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INDOLE ALKALOIDS FROM *RAUWOLFIA SELLOWII*

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Key Word Index—*Rauwolfia sellowii*; Apocynaceae; leaves; indole alkaloids; sellowiine.

Abstract—A new alkaloid, sellowiine (N_1-demethyl-20-deethyl suaveoline), was obtained from leaves of *Rauwolfia sellowii* collected at two different locations in southern Brazil. Also obtained were the known alkaloids, perakine, raucaffrinoline, vomilenine, 19α,20α-epoxy-akuammicine, picrinine and 12-demethoxytabernulosine. The NMR spectra of the alkaloids were assigned completely.

INTRODUCTION

Species of *Rauwolfia* are rich sources of bioactive indole alkaloids, such as reserpine, included in the WHO Model List of Essential Drugs as an antihypertensive agent [1]. *Rauwolfia sellowii*, commonly known as 'Jasmim-Grado', is a rare tree, that grows up to 15 m in height in forests from southeast to south Brazil [2]. Roots of this species are used in folk medicine as an antihypertensive and the analysis of the crude extract has demonstrated some hypotensive activity in dogs [3]. Several indole alkaloids have been isolated from the root bark [4–7] and some of them have been evaluated for genotoxic effects [8, 9]. In the present paper, results of an investigation of *R. sellowii* leaves are reported describing the chemical characterization of seven indole alkaloids.

RESULTS AND DISCUSSION

Plant material collected at two different locations was investigated. Material collected from Curitiba (Paraná, Brazil) yielded three alkaloids, the known alkaloids raucaffrinoline (**2**) [10, 11] and perakine (**3**) [12], the new alkaloid sellowiine (**1**) and perakine dimethyl acetal (**4**), which is considered to be an artefact from the isolation procedure. Material collected from Marcelino Ramos (Rio Grande do Sul, Brazil) yielded the known alkaloids vomilenine (**5**) [11, 12], 12-demethoxytabernulosine (**6**) [13] and picrinine (**7**) [14, 15], the new alkaloid **1** and 19α,20α-epoxy-akuammicine (**8**), which has not been reported before from a natural source.

The mass spectrum of **1** indicated a M_r of 261. In the down-field ^{1}H NMR spectrum (Table 1), besides signals for an indolic nucleus, three additional signals were observed. Two doublets and a singlet indicated the presence of a 3,4-disubstituted pyridine moiety. In the aliphatic part of the spectrum, seven additional signals derived from CH_2–CH units, with geminal coupling constants of 16.9 and 16 Hz, respectively, and both with only one vicinal coupling of 5 Hz, indicated the similarity of the structure with suaveoline, an indole alkaloid isolated previously from *R. suaveolens* [16]. The structure of sellowiine was thus suggested as N_1-demethyl-20-deethyl suaveoline.

A crude fresh ethanolic extract of the leaves, without previous acid or basic treatment, was analysed by HPLC and the presence of sellowiine in this extract confirms its natural occurrence. This finding and the loss of the ethyl side-chain in sellowiine do not agree with the theory that related structures, like suaveoline, have their origin from an ajmaline-type precursor in a basic medium [17].

The aromatic pattern from the ^{1}H NMR spectrum of **8** indicated that this compound contained a non-substituted α-methylene indolenine nucleus [18]. The ^{13}C NMR signals (Table 2) of the aliphatic part were identical to those reported for 12-methoxy-19α, 20α-epoxyakuammicine [19]. Thus, the structure of **8** was established as 19α,20α-epoxy-akuammicine.

In material from one collection, perakine and raucaffrinoline were found, while in the material from the other collection, vomilenine was found. Taylor *et al.* [12] reported that vomilenine could be easily converted into perakine and they raised the question of whether perakine could be formed from vomilenine during the isolation procedure. However, the presence of raucaffrinoline, a reduction product of perakine, does not support this hypothesis.

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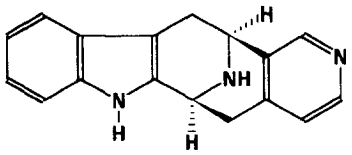
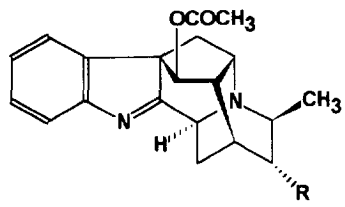
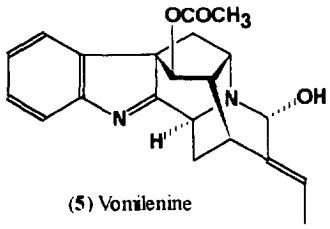


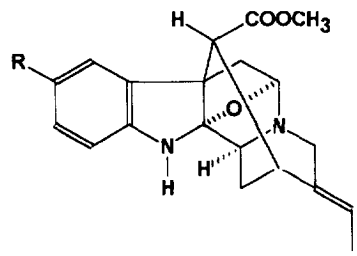
(1) Sellowiine



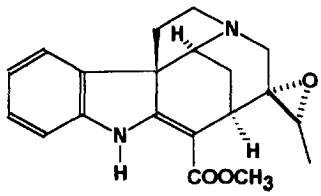
	R
(2) Raucaffrinoline	CH_2OH
(3) Perakine	CHO
(4) Perakine dimethyl acetal	$CH(OCH_3)_2$



(5) Vomilenine



	R
(6) 12- Demethoxytabernulosine	OCH_3
(7) Picrinine	H



(8) 19α, 20α - Epoxyakuammicine

EXPERIMENTAL

General. MS were recorded on an instrument fitted with a thermospray interface. Samples were introduced in column by-pass mode at 1 μg per injection. The eluent was MeOH–0.1 mM NaOAc at a flow rate of 1.2 ml min^{-1}. Vaporizer, repeller and source temp. were set at 70°, 50 V and 250°, respectively.

Plant material. Leaves of *R. sellowii* Müll. Arg. were collected in Marcelino Ramos, RS, and Curitiba, PR, in 1993 and 1991, respectively. Voucher specimens (ICN 2744) are kept in the herbarium of the Universidade Federal do Rio Grande do Sul, Brazil.

Extraction and isolation of alkaloids. Powdered air-dried leaves (500 g) collected in Marcelino Ramos were homogenized in an Ultra-turrax with 10 l of EtOH. The soln was filtered off and concd to dryness *in vacuo* to yield 380 g crude extract, which was dild with 0.5 ml MeOH and 5.5 ml 5% HCl. The suspension was washed once with 3 l hexane, which removed 20 g of a non-alkaloid fr. The acid aq. layer was adjusted to pH 9.0 with NH_4OH and partitioned against CH_2Cl_2. The combined

Table 1. ^{1}H NMR spectral data for compounds **1–8** (δ in $CDCl_3$)

H	Sellowiine	Raucaffrinoline	Perakine	Perakine dimethyl acetal	Vomilenine	12-Demethoxy tabernulosine	Picrinine	19α,20α-Epoxy akuammicine
3	4.36 *d* (5.0)	4.13 *d* (9.3)	4.20 *d* (9.1)	4.17 *d* (9.4)	4.41 *sbr*	3.57 *d* (4.5)	3.60 *d* (4.7)	4.04 *td* (3.0; 0.8)
5	4.71 *d* (5.0)	3.66 *dd* (6.5; 5.1)	3.64 *dd* (6.7; 5.3)	3.65 *dd* (6.5; 5.5)	2.46 *dd* (5.7; 6.1)	4.84 *d* (2.2)	4.84 *d* (2.2)	3.17 *ddd* (12.5; 12.1; 6.2)
								2.86 *dd* (12.5; 7.0)
6	3.36 *dd* (16.9; 5.7)	2.81 *dd* (12.1; 5.1)	2.82 *dd* (12.1; 5.3)	2.79 *dd* (11.85; 5.0)	2.80 *dd* (12.2; 4.8)	3.40 *d* (13.8)	3.41 *d* (13.8)	2.68 *ddd* (13.1; 12.1; 7.0)
	2.86 *d* (16.9)	1.64 *d* (12.1)	1.66 *d* (12.1)	1.63 *d* (12)	1.72 *dd* (6.9; 1.5)	2.25 *dd* (13.8; 2.8)	2.26 *dd* (13.8; 2.8)	1.92 *dd* (13.1; 6.2)
9	7.39 *d* (7.0)	7.47 *dd* (7.0; 1.5)	7.49 *dd* (7.2; 1.5)	7.46 *dd* (7.8; 1.2)	7.47 *d* (7.8)	6.77 *d* (2.2)	7.14 *dd* (7.6; 1.3)	7.23 *d* (7.0)
10	7.14 *dd* (7.6; 7.0)	7.22 *ddd* (7.3; 7.3; 1.2)	7.23 *ddd* (7.7; 7.5; 1.1)	7.21 *ddd* (7.5; 7.2; 1.2)	7.25 *ddd* (7.5; 7.3; 1.0)		6.78 *ddd* (7.6; 7.6; 1.2)	6.92 *dd* (8.0; 7.0)
11	7.07 *dd* (7.6; 7.6)	7.39 *ddd* (8.0; 8.0; 1.2)	7.40 *ddd* (7.8; 7.5; 1.0)	7.39 *ddd* (7.7; 7.5; 1.6)	7.42 *ddd* (7.7; 7.6; 1.3)	6.63 *dd* (8.0; 2.2)	7.08 *ddd* (7.6; 7.6; 1.4)	7.16 *dd* (8.0; 7.8)
12	7.30 *d* (7.6)	7.61 *d* (7.6)	7.62 *d* (7.8)	7.61 *d* (7.8)	7.64 *d* (7.6)	6.67 *d* (8.0)	6.75 *d* (7.8)	6.84 *d* (7.8)
14	3.25 *dd* (16.0; 5.0)	1.95 *dd* (14.7; 9.3)	1.77 *dddd* (14.8; 9.2; 2.0; 1.0)	1.96 *dd* (15.0; 9.4)	1.95 *d*	2.14 *ddd* (14.2; 4.5; 1.0)	2.14 *ddd* (14.2)	2.54 *ddd* (13.5; 3.1; 3.1)
	2.67 *d* (16.0)	1.54 *dd* (14.7; 5.5)	1.60 *dddd* (14.8; 5.2; 2.0; 1.0)	1.54 *dd* (14.8; 5.5)		1.85 *dd* (14.2; 3.4)	1.85 *dd* (14.5; 3.4)	1.39 *ddd* (13.5; 3.2; 3.1)
15		2.49 *dd* (5.5; 5.5)	2.91 *dd* (5.5; 5.2)	2.47 *dd* (5.5; 5.0)		3.28 *dbr* (2.8)	3.28 *dbr* (2.8)	2.99 *ddbr* (3.2; 3.1)
16		2.38 *ddd* (6.5; 5.5; 1.5)	2.48 *ddd* (7.0; 5.5; 1.7)	2.35 *ddd* (7.0; 5.0; 1.5)	3.33 *sbr*	2.45 *d* (3.7)	2.45 *d* (3.7)	
17	8.39 *sbr*	5.00 *d* (1.2)	4.95 *d* (1.7)	5.00 *d* (1.2)	4.99 *d*			
18		1.28 *d* (6.8)	1.30 *d* (6.7)	1.34 *d* (6.7)	1.25 *s*	1.48 *dd* (7.0; 2.4)	1.48 *dd* (7.0; 2.4)	1.42 *d* (5.7)
19		2.54 *dd* (9.3; 6.7)	3.34 *dq* (9.3; 6.7)	2.77 *dq* (8.8; 6.7)	3.90 *sbr*	5.40 *qbr* (7.0)	5.40 *qbr* (7.0)	2.93 *q* (5.7)
20	6.94 *d* (5.1)	1.51 *ddd* (9.3; 8.6; 5.1)	2.17 *d* (9.3)	1.54 *dd* (8.8; 7.8)				
21	8.25 *d* (5.1)	3.75 *dd* (11.0; 5.2)			5.13 *sbr*	3.77 *dbr* (17.8)	3.77 *dbr* (17.8)	2.81 *d* (13.3)
						3.09 *d* (17.8)	3.09 *d* (17.8)	2.73 *d* (13.3)
N-H	7.82 *sbr*					4.67 *sbr*	4.90 *sbr*	
OAc			2.19 *s*	2.17 *s*	2.18 s			
OMe				3.43 *s*		3.66 *s*	3.65 *s*	3.75 *s*
OMe				3.35 *s*		3.71 *s*		

Coupling constants (*J* in Hz) given in parentheses.
Signal assignments based on ^{13}C–^{1}H COSY.

Table 2. ^{13}C NMR spectral data for compounds **1–8** (δ in $CDCl_3$, 75, 47 MHz)

C	1	2	3	4	5	6	7	8
2	133.3	183.4	182.6	183.4	181.5	107.0	106.3	170.8
3	52.7	57.2	56.9	57.1	54.1	52.0	52.0	60.8
5	47.7	51.2	51.6	51.1	49.1	87.4	87.3	54.1
6	34.7	37.5	37.3	37.5	36.4	40.5	40.5	44.4
7	105.3	65.0	64.8	65.0	64.5	51.8	51.8	57.2
8	127.1	136.5	136.1	136.5	136.0	136.4	135.1	135.8
9	118.1	123.8	123.9	123.7	123.8	111.9	125.0	120.2
10	121.9	125.4	125.6	125.4	125.8	154.5	120.7	121.3
11	119.7	128.7	128.8	128.6	128.9	112.9	127.9	127.8
12	110.9	121.0	121.1	121.0	121.3	110.9	110.6	109.7
13	135.9	156.6	156.5	156.6	156.5	141.3	147.4	143.8
14	26.6	21.7	22.6	22.7	26.3	25.9	25.9	29.1
15	142.6	26.6	26.2	26.5	28.3	31.1	31.1	30.9
16	136.0	49.3	48.7	49.1	49.0	51.7	51.7	98.8
17	148.2	78.2	78.0	78.1	79.6	172.4	172.4	168.1
18	—	18.3	18.9	19.3	13.2	12.6	12.7	14.5
19	—	53.2	49.9	53.3	120.0	120.4	120.4	61.4
20	124.1	45.7	56.3	44.9	128.5	136.1	136.1	62.0
21	147.3	62.0	201.5	105.6	82.5	46.3	46.3	54.6
OAc	—	21.1	21.1	21.1	21.1	—	—	—
OAc	—	170.0	170.1	169.9	169.9	—	—	—
OMe	—	—	—	52.8	—	—	—	—
OMe	—	—	—	55.1	—	55.9	—	—
17-OMe	—	—	—	—	—	51.4	51.4	50.9

Signal assignments based on ^{13}C-^{1}H COSY.

organic layers were washed with H_2O and concd *in vacuo* to yield 6.3 g crude alkaloid fr. This was dissolved in 120 ml CH_2Cl_2–MeOH (9:1) and treated with 200 ml petrol. The pptd deposit was filtered and dried to give 3.5 g of an alkaloid fr. I (AF-I). The soln was concd to dryness *in vacuo* yielding 2.6 g of alkaloid fr. II (AF-II). AF-I was subjected to prep. TLC on silica gel GF_{254} (CH_2Cl_2–MeOH, 7:3) to yield alkaloid **1**, which recrystallized from cool MeOH. AF-II was sepd by prep. TLC runs on silica gel GF_{254} in $CHCl_3$–MeOH (7:1). Alkaloids were repurified on TLC using $CHCl_3$–MeOH (17:3), EtOAc–MeOH (9:1), $CHCl_3$–MeOH (23:2) and $CHCl_3$–EtOAc–MeOH (11:8:1) to produce **5–8**, respectively.

Leaves (78 g) collected in Curitiba (PR) were analysed in a similar fashion. AF-1 (PR) provided alkaloid **1** and AF-II was submitted to a prep. TLC, on silica gel GF_{254} layers with $CHCl_3$–MeOH (7:1) as mobile phase, to afford compound **2**.

Alkaloids **3** and **4** were purified by prep. TLC using $CHCl_3$–MeOH (23:2). Yields of alkaloids **1–4** were 38, 37, 18 and 15 mg, respectively.

HPLC analysis. The crude alkaloid extract was dissolved in MeCN–MeOH (9:1) and 20 μl was injected. A Lichrosorb OS (125 × 4.0 i.d. mm) column was eluted isocratically with MeCN–H_2O (pH 7.8 adjusted with NH_4OH) (2:1). The flow rate was 1 ml min^{-1} throughout. Compounds were detected by UV at 222 nm. The sellowiine content of the sample determined by the ext. standard method was 8.47%.

Sellowine. (**1**). Mp 202–206° (MeOH). $[\alpha]_D$ − 135° ($CHCl_3$; *c* 0.54). UV-VIS λ_{max} (MeOH) nm: 222, 268, 282, 290. FAB-MS *m/z* 262 $[M + H]^+$, 284 $[M + Na]^+$, 300 $[M + K]^+$. EIMS (70 ev, direct inlet) *m/z* (rel. int.): 261 (10), 245 (100), 218 (73), 169 (26), 132 (26), 131 (32), 94 (10).

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