Guiaflavine, a New Bisindole Quaternary Alkaloid from the Stem Bark of *Strychnos guianensis*

Jacques Penelle,[†] Monique Tits,[†] Philippe Christen,[‡] Viviane Brandt,[†] Michel Frédérich,[†] and Luc Angenot*,[†]

Laboratory of Pharmacognosy, Institute of Pharmacy, University of Liège, CHU, Tour 4, Avenue de l'Hôpital 1, B-4000 Liège 1, Belgium, and Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva 4, 20 Bd d'Yvoy, CH-1211 Geneva 4, Switzerland

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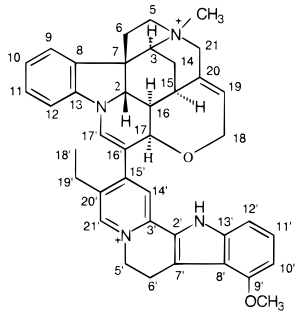
A reinvestigation of *Strychnos guianensis* resulted in the isolation of a colored quaternary bisindole alkaloid from the stem bark. The structure of this new substance, guiaflavine (**1**), was defined by detailed spectroscopic methods and comparison with model compounds.

Strychnos guianensis (Aubl.) Mart. (Loganiaceae) is a moderate-sized liana that occurs widely throughout the middle and upper Rio Orinoco basins and the entire Amazon basin. It is one of the most frequently collected species of this genus in South America and was the first botanically identified source of curare, of which it is a frequent ingredient.[1−3] In the 1950s, chemical investigation of the plant led to the isolation of small amounts of many colored alkaloidal products of unknown structure.[4] We have reported the structures of guianensine[5] and 9-methoxygeissoschizol[6] from the tertiary alkaloidal fractions of the stem bark of *S. guianensis*.

In a continuation of our search for bioactive compounds from the genus *Strychnos*, we have studied the quaternary bases of *S. guianensis*, which resulted in the isolation of several yellow, orange, and red alkaloids. Among these components is an alkaloid that was named guiaflavine (**1**), after the Latin word "flavus" for yellow. In this paper, we report the purification and structure determination of this new natural product (**1**).



1 Guiaflavine

After elimination of the tertiary alkaloids, the quaternary fraction, precipitated with Mayer's reagent and converted to the chloride form, was separated by MPLC on a Lichrospher RP select B column. Fractions containing guiaflavine (**1**) were then purified by liquid chromatography on a cellulose column, using system C of Karrer and Schmid[7] to afford this compound in pure form.

Guiaflavine (**1**) is an asymmetrical bisindole quaternary alkaloid that gave a blue-green color with ceric sulfate−sulfuric acid reagent. The same coloration is observed by spraying with this reagent for guianensine,[5] afrocurarine,[8] and strychnochrysine.[9] Compound **1** showed UV spectral maxima at 208, 252, 321, and 433 nm. This highly conjugated chromophore is similar to that of afrocurarine and strychnochrysine because it is not modified in alkali and therefore differs from an anhydronium base.[10] The molecular weight, 610.3296, established by high resolution ESIMS corresponds to the elemental composition $C_{40}H_{42}N_4O_2$, and the structure of **1** was deduced from interpretation of its 2D NMR spectra (Table 1) and by data comparison with model compounds.[5,8,9,14,15,21,25]

In the aromatic region of the ^{1}H NMR spectrum of **1**, two deshielded pyridine protons at δ 8.13 and 8.48, due to H-14′ and H-21′, were observed, and therefore, the shifts of C-14′ and C-21′ could be established from the HSQC spectrum at δ 120.1 and 144.7, respectively. In the ^{1}H−^{1}H COSY spectrum of the aromatic region, one set of four aromatic protons was observed from an unsubstituted indole and another set of three protons from a C-9 or C-12 substituted indole nucleus was also apparent. The protons at δ 7.22 (t), 7.06 (m), and 6.56 (d) could be attributed to a corynanium portion of **1**. Indeed, comparison with the spectra of guianensine,[5] 9-methoxygeissoschizol,[6] and *C*-alkaloid O[11] indicated that the methoxyl group, corresponding to the three-proton singlet appearing at δ 3.94 ppm, could be at C-9 of this corynanium unit.[12] Consequently, the four remaining aromatic protons at δ 7.53 (d), 7.29 (t), 7.055 (m), and 7.05 (m) were assigned to a strychnan ring. The protonated aromatic carbons were assigned by their direct correlations observed in the HSQC and DEPT spectra (Table 1). The HMBC spectrum confirmed these assignments and also allowed the assignments made for the quaternary carbons. The H-11′ signal correlated to C-13′ and C-9′ (δ 142.5 and 156.9), while H-10′ correlated to C-8′ and C-12′ (δ 117.5 and 106.3); H-12′ was coupled to C-8′ and C-10′ (δ 117.5 and 101.3). Similar correlations were observed for the indoline ring: H-11, H-9, and H-2 correlated to C-13 (δ 146.0), while H-10 and H-12 correlated to C-8 (δ 130.5), as shown in Table 1.

Examination of its NMR spectra revealed for the corynanium portion of **1**, three methylene groups at δ 56.2, 22.3, and 25.3, which were assigned to C-5′, C-6′, and C-19′, respectively. In the COSY spectrum, a strong correlation between H_2-5′ and H_2-6′, indicating a dihydroflavopereirine skeleton,[13] was observed. Similarly, a connectivity was observed in the HMBC spectrum between H_2-5′ and C-7′ (δ 118.3). The third methylene unit H_2-19′ was clearly

* To whom correspondence should be addressed. Tel.: ++32-4-3664331. Fax: ++32-4-3664332. E-mail: L.Angenot@ulg.ac.be.
[†] University of Liège.
[‡] University of Geneva.

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Table 1. ^{1}H and ^{13}C NMR Data of Compound **1** (recorded in 400/100 and 600/150 MHz in CD_3OD)

position	^{1}H[a]	homonuclear correlation (H/H)	^{13}C (by HSQC, DEPT, and CPD)[b]	HMBC (H/C correlations)[c]
2	4.20 (d, 12)	16	60.5 (CH)	C-6, C-8, C-13, C-17
3	4.47 (s)	14 A	76.1 (CH)	C-2
5	3.8 (m)	6	63.6 (CH_2)	
6	2.35 (m)	5	38.7 (CH_2)	C-2
7			54.0 (C)	
8			130.5 (C)	
9	7.53 (d)	10	124.6 (CH)	C-7, C-11, C-13
10	7.05 (m)	9	123.6 (CH)	C-8, C-12
11	7.29 (t)	12	131.4 (CH)	C-9, C-13
12	7.055 (m)	11	111.6 (CH)	C-8, C-10
13			146.0 (C)	
14A	2.75 (d, 14)	3, 14 B	25.8 (CH_2)	
14B	1.84 (d, 14)	14 A		
15	3.67 (br s)		31.1 (CH)	
16	1.9 (d, 12)	2	43.9 (CH)	
17	5.0 (s)		79.3 (CH)	C-2, C-16′
18A	4.32 (dd, 14, 6)	18B, 19	65.3 (CH_2)	
18B	4.15 (dd, 14, 6)	18A, 19		C-17, C-19, C-20
19	6.46 (t, 6)	18A, 18B	137.2 (CH)	
20			135.0 (C)	
21A	4.24 (d, 16)	21B	65.7 (CH_2)	
21B	3.96 (d, 16)	21A		C-3, C-15, C-19
2′			124.9 (C)	
3′			141.5 (C)	
5′	4.74 (t)	6′	56.2 (CH_2)	C-7′
6′	3.54 (t)	5′	22.3 (CH_2)	
7′			118.3 (C)	
8′			117.5 (C)	
9′			156.9 (C)	
10′	6.56 (d)	11′	101.3 (CH)	C-8′, C-12′
11′	7.22 (t)	10′, 12′	128.7 (CH)	C-9′, C-13′
12′	7.06 (m)	11′	106.3 (CH)	C-8′, C-10′
13′			142.5 (C)	
14′	8.13 (s)		120.1 (CH)	C-2′, C-3′, C-16′,C-20′
15′			157.7 (C)	
16′			115.8 (C)	
17′	7.40 (s)		135.5 (CH)	C-2, C-15′, C-16′, C-17
18′	1.35 (t)	19′A, 19′B	14.8 (CH_3)	C-19′, C-20′
19′A	3.05 (m)	18′, 19′B	25.3 (CH_2)	C-15′, C-18′, C-20′, C-21′
19′B	2.89 (m)	18′, 19′A		C-15′, C-18′, C-20′, C-21′
20′			138.6 (C)	
21′	8.48 (s)		144.7 (CH)	C-3′, C-5′, C-15′, C-19′, C-20′
OCH_3	3.94 (s)		55.8 (CH_3)	C-9′
NCH_3	3.48 (s)		55.4 (CH_3)	C-3, C-5, C-18, C-21

[a] Chemical shift (δ) in ppm from TMS, with multiplicities and coupling constants in Hz in parentheses, and overlapped signals *J* unresolved. [b] Chemical shift (δ) in ppm from TMS. [c] Correlation from H to the indicated carbons.

coupled to the methyl protons H_3-18′ (δ 1.35). In the HMBC spectrum, H_3-18′ correlated to C-19′ and C-20′ (δ 25.3, 138.6).

The other half of the structure of **1** was also deduced from NMR data. The chemical shifts of the protons and the carbons (Table 1) supported the structure of a strychnan skeleton with a N_b-methyl group (δ 55.4), five methylene groups (C-5, C-6, C-14, C-18, and C-21), and five methine groups (C-2, C-3, C-15, C-16, and C-17). The chemical shifts of the C-19−C-20 double bond (δ 137.2 and 135.0) were similar to those of strychnine methiodide.[14] The chemical shift of H-17 at δ 5.0 is in accordance with a linkage to an oxygen atom.[16] Moreover, H_2-18 showed long-range correlations in the HMBC spectrum to C-19, C-20, and C-17 and confirmed the presence of a seven-membered ring in **1**.

The linkage between the two moieties of **1** was deduced from the presence of a lowfield singlet at δ 7.40 (H-17′),[17] which correlated not only to C-16′ and C-15′ (δ 115.8, 157.7) but also to C-2 and C-17 (δ 60.5, 79.3). The deshielding of the isolated H-17′ enamine signal could be explained by the presence of a highly conjugated chromophore. As compared to the spectrum of strychnochrysine,[9] the H-17′ signal was shifted to downfield in **1**, a result of an incremental effect of the methoxyl substituent on the dihydroflavopereirine chromophore.

The stereochemistry of **1** must still be considered. The proposed relative configurations of C-2, C-7, C-3, and C-15 were assigned on biogenetic grounds: H-2β (2*S*), 7β (7*R*), H-3α (3*S*), H-15α (15*R*).[18] The large coupling constant observed between H-16 and H-2 (J = 12 Hz) indicated that guiaflavine (**1**) belongs to the isoretuline series with a H-16α (16*R*) configuration.[14,15,19] The stereochemistry of H-17 was deduced from the absence of coupling between H-17 and H-16 in the 2D homonuclear correlation NMR spectrum. The dihedral angle of ca. 90°, observed in the Dreiding stereomodel between H-16 and H-17, was in good agreement with a H-17α (17*R*) configuration, which is also encountered in longicaudatine.[20,21] Another dihedral angle of ca. 90° in the stereomodel between H-16 and H-15 accounts for the absence of coupling between these two protons and supports the configuration proposed for **1**.

Biological investigation of **1** has been initiated. In mice, guiaflavine administered intraperitoneally possesses an LD_{100} of about 3 mg/kg. The poisonous effects appear fairly rapidly, and death occurs after 5−10 min. Further studies to investigate the basis for this toxicity would be necessary. Nevertheless, the ethnobotanical use of *S. guianensis* in

the preparation of arrow poisons could at least be partially explained by the presence of **1**.

Experimental Section

General Experimental Procedures. UV and visible spectra were recorded on a Kontron Uvikon 922 spectrophotometer, and the IR spectrum was recorded as a KBr pellet on a Perkin-Elmer 1750 FTIR spectrometer. 1D and 2D NMR, ^{13}C, $^{1}H-^{13}C$ short- and long-range correlation HSQC, and HMBC spectra were obtained at 400/100 and 600/150 MHz on Bruker AMX-400 and Bruker AMX-II-600 spectrometers. The chemical shifts are recorded in δ (ppm) based on δ TMS = 0, and the coupling constants (J) are in Hertz. The ESMS was recorded on a Finnigan MAT SSQ 7000 spectrometer. High resolution ESIMS was conducted with the Bruker BioAPEX 4.7 T FTMS spectrometer.

Analytical TLC was performed in precoated Si gel F_{254} (Art. 1.05735, E. Merck) plates. After development, the dried plates were examined under short-wave (254 nm) or long-wave (366 nm) UV light and sprayed with 1% ceric sulfate in 10% H_2SO_4. LiChrospher 60 RP-select B, 12 μm (Art. 1.19655.0100, E. Merck) was selected as stationary support for the first purification, while Whatman CC31 Cellulose was used for the last purification. All solvents used were analytical grade (E. Merck).

Plant Material. Stem bark of *S. guianensis* (Aubl.) Mart. was collected in April 1988, by L. A. at Manaus, near Rio Taruma in Brazilian Amazonia. Identification of the plant was confirmed by Dr. A. J. M. Leeuwenberg (Wageningen); voucher specimens (INPA Herb. no. 150,295) are deposited at the INPA (Instituto Nacional de Pesquisas da Amazônia) at Manaus, at the Pharmaceutical Institute at Liège (Belgium), and at the Agricultural University at Wageningen (The Netherlands).

Extraction and Isolation. The powdered stem bark (600 g) was macerated for 24 h with MeOH−HOAc (99:1) and percolated with the same mixture. After concentration of the extract under reduced pressure, precipitation by addition of H_2O and filtration, the aqueous solution was then extracted with $CHCl_3$. After separation of $CHCl_3$, the aqueous solution was basified to pH 10−11 and again extracted with $CHCl_3$. During this last operation, a precipitate was obtained. The $CHCl_3$ extracts contained tertiary alkaloids, and the terminal aqueous solution, quaternary alkaloids. This last solution was acidified with 5 N HCl to pH 5 and precipitated by Mayer's reagent.[22] The precipitate was then dissolved in MeOH−Me_2CO−H_2O (6:2:1) and the alkaloids converted to the chlorides by passage through a column of Amberlite IRA-420. The residue (6.7 g) obtained after evaporation of the solvents was chromatographed over a Lichrospher RP-select B Merck column. The mobile phase was a mixture of two solvents; solvent A was an aqueous solution of heptanesulfonic acid (sodium salt) (1 g in 420 mL) adjusted to pH 2 with phosphoric acid, and solvent B was MeCN.[23] Because of the complexity of this extract, a linear gradient system of elution was selected from 15% to 35% B in A. After TLC controls, using the solvent system MeOH−25% aqueous MeCOONa solution−Me_2CO (65:35:20),[24] the eluates with similar profiles were combined and precipitated by Mayer's reagent. Each precipitate was dissolved in MeOH−Me_2CO−H_2O (6:2:1) and the alkaloids converted to the chlorides by passage through a column of Amberlite IRA-420. Fractions containing guiaflavine (**1**) (detected when the gradient was 25% B in A) were pooled and submitted to column chromatography on cellulose, eluted with system C of Karrer and Schmid (methyl ethyl ketone saturated with H_2O and 1−3% MeOH).[7] This last purification afforded the pure compound **1** (20 mg).

Guiaflavine (**1**): yellowish-brown amorphous solid; UV (MeOH) λ_{max} (log ϵ) 208 (4.32), 252 (4.07), 321 (3.93), 433 (3.99) nm; IR (KBr) ν_{max} 3427, 2924, 1637, 1561, 1516, 1484, 1462, 1352, 1260, 1104, 1045, 1028, 964, 898, 753 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS m/z 610 (9) [M^+] corresponding to $C_{40}H_{42}N_4O_2$, 611 (1) [MH^+], 609 [$M^+ - H$] (40), 305 [M^{2+}] (100). HRESIMS m/z 610.3296 (calcd for $C_{40}H_{42}N_4O_2$, 610.3296).

Biological Material. The biological experiments were conducted using NMRI male mice (29−43 g). The mice were housed in standard metal cages and provided food and water ad libitum.

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