Monoterpene Indole Alkaloids from *Palicourea crocea*

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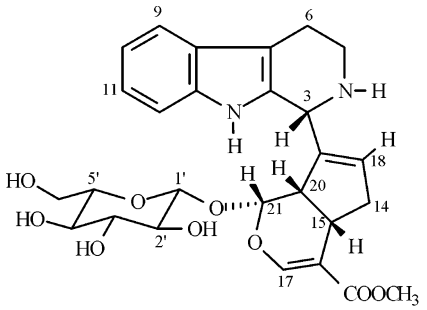
Two new monoterpene indole alkaloids, named croceaines A (**1**) and B (**2**), were isolated from the leaves of *Palicourea crocea*. The structures of **1** and **2** were elucidated by means of spectroscopic methods.

The genus *Palicourea* (Rubiaceae) is known to biosynthesize indole alkaloids with a secologanin moiety, as in the compounds palinine, palicoside, and lyaloside and its hydroxycinnamic acid derivatives, (*E*)-*O*-cinnamoyl-4″-hydroxy-3″-methoxylyaloside and (*E*)-*O*-cinnamoyl-4″-hydroxy-3″,5″-dimethoxylyaloside.[1−3] Additionally, pyrrolidinoindoline alkaloids, terpenoids, coumarins, and phenolic compounds have also been reported from this genus.[4−9] *Palicourea* species have been used in traditional medicine to treat cancer.[10] Recently, the isolation of a novel anti-HIV macrocyclic peptide from *P. condensata* was reported.[11]

The biogenetic importance of monoterpenoid indole alkaloids and the activity reported for some plants of the genus *Palicourea* led us to investigate phytochemically *P. crocea* (Sw.) Roem. et Schultes, a medium to large shrub known as "red palicourea" and "yellow palicourea", occurring from southern Mexico through Central America and South America to southern Brazil and Paraguay.[12] Herein, we report the isolation and structure elucidation of two new monoterpene indole alkaloids, named croceaines A (**1**) and B (**2**), from the leaves of *P. crocea*.

Croceaine A (**1**) was isolated as amorphous solid. Its IR spectrum showed absorption bands of an α,β-unsaturated carbonyl group (1695 cm^{-1}) and an aromatic moiety (1633, 1560, 1441 cm^{-1}). The HRESIMS of **1**, in the positive-ion mode, exhibited a molecular ion peak $[M + H]^+$ at *m/z* 529.2256, consistent with the molecular formula $C_{27}H_{32}N_2O_9$. The ^1H NMR spectrum (Table 1) revealed the presence of a tetrahydro-β-carboline system due the signals at δ 3.08−3.14 (m, H-5), 2.78−2.84 (m, H-6), 7.40 (d, $J = 7.5$ Hz, H-9), 6.97 (td, $J = 7.5$, 1.2 Hz, H-10), 7.05 (td, $J = 7.5$, 1.2 Hz, H-11), and 7.28 (d, $J = 7.5$ Hz, H-12). This spectrum also showed signals for a glucosylated secoiridoid unit at δ 2.14 (ddd, $J = 15.9$, 9.3, 1.0 Hz, H-14α) and 2.92 (ddd, $J = 15.9$, 8.0, 2.7 Hz, H-14β), 3.30 (m, H-15), 7.55 (s, H-17), 2.82 (m, H-20), 5.16 (d, $J = 9.0$ Hz, H-21), and 3.07−4.80 (sugar moiety). The sugar moiety was assigned as β-glucopyranose on the basis of ^1H and ^{13}C NMR data and the *J* value for the anomeric proton at δ 4.80 (d, $J = 7.8$ Hz). The ^{13}C NMR spectrum (Table 1) confirmed a tetrahydro-β-carboline monoterpenoid structure for **1**. Comparison of the NMR data with those reported for monoterpene indole glucosides containing a secologanin unit[1−3] showed the absence of a terminal vinyl group for **1**. Instead, the presence of the



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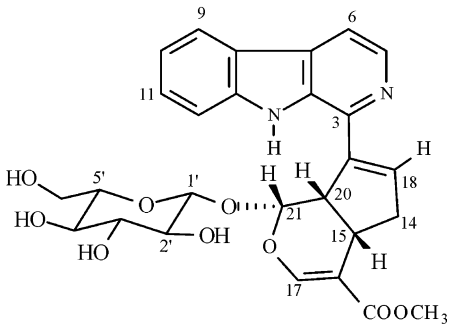

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signals for a quaternary carbon (δ 142.9) and a methine sp^2 carbon (δ 135.1) was observed, indicating a modified monoterpenoid unity for **1**. The nature of the monoterpenoid unit was deduced by analysis of the $^1H-^1H$ COSY and HMBC spectra. The $^1H-^1H$ COSY spectra exhibited correlations for H-21 with H-20 and for H-15 with H-14 and H-20, whereas no correlation was observed for H-3 (δ 4.94, s). These data, together with the correlations observed in the HMBC spectrum for the signals at δ 5.85 (H-18) with C-3 (δ 53.7), C-20 (δ 49.0), and C-15 (δ 37.5) and at δ 5.16 (H-21) with δ 142.9 (C-19), 37.5 (C-15) and 153.5 (C-17) were in agreement with the presence of a cyclopentenedihydropyrone ring linked to C-3 of the tetrahydro-β-carboline system. The coupling constant value for H-21 ($J = 9.0$ Hz) together with the strong NOESY cross-peaks observed between H-21 and H-20 indicated that the monoterpenoid fragment consists of an iridoid derivative of the 1-*epi* series. The correlation between H-3 and H-20 and between H-20 and H-15 in the NOESY spectrum of **1** suggested that these protons must be on the same face of the molecule. An unexpected NOESY correlation was observed between H-3 and H-18. To determine the config-

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Table 1. ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD) Spectral Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C
2		134.3		138.8
3	4.94 s	53.7		141.7
5	3.08−3.14 m	41.8	8.24 d (5.2)	138.0
6	2.78−2.84 m	22.0	7.96 d (5.2)	114.8
7		108.6		127.2
8		128.4		122.5
9	7.40 d (7.5)	118.8	8.16 d (8.0)	122.7
10	6.97 td (7.5, 1.2)	119.9	7.24 t (8.0)	120.9
11	7.05 td (7.5, 1.2)	122.4	7.50 t (8.0)	129.6
12	7.28 d (7.5)	112.4	7.61 d (8.0)	113.2
13		138.0		140.8
14α	2.14 ddd (15.9, 9.3, 1.0)	40.1	2.64 m	41.1
14β	2.92 ddd (15.9, 8.0, 2.7)		3.12 m	
15	3.30 m	37.5	3.50 m	35.0
16		112.2		112.7
17	7.55 s	153.5	7.51 s	153.9
18	5.85 brs	135.1	6.59 brs	136.0
19		142.9		143.1
20	2.82 m	49.0	3.71 m	49.0
21	5.16 d (9.0)	98.9	5.50 d (6.3)	97.1
22		169.5		169.5
1′	4.80 d (7.8)	100.4	4.43 d (7.8)	99.9
2′	3.16 dd (9.0, 7.8)	74.7	2.47 dd (9.0; 7.8)	74.5
3′	3.36 t (9.0)	77.8	3.12 t (9.0)	77.6
4′	3.07 t (9.0)	71.4	2.64 t (9.0)	71.6
5′	3.27 m	78.6	3.24 m	77.9
6′	3.58 dd (12.0, 6.6) 3.84 dd (12.0, 2.4)	62.6	3.67 dd (11.7; 6.6) 3.86 m	62.8
$COOCH_3$	3.71 s	52.0	3.75 s	51.8

[a] Assignments based on ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra.

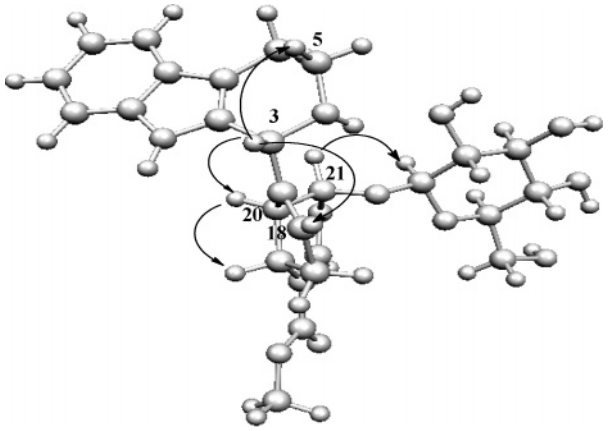

Figure 1. Lowest energy conformation of **1** and significant NOESY correlations.

uration of the C-3 of **1**, circular dichroism measurements were carried out. The CD curve showed a positive Cotton effect in the 230−250 nm region and a negative Cotton effect at 290 nm, characteristic of a tetrahydro-β-carboline-type glycoalkaloid with *R*-configuration at C-3.[13] On the basis of this assumption and taking into account the NOESY spectrum and coupling constant data, molecular modeling studies were performed on compound **1**. The ab initio HF (Hartree−Fock) method was employed for geometry optimization, using the 6-31G* basis set on Gaussian 98.[14] The lowest energy conformation adopted by the molecule is shown in Figure 1. The stability of this conformation is probably due to a hydrogen bridge between N_β-H and the oxygen atom of the hydroxyl group at C-2′ of the glucose moiety. A detailed analysis of the NOESY spectrum associated with the lowest energy conformation (Figure 1) obtained showed consistency for the calculated distance between the hydrogen atoms (less than 4 Å) and all NOE correlations observed.

Croceaine B (**2**) was obtained as an amorphous solid. Its IR spectrum was similar to that of **1**. The HRESIMS for **2**, in the positive-ion mode, exhibited a molecular ion peak $[M + H]^+$ at *m/z* 525.3324, corresponding to the molecular formula $C_{27}H_{28}N_2O_9$. Analysis of the ^{1}H, ^{13}C, and 2D NMR (^{1}H−^{1}H COSY, HMQC) data of **2** pointed to a monoterpenoid indole alkaloid skeleton as established for **1**. The ^{1}H and ^{13}C NMR data (Table 1) for the monoterpene glucoside moiety of **2** were closely related to those **1**, indicating the same cyclopentene-dihydropyrone system. The most remarkable differences in the NMR data compared with those **1** were relative to the β-carboline system. The absence of the signals for H-3 and of the methylene hydrogens H-5 and H-6 and the downfield signals at δ 8.24 (d, J = 5.2 Hz) and 7.96 (d, J = 5.2 Hz) confirmed the presence of double bonds at C-3 and C-5 in ring C of **2**. These data, together with the signals for aromatic hydrogens at δ 8.16 (d, J = 8.0 Hz, H-9), 7.24 (t, J = 8.0 Hz, H-10), 7.50 (t, J = 8.0 Hz, H-11), and 7.61 (d, J = 8.0 Hz, H-12), were consistent with a β-carboline system in **2**. The signals for a C=N group at δ 141.7 (C-3), six methine sp^2 carbons at δ 138.0 (C-5), 114.8 (C-6), 122.7 (C-9), 120.9 (C-10), 129.6 (C-11), and 113.2 (C-12), and four quaternary carbons at 138.8 (C-2), 127.2 (C-7), 122.5 (C-8), and 140.8 (C-13) in the ^{13}C NMR spectrum of **2** (Table 1) confirmed the β-carboline system. The assignment of the chemical shifts for the β-carboline unit of **2** was based on ^{1}H−^{1}H COSY and HMQC data and comparison with those reported for lyaloside.[3]

Indole alkaloids containing a monoterpenoid moiety as in croceaines A (**1**) and B (**2**) are unusual. The isolation and structure elucidation of brachycerine, a monoterpenoid indole alkaloid exhibiting a cyclopentane-dihydropyrone ring linked to the tetrahydro-β-carboline moiety, was recently reported.[15a,b] Croceaines A (**1**) and B (**2**) isolated from *P. crocea* can be therefore included in the same class as the indole alkaloid brachycerine.

Experimental Section

General Experimental Procedures. Optical rotation and CD spectra were obtained on a JASCO J-720 spectropolarimeter with a RD-306 coupled unit. IR spectra were recorded on a Bomem model MB 100 spectrophotometer. ^{1}H (300 MHz), ^{13}C (75.5 MHz), and 2D (COSY, NOESY, HMQC, HMBC) NMR spectra were recorded in a Varian spectrometer model Mercury plus BB 300 MHz, using CD_3OD as solvent and TMS as internal standard. High-resolution (7000) and high-accuracy (5 ppm) ESIMS in the positive-ion mode were acquired on a QTOF (Micromass, Manchester, UK) hybrid quadrupole orthogonal time-of-flight mass spectrometer, using an ESI capillary voltage of 3 kV and a cone voltage of 10 V. TLC was carried out on silica gel 60 GF_{254}, and spots were visualized by spraying with Dragendorff reagent. Silica gel 60 G (0.063−0.200 mm) was used for column chromatography.

Plant Material. Leaves of *P. crocea* were collected in the forest near the Paraná River, Porto Rico, Paraná State, Brazil, by M.C.S. (Departamento de Biologia/NUPELIA, Universidade Estadual de Maringá, PR, Brazil) in October 2000. A voucher specimen (HNPU 2060) is deposited in the herbarium of the Universidade Estadual de Maringá.

Extraction and Isolation. Dried leaves (850 g) were exhaustively extracted with MeOH at room temperature. Evaporation of the solvent under vacuum furnished the methanolic extract. Part of this extract (12 g) was treated with 5% aqueous HCl and the acidic solution extracted with $CHCl_3$.

The aqueous phase was basified with NH_4OH to pH 9−10 and extracted with $CHCl_3$ and $CHCl_3$−MeOH (2:1). The organic fractions were combined and the solvent was evaporated to furnish the alkaloid fraction (715.8 mg), which was purified by repeated preparative TLC ($CHCl_3$−MeOH 20%) to afford **1** (30.3 mg) and **2** (5.0 mg). Compounds **1** and **2** were also obtained by dissolution of the crude extract (10 g) in H_2O−MeOH (1:1) and successive partition with *n*-hexane, $CHCl_3$, and EtOAc. The EtOAc fraction (2.5 g) was subjected to column chromatography over silica gel using $CHCl_3$ and MeOH mixtures of increasing polarity. Purification of the fraction eluted with 10% MeOH−$CHCl_3$ by repeated preparative TLC ($CHCl_3$−MeOH 20%) afforded compounds **1** (25.0 mg) and **2** (3.0 mg).

Croceaine A (1): amorphous solid; $[\alpha]^{25}_D$ −11° (*c* 0.003, MeOH); IR (KBr) ν_{max} 3378, 1695, 1633, 1560, 1441 cm^{-1}; NMR data are shown in Table 1; HRESIMS *m/z* 529.2256 $[M + 1]^+$ (calcd for $C_{27}H_{32}N_2O_9$, 528.21078).

Croceaine B (2): amorphous solid; $[\alpha]^{25}_D$ −40° (*c* 0.002, MeOH); IR (KBr) ν_{max} 3377, 1693, 1641, 1558, 1440 cm^{-1}; ^1H and ^{13}C NMR data are shown in Table 1; HRESIMS *m/z* 525.3324 $[M + 1]^+$ (calcd for $C_{27}H_{28}N_2O_9$, 524.17948).

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