Brachycerine, a Novel Monoterpene Indole Alkaloid from *Psychotria brachyceras*

Vitor A. Kerber,[†] Tatiana S. Gregianini,[‡] Juçara T. Paranhos,[‡] Joséli Schwambach,[‡] Fabiane Farias,[†] Janette P. Fett,[‡] Arthur G. Fett-Neto,[‡] José Ângelo S. Zuanazzi,[†] Jean-Charles Quirion,[§] Elaine Elizabetsky,[†] and Amélia T. Henriques*,[†]

Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga 2752, Porto Alegre, RS, CEP: 90 610 000, Brazil, Laboratório de Fisiologia Vegetal, Departamento de Botânica, UFRGS, Av. Bento Gonçalves 9500, Prédio 43423, Bloco 4, Porto Alegre, RS, 91509-900, Brazil, and Laboratoire d'Hétérochimie Organique, Institut de Recherche en Chimie Organique Fine, Institut National des Sciences Appliquées de Rouen, Pl. E. Blondel, BP 08, 76131 Mont-Saint-Aignan Cedex, France

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Brachycerine (**1**), an unusual alkaloid from the leaves of *Psychotria brachyceras*, was characterized through spectroscopic data interpretation and its stereochemistry established by NOE difference techniques. Brachycerine (**1**) was found to be restricted to shoots in rooted cuttings of *P. brachyceras* (0.018 ± 0.004% dry weight), and accumulation was unaffected by root induction treatment with auxin.

Psychotria species (Rubiaceae), one of the largest genera of angiosperms (1000 to 1650 species worldwide), is taxonomically complex due to the unwieldy number of species and the relative lack of morphological characteristics available to establish subgroupings.[1] This mostly tropical and subtropical genus is categorized with *Calycodendrum* and is closely related to *Cephaelis* and *Palicourea*.[2] Recently, *Cephaelis* species were transferred to *Psychotria*.[3] Segregation of some species currently within *Psychotria* has been proposed based on both morphological and molecular (rDNA and *rbcL* sequence) analyses.[1,2] The chemical analysis of *Psychotria* species may aid in the establishment of new limits for the genus. *Psychotria brachyceras* Muell. Arg. is a shrub (1−3 m in height) widely distributed in tropical and subtropical forests of Brazil.[3] It ranges from the state of Rio de Janeiro to Rio Grande do Sul, where it occurs sparsely throughout the northern half of the state.[4] A previous study showed nonspecific analgesic activity for an alkaloid extract of the species.[5]

As part of our continued interest in Rubiaceae alkaloids, in the present work we report the structure elucidation of brachycerine (**1**), the main alkaloid isolated from *P. brachyceras* leaves, and its distribution in rooted cuttings generated by different procedures.

The alkaloid extract (720 mg) of *P. brachyceras*, corresponding to ca. 0.16% w/w of the dry leaves, comprised one main and two minor alkaloids. The latter were not isolated due to their chemical instability. The mass spectrum of the isolated alkaloid (**1**) displayed a peak at *m/z* 547 $[M + H]^+$, and in the HREIMS, two major ions were observed at *m/z* 366.17780 ($C_{21}H_{22}N_2O_4$) and 180.08510 ($C_6H_{12}O_6$), related to the loss of the glucose residue. The proton-bearing carbons were assigned from the $^1H-^{13}C$ correlation spectrum (HMQC). The ^1H NMR spectrum (Table 1) displayed a characteristic pattern for a nonsubstituted glucosylated monoterpenoid indole alkaloid. The UV spectrum showed maximum absorptions at 226 and 280 nm, typical of an indole chromophore. The absence of ^1H NMR signals for a terminal vinyl suggested that the terpenoid structure was not that of a glucosylated secoiridoid such as isodolichantoside, occurring in *Psychotria correae*.[6] The ^1H COSY experiment exhibited correlations of the signal at δ 2.33 (H-20) with those at δ 4.89 (H-21), 2.72 (H-19), and 3.42 (H-15), whereas that at δ 4.78 (H-3) displayed a correlation with the signal at δ 2.72 (H-19). The signal at δ 4.36 (H-18) exhibited a correlation with that of H-19 (δ 2.72). The signals at δ 3.42 (H-15) and 4.36 (H-18) had correlations with the signals at δ 1.50 and 2.44 (H-14 α and β). These observations were in agreement with a cyclopentane−dihydropyrone ring linked to the N_b-methyltetrahydro-β-carboline moiety, supporting the absence of a secoiridoid portion in this molecule. The resonance for H-18 (δ 4.36) correlated with a carbon at δ 74.0 (HMQC spectrum), suggesting the presence of a C−OR bond. The determination of the relative configurations is based mainly on the results of nuclear Overhauser experiments and on the observed $^1H/^1H$ coupling constants. The coupling constant of 6 Hz between protons H-14β and H-15, together with a stronger NOE than that observed with H-14α, supported a pseudo-β-configuration for H-15. The coupling constant of 8.2 Hz between H-15 and H-20, together with a NOE, indicated a close to 0° dihedral angle between them. A similar angle can be proposed between H-20 and H-21, based on the coupling constant (J = 8.4 Hz) and NOE analysis. Proton-18 had a coupling constant of 3.8 Hz with H-14α, together with a stronger NOE than that observed for H-14β, suggesting a pseudo-α-configuration. The coupling constant of 8.2 Hz and the NOE found between H-18 and H-19 support a close to 0° dihedral angle. The coupling constant of 5 Hz between H-19 and H-20 with almost no NOE observed suggested they are on the opposite side of the molecule, in agreement with what was observed for H-15 and H-18. H-3 and H-19 displayed a coupling constant of 8.4 Hz and no NOE was observed, indicating a close to 180° dihedral angle, which was reinforced by the NOE observed between H-3 and H-20. This allowed us to suggest a *trans* relation between H-3 and H-19. These spectroscopic data revealed the structural elements of *epi*-loganin (**2**). The combined results of the COSY, HMQC, analysis of correlation constants, and NOE experiments

* To whom correspondence should be addressed. Tel: 55 51 3165417. Fax: 55 51 3165243. E-mail: amelia@farmacia.ufrgs.br.
[†] Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul.
[‡] Departamento de Botânica, Universidade Federal do Rio Grande do Sul.
[§] Laboratoire d'Hétérochimie Organique, Institut National des Sciences Appliquées de Rouen.

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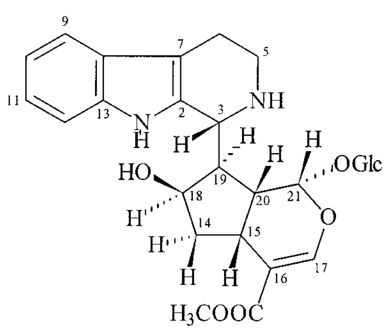
Table 1. ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, and NOE Difference NMR Data for Brachycerine (**1**) (300 MHz, CD_3OD)

position	^{1}H NMR (J = Hz)	^{13}C NMR	COSY	NOE difference
2		130.7		
3	4.78 d (8.2)	54.7	19	
5α	3.40 m	41.8		
5β	3.69 d (5.8)	41.8		
6(α, β)	2.98 m	24.4		
7		108.3		
8		127.7		
9	7.45 dd (1, 7.7)	118.9		
10	7.00 ddd (0.9, 7.4, 7.7)	120.2		
11	7.13 ddd (1.1, 7.4, 8.1)	123.2		
12	7.37 dd (1, 8.1)	112.3		
13		137.4		
14α	1.50 ddd (13.2, 12.7. 3.8)	43.5	18, 15	18, 14β, 15 (w)
14β	2.44 dd (13.2, 6)	43.5	18, 15	18 (w), 14α, 15
15	3.42 m	35.5	20, 14α,β	
16		111.8		
17	7.53 d (1)	153.5		
18	4.36 dd (8.2, 3.8)	74.3	19, 14α	19, 14α, β(w)
19	2.71 ddd (8.2, 8.2, 5)	49.0	20, 18, 3	18
20	2.33 ddd (8.4, 8.2, 5)	41.9	21, 19, 15	21, 15, 3
21	4.89 d (8.4)	99.0	20	
22		169.1		
23	3.72 s	51.8		
1′	4.70 d (8)	100.6		
2′	3.18 m	74.0		
3′	3.38 m	71.1		
4′	3.24 m	78.3		
5′	3.36 m	77.7		
6′α	3.65 d (12, 5.3)	62.1		
6′β	3.82 dd (12, 2.4)	62.1		

suggested a structure formed by the combination of a 1-*epi*-loganin (**2**) derivative and tryptamine (**3**), resulting in a new alkaloid skeleton named brachycerine (**1**). Some examples of *epi*-1 iridoids have already been described, as observed for 1,5,9-*epi*-loganin.[7]



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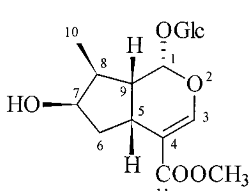

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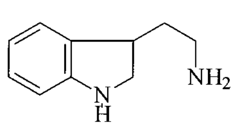

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Brachycerine (**1**) eluted at a retention time of 10.9 min by HPLC, and it was detected in the shoots of rooted cuttings of *P. brachyceras*, mainly in the leaves, two months after a harvest of tip cuttings from natural stands and culture in hydroponic solution. The alkaloid was not detected in adventitious roots, suggesting that its biosynthesis occurs in shoots or that it undergoes rapid transport to aerial parts. The presence of brachycerine (**1**) in freshly extracted plant material (from field-grown leaves and cuttings) indicates that it is not a post-harvest catabolite formed during leaf drying for alkaloid extraction. The procedures for adventitious root induction, involving different exposures to the root-inducing auxin indole butyric acid, did not affect significantly the content of brachycerine (**1**), at least after two months of culture under the various rooting treatments. The biosynthesis of many alkaloids, including some of the tropane and monoterpene indole families, is down-regulated by auxin.[8] The lack of effects of auxin on brachycerine (**1**) accumulation may reflect the long time of exposure to auxin solution, possibly allowing the plants to adequately control auxin activity by degradation or conjugation.[9] Shorter time courses after transfer to auxin may reveal effects on brachycerine (**1**) accumulation. The overall average amount of brachycerine (**1**) in leaves of rooted cuttings was 0.018 ± 0.004% dry weight. Leaves of field-grown trees (from which cuttings were obtained) harvested in spring contained 0.034 ± 0.012% dry weight of **1**, which is a content statistically equivalent to that of leaves from rooted cuttings. The presence of brachycerine (**1**) in hydroponically cultured cuttings suggests that these may be adequate systems for studying the regulation of the accumulation of this alkaloid.

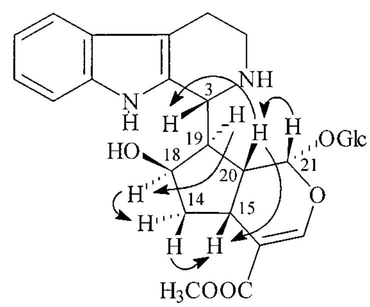

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Figure 1. NOE correlations for brachycerine (**1**).

In conclusion, although it is well accepted that nearly all monoterpenoid indole alkaloids are derived from strictosidine,[10] the isolation and identification of brachycerine (**1**) suggests the existence of an alternative biosynthetic pathway for these secondary metabolites in the genus, leading to a new class of indole alkaloids.

Experimental Section

General Experimental Procedures. Optical rotation and UV spectra were obtained in MeOH, using a Perkin-Elmer 241 polarimeter and a Cintra 5 spectrophotometer, respectively. IR spectra were recorded on a Perkin-Elmer spectrophotometer model 684. ^{1}H NMR spectra were recorded in CD_3OD at 300 MHz using the residual MeOH as internal standard. ^{13}C NMR spectra were recorded at 75.5 MHz in CD_3OD. 2D (COSY, HMQC) and NOE difference experiments were performed using standard microprograms. NMR spectra were obtained on a Bruker AMX-300 spectrometer. The five quaternary carbons were assigned in comparison with those of several indole alkaloids of the β-carboline kind.[6,11] Mass spectra were recorded in a Finnigan MAT TQS-70 double quadruple spectrometer with an electrospray ionization interface. The purity of compound **1** was checked by HPLC. HPLC analyses were carried out on a 2690 Waters Alliance Analytical Chromatograph with a Hibar RP-8 column (5 μm, 4 × 250 mm, Merck). Eluting compounds were monitored with a Waters Millennium (version 2.15.01) detector, which measured absorbance (200−600 nm) every 1 s with 4.8 nm resolution. The mobile phase consisted of a linear gradient that started with solvent A (MeOH−H_2O−TFA, 35:65:0.05) and ended with solvent B (MeOH−TFA, 100:0.05) within 20 min; the flow rate was 1 mL min^{-1}.

Plant Material. Fresh leaves and shoot tips of *P. brachyceras* were collected from the native forest of Morro Santana, Porto Alegre, Brazil (October 1997) and identified by Marcos Sobral from the Federal University of Rio Grande do Sul (UFRGS). A voucher specimen is deposited in the University herbarium (ICN Sobral & Kerber 7899).

Extraction and Isolation. Dried leaves (454 g) were extracted with EtOH at room temperature (three times for a week). The extract was concentrated under vacuum at 40 °C, and the crude alkaloid fraction was obtained by classical means.[12] The resulting CH_2Cl_2 extract (1.74 g) was submitted to vacuum-liquid chromatography on silica gel (10 g, 3 cm diameter) and eluted using mixtures of $CHCl_3$−MeOH of increasing polarities. The fraction collected with $CHCl_3$−MeOH (9:1) yielded **1** (720 mg). Brachycerine (**1**) was obtained as a pure colorless powder: $[\alpha]^{20}_D$ −24.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.59), 280 (3.88) nm; IR KBr ν_{max} 3375, 2924, 2854, 1693, 1635, 1562, 1439, 1307, 1284, 1161, 1099, 1076, 748 cm^{-1}; NMR data are shown in Table 1; CIMS *m*/*z* 547 $(M + H)^+$, 385 (2), 367 (100) 356 (1), 262 (2), 211 (2), and 180 (51); HREIMS *m*/*z* 366.17780 $[M - C_6H_{12}O_6]^+$ (calcd for $C_{21}H_{22}N_2O_4$, 366.15796), 209.11604 (calcd for $C_{14}H_{13}N_2$, 209.10787), 180.08510 (calcd for $C_6H_{12}O_6$, 180.06339), 171.10139 (calcd for $C_{11}H_{11}N_2$, 171.09222).

Rooting Protocols and Alkaloid Analysis. Tip cuttings were harvested from three field-grown trees in the spring (November 1999) and washed and trimmed under distilled water to prevent xylem cavitation. Approximately 10 cm cuttings with 3 to 4 leaves each were cultured in a solution of 0.1 × salt of Murashige and Skoog,[13] with or without 10 mg L^{-1} of 4-(3-indolyl)butyric acid (IBA, Sigma Chemical Co, St. Louis, MO). Cuttings were exposed to IBA for 7 days or throughout the experiment. After 8 weeks, three replicates of rooted cuttings from each treatment were harvested, separated into leaves and roots, and quick frozen in liquid nitrogen. Methanol extracts of the various organs were analyzed by HPLC. Quantification was conducted using an external standard curve; identity and purity were based on retention time, UV spectra, and co-chromatography with authentic brachycerine (**1**). Statistical analysis of brachycerine (**1**) content was performed by two-way analysis of variance (auxin × culture system) at $p \leq 0.05$.

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