phytochemical studies on this species.

From *G. stenopetala*, we have isolated three aporphine alkaloids: dicentrinone (**1**), *N*-methyldicentrinone (**2**), and 4,5-dioxodehydrodicentrine (**3**). The structures of compounds **2** and **3** have not been reported previously.

Dicentrinone (**1**), isolated as fine yellow needles, is an oxoaporphine alkaloid that has been isolated from various families, such as Annonaceae, including *G. scandens* [5] and *G. maypurensis* (our unreported study), Hernandiaceae, Lauraceae, Magnoliaceae, Menispermaceae, and Papaveraceae. The molecular ion in the EIMS was observed at *m/z* 335.1, corresponding to the molecular formula $C_{20}H_{13}NO_5$. The structure identification was established by comparison of its spectroscopic data with those reported in the literature [6].

N-Methyldicentrinone (**2**) was isolated as an amorphous, purple-brown colored solid in small quantity (3 mg). The molecular ion at m/z 350.3 in the ESIMS suggested the addition of a methyl group to the dicentrinone structure, i.e. $C_{20}H_{16}NO_5^+$ as the molecular ion. The ^{1}H-NMR spectrum of **2** (Table 1) was similar to that of dicentrinone. A pair of doublets at δ 8.40 (1H, d, *J* = 6.5 Hz) and 8.75 (1H, d, *J* = 6.5 Hz) can be assigned to H-4 and H-5 in an oxoaporphine skeleton. Three singlets at δ 7.56, 7.77, and 8.08, corresponding to 1H each, indicate a similar substitution pattern of this alkaloid to that of dicentrinone, i.e. the singlet at δ 7.56 corresponds to H-3 because of its chemical shift in the relatively high field, and is correlated to H-4 at δ 8.40 (NOESY), and the singlets at δ 7.77 and 8.08, which are assigned to H-8 and H-11, respectively. The singlet at δ 7.77 was correlated to the C-7 carbonyl carbon at δ 176.5 (HMBC). Four oxygenated substituents were observed, comprising one dioxymethylene group (δ 6.62, 2H, s) and two methoxy groups (δ 3.98, 3H, s, and δ 4.02, 3H, s), which could be located at C-1, C-2, C-9, and C-10 on rings A and C. The location of the methoxy groups at C-9 and C-10, but not at C-1 and C-2, as in the case of oxonantenine (1,2-dimethoxy-9,10-methylenedioxyoxoaporphine), was confirmed by NOESY correlations of these groups to H-8 and H-11. In addition, *N*-CH$_3$ was correlated to H-5 by NOESY experiment, thus establishing the structure of this alkaloid as **2**. HMBC and NOESY correlations (Table 1) are depicted in the figure. Finally, dicentrinone (**1**), by treatment with methyl iodide, was converted to its *N*-methyl derivative, which was identical to alkaloid **2**.

4,5-Dioxodehydrodicentrine (**3**) was isolated as a pink-orange colored crystalline solid in small quantity (2 mg). Its IR spectrum presented two carbonyl groups at 1644 and 1674 cm^{-1}, suggesting the 4,5-dioxoaporphine skeleton. It has been reported that 4,5-dioxoaporphines are of orange to red color [7]. In contrast with the ^{1}H-NMR spectra of dicentrinone (**1**) and *N*-methyldicentrinone (**2**), alkaloid **3** did not have a pair of doublets corresponding to H-4 and H-5, and instead, presented one additional singlet (1H) in the aromatic region (Table 2). HMQC, HMBC, and NOESY experiments established its structure as shown in the figure, while the molecular ion in the EIMS spectrum at m/z 365.1 was consistent with the molecular formula

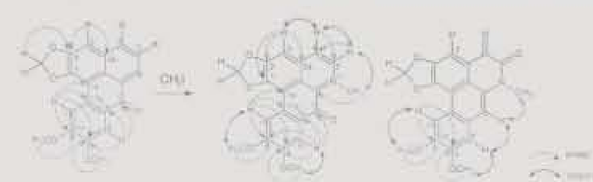
Table 1: ^{1}H, ^{13}C (HMQC), HMBC and NOESY spectroscopic data of compound **2** (CD$_3$OD, 500 MHz).

Position	δ_H	δ_C (HMQC)	HMBC	NOESY
3	7.56 (s)	[illegible]	[illegible] [illegible] [illegible] (C3a)	8.40 (H4)
4	8.40 (d, *J*=6.5 Hz)	127.5	140.8 (C3)	8.75 (H5)
5	8.75 (d, *J*=6.5 Hz)	142.0	127.5 (C4)	8.40 (H4)
8	7.77 (s)	110.0	176.5 (C7) 155.5 (C9) 149.8 (C10) 120.0 (C7a)	
11	8.08 (s)	109.4	155.5 (C9) 149.8 (C10) 124.7 (C11a) 108.0 (C1a)	
-OCH$_2$O-	6.62 (s)	106.8		
9-OCH$_3$	3.98 (s)	56.4	149.8 (C10)	7.77 (H8)
10-OCH$_3$	4.02 (s)	56.8	155.5 (C9)	8.08 (H11)
N-CH$_3$	4.78 (s)	51.0	140.0 (C6a)	8.75 (H5)

*Not assigned

Table 2: ^{1}H, ^{13}C (HMQC), HMBC and NOESY spectroscopic data of compound **3** (CF$_3$COOD, 500 MHz).

Position	δ_H	δ_C (HMQC)	HMBC	NOESY
3	8.31 (s)	110.6		
7	8.60 (s)			7.69 (H8)
8	7.69 (s)	111.1		8.60 (H7)
11	8.70 (s)	108.2	114.5* 120.5* 151.5 (C9)	
-OCH$_2$O-	6.78 (s)	107.5		
N-CH$_3$	4.27 (s)	32.0		8.60 (H7)
10-OCH$_3$	4.25 (s)	56.0	157.0 (C10)	8.70 (H11)
9-OCH$_3$	4.20 (s)	56.5	151.5 (C9)	7.69 (H8)



1 2 3

$C_{20}H_{21}NO_4$. 4,5-Dioxoaporphines have been isolated from various sources [8]. These three structurally somewhat different aporphine alkaloids of the same substituent pattern may be correlated with each other, and are interesting from the biosynthetic point of view.

The alkaloids **1**, **2**, and **3** were subjected to MAO-B inhibitory bioassay on TLC plates, as described in the experimental section, giving an reddish spot on spraying with Nile Blue solution, in contrast to a selective MAO-B inhibitor, L-deprenil, which gave a reddish color.

Experimental

General: UV/Vis spectra were recorded on an Ocean Optics PC2000 spectrophotometer. IR spectra were registered on a FT Nicolet Nexus 470 spectrophotometer. A Bruker Avance 500 spectrometer was used for the NMR experiments. ESIMS (positive mode) and EIMS were obtained using Finnigan LCQDL 10 and Jeol LMS-AX505WA spectrometers, respectively. TLC was performed on precoated Macherey-Nagel Alugram SilG/UV254 plates; alkaloid spots were detected with Dragendorff and chloroplatinic acid reagents. Meyer's reagent was used for the detection of alkaloids in aqueous solutions. Column chromatography was performed on Scharlau silica gel G60 (0.06-0.2 mm/70-230 mesh ASTM).

Plant material: The leaves of *Guatteria stenopetala* were collected in May, 1998, in the rainforest along the Cuao-Sipapo rivers located in Autana municipality of Amazonas State, Venezuela, cartographically between 4°54' - 5°3' latitude N, 67°37 - 67°46' longitude W. A voucher specimen is deposited in the National Herbarium of Venezuela (VEN) under the code AC5677.

Extraction and isolation: The air-dried powdered leaves (470 g) were extracted by exhaustive maceration with MeOH at room temperature. The extract was concentrated under reduced pressure, followed by extraction with 5% aq. HCl solution. This acidic solution, after being extracted successively with *n*-hexane and Et_2O, was basified with aq. NH_4OH at pH 8, and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was concentrated to dryness, and 5.0 g of the residue was chromatographed over a silica gel column (500 g) with CH_2Cl_2, CH_2Cl_2-MeOH mixtures, and MeOH. Elution with the mixture of CH_2Cl_2-MeOH (9:1, 1:1) yielded alkaloid-rich fractions (965 mg). These were combined, and chromatographed repeatedly on silica gel columns using CH_2Cl_2, CH_2Cl_2-MeOH, and CH_2Cl_2-MeOH-NH$_4$OH as eluents, monitoring the fractions by TLC; similar fractions were combined according to TLC analysis. Finally, three fractions: composed **1**-rich (39 mg), composed **2**-rich (11 mg), and composed **3**-rich (28 mg) were obtained. Each fraction was purified on a silica gel column with CH_2Cl_2-MeOH mixtures, affording 13 mg of pure alkaloid **1**, 5 mg of **2**, and 2 mg of **3**.

MAO assay

Materials: Mouse liver mitochondrial monoamine oxidase (EC 1.4.3.4) was partially purified by the method of Holt *et al.* [9], with a slight modification. MAO activity was adjusted to 8.9102 +/- 0.4705 nmol/h/mg of protein for the assays. Protein levels were determined by the modified method of Lowry *et al.* [10] using bovine serum albumin as a standard. Monobasic and dibasic phosphates were purchased from Riedel de Haën. Bovine serum albumin was obtained from Merck. The Nile Blue chloride salt was purchased from Aldrich.

Samples tested: Alkaloids were dissolved in methanol and applied to TLC plates in varying concentrations.

Bioautography: Mitochondrial monoamine oxidase, partially purified (1 mL), was dissolved in 1 mL of either dibasic phosphate or tris-hydrochloride acid buffer solution at pH 7.2 or 9.0, respectively; bovine serum albumin (0.8 mg) was added to the solution in order to stabilize the enzyme during the bioassay. The TLC plates were washed with methanol and then dried before use. After direct application of samples, the TLC plate was dried with a hair dryer for total removal of the solvent. The plate was then sprayed with benzylamine solution (1 mL in 3 mL of dibasic phosphate buffer solution, pH 7.2), dried, subsequently sprayed with the enzyme solution, and thoroughly dried again. For the incubation of the enzyme, the plate was laid flat in an incubator with a humid atmosphere at 37°C for 20 min. The enzyme was successfully stabilized under these conditions. For the detection of enzyme activity, a saturated Nile Blue solution in distilled water, freshly prepared in each experiment, was sprayed onto the TLC plate. Reddish colored spots were observed in the inhibition zone in a few seconds for known MAO-B inhibitors, such as L-deprenil (selegiline). Under the same

conditions, compounds **1-3** showed no reaction with Nile Blue solution.

Dicentrinone (1)

Yellow crystalline needles (13 mg).
Partially soluble in CH_2Cl_2, $CHCl_3$, and MeOH, giving a yellow color, that changed to red in the presence of hydrochloric acid.
IR (KBr): 1636 (C=O), 1057, 970 cm^{-1}.
UV/Vis λ_{max} (MeOH): 210, 248, 270, 314, 348, 390 nm.
^1H NMR ($CDCl_3$): 4.03 (3H, s, C9-OMe), 4.06 (3H, s, C10-OMe), 6.36 (2H, s, OCH_2O), 7.14 (1H, s, H3), 7.75 (1H, d, *J* = 5.2 Hz, H4), 7.98 (1H, s, H8), 8.03 (1H, s, H11), 8.88 (1H, d, *J* = 5.2 Hz, H5).
^1H NMR (CF_3COOD): 4.16 (3H, s, C9-OMe), 4.20 (3H, s, C10-OMe), 6.66 (2H, s, OCH_2O), 7.54 (1H, s, H3), 8.06 (1H, s, H8), 8.36 (1H, s, H11), 8.46 (1H, d, *J* = 6.6 Hz, H4), 8.75 (1H, d, *J* = 6.6 Hz, H5).
MS (EI, 70 eV): m/z 335.1 $[M]^+$.

***N*-Methyldicentrinone (2)**

Amorphous, purple-brown colored solid (3 mg).
Soluble in MeOH, and partially soluble in CH_2Cl_2 and $CHCl_3$, giving intense red color.
IR (KBr): 1649 (C=O) cm^{-1}.
UV/Vis λ_{max} (MeOH) nm: 240, 261, 289, 380.
NMR: Table 1.
ESIMS: m/z 350.3 $[M]^+$.

Dicentrinone (**1**) was kept in neat MeI in a refrigerator for several days, checking its transformation to the *N*-methyl derivative by TLC. The resultant product was identical to compound **2** by comparison of their TLC and ^1H NMR spectroscopic data.

4,5-Dioxodehydrodicentrine (3)

Pink-orange colored crystalline solid (2 mg).
Partially soluble in CH_2Cl_2, $CHCl_3$, and MeOH, giving an orange color, that changed to purple in the presence of hydrochloric acid.

Detection of this alkaloid on TLC plates was better achieved with chloroplatinic acid reagent than by Dragendorff's reagent.

IR (KBr): 1674, 1644 (C=O) cm^{-1}.
NMR: Table 2.
MS (EI, 70 eV): m/z 365.1 $[M]^+$.

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