

FLAVONOIDS AND ALKALOIDS FROM *STRYCHNOS PSEUDOQUINA*

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ABSTRACT.—From the leaves of *Strychnos pseudoquina* (Loganiaceae), isorhamnetin (**1**) and a new biflavone (**2**) named strychnobiflavone were isolated together with the alkaloids diaboline and 11-methoxydiaboline.

Strychnos pseudoquina St. Hil. (Loganiaceae) is a medium-size tree that grows in the Brazilian "cerrado" where it is well known by the trivial names of "quina do campo" and "falsa quina." It is used in traditional medicine for the treatment of malaria and for the preparation of the so-called "aqua inglesa," largely used in Brazil as a tonic and febrifuge and also to relieve stomach pains (1, 2).

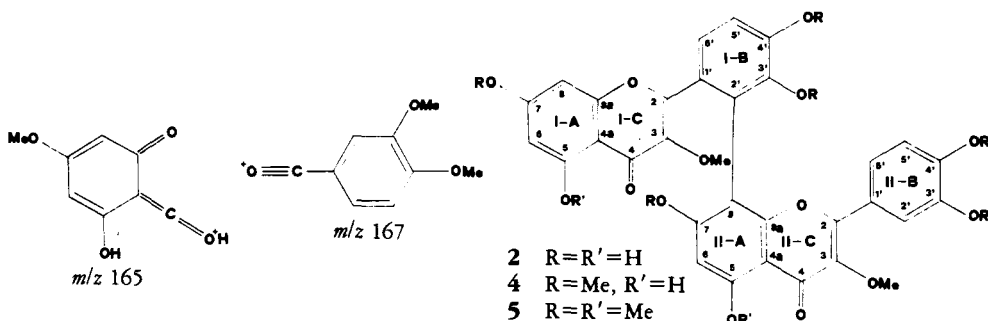
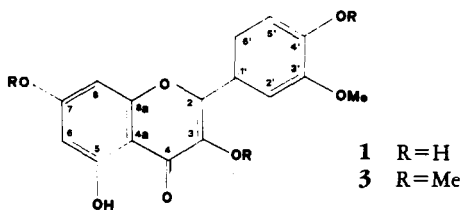
In a previous paper (3) it was demonstrated that no quinine was present in the bark of the roots, of the trunk, and of the branches, but a number of indole alkaloids, mainly nor-dihydrotoxiferine, were present. Later, 11-methoxydiaboline and 11-methoxy-Wieland-Gumlich aldehyde were found in the bark (4).

In the present paper we have investigated the leaves of *S. pseudoquina*, which contain both alkaloids and flavonoids.

RESULTS AND DISCUSSION

Ground and dried leaves of *S. pseudoquina* were exhaustively extracted with EtOH. Part of the residue was reextracted according to the usual procedure for alkaloids. Two main alkaloids were isolated by counter-current distribution (ccd) at discontinuously decreasing pH (5) and were identified as diaboline and 11-methoxydiaboline by direct comparison and analysis of the spectral data. Diaboline and 11-methoxydiaboline have been independently found in several *Strychnos* species of American and African origins (6), but their concomitant occurrence is not common. They are precursors of quaternary alkaloids, such as nordihydrotoxiferine, found in other parts of the plant. In the leaves of this sample, quaternary alkaloids were absent, confirming their usual absence in aerial parts.

Another part of the residue was extracted with EtOAc. In this extract, five flavonoids were evident by tlc; the two main ones were isolated by column chromatography. The less polar was identified as isorhamnetin (**1**) by analysis of chemico-physical and spectral data (7). The second product, **2**, mp 217-219°, showed an uv spectrum similar to that of isorhamnetin (**1**) and analogous shifts after addition of AlCl₃ and AlCl₃/HCl. ¹H-nmr spectra of **2** (in Me₂CO-*d*₆) showed the following sets of signals: (a) two methoxy groups (δ 3.82 and 3.63); (b) two doublets (δ 5.84 and 6.10, *J* = 2 Hz) and a sharp singlet (δ 6.26) attributed to the protons of A rings, showing that one of the ligation sites was in the 6 or 8 position; (c) the other signals in the aromatic region showed a trisubstituted and a tetrasubstituted system pertinent to I-B and II-B rings; and (d) two chelated hydroxy groups (δ 12.6 and 12.8), indicating a possible



biflavonoid structure. All these indications were well confirmed by the ^{13}C -nmr spectrum of **2** (in $\text{Me}_2\text{CO}-d_6$, see Table 1). The majority of the peaks were duplicated, in comparison with those in the cmr spectrum of the 3,7,4' trimethyl derivative of

TABLE 1. ^{13}C -nmr Chemical Shifts of **2-5**^a

	Compound : Solvent			
	2 $\text{Me}_2\text{CO}-d_6$	3 (CDCl_3)	4 (CDCl_3)	5 (CDCl_3)
I-A, II-A				
C(4a)	105.5	105.2	110.3	109.1
C(5)	161.9, 162.0	161.7	161.9, 162.0	160.4, 160.7
C(6)	98.9, 98.8	97.7	94.7, 97.8	91.6 ^b , 95.8
C(7)	162.5, 164.2	165.2	162.1, 165.3	161.0, 163.8
C(8)	93.6, 103.6	92.0	91.6, 102.6	91.2 ^b , 106.7
C(8a)	156.4, 155.5	156.4	154.9, 155.6	154.3, 155.5
I-B, II-B				
C(1')	122.9, 123.0	122.8	123.9	126.3, 124.9
C(2')	116.2, 124.0	111.2 ^b	111.5, 126.3	110.2, 127.7
C(3')	147.9, 148.7	151.0	151.2, 153.8	150.6, 151.9
C(4')	144.8, 145.3	148.5	148.0	148.0, 148.7
C(5')	114.9, 115.7	110.9 ^b	110.9	111.0, 111.5
C(6')	119.8, 121.7	122.0	122.7, 122.9	122.2, 123.5
I-C, II-C				
C(2)	157.4	155.3	157.6	158.4
C(3)	138.7	138.8	138.9	140.9, 141.6
C(4)	179.0, 179.1	178.5	178.1, 178.8	174.1, 174.2
OMe	59.9, 60.3	55.6	55.4, 55.6	55.3, 55.5
		55.8	55.8, 55.9	55.7, 55.7
		56.0	60.0, 60.5	55.8, 56.4
		60.0	60.7	59.8, 60.4
				60.7

^aValues in ppm from TMS.

^bWithin the same column these assignments may be interchanged.

isorhamnetin (**3**). Each of the side phenyl rings appeared to be substituted by two phenolic hydroxyls, *ortho* to each other. The signals of two methoxy groups were also present and, by their chemical shifts, they seemed *ortho*, *ortho'* disubstituted; methoxy carbon atoms *ortho*, *ortho'* disubstituted resonate at lower fields (~ 60 ppm), whereas ordinary OMe groups are usually found between 55-57 ppm (8). In **2**, OMe groups could be assigned to position 3 (rings I-C and II-C) in consideration of the chemical shifts of carbon atoms of rings A and by comparison of **3** with literature data (9).

Thus, the structure of a biflavonoid methoxylated at position 3 could be assigned to compound **2**, to which we propose the name of strychnobiflavone.

By reaction with ethereal CH_2N_2 , the hexamethyl derivative of **2** (**4**), mp 233-234°, was obtained.

The ms spectrum of **4** showed, besides the molecular peak at m/z 714 (47%), the $M^+ - 1$ peak characteristic of the fragmentation pattern of flavonols. The most significant ions were at m/z 357 (30) corresponding to either the doubly charged molecular ion, M^{++} , or the fission product $M/2^{1+}$, and at m/z 167 (15) and 165 (58) attributable to ions related to I-A and II-B rings, respectively, by analogy with other flavonoids (7).

The ^1H -nmr spectrum of **4** in CDCl_3 showed two sets of methoxy groups (three at ~ 4.0 ppm and five between 3.4 and 3.8 ppm) partially overlapped, whereas in $\text{Me}_2\text{CO}-d_6$ all eight signals were located between δ 3.56 and 4.00. Two low-field signals were present (δ 13.20 and 13.54, exchangeable with D_2O), and they could again be assigned to the two hydroxy groups in position 5 (rings I-A and II-A) chelated by the carboxyl groups at position 4 (rings I-C and II-C). The aromatic signals were now clearly separated, and on the basis of their multiplicities the first site of attachment of the two monomers could be assigned to position 2' of one of the benzene rings. The ^{13}C -nmr spectrum of **4**, in comparison with that of **2**, showed as the main difference, aside from the effects due to the methylation, the pattern of the aromatic substitution. Finally, by reaction of **2** with $\text{K}_2\text{CO}_3/\text{Me}_2\text{SO}_4$ the octamethyl derivative (**5**, mp 269-271°) was obtained in order to methylate also the hydroxyl groups in position 5 (rings I-A and II-A), and thus assign the second coupling site. Cmr data of **5**, in comparison with those of **4**, showed the effect of methylation at C(5) in accordance with reported data for flavonoids (9), i.e., strong shielding of C(4), deshielding of C(3) and shielding of C(2). Two signals of the three aromatic methynes of A rings appeared shielded by the effect of the methylation, assigning the second site of attachment to the 8 position. As an independent confirmation, no additional OMe peaks appeared at ~ 60 ppm and seven OMe peaks were present at higher field.

Thus the structure of I-5, I-7, I-3', I-4', II-5, II-7, II-3', II-4'-octahydroxy-I-3, II-3-dimethoxy [I-2'; II-8] biflavone was assigned to strychnobiflavone (**2**). Only in a chromatographic study on African species was the presence of flavonoids and related compounds in *Strychnos* previously reported (10). Therefore, the finding of flavonoids in *S. pseudoquina* is interesting from a chemotaxonomic standpoint.

EXPERIMENTAL

GENERAL EXPERIMENTAL.—A Craig-Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for ccd. ^1H -nmr and ^{13}C -nmr spectra were recorded with a Varian XL 100 spectrometer (using TMS as internal reference). Ms spectra were registered on a LKB 9000 S spectrometer.

PLANT MATERIAL.—Leaves of *S. pseudoquina* were collected near Brasilia, Brazil, and identified at the Universidade Federal de Alagoas, Maceio, Brazil, where a voucher specimen (no. SPQ 81) is deposited. The material (3 kg) was dried at 40°, powdered, and exhaustively extracted with EtOH to yield, after evaporation of the solvent, a residue that amounted to $\sim 12\%$ of the starting material.

ISOLATION AND IDENTIFICATION OF THE ALKALOIDS.—Part of the residue (50 g) was extracted with a 2% HOAc until a negative Dragendorff reaction occurred. The aqueous extract was made alkaline by adding NaHCO_3 and extracted twice with CHCl_3 . The organic phase, after evaporation of the solvent,

yielded a residue (2.5 g) which, by analysis on silica gel (C_6H_6 -EtOAc-diethylamine, 5:4:1), resulted in two main compounds. In the aqueous phase quaternary alkaloids were absent. The alkaloidal mixture was separated by ccd between $CHCl_3$ and a phosphate-citric acid buffer: at pH 7.0, diaboline ($k_b, k_s = 9 \times 10^{-8}$), and at pH 6.0, 11-methoxydiaboline ($k_b, k_s = 6 \times 10^{-8}$) were isolated.

Diaboline: crystals from EtOAc (900 mg), mp 185-187; $[\alpha]^{20}_D + 36$ (c 0.8, $CHCl_3$), M^+ at m/z 352.

11-Methoxydiaboline: crystals from EtOAc and *n*-hexane (450 mg), mp 214-216°; $[\alpha]^{20}_D + 20$ (c 1, $CHCl_3$), M^+ at m/z 382.

ISOLATION AND IDENTIFICATION OF THE FLAVONOIDS.—Another part of the ethanolic residue (100 g) was extracted with EtOAc in a Soxhlet apparatus. The extract (20 g) by tlc analysis on silica gel (EtOAc-toluene, 3:2) resulted in two main compounds. By chromatography on silica gel in EtOAc-toluene (3:2), pure isorhamnetin (**1**, 380 mg) and strychnobiflavone (**2**, 210 mg) were obtained from 5 g of the extract.

Isorhamnetin (**1**): yellow crystals from EtOAc and C_6H_6 , mp 302-304°. The compound was identified by comparison of uv, ir, and 1H -nmr spectra with reported data (7). By methylation with ethereal CH_2N_2 , isorhamnetin (**1**) yielded the 3,7,4'-trimethyl derivative **3**, purified by silica gel in toluene-EtOAc (4:1) and crystallized as yellow needles from EtOAc and *n*-hexane, mp 156-159°, M^+ at m/z 358.

Strychnobiflavone (**2**): yellow needles from EtOAc and C_6H_6 , mp 217-219°; λ max (MeOH) 352, 302 (sh), 265 (sh), 256 nm (log ϵ 3.98, 3.49, 3.92, 4.27); (MeOH/ $AlCl_3$) 420, 356 (sh), 308 (sh), 263 (log ϵ 4.43, 3.94, 3.98, 4.09); (MeOH/ $AlCl_3$ /HCl) 400, 360, 302 (sh), 265 (log ϵ 3.95, 3.47, 3.63, 3.54); ν max 3400, 1640, 1600 cm^{-1} ; 1H -nmr δ (DMSO- d_6) 3.43 and 3.80 (6H, 2s; 2 \times OMe), 5.66 (1H, d, $J=2$ Hz; I-A, H-6), 6.06 (1H, d, $J=2$ Hz; I-A, H-8), 6.16 (1H, s; II-A, H-6), 6.69 (1H, d, $J=8$ Hz; I-B, H-5'), 7-7.1 (3H, m; II-B, H-5', H-6' and I-B, H-6'), 7.40 (1H, d, $J=2$ Hz; II-B, H-2'); δ (Me $_2$ CO- d_6) 3.63 and 3.82 (6H, 2s; 2 \times OMe), 5.84 (1H, d, $J=2$ Hz; I-A, H-6), 6.10 (1H, d, $J=2$ Hz; I-A, H-8), 6.26 (1H, s; II-A, H-6), 6.82 (1H, d, $J=8$ Hz; I-B, H-5'), 7.0-7.1 (2H, m; II-B, H-5' and I-B, H-6', partially overlapped), 7.29 (1H, dd, $J=2$ and 8 Hz; II-B, H-6'), 7.60 (1H, d, $J=2$ Hz; II-B, H-2'), 12.6 and 12.8 (2H, 2s, exchangeable with D_2O ; 2 \times OH).

HEXAMETHYL DERIVATIVE OF 2 (4).—Compound **2** (300 mg) was dissolved in MeOH (2 ml) and treated with ethereal CH_2N_2 for 2 days. The solvents were evaporated and the residue chromatographed on silica gel in EtOAc-toluene (1:4), obtaining pure **4** (250 mg), which crystallized from EtOAc and *n*-hexane as yellow needles, mp 233-234°; λ max (MeOH) 342, 299 (sh), 272 (sh), 261 nm (log ϵ 4.33, 3.98, 4.27, 4.52); 1H -nmr δ (Me $_2$ CO- d_6) 3.56, 3.60, 3.62, 3.73, 3.80, 3.88, 3.92, 4.00 (24H, 8s; 8 \times OMe), 5.83 (1H, d, $J=2$ Hz; I-A, H-6), 6.19 (1H, d, $J=2$ Hz; I-A, H-8), 6.50 (1H, s; II-A, H-6), 7.07 (1H, d, $J=8$ Hz; I-B, H-5'), 7.30 (1H, d, $J=2$ Hz; II-B, H-2'), 7.36 (1H, d, $J=8$ Hz; II-B, H-5'), 7.58 (1H, d, $J=8$ Hz; I-B, H-6'), 7.85 (1H, dd, $J=2$ and 8 Hz; II-B, H-6'); δ ($CDCl_3$) 3.44 (3H, s; OMe), 3.62 (6H, s; 2 \times OMe), 3.67 (3H, s; OMe), 3.79 (3H, s; OMe), 3.82 (6H, s; 2 \times OMe), 3.97 (3H, s; OMe), 5.64 (1H, d, $J=2$ Hz; I-A, H-6), 6.19 (1H, d, $J=2$ Hz; I-A, H-8), 6.32 (1H, s; II-A, H-6), 6.85 (1H, d, $J=8$ Hz; I-B, H-5'), 7.10 (1H, d, $J=8$ Hz; II-B, H-5'), 7.34 (1H, d, $J=2$ Hz; II-B, H-2'), 7.49 (1H, d, $J=8$ Hz; I-B, H-6'), 7.79 (1H, dd, $J=2$ and 8 Hz; II-B, H-6'), 13.20 and 13.54 (2H, 2s, exchangeable with D_2O ; 2 \times OH); m/z (%): 714 (47, M^+), 713 (42), 683 (100), 682 (83), 357 (30), 167 (15), 165 (58).

METHYLATION OF 4.—Hexamethylstrychnobiflavone, **4**, (100 mg) in Me $_2$ CO (25 ml), K_2CO_3 (1 g) and Me $_2$ SO $_4$ (2 ml) was refluxed. After 4 h, more Me $_2$ SO $_4$ (1 ml) was added and the mixture refluxed for additional 4 h. After standard work-up and chromatographic separation on silica gel in $CHCl_3$, octamethyl strychnobiflavone (**5**, 90 mg) was obtained. Crystals from EtOAc, mp 269-271°; 1H -nmr ($CDCl_3$), δ : 3.5-4.0 (30H; 10 \times OMe), 5.60 (1H, d, $J=2$ Hz; I-A, H-6); 6.16 (1H, d, $J=2$ Hz; I-A, H-8); 6.33 (1H, s; II-A, H-6); 6.79 (1H, D, $J=8$ Hz; I-B, H-5'); 6.94 (1H, d, $J=8$ Hz; II-B, H-5'); 7.28 (1H, d, $J=2$ Hz; II-B, H-2'); 7.42 (1H, d, $J=8$ Hz; I-B, H-6'); 7.80 (1H, dd, $J=2$ and 8 Hz; II-B, H-6').

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