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# GEOVANINE,* A NEW AZAANTHRACENE ALKALOID FROM *ANNONA AMBOTAY* AUBL.†

ALAÍDE B. DE OLIVEIRA,‡ GEOVANE G. DE OLIVEIRA, FERNANDO CARAZZA and JOSE GUILHERME S. MAIA

Departamento de Química, Instituto de Ciências Exatas Universidade Federal de Minas Gerais, 30.161, Belo Horizonte, Brazil; Departamento de Produtos Naturais, INPA, 69.000, Manaus, Brazil

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**Key Word Index**—*Annona ambotay*; Annonaceae; alkaloids; flavanoids; geovanine; azaanthracene; oxoaporphines.

**Abstract**—The new azaanthracene geovanine was isolated from the trunkwood of *Annona ambotay* Aubl. together with liriodenine, *O*-methylmoschatoline and other known substances, the flavonoids kaempferol, quercetin, (+)-dihydrokaempferol, (+)-dihydroquercetin, (±)-eriodictiol and (+)-catechin and the steroids sitosterol and 5α-stigmastan-3,6-dione.

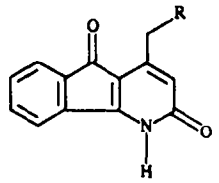
## INTRODUCTION

*Annona ambotay* Aubl. (Annonaceae) is a shrub of 2–3 m in height with a fibrous and fragrant bark, it is known as 'enviratataia'. The present paper describes the chemical examination of the trunkwood of a specimen collected at the Reserva Florestal Ducke (CNPq/INPA) in Manaus, Amazonas State. A voucher is deposited at the INPA herbarium (No. 46 803). Previous investigation on this species reports the analysis of the essential oil from the bark which showed the presence of the sesquiterpenes β- and γ-elemene, β-caryophyllene, γ-muurolene and muurolol as the predominant volatile constituents [1].

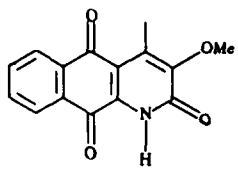
## RESULTS AND DISCUSSION

A trunkwood sample of *A. ambotay* was successively extracted with benzene and ethanol in a Soxhlet apparatus. The ethanol extract was submitted to exhaustive extraction with cold ethyl acetate. The benzene extract and the ethyl acetate soluble fraction from the ethanol extract were fractionated by column chromatography. The benzene extract afforded the oxoaporphine alkaloids liriodenine and *O*-methylmoschatoline, the flavonoids kaempferol and quercetin, the steroids sitosterol and 5α-stigmastan-3,6-dione besides a new azaanthracene alkaloid which was named geovanine. The ethyl acetate soluble fraction led to the isolation of further amounts of kaempferol and quercetin besides (+)-dihydroquercetin, (+)-dihydrokaempferol, (±)-eriodictiol and (+)-catechin.

Geovanine is a yellow alkaloid (0.00007 g% in the trunkwood) that gives a brownish yellow Dragendorff reaction in TLC. HREIMS showed a molecular ion with composition $C_{17}H_{17}NO_4$ (*m/z* 299.1185) (73%) and fragmentary ion peaks for $[M-Me]^+$ (284.0866) (100%) and $[M-2Me]^+$ (269.0715) (51%). The $^1H$ NMR spectrum revealed a methyl group (δ2.78) linked to an $sp^2$ carbon, an olefinic proton as a broad singlet (δ6.48) due to long-range coupling with the methyl group, three methoxyl groups (δ3.91, 3.93 and 4.04) and three vicinal aromatic protons (6.91 *d J* = 8.0 Hz; 7.35 *t J* = 8.0 Hz and 7.77 *d J*



**1** R = H
**2** R = OH



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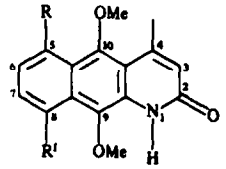
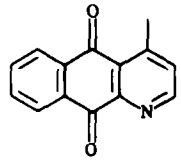


**6** R = H, R¹ = OMe
**7** R = OMe, R¹ = H



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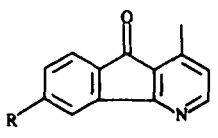
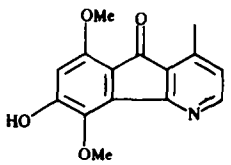


**4** R = H
**5** R = OMe



**9**

* In memory of Prof. Geovane G. de Oliveira who passed away on 17 July, 1984.

†Part of the doctorate thesis submitted by F. Carazza at Universidade Federal de Minas Gerais.

‡Author to whom correspondence should be addressed.

= 8.0 Hz). A low frequency carbonyl group (1650 $cm^{-1}$) and broad bands at 3390 and 3110 $cm^{-1}$ in the IR spectrum indicated the presence of a lactam group (NHCO free and dimeric). The UV-Vis spectrum underwent modification only upon addition of sodium hydroxide (2.5 M). This behaviour is consistent with a pyridone derivative, as previously observed for dielsine (1) and dielsinol (2) while even a weak base (sodium acetate) causes bathochromic shifts in the spectrum of dielsiquinone (3) and, as expected, the spectra of onychine (4) and 6-methoxyonychine (5) are shifted only upon acidification [2, 3]. The discussed data indicated that this new compound is a 1-azaanthracene but did not allow a distinction between the isomeric structure 6 or 7 and geovanine must be 1-aza-5(or 8), 9,10-trimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene. It is the first 1-azaanthracene alkaloid to be characterized as such. The previously described representatives of this group are the quinones cleistopholine (8) from *Cleistopholis patens* [4] and dielsiquinone (3) from *Guatteria dielsiana* [2, 3] respectively an African and a Brazilian Annonaceae.

The isolation of liriodenine and geovanine from *A. ambotay* together with the previously reported co-occurrence of liriodenine, cleistopholine (8) and onychine (4) in *C. patens* [4] as well as of liriodenine, dielsiquinone (3), onychine (4), 6-methoxyonychine (5) and dielsinol (2) in *G. dielsiana* [2, 3] and of liriodenine, cleistopholine (8) and kinabaline (9) in *Meiogyne virgata* [5] is further support to the proposal that 1-azaanthracene and 4-azafluorene alkaloids are aporphine derived [5, 6]. Cleistopholine (8) and onychine (4) are the simplest representatives of these two new classes of azapolycyclic alkaloids. Further elaboration of the basic structures can occur by oxidation of the α-carbon or of both α- and β-carbons of the pyridine nucleus as disclosed in dielsine (1), dielsinol (2) and dielsiquinone (8). Geovanine would be the result of a further step in the recently proposed biosynthetic pathway which leads to 1-azaanthraquinones [6] since its derivation requires the reduction of the quinone and *O*-methylation of the corresponding hydroquinone.

The up-to-now restricted group of 1-azaanthracene and 4-azafluorene alkaloids, when considered as derived from aporphines [5, 6], the main alkaloidal constituents of the Annonaceae [7], may be of particular significance in chemosystematics since they would represent a new biosynthetic trend in the evolution of benzylisoquinoline alkaloids [8].

## EXPERIMENTAL

Mps are uncorr. EIMS, HREIMS and $^1H$ NMR were recorded at the Central Analitica (NPPN/UFRJ). TLC spots were developed by 2% ceric sulphate soln in dil. $H_2SO_4$ and heating at 100°, or by Dragendorff reagent. UV: EtOH–$H_2O$ (9:1). IR: KBr discs.

*Extraction and isolation.* A trunkwood sample (11 kg) of *A. ambotay* was ground and extracted successively with $C_6H_6$ and EtOH in a Soxhlet. The $C_6H_6$ extract (43 g) was chromatographed over silica gel (500 g) affording the following useful fractions with the indicated eluents: A ($C_6H_6$), B ($C_6H_6$–$CHCl_3$, 1:1), C ($CHCl_3$), D ($CHCl_3$–MeOH, 9:1), E ($CHCl_3$–MeOH, 1:1). A was chromatographed on a polyamid column: $C_6H_6$ + MeOH (23:2) eluted kaempferol (0.01 g) and quercetin (0.02 g). B was crystallized (EtOH) giving sitosterol (0.06 g). Silica gel column chromatography of C gave, upon elution with $C_6H_6$ + $CHCl_3$ (8:2), 5α-stigmastan-3,6-dione (0.05 g). D was chromatographed over silica gel affording *O*-methylmoschatoline (0.01 g), liriodenine (0.34 g) upon elution with $CHCl_3$ and geovanine (0.008 g) was isolated after elution with $CHCl_3$–MeOH (9:1) followed by prep. TLC. E was dissolved in EtOAc, the soln was extracted with 6% aq. HCl. The aq. acidic fraction was made alkaline with conc $NH_4OH$ and extracted with $CHCl_3$. The combined organic layers were dried ($Na_2SO_4$) and removal of the solvent gave a residue which was chromatographed over alumina giving liriodenine (0.02 g). The EtOH extract (140 g) was exhaustively extracted with cold EtOAc yielding an insoluble fraction (111 g) and a soluble fraction (17 g). The last one was chromatographed over silica gel (340 g) yielding three useful fractions: F and G ($CHCl_3$–$Me_2CO$, 3:1), H ($CHCl_3$–$Me_2CO$, 1:1). F was crystallized from $C_6H_6$–EtOH (1:1) affording quercetin (0.22 g). G was chromatographed on a polyamid column: $C_6H_6$–MeOH (9:1) eluted kaempferol (0.07 g), quercetin (0.05 g), (+)-dihydroquercetin (0.23), (+)-dihydrokaemferol (0.03 g) and (±)-eridictiol (0.04 g). Chromatography of H over Sephadex LH-20 (MeOH) gave (+)-catechin (0.35 g).

Geovanine [1-aza-5(or 8),9,10-trimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene] (6 or 7). Yellow powder, mp 190–192°. EIMS *m/z* (rel. int.): 300 $[M+H]^+$ (14), 299 $[M]^+$ (73), 298 $[M-H]^+$ (73), 285 $[M+H-Me]^+$ (19), 284 $[M-Me]^+$ (100), 270 $[M+H-2Me]^+$ (8), 269 $[M-2Me]^+$ (8), 268 $[M-H-2Me]^+$ (41), 252 $[M-2Me-OH]^+$ (7). HREIMS *m/z* (rel. int.): 299.1185 $[M]^+$ (79) corresp. to $C_{17}H_{17}NO_4$, calc. 299.1187; 284.0866 $[M-Me]^+$ (100), corresp. to $C_{16}H_{14}NO_4$, calc. 284.0861; 269.0715 $[M-2Me]^+$ (51), corresp. to $C_{15}H_{11}NO_4$, calc. 269.0717; 252.0563 $[M-2Me-OH]^+$ (5), corresp. to $C_{15}H_{10}NO_3$, calc. 252.0554. $^1H$ NMR (270 MHz, $CDCl_3$, δ): 2.78 (*s*, Me), 3.91 (*s*, OMe), 3.93 (*s*, OMe), 4.04 (*s*, OMe), 6.48 (*s*, H-3), 6.91 (*d*, *J* = 8.0 Hz, 1H), 7.35 (*t*, *J* = 8.0 Hz, 1H), 7.77 (*d*, *J* = 8.0 Hz, 1H). UV $\lambda_{max}^{EtOH}$ nm (log ε): 242 (4.70), 282 (4.79), 293 (4.78), 331 (4.19), 348 (4.11), $\lambda_{max}^{EtOH+2.5\,M\,NaOH}$ nm (log ε): 287 (4.72), 341 (3.98), 358 (3.85); IR $\nu_{max}$ $cm^{-1}$: 3390, 3110, 2890, 2810, 1650, 1585, 1550, 1500, 1465, 1450, 1360, 1250, 1060, 1000, 850, 760.

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