Turbinatine, a Potential Key Intermediate in the Biosynthesis of Corynanthean-Type Indole Alkaloids

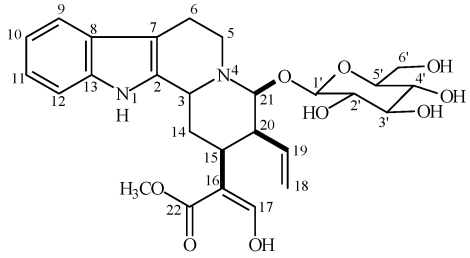
Carmen Lucia Cardoso,[†] Dulce Helena Siqueira Silva,[†] Daniela Maria Tomazela,[‡] Hugo Verli,[§] Maria Claudia Marx Young,[⊥] Maysa Furlan,[†] Marcos Nogueira Eberlin,[‡] and Vanderlan da Silva Bolzani*[,†]

Instituto de Química, Universidade Estadual Paulista, UNESP, CP 355, CEP 14-801-970, Araraquara, São Paulo, Brazil, Thompson Mass Spectroscopy Laboratory, Instituto de Química, Universidade Estadual de Campinas, UNICAMP, CP 6154, CEP 13083-970, Campinas, São Paulo, Brazil, Seção de Fisiologia e Bioquímica de Plantas, Instituto de Botânica, CP 4005, CEP 10051, São Paulo-SP, Brazil, and Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS, CP 15005, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

Received November 25, 2002

Extraction of the leaves of *Chimarrhis turbinata* has led to the isolation of turbinatine (**1**), a new corynanthean-type indole alkaloid, besides four known indole alkaloids, strictosidine, 5α-carboxystrictosidine, vallesiachotamine, and isovallesiachotamine. The structural determination of **1** was based on 1D and 2D spectroscopic data. An evaluation of the DNA-damaging activities of the isolates was performed by means of a bioassay using mutant strains of *Saccharomyces cerevisiae*, which indicated these compounds were weakly active.

Chimarrhis turbinata (DC.) Prodr. (Rubiaceae) belongs to the subfamily Cinchonoideae and the tribe Condaminae.[1,2] This tree is distributed from Central America and the Caribbean Islands through tropical South America, especially in the Amazon region. Its light and resistant wood is used for making tools, including pawls, which is the reason for its popular name "pau-de-remo" in the Amazon.[3] The present work describes the first study on a plant in the genus *Chimarrhis*, which has resulted in the isolation and characterization of a new indole monoterpenoid glucoalkaloid, turbinatine (**1**), along with the known compounds strictosidine, 5α-carboxystrictosidine, vallesiachotamine, and isovallesiachotamine. In addition, the potential chemotaxonomic significance of these alkaloids and evidence for turbinatine (**1**) as a key intermediate in the biosynthesis of corynanthean-type alkaloids are briefly discussed.



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Five indole alkaloids were isolated from extracts of *Chimarrhis turbinata* leaves by liquid−liquid partition followed by Sephadex LH-20 and reversed-phase HPLC. The identification of the known alkaloids was based on their spectroscopic data, mainly 1D NMR, and comparison with literature data,[4] with the known compounds identified as strictosidine,[5,6] 5-carboxystrictosidine,[7] vallesiachotamine,[8] and isovallesiachotamine,[8] respectively. Turbinatine (**1**) was obtained as a stable yellow powder. The molecular formula $C_{27}H_{34}N_2O_9$ for **1** was assigned from the molecular ion at *m*/*z* $[M + 2]^+$ 532.3070 in the HREISMS, in combination with its ^{13}C NMR data. The ^{13}C NMR spectral data of compound **1** (Table 1) showed signals at δ 108.5, 111.7, 118.6, 120.0, 122.8, 127.2, 138.0, and 138.6, typical of a 2,3-disubstituted-indole system, which were assigned to C-7, C-12, C-9, C-10, C-11, C-8, C-2, and C-13, respectively. The signals at δ 52.6, 48.5, and 23.8 were attributed to C-3, C-5, and C-6, respectively, of a strictosidine-type compound. These assignments were based on comparison with literature data[4−6] as well as with the standard compound strictosidine, also isolated during this work. Signals at δ 34.4, 32.4, 45.0, and 97.0 were assigned to C-14, C-15, C-20, and C-21, respectively, of the D ring. The ^{13}C NMR spectrum also showed signals for a glucosyl moiety, which appeared at δ 71.3, 74.2, 77.5, and 78.0. Signals were also observed for the hydroxymethine carbons C-4′, C-2′, C-3′, and C-5′, respectively, and at δ 62.6 for the hydroxymethylene C-6′ and at δ 100.0 for the anomeric C-1′. The remaining signals of the ^{13}C NMR spectrum were assigned to the *seco*-iridoid moiety of the alkaloid which comprised a terminal olefinic system attached to C-20 with signals at δ 119.2 (C-18) and 134.8 (C-19) and a β-hydroxy-α,β-unsaturated carbomethoxy system attached to C-15 of the D ring. The signals in the latter unit appeared at δ 52.3, 108.1, 156.5, and 171.1 and were assigned to CH_3O, C-α, C-β, and the carbonyl, respectively. The ^1H NMR spectrum showed two doublets at δ 7.47 and δ 7.29 and two doublets of doublets at δ 7.03 and 7.11, attributed to the aromatic hydrogens H-9, H-12, H-10, and H-11, respectively. Correlations observed in the HOMOCOSY, NOESY, and TOCSY NMR spectra (Table 1) confirmed the structure of the indole moiety of compound **1**. The HOMOCOSY and TOCSY spectra also showed correlations of signals at δ 5.25, 5.37, and 5.85, which were then assigned to H-18$_{cis}$, H-18$_{trans}$, and H-19, respectively, on the basis of their chemical shifts and coupling constants (Table 1). Further analysis of the HOMOCOSY spectrum showed correlations of H-19 to the signal at δ 2.77 (H-20) and correlations of the latter to the signal at δ 3.10 (H-15) and to the doublet at δ 5.90, which was assigned to the

* To whom correspondence should be addressed. Tel: 55(16)2016660. Fax: 55(16)2227932. E-mail: bolzaniv@iq.unesp.br.
[†] Instituto de Química, Universidade Estadual Paulista, UNESP.
[‡] Instituto de Química, Universidade Estadual de Campinas, UNICAMP.
[§] Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS.
[⊥] Seção de Fisiologia e Bioquímica de Plantas, Instituto de Botânica.

10.1021/np020547m CCC: $25.00
Published on Web 06/17/2003

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Turbinatine (**1**)[a–c]

position	δ_H (mult., J (Hz))	δ_C (mult.)	HMBC	NOESY
2		138.0 s		
3	4.60 br d (13.0)	52.6 d		H-14α
5	3.42 m	48.5 t		H-6
	3.90 m			H-6; H-5
6	3.00 br t (12.2)	23.8 t		H-5a, H-5b
	3.26 m			
7		108.5 s		
8		127.2 s	H-10	
9	7.47 d (8.0)	118.6 d		H-10
10	7.03 dd (8.0, 8.0)	120.0 d	H-12	H-9; H-11
11	7.11 dd (8.0, 8.0)	122.8 d		H-12; H-10
12	7.29 d (8.0)	111.7 d		H-11
13		138.6 s	H-9	
14_{ax}	2.40 br ddd (13.0, 11.5)	34.4 t		H-15; H-14_{eq}
14_{eq}	2.20 br dd (4.0, 11.5)			H-3; H-14_{ax}
15	3.10 br ddd (4.0, 4.0, 13.0)	32.4 d	H-17	H-14_{ax}; H-20
16		108.1 s	H-17	
17	7.80 s	156.5 d		
18_{cis}	5.25 d (10.5)	119.2 t		H-18_{trans}
18_{trans}	5.37 d (18.0)			H-18_{cis}
19	5.85 ddd (7.5; 10.5; 18.0)	134.8 d		H-18_{cis}; H-18_{trans}
20	2.77 br ddd (4.0, 7.5, 9.5)	45.0 d		H-15; H-21
21	5.90 d (9.5)	97.0 d		H-20
22		171.1 s	H-17; MeO-22	
OCH_3	3.79 s	52.3 q		
1′	4.80 br d (8.0)	100.0 d		
2′	3.25 dd (9.0, 14.5)	74.2 d		
3′	3.40 m	77.5 d		H-6′a
4′	3.65 m	71.3 d		
5′	3.30 m	78.0 d		
6′	3.67 dd (7.0, 11.8)	62.6 t		H-3′; H-6′b
	4.01 dd (2.0, 11.8)			H-6′a

[a] ^{1}H NMR assignments are based on 2D ^{1}H NMR and HMQC correlations. [b] Internal standard: TMS. [c] Measured in CD_3OD at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively.

aminohydroxymethine H-21 signal. Subsequent correlations of H-15 to signals at δ 2.20 and 2.40 (H-14_{eq} and H-14_{ax}) and of H-14 to the broad doublet at δ 4.60 (H-3), as well as the correlations between signals at δ 3.26 (H-6) and 3.00 (H-6), led to the complete assignment of the aglycon aliphatic hydrogens of turbinatine (**1**). The TOCSY spectrum gave additional support to these assignments by showing correlations of signals of H-3, H-14, H-15, H-20, H-21, H-19, and H-18, and TOCSY-1D experiments allowed the assignments of the sugar moiety hydrogens (Table 1). The HMBC spectrum of **1** showed correlations from C-22 (δ 171.1) to the methoxyl group (δ 3.79) and to H-17 (δ 7.80). Additionally, correlations of C-15 (δ 32.4) and C-16 (δ 108.1) to H-17 confirmed the presence of the β-hydroxy-α,β-unsaturated caboxymethoxy system, as well as its attachment to C-15. The relative configurations of the asymmetric centers C-3, C-15, C-20, and C-21 were established by coupling constant measurements and NOESY experiments as well as through comparison with ^{13}C NMR data of model compounds.[9,10]

Corynanthean-type indole alkaloids may be classified into the *normal*, *pseudo*, *allo*, or *epiallo* series depending on their C-3, C-15, and C-20 stereochemistry. The detailed analysis of coupling constants of H-3 with H-14_{ax}; H-14_{ax} with H-15; H-15 with H-20; and H-20 with H-21 signals ($J_{3,14ax}$ = 13.0 Hz; $J_{14ax,15}$ = 13.0 Hz; $J_{15,20}$ = 4.0 Hz; $J_{20,21}$ = 9.5 Hz) for compound **1** suggested a stereochemical arrangement in which H-20 is *cis* to both H-15 and H-21, and H-3 is *trans* to H-14_{ax} and *cis* to H-15. These observations defined the *allo* configuration of the stereocenters C-3, C-15, and C-20. Additional information on the relative stereochemistry at the asymmetric centers of **1** was obtained through analysis of the NOESY spectrum. In this spectrum, interactions observed between H-3 and H-14_{eq} as well as of H-15 with H-14_{eq} and H-20 confirmed that the orientations of these hydrogens are all in the same plane. These findings corroborated the positions of H-3, H-15, and H-20 as α, α, and α, indicative of an *allo* configuration for compound **1**. The 9.5 Hz value measured for the coupling constant (J) between H-21 and H-20 is anomalous for this configuration, and the only explanation to support this constant value is a twist-boat conformation adopted by the D ring. Further analysis from a Karplus curve by H-20 and H-21 revealed a near 0° angle for these hydrogens (28° calcd by molecular modeling, Table 2), predicting a large value for the coupling constant. This is a plausible hypothesis due to the stereoelectronic effects from the nitrogen and the glucose at C-21 and the minimization of steric hindrance effects assumed by this conformation. This observation was confirmed by NOESY interactions. From these experiments a correlation between H_2-18 (δ 5.25 and 5.37) and H-1′ (δ 4.76) established a β-stereochemistry for the substituents at C-19 and C-21. In addition, a strong interaction of H-21 with H-20 confirmed the orientation of these hydrogens in the same face and corroborated the β-glycosidic linkage at C-21. An extensive analysis of the ^{13}C NMR data of the corynanthean-type alkaloids, especially for C-3, C-5, and C-6, has also been used for definition of their stereochemistry and inclusion in the *normal*, *pseudo*, *allo*, or *epiallo* series.[9] Analysis of the ^{13}C NMR data for C-3, C-5, and C-6 (δ 52.6, 48.5, and 23.8, respectively) and comparison with model compounds[16] strongly supported the *allo* configuration for turbinatine (**1**), if we consider the γ-effect of the glucosyl moiety at C-21 on C-3 and C-5, whose signals were shifted upfield ($\Delta\delta$ ~5 ppm).

To elucidate the anomalous values of the J-coupling and the strong NOE effects between H-20 and H-21, compound **1** was subjected to theoretical study using semiempirical PM5 and AM1 methods at the SCF-MO level in the gas and solvent phases, with full geometry optimization,[17] as described previously.[18] A conformational analysis of **1** was performed with the BioMedCAChe 5.0 program.[19] From this analysis, three minimal energy conformations were obtained,[20] namely, **1a**, **1b**, and **1c**, and the heats of formation for each conformation are summarized in Table 2. Differences in conformational energy (Table 2) are due mainly to the conformations adopted by ring D, e.g., chair (**1b**) or twist-boat (**1a** and **1c**), and by N-4, i.e., the lone pair above or below the ring D plane due to pyramidal inversion. Variations in conformations of groups attached at C-15, C-20, and C-21 are limited because of steric hindrance. With the exception of calculations made with the AM1 Hamiltonian in vacuum, all the methods used showed correlated values reinforcing both the reliability of the results and the importance of simulating solvent effects in solute conformation and stability. The conformation **1a** is the most stable by ~5 kcal/mol, with the closure of the dihedral angle H-21−C-21−C-20−H-20 being about 28° (Figure 2). This approximation of H-21 and H-20 to a plane and the torsion of ring D (consequences of compound **1** stabilization) are in agreement with the high J-coupling constant actually observed.

Strictosidine can be enzymatically transformed to give a variety of indole alkaloids. Such reactions usually involve

Table 2. Heats of Formation (kcal/mol) for the Conformations Obtained from Conformational Analysis Using the AM1 and PM5 Semiempirical Methods

	heats of formation[a]			
	AM1		PM5	
conformation	vacuum	methanol[b]	vacuum	methanol[b]
1a	−321.0 (42.5°)	−343.6 (38.8°)	−336.6 (28.3°)	−364.7 (28.7°)
1b	−319.5 (51.5°)	−341.5 (47.3°)	−330.2 (49.0°)	−359.4 (48.0°)
1c	−321.1 (59.7°)	−339.4 (55.1°)	−329.3 (63.1°)	−358.6 (63.4°)

[a] In parentheses are the values for the dihedral angle formed by H-21−C-21−C-20−H-20. [b] The COSMO solvation model was used as described previously.[18]

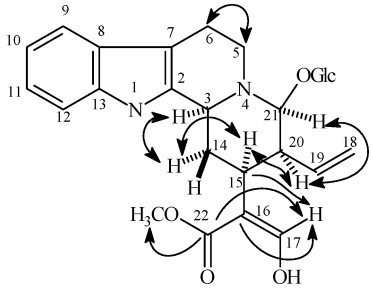


Figure 1. Selected HMBC (C → H) and NOESY (H ↔ H) correlations for turbinatine (**1**).

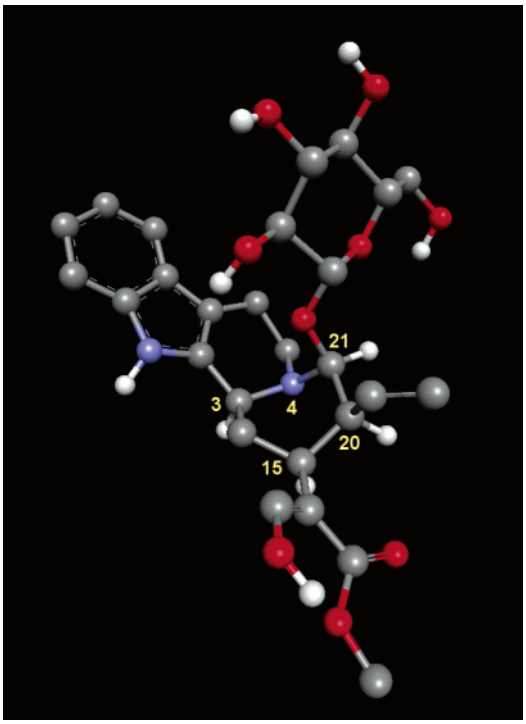


Figure 2. Structure of conformation **1a**. With the exception of hydrogen atoms attached to carbon atoms C-3, C-15, C-20, and C-21 the nonpolar hydrogen atoms were omitted in order to clarify the image.

the initial deglucosylation by the specific strictosidine *β*-glucosidase to give a highly unstable hemiketal aglycon (**2**), which has its secologanin ring then opened to afford the reactive dialdehyde **3**[11,12] (Scheme 1). The proposed biogenetic pathway for corynanthean-type alkaloids requires the subsequent rotation of the C-15−C-20 bond followed by the nucleophilic attack of N-4 on the aldehydic C-21, leading to intermediate **4**. This carbinolamine has been proposed as a putative intermediate in the biosynthesis of several corynanthean-type indole alkaloids, among these being 4,21-dehydrogeissoschizine and cathenamine.[15,16] Despite the large number of known indole alkaloids, the detection or isolation of carbinolamine **4** has not yet been reported probably due to its high unstability and rapid dehydration to the iminium aldehyde 4,21-dehydrocorynantheine (**5**). Subsequent reduction steps could lead to cathenamine (**6**) and further to corynantheine, through dehydration followed by reduction steps, as well as to other corynanthean-type alkaloids.[13−16] Alternatively, stabilization of carbinolamine **4** could be achieved through HO-21 glucosylation, leading to turbinatine (**1**). Therefore, the isolation of turbinatine (**1**) from the leaves of *Chimarrhis turbinata* may represent additional support for a biogenetic proposal for corynanthean-type alkaloids in which carbinolamine **4** could be involved.

Recent taxonomic studies on Rubiaceae[21] have included a series of other distinctive characters such as placentation, fruit and seed morphology, anatomy, and their combinations and tendencies, leading to its organization into four subfamilies: Ixoroideae, Cinchonoideae, Antirheroideae, and Rubioideae. The definitions of these subfamilies and the limits of some tribes remain problematic, mostly due to the lack of morphoanatomical characters of many taxa and detailed chemical studies. The presence of indole alkaloids in *Chimarrhis* emphasizes the importance of chemotaxonomic data to help better understand the morphoanatomical classification of this complex plant family.

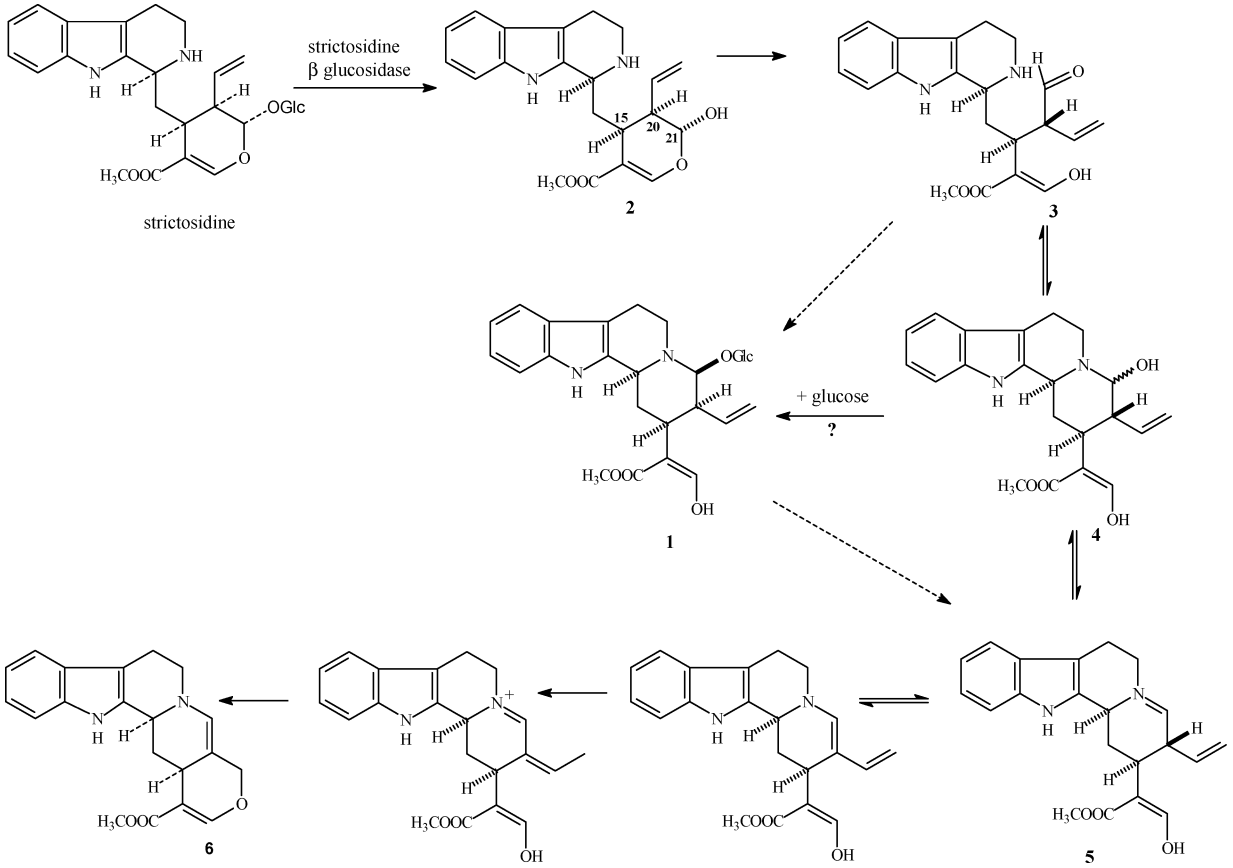
Although the initial crude extract from *C. turbinata* showed weak DNA-damaging activity,[22] to the best of our knowledge none of the known indole alkaloids isolated have been reported to show any effect on DNA. Compound **1** and the five known compounds isolated exhibited weak but selective activity with IC_{12} values around >250 and 100 *μ*g/mL or higher, in the mutant yeast strains RS 188N (RAD^+) and RS 322YK (rad 52Y), respectively.

Experimental Section

General Experimental Procedures. Optical rotation measurements were conducted on a Perkin-Elmer 241 polarimeter using a quartz cuvette (length 1 cm). IR spectra were measured on a Perkin-Elmer 1600 or Nicolet EMACT-40 FTIR spectrophotometer. NMR spectra were recorded on a Bruker AC 200, a Varian INOVA 300, or a Varian INOVA 500 NMR spectrometer operating at 200, 300, and 500 MHz for ^1H, respectively, and at 50, 75, and 125 MHz for ^{13}C, respectively. TMS was used as internal standard. Mass spectra were recorded at high resolution on a Micromass Q-TOF spectrometer. For chromatographic procedures, silica gel PF254, silica gel (230−400 mesh or 60−230 mesh) (Merck), Sephadex LH-20 (Pharmacia Biotech), and Amberlite resin XAD-16 (Sigma) were used. HPLC separations were performed on a Waters Prep LC 4000 System, and C_{18} Luna (Phenomenex) columns and precolumns were used. All solvents (Merck or Mallinckrodt) were of analytical or HPLC grade. The theoretical study was done using the semiempirical PM5 and AM1 methods on SCF-MO-MOPAC-2000 and BioMedCAChe software.

Plant Material. *Chimarrhis turbinata* was collected in Reserva do Viro, Belém, PA, Brazil, in October 1996 and identified by Dr. Marina Thereza V. do A. Campos. A voucher specimen is deposited in the Herbarium of the Botanic Garden, São Paulo, and catalogued as Lopes-51.

Scheme 1. Biogenetic Proposal for Corynanthean-Type Indole Alkaloids[15,16]



Extraction and Isolation. Dried and powdered leaves of *C. turbinata* (1.0 kg) were extracted with $CHCl_3$−MeOH and EtOH, successively, and afforded extracts A and B, respectively, after solvent evaporation under reduced pressure. Extract B was dissolved in MeOH−H_2O (8:2) and partitioned with hexane. The hydro alcoholic fraction was partially evaporated to MeOH−H_2O (6:4) and then partitioned successively with CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-BuOH fraction (0.5 g, after solvent evaporation) was dissolved into MeOH (5 mL) and submitted to gel filtration over Sephadex LH-20 eluted with MeOH. The subfractions obtained were compared by TLC analysis and pooled into fractions A−H. Fractions B (27 mg), C (34 mg), and D (39 mg) were purified by HPLC (Phenomenex C_{18}, 25.0 × 21.2 cm, 10 μm; eluent: MeCN−H_2O (1:4); 12 mL/min; UV detection at 237 nm) and afforded compound **1** (7 mg) (retention time 28.7 min). Extract A was submitted to the same partition procedures, and the resulting *n*-BuOH fraction (1.53 g) was chromatographed by column chromatography over silica gel [$CHCl_3$−MeOH−H_2O (70:28:2) and $CHCl_3$−MeOH−H_2O (65:30:5)], affording 29 subfractions, which were pooled into five fractions (A′−E′) after comparison by TLC analysis. Gel filtration over Sephadex LH-20 of fraction B′ (209 mg) led to the purification of strictosidine (79 mg) and a mixture of vallesiachotamine (6 mg) and isovallesiachotamine (60 mg). The separation of this mixture was subsequently undertaken by passage over Sephadex LH-20 and afforded pure vallesiachotamine (6 mg) and a further mixture of vallesiachotamine and isovallesiachotamine (24 mg). Gel filtration over Sephadex LH-20 of fraction D (160 mg) led to the purification of 5α-carboxystrictosidine (39 mg).

Bioassay. Identical to those reported in ref 22.

Turbinatine (1): amorphous yellow powder; $[\alpha]^{27}_D$ −8.5° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 235 nm (ϵ 5875); IR (KBr) 3496 (OH), 3370 (NH), 1710 (C=O) cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 532.3070 $[M + 2]^+$ (calcd for $C_{27}H_{34}N_2O_9$, 532.2409).

Strictosidine: colorless crystals; mp 161−165 °C; $[\alpha]^{27}_D$ −7.27° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 241 nm (ϵ 6025) and 474 nm (ϵ 11 800); IR (KBr) 3450 (OH), 1720 (C=O) cm^{-1}; the ^1H and ^{13}C NMR and EIMS were comparable with literature values.[5,6]

5α-Carboxystrictosidine: amorphous yellow powder; $[\alpha]^{27}_D$ −9.8° (*c* 0.053, MeOH); UV (MeOH) λ_{max} 240 nm (ϵ 4528) and 450 nm (ϵ 8490); IR (KBr) 3400 (OH), 1734 (C=O), 1650 (C=O) cm^{-1}; the ^1H and ^{13}C NMR and EIMS were comparable with literature values.[7]

Vallesiachotamine: colorless crystals; mp 249−251 °C; $[\alpha]^{25}_D$ +204° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} 222 nm (ϵ 22 200), 285 sh (ϵ 28 500), 291 nm (ϵ 29 100); IR (KBr) 3260 (N−H), 1680 (C=O), 1660 (C=O) cm^{-1}; the ^1H and ^{13}C NMR and EIMS were comparable with literature values.[8]

Isovallesiachotamine: amorphous yellow powder; $[\alpha]^{25}_D$ −54° (*c* 0.06, $CHCl_3$); UV, IR, and EIMS were the same as observed for vallesiachotamine; the ^1H and ^{13}C NMR data were comparable with literature values.[8]

Acknowledgment. This work was supported by the State of São Paulo Research Foundation (FAPESP) within the BIOTA/FAPESP (Biodiversity Virtual Institute Program) (www.biota.org.br), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). C.L.C. thanks FAPESP for a scholarship, and M.C.M.Y., M.F., and V.S.B. thank CNPq for research fellowships. We thank Prof. Dr. Jorge Guimarães (Centro de Biotecnologia-UFRGS) for the use of BioMedCAChe software.

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(18) (a) Peçanha, E. P.; Verli, H.; Rodrigues, C. R.; Barreiro, E. J.; Fraga, C. A. M. *Tetrahedron Lett.* **2002**, *43*, 1607−1611. (b) Due to solvent characteristics we take the relative permittivity value of methanol as $\epsilon = 32.6$ and the effective VDW radius of the solvent molecule as 2.52 Å.
(19) (a) *BioMedCAChe 5.0*; Fujitsu Ltd. and Oxford Molecular Ltd., 2001. (b) The dihedral angles C-14−C-15−C-16−C-17, C-15−C-20−C-19−C-18, C-20−C-21−O−C1′, and C-21−O−C-1′−C-2′ were independently searched between 0° and 360° in 30° steps. (c) The conformations of ring D were evaluated as chair or twist-boat conformations, and the N-4 atom lone pair was evaluated to be above or below the plane of ring D.
(20) Hessian matrix analyses were employed to unequivocally characterize them as true potential energy surface minima.
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NP020547M