Pergamon

0031-9422(95)00502-1

Phytochemistry, Vol. 40, No. 5, pp. 1557–1560, 1995

0031-9422/95 $9.50 + 0.00

GUIANENSINE, A ZWITTERIONIC ALKALOID FROM *STRYCHNOS GUIANENSIS**

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(*Received in revised form* 9 *May* 1995)

Key Word Index—*Strychnos guianensis*; Strychnaceae; bark; *bis*-indole alkaloid; zwitterion; curare; guianensine; 2D-NMR.

Abstract—The isolation and structural determination of an alkaloid isolated from the stem bark of *Strychnos guianensis* is described. Elucidation of its structure is based mainly on 1D and 2D NMR studies. The new alkaloid has a zwitterionic asymmetrical *bis*-indole structure and is named guianensine.

INTRODUCTION

Strychnos guianensis is a moderately sized liane well distributed in the basin of the middle and upper Rio Orinoco and throughout the entire Amazon basin [1]. It was the first plant source of curare to be collected and identified botanically, and the use of this species in the preparation of curare is very widespread from Colombia to Surinam and in Ecuador and Brazil. Furthermore, it is often the main ingredient [2]. *Strychnos guianensis* was studied pharmacologically by King [3], West [4] and also by Marini-Bettolo and co-workers [5, 6], who also carried out chemical studies [7, 8]. They showed that crude extracts from the root and stem bark displayed muscle-relaxant activity [3–6]. Several alkaloids (including anhydronium bases), named guiacurarines I–VIII, guianine, guiacurine or erythrocurine were characterized by certain of their chromatographic properties on paper, the colour given with cerium sulphate reagent and sometimes their UV spectra [7, 8].

This paper describes the purification and structural determination of an alkaloid isolated from the stem bark of *S. guianensis*. This new product has been named guianensine.

RESULTS AND DISCUSSION

Guianensine is a yellow fluorescent alkaloid which is generally poorly soluble in organic solvents. Its UV spectrum, showing maxima at 207, 258, 280, 318 and 423 nm, indicates a highly conjugated chromophoric group. Furthermore, bathochromic shifts are observed in alkali, suggesting an anhydronium (β-carbolinium) moiety. These UV spectra are similar to those of guiacurarine III, isolated by Marini-Bettolo *et al.* from *S. guianensis* [8], and xanthocurine, isolated by Giesbrecht *et al.* from a calabash curare [9]. Nevertheless, paper chromatography in system D from Karrer and Schmid [10] have shown that guianensine was less polar than these alkaloids ($R_c > 6$). The ESI mass spectrum of guianensine showed a $[M + 1]^+$ at m/z 625 corresponding to the elemental composition $C_{39}H_{36}N_4O_4$. Spectroscopic studies led us to propose for guianensine the structure **1** based on a longicaudatine or afrocurarine skeleton. The presence of a flavopereirine-type moeity in the molecule, as suggested by the UV spectra, was confirmed by ^{1}H NMR spectra, particularly by the four deshielded protons at $\delta > 8$ (two singlets and two doublets) which could correspond to the pyridinium protons of rings C and D of a flavopereirine moiety [11]. The absence of vinylic protons and the presence of a CH_2–CH_3 unit related to the methyl group at δ1.37 and the methylene at δ2.8 indicates that the side-chain is an ethyl group. Mass spectral fragmentation of guianensine did not give a flavopereirine peak at m/z 247, but weak peaks were observed a m/z 277, indicative of a methoxy-flavopereirine, and another one at m/z 262 $[277\text{-Me}]^+$. In the 2D-COSY spectrum of the aromatic region, we detected one set of four benzenic protons from an unsubstituted indole nucleus at δ6.9, 7.15 (2*H*) and 7.3 and another set of three protons from a 9 or 12 substituted indole nucleus at δ6.6 (*d*) and 7.4 (2*H*, *m*). The substituent should be the methoxyl group corresponding to the

*This paper is dedicated to the memory of the late Prof. Norman G. Bisset, London, whose researches over many years on arrow poisons have contributed so much to our present understanding of curare and other dart poisons.

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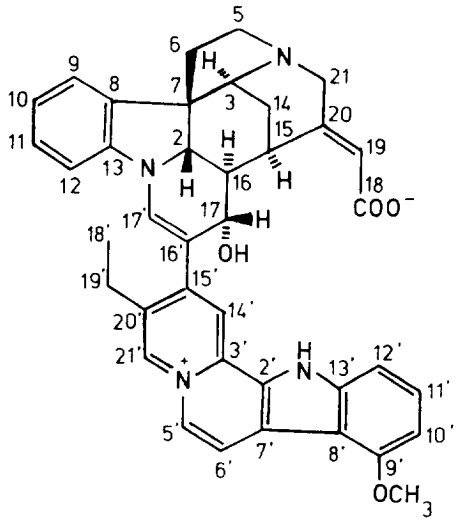

three proton singlet at δ4.02. The position of this substituent was assessed by analysis of the ^{1}H and mainly ^{13}C NMR chemical shifts of the atoms concerned. Comparison of the data with the substituent-induced shifts given by Verpoorte *et al.* [12] indicated that the methoxyl group must be at the 9-position of the indole ring. The chemical shifts of H-10′, H-11′ and H-12′ are also in accordance with a 9-methoxyl substitution as compared with other 9-substituted indole nuclei [13, 14], but not with 12-substituted molecules [15].

The other part of the molecule was deduced mainly from the 2D-COSY spectrum where we could assemble three mutually non-interacting spin systems; the first fragment assembles five methines and one methylene. A convenient entry point in this system is afforded by the δ3.8 broadened singlet. The connectivities provide the means of assembling the multispin substructure –CH–CH$_2$–CH–CH(CH)–CH. The chemical shifts of the protons of this fragment show that it could correspond to the C(3)H–C(14)H$_2$–C(15)H–C(16)H(C(2)H)–C(17)H–O of a strychnane-type skeleton. The chemical shift of H-17 at δ4.5 is in accordance with a linkage to an oxygen atom, as found in strychnine. The second fragment, a CH$_2$–CH$_2$ unit, is related to the four protons at 1.95, 2.58, 3.68 (2H). This fragment probably arises from the tryptamine part of this moiety and should correspond to a N–C(5)H$_2$–C(6)H$_2$–. The last spin-system belongs to an isolated and quite deshielded (δ 4.86 and 4.37) methylene group.

The linkage between the two moeities in an afrocurarine or longicaudatine-like skeleton is also supported by the presence of a singlet at δ7.59 assigned to H-17′, more deshielded than in afrocurarine because of the higher number of conjugated double bonds from the methoxyflavopereirine part of the molecule, and by the major mass spectral fragments found at *m/z* 303, corresponding to a vinyl-methoxyflavopereirine, and *m/z* 288 [303-Me]$^+$.

The ^{13}C NMR spectra (total decoupling and DEPT) show the presence, in the aliphatic region, of two methyl groups (one at δ13.4 and the methoxyl at δ55.5), five methines and one quaternary carbon atom whose chemical shift is indicative of a C-7 from a strychnane skeleton. In the aromatic region, we observed the presence of 13 methines and several quaternary carbon atoms. Among them, a signal at δ167.5 suggested the presence of a conjugated carbonyl group. This was also supported by the intense absorptions at 1592 and 1385 cm^{-1} in the IR spectrum characteristic of a carboxylate ion [16]. The presence of a carboxyl group was also confirmed by the peaks at *m/z* 579 [M – COOH]$^+$, 564 [M – COOH – Me]$^+$ and 45 [CO$_2$ + 1]$^+$ in the mass spectrum. Assignments of carbons were mainly determined by 2D-NMR experiments (X–H CORR). Quaternary carbon atoms were identified by a quaternary-only sequence [17]. All the chemical shifts of the protons and carbons fully support this structure as compared with data from known compounds (longicaudatine *Z*, afrocurarine, flavopereirine or dihydroflavopereirine [11, 18–20]). The deshielding observed for C-17′ can, as for H-17′ be explained by the conjugation between the aromatic ring of the strychnane part of the molecule and the flavopereirine moiety.

The stereochemistry remains to be considered. The proposed relative configurations of C-2, C-7, C-3 and C-15 are those commonly accepted from the biogenetical hypothesis: 2β, 7β, 3α and 15α [21, 22]. The large coupling constant observed between H-16 and H-2 (10.7 Hz) indicates that guianensine belongs to the isoretuline series with 16α [23, 24]. The *ca* 90° dihedral angle observed in the Dreiding stereomodel between H-15 and H-16 accounts for the very weak coupling observed between these two protons and supports the proposed configuration. The value of $^3J_{16-17}$ (9.5 Hz) indicates that C-17 is β, as found in longicaudatine Y [24]. It is interesting to note that the configuration of C-16 and C-17 are opposite to those of afrocurarine [16]. The orientation of the side-chain bearing the carboxylate function is proposed to be *E* because of the deshielding observed for C-21 [25].

To our knowledge, guianensine is the first natural alkaloid possessing such a zwitterionic structure with the negative and positive charges on the two parts of an asymmetrical *bis*-indole alkaloid. This very complex structure and the heterogeneity of the alkaloidal extracts could explain why no structural determinations were carried out earlier when the new chromatographic and spectroscopic methods we used were not available. Moreover, 9-methoxy-substituted alkaloids are not common in *Strychnos* species; only two have been identified up to now, viz., strychnorubigine from *S. rubiginosa* [26] and C-alkaloid-O from a *Strychnos*-based curare [27].

EXPERIMENTAL

Plant material. Stem bark of *S. guianensis* (Aubl.) Mart. was collected in April 1988 by two of the authors (L. A. and M.-L. B.-P.) at Manaus, near Rio Taruma in Amazonia (Brazil). The plant was identified by Prof. A. Imbiriba da Rocha (Manaus) and Dr A. J. M. Leeuwenberg (Wageningen). Voucher specimens (INPA Herb. no

150,295) are deposited not only in the INPA at Manaus, but also in the Pharmaceutical Institute at Liège and the Agricultural University at Wageningen (The Netherlands).

Extraction and isolation. Powdered bark was macerated for 24 hr with MeOH–HOAc (99:1) and percolated with the same mixt. After concn of the extract under red. pres. and filtration, the soln was washed with Et_2O. Na_2CO_3 was then added to pH8 and the soln extracted with $CHCl_3$. The aq. soln was basified to pH12 and re-extracted with $CHCl_3$. During this operation, a ppt was obtained containing guianensine. The $CHCl_3$ extract also contains guianensine, but it is accompanied in this fr. by many other alkaloids. After filtration, the ppt was partitioned between the two phases of a mixt. of $CHCl_3$–MeOH–H_2O (4:4:3). The lower organic phase was evapd. The residue was fractionated firstly by HSCCC in a multilayer-coil separator–extractor fitted with 2.6 mm i.d. coiled tubing and EtOAc–MeOH–H_2O (4:1:3) as solvent. The lower aq. phase was used as stationary phase and the upper organic phase was pumped from the tail of the column to the head. Frs containing guianensine were purified by an MPLC on silica gel 60 using $CHCl_3$–MeOH (9:1) as solvent system. Pure guianensine was finally obtained after prep. TLC on silica gel (1 mm) in EtOAc–*iso*PrOH–NH_4OH 17% (12:5:3).

Guianensine **1**. Orange-coloured powder. Blue–green colour with cerium sulphate reagent on TLC. PC (System D) $R_f > 6$. UV (MeOH) λ_{max} (log ε): 207 (3.97), 258 (3.91), 280 (3.80), 318 (3.73), 423 (3.56); (MeONa) λ_{max} (log ε): 217 (4), 268 (sh), 285 (3.93), 310 (sh), 385 (3.52),

Table 1. ^1H and ^{13}C NMR data of compound **1** ($CDCl_3$)

H	δ	Correlations*	C	δ
2	3.53 (*d*: 10.7 Hz)	16	2	66.5
3	3.8 (*s*)	15, 14A, 14B	3	58.1
5A	3.68 (*m*)	6A, 6B	5	50.2
5B	3.68 (*m*)	6A, 6B	6	41.9
6A	2.58 (*m*)	5A, 5B, 6B	7	53.0
6B	1.95 (*m*)	5A, 5B, 6A	8	129.9†
9	7.15 (*d*)	10, 11	9	122.6
10	6.9 (*t*: 7.2 Hz)	9, 11, 12	10	121.3
11	7.3 (*t*)	9, 10, 12	11	129.3
12	7.15 (*d*)	12-11, 12-10	12	121.3
14A	2.25 (*br d*: 13.1 Hz)	3, 15, 14B	13	147.1
14B	2.05 (*br d*: 13.1 Hz)	3, 15, 14A	14	26.4
15	3.21 (*s*)	3, 14A, 14B, 16	15	22.7
16	2.01 (*dd*: 9.5, 10.7 Hz)	17, 2, 15	16	49.4
17	4.55 (*d*: 9.5 Hz)	16	17	69.5
19	7.15 (*s*)		18	167.5
21A	4.86 (*d*: 16 Hz)	21B	19	152.5
21B	4.37 (*d*: 16 Hz)	21A	20	131.1†
5′	8.22 (*d*)	6′	21	63.4
6′	8.18 (*d*)	5′	2′	130.4†
10′	6.6 (*d*: 6.7 Hz)	11′, 12′	3′	143.4
11′	7.43 (*m*)	10′, 12′	5′	124
12′	7.43 (*m*)	11′, 10′	6′	117.4
14′	8.37 (*s*)		7′	119.9
17′	7.59 (*s*)		8′	111.1
18′	1.37 (*t*: 6.9 Hz)	19′A, 19′B	9′	155.9
19′A	2.89 (*m*)	19′B, 18′	10′	101
19′B	2.83 (*m*)	19′A, 18′	11′	130.2
21′	9.1 (*s*)		12′	106.5
OCH_3	4.02 (*s*)		13′	136.6
			14′	131.9
			15′	147
			16′	110.5
			17′	132.5
			18′	13.4
			19′	24.5
			20′	121.3
			21′	121.9
			OCH_3	55.5

*Observed by means of 2D-COSY spectrum.
†These values may be interchanged.

460 (3.31). EI–MS (120 eV) *m/z* (rel. int.): 625 (100) $[M + 1]^+$, 624 (78), 626 (46), 607 (3), 592 (2), 579 (1), 564 (2.1), 327 (2), 312 (2.5), 303 (9.5), 288 (9.6), 277 (2.3), 262 (2), 144 (5.1), 143 (4), 45 (2). IR (KBr) ν_{max} cm^{-1}: 3400, 3171, 2826, 2740, 1592, 1480, 1462, 1385, 1352, 1244, 1205, 1100, 1049, 914, 767. ^1H and ^{13}C NMR in Table 1.

Acknowledgements—The authors thank Dr E. De Pauw (Chimie Physique, Université de Liége) for providing the MS. Prof. A. Imbiriba da Rocha (Universidade do Amazonas, Manaus) and Dr A. J. M. Leeuwenberg (Agricultural University, Wageningen) gave help in the identification of plant material. The NMR spectra were recorded on the Bruker 400 MHz. ^1H spectrometer in the CREMAN (Centre de Résonance Magnétique Nucléaire de l'Université de Liége). This research was supported by FNRS and CNPq grants (Belgo–Brazilian Cooperation Program). J. Q.-L. is a Research Associate of the Belgian National Fund for Scientific Research (FNRS).

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