

Alkaloids from *Palicourea coriacea* (Cham.) K. Schum.

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Two new β -carboline alkaloids, *epi*-strictosidinic acid (**1**) and strictosidinic ketone (**2**), together with two known alkaloids, calycanthine (**3**) and strictosidinic acid (**4**) besides the triterpene ursolic acid (**5**) were isolated from the roots and leaves of *Palicourea coriacea*. The structures of the isolates were elucidated on the basis of spectral data, particularly COSY, HMQC and HMBC experiments, and HR mass spectra.

Key words: Alkaloid, β -Carbolines, *Palicourea coriacea*, *epi*-Strictosidinic Acid, Strictosidinic Ketone

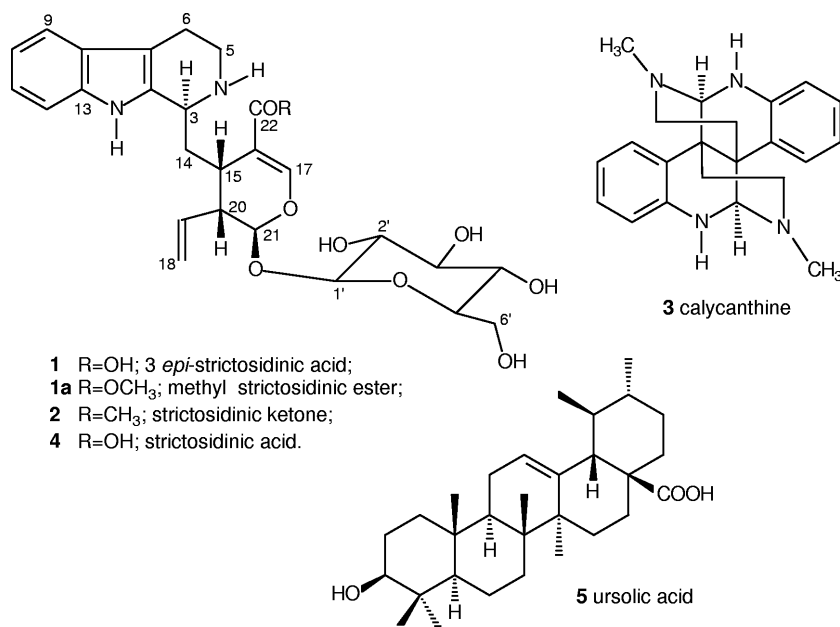
Introduction

Palicourea Aubl. (Rubiaceae) includes about 200 species of shrubs and small trees distributed widely from Mexico to Argentina. The isolation of interesting compounds, mainly triterpenes [1], alkaloids [2], fluoroacetate [3], and cyclotides [4], many of them possessing important biological properties prompted us to investigate *Palicourea coriacea* (Cham.) K. Schum. which is well known in Brazilian Cerrado folk medicine as potent diuretic. Since Dragendorff analyses indicated the presence of alkaloids in the crude extract, this was fractionated by acid-base treatment to yield two new alkaloids, *epi*-strictosidinic acid (**1**) and strictosidinic ketone (**2**), besides the known calycanthine (**3**), strictosidinic acid (**4**) and the triterpene ursolic acid (**5**). We now report the isolation and structural elucidation of (**1**–**2**).

Results and Discussion

By CC or preparative TLC on silica gel 60 using a gradient of MeOH in CHCl₃ four alkaloids (**1**–**4**) and one triterpene (**5**) were isolated from the crude ethanolic extract of leaves and roots of *P. coriacea*. The UV, IR, MS, ¹H and ¹³C NMR data of compounds **3**, **4** and **5** agreed with those published for calycanthine [5], strictosidinic acid [6] and ursolic acid [7], respectively.

Compound **1** and **4** were isolated as a mixture with very close $R_{F's}$ which was submitted to chromatography by preparative TLC on silica gel. Compound **1** was obtained as a brownish solid, $[\alpha]_D^{25}$: –72 (c 8.2, MeOH) and its HREIMS gave a $[M]^+$ at $m/z = 516.21078$ (calculated $m/z = 516.21078$) consistent with the molecular formula C₂₆H₃₂N₂O₉. Its IR spectrum showed absorbance of hydrogen-bonded –OH/NH (3400 cm^{–1}), α,β -unsaturated C=O (1639 cm^{–1}), and aromatic (1554 cm^{–1}) groups. ¹H and ¹³C NMR chemical shift assignments (Table 1) were carried out by 2D NMR techniques, ¹H–¹H COSY and NOESY, ¹H–¹³C HMQC and HMBC, and reinforced by comparison with ¹³C NMR literature data for the tetrahydro- β -carboline alkaloids [8]. The tetrahydrocarboline unit was characterised in the ¹H NMR (Table 1) by vicinal aromatic hydrogen at $\delta = 7.46$ (*d*, $J = 7.8$ Hz, 1H), 7.05 (*dt*; $J = 7.8$, 0.9 Hz, 1H), 7.12 (*dt*, $J = 7.8$, 0.9 Hz, 1H) and 7.32 (*d*, $J = 7.8$ Hz, 1H), a broad singlet at $\delta = 4.54$ (1H), and three multiplets at $\delta = 3.02$ (2H), 3.40 (1H) and 3.60 (1H). The ¹³C NMR spectrum showed peaks corresponding to sp^2 quaternary carbons at $\delta = 106.0$, 127.5, 129.5 and 138.3, four sp^2 tertiary carbons at $\delta = 112.3$, 119.0, 120.6 and 123.3, besides one sp^3 tertiary carbon at $\delta = 59.5$ and two sp^3 secondary carbons at $\delta = 17.3$ and 51.0. According to the HMQC correlations, the presence of a sp^2 tertiary carbon at

Table 1. NMR spectral data of **1** (CD₃OD).

Carbons	¹³ C (δ)	¹ H (δ)	HMBC couplings
C-2	129.5	—	6H
C-3	59.5	4.54 (<i>brs</i> , 1H)	
C-5	51.0	3.40 (<i>m</i> , 1H)	
		3.60 (<i>m</i> , 1H)	
C-6	17.3	3.02 (<i>m</i> , 2H)	
C-7	106.0	—	6-H; 9-H
C-8	127.5	—	10-H; 12-H
C-9	119.0	7.46 (<i>d</i> , 1H, <i>J</i> = 7.8 Hz)	
C-10	120.6	7.05 (<i>dt</i> , 1H, <i>J</i> = 7.8 and 0.9 Hz)	12-H
C-11	123.3	7.12 (<i>dt</i> , 1H, <i>J</i> = 7.8 and 0.9 Hz)	9-H
C-12	112.3	7.32 (<i>d</i> , 1H, <i>J</i> = 7.8 Hz)	10-H
C-13	138.3	—	9-H; 11-H
C-14	33.0	2.25 (<i>dt</i> , 2 H, <i>J</i> = 11.4 and 4.2 Hz)	
C-15	33.6	2.94 (<i>m</i> , 1H)	17-H
C-16	114.5	—	17-H
C-17	152.4	7.48 (<i>brs</i> , 1H)	
C-18	119.0	5.22 (<i>d</i> , 1H, <i>J</i> = 10.5 Hz)	
		5.30 (<i>d</i> , 1H, <i>J</i> = 17.4 Hz)	11-H
C-19	136.5	5.87 (<i>ddd</i> , 1H, <i>J</i> = 17.4, 10.5, 7.8 Hz)	
C-20	45.8	2.68 (<i>m</i> , 1H)	18-H
C-21	96.8	5.72 (<i>d</i> , 1H, <i>J</i> = 8.1 Hz)	1'-H; 17-H; 19-H
C-22	175.0	—	17H
C-1'	100.3	4.76 (<i>d</i> , 1H, <i>J</i> = 7.8 Hz)	
C-2'	74.7	3.20 (<i>m</i> , 1H)	2'-H
C-3'	77.9	3.40 (<i>m</i> , 1H)	
C-4'	71.8	3.24 (<i>m</i> , 1H)	
C-5'	78.6	3.36 (<i>m</i> , 1H)	6-H'
C-6'	63.1	3.66 (<i>m</i> , 1H)	
		3.96 (<i>brd</i> , 1H, <i>J</i> = 10.5 Hz)	

Table 2. NMR spectral data of **2** (D₂O).

Carbons	¹³ C (δ)	¹ H (δ)	HMBC couplings
C-2	130.5	—	—
C-3	absent	4.54 (<i>brs</i> , 1H)	
C-5	52.8	3.40 (<i>m</i> , 1H)	
		3.60 (<i>m</i> , 1H)	
C-6	19.1	2.98–3.10 (<i>brs</i> , 2H)	
C-7	108.5	—	
C-8	128.4	—	10-H; 12-H
C-9	121.2	7.58 (<i>d</i> , 1H, <i>J</i> = 7.5 Hz)	11-H
C-10	122.7	7.17 (<i>dt</i> , 1H, <i>J</i> = 7.5 Hz)	12-H
C-11	125.5	7.26 (<i>t</i> , 1H, <i>J</i> = 7.5 Hz)	9-H
C-12	114.6	7.47 (<i>d</i> , 1H, <i>J</i> = 7.5 Hz)	10-H
C-13	139.3	—	9-H; 11-H
C-14	34.3	2.24 (<i>brs</i> , 1H)	
		2.78 (<i>brs</i> , 1H)	
C-15	32.8	1.34 (<i>brs</i> , 1H)	
C-16	114.6	—	
C-17	153.2	7.35 (<i>s</i> , 1H)	
C-18	122.7	5.32 (<i>d</i> , 1H, <i>J</i> = 11.7 Hz)	
		5.30 (<i>d</i> , 1H, <i>J</i> = 17.4 Hz)	
C-19	137.0	5.85 (<i>q</i> , 1H, <i>J</i> = 8.7 Hz)	
C-20	46.9	2.70 (<i>m</i> , 1H)	
C-21	101.9	4.89 (<i>d</i> , 1H, <i>J</i> = 7.8 Hz)	
C-22	184.4	—	
C-23 (CH ₃)	26.1	1.92 (<i>s</i> , 3H)	23-H
C-1'	99.9	5.64 (<i>d</i> , 1H, <i>J</i> = 6.6 Hz)	3'-H
C-2'	78.5	3.40–3.57 (<i>m</i> , 1 H)	3'-H
C-3'	77.5	3.30 (<i>m</i> , 1H)	2'-H
C-4'	72.4	3.43–3.51 (<i>m</i> , 1H)	5'-H
C-5'	79.2	3.40–3.57 (<i>m</i> , 1 H)	
C-6'	63.6	3.72 (<i>m</i> , 1H)	
		3.96 (<i>d</i> , 1H, <i>J</i> = 10.5 Hz)	

$\delta = 152.4$ assigned to $\delta = 7.48$ (1H, *brs*) and a sp^3 tertiary carbon at $\delta = 96.8$ assigned to $\delta = 5.72$ (1H, *d*, 8.1 Hz), besides the sp^2 carbon absorptions of the methylenedioxy group at $\delta = 119.0$ assigned to $\delta = 5.22$ (*d*, 1H, 10.5 Hz) and 5.30 (*d*, 1H, 17.4 Hz) together with a resonance of a CH at $\delta = 136.5$ assigned to $\delta = 5.87$ (*ddd*, 1H, 17.4, 10.5 and 7.8 Hz) indicate a secologaninic unit. These signals were very close or closely related to those obtained in this study for strictosidinic acid (**4**) and to those described in the literature [6].

Compounds **1** and **4** exhibited an identical series of signals basically differing in the chemical shifts of carbons C-3, C-5, C-6 and C-14 and in the chemical shifts of their hydrogens. Coalescence of 3-H and 14-H signals in the ^1H NMR of compound **1** when compared to **4** is also observed. A survey of representative literature revealed that the chemical shifts of C-5, C-14 and specially C-3 of the tetrahydro- β -carboline alkaloids have been used to distinguish the epimers in C-3 [9]. These evidences permitted the identification of **1** as *epi*-strictosidinic acid. To confirm the presence of the carboxylic acid group, methylation was carried out with CH_2N_2 in diethyl ether yielding the methyl ester derivative **1a**. The ^1H NMR data for **1a** corresponded well to that of **1**, except for the presence of one methyl group at $\delta = 3.70$ (*s*, 3H) and the carbon corresponding to the methyl at $\delta = 58.8$.

Compound **2** was isolated as an amorphous yellowish solid and has the molecular formula $\text{C}_{27}\text{H}_{34}\text{N}_2\text{O}_8$ as established by HREIMS showing a $[\text{M}]^+$ peak at $m/z = 514.23152$ (calculated $m/z = 514.23152$). Its IR spectrum showed absorbance of NH ($3500\text{--}3300\text{ cm}^{-1}$), α,β -unsaturated C=O (1643 cm^{-1}), and aromatic (1564 cm^{-1}) groups. The ^1H and ^{13}C NMR chemical shift assignments of **2** (Table 2) were carried out by 2D NMR techniques, $^1\text{H}\text{--}^1\text{H}$ COSY and NOESY, $^1\text{H}\text{--}^{13}\text{C}$ HMQC and HMBC, and reinforced by comparison with NMR data for alkaloids **1** and **4**. The spectral data revealed that compound **2** had the same strictosidinic skeleton, except for the presence of a ketone methyl group indicated by a singlet at $\delta = 1.92$ (*s*, 3H), and the carbon corresponding to the ketone carbonyl group at $\delta = 184.0$. These data indicated that compound **2** differed from **4** only in C-22, by replacement of the acid group by a ketone. This was further confirmed by HMBC correlations observed between the carbon carbonyl at (C-22) with the methyl group at (C-23) which assured the unequivocal structure of the strictosidinic ketone **2**.

Experimental Section

General experimental procedures

NMR spectra were recorded with a Varian Gemini 2000 spectrometer (300 MHz for ^1H , 75.5 MHz for ^{13}C). CDCl_3 and CD_3OD were used as solvents, with TMS as internal standard for the ^1H NMR spectra and CDCl_3 as internal standard for the ^{13}C NMR spectra. Methyl, methylene, methyne, and carbon non-bonded to hydrogen were discriminated using DEPT 135° and DEPT 90° spectra. 2D NMR spectroscopy was performed with standard H, H correlation and H, C correlation pulse sequences. Optical rotations were measured with a Perkin Elmer 341 polarimeter. HRMS were carried out using a Micromass VG AutoSpec spectrometer operating at 70 eV. IR spectra were recorded with a FTIR Bomem, MB100 using KBr pellets.

Plant material: Aerial parts of *P. coriacea* were collected in Goiânia city, Goiás, Brazil and authenticated by Professor Heleno Dias Ferreira, Departamento de Botânica, Universidade Federal de Goiás. Voucher specimens are deposited at the herbarium of the Universidade Federal de Goiás under the number ($\neq 27153$).

Extraction and isolation of compounds

The air-dried and powdered leaves (362 g) were successively extracted with EtOH (1.5 L for 48 h). The resulting extract was filtered and concentrated under reduce pressure to give 41.3 g of a residue to which was then added a 10% HOAc soln. (250 mL) and the suspension was kept at 5°C overnight. The suspension was filtered and the acidic aqueous phase was partitioned with CH_2Cl_2 . The resulting aqueous layer was basified (pH 8–9) with a satd. NaHCO_3 solution, and then again extracted with CH_2Cl_2 . The combined organic layers were treated with Na_2SO_4 and filtered, affording the CH_2Cl_2 basic fraction. The calycanthine (29.6 mg) was obtained from this fraction by preparative TLC on silica gel with a mixture of $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (37:1:2–organic phase). The alkaloids strictosidinic acid (29 mg), *epi*-strictosidinic acid (24 mg), and strictosidinic ketone (11 mg) were obtained from the crude methanolic extract of the roots after repeated fractionation using column chromatography ($\text{CHCl}_3/\text{MeOH}$ eluent system on gradient form) followed by preparative TLC on silica gel (*n*-butanol/HOAc/ H_2O (4:1:5–organic phase)). The ursolic acid from the leaves and roots crude extracts precipitated as a solid which was further recrystallized to give colourless crystals (150 mg).

epi-Strictosidinic acid (**1**)

Brownish crystals, m.p. $190\text{--}194^\circ\text{C}$. – $[\alpha]_{\text{D}}^{25}$: -72 (MeOH; *c* 8.2). – IR (KBr): ν_{max} 3400 (OH), 1639 (C=O), 1537, 1454, 1074 cm^{-1} . – ^1H NMR (300.06 MHz, CD_3OD), ^{13}C NMR (75.45 MHz, CD_3OD) and HMBC: Table 1. –

HREIMS m/z = 516.21078 (calcd. for $C_{26}H_{32}N_2O_9$, 516.21078).

Strictosidinic ketone (2)

Yellowish crystals, $[\alpha]_D -60$ (MeOH; c 10). – IR (KBr): ν_{\max} 3600 (NH), 3300 (OH), 1643 (C=O), 1564, 1058 cm^{-1} . – ^1H NMR (300.06 MHz, D_2O), ^{13}C NMR (75.45 MHz, D_2O) and HMBC: Table 2. – HREIMS m/z = 514.23152 (calcd. for $C_{27}H_{34}N_2O_8$, 514.23152).

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- [1] H. R. El-Seedi, *Rev. Latinoamer. Quim.* **27**, 13 (1999).
[2] L. T. Dusman, T. C. M. Jorge, M. C. Souza, M. N. Eberlin, E. C. Meurer, C. C. Bocca, E. A. Basso, M. H. Saragiotto, *J. Nat. Prod.* **67**, 1886 (2004).
[3] W. Kemmerling, *Z. Naturforsch.* **51c**, 59 (1996).
[4] H. R. Bokesch, L. Pannell, P. K. Cochran, R. C. Sowder, T. C. Mickee, M. R. Boyd, *J. Nat. Prod.* **64**, 249 (2001).
[5] I. Vencato, C. Lariucci, C. M. A. de Oliveira, L. Kato, C. A. do Nascimento, *Acta Crystallogr.* **E 60**, O1023 (2004).
[6] D. Arbain, D. P. Putra, M. V. Sargent, *Phytochemistry* **46**, 977 (1993).
[7] A. V. Tkachev, A. Y. Denisov, Y. V. Gatilov, I. Y. Bagryanskaya, S. A. Shevtsov, T. V. Rybalova, *Tetrahedron* **50**, 11459 (1994).
[8] P. A. Lukáts, L. Károlyhazy, L. F. Szabó, B. Podányi, *J. Nat. Prod.* **60**, 69 (1997).
[9] Z. Z. Zhang, H. N. Elsohly, M. R. Jacob, D. S. Pasco, L. A. Walker, A. M. Clark, *J. Nat. Prod.* **64**, 1001 (2001).