Triterpenoid Constituents of *Raulinoa echinata*

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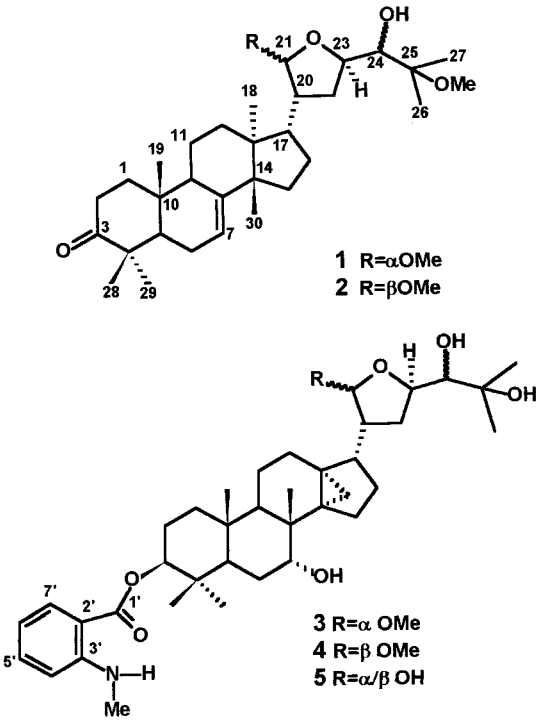
A phytochemical investigation of the stems of the South Brazilian endemic species *Raulinoa echinata* has led to the isolation of two new methoxylated protolimonoid epimers (**1** and **2**) together with the known melianone and melianodiol. The leaves afforded three glabretal-type triterpene derivatives esterified by *N*-methylanthranilic acid (**3**−**5**). Compounds **1** and **2** displayed weak inhibitory activity when assayed in vitro against trypomastigote forms of *Trypanosoma cruzi*. Compounds **1**−**5** were inactive in a brine shrimp (*Artemia salina*) lethality test.

Raulinoa is a monospecific genus, and the species *Raulinoa echinata* Cowan (Rutaceae) is endemic to the Itajaí Valley, Santa Catarina, Brazil.[1] This perennial woody shrub is characterized by the presence of spines and has been found only in a restricted location (1000 m) on the frequently inundated banks of the Itajaí River at an approximate altitude of 100 m, showing a high degree of adaptation to the environment. In continuation of our research on the chemistry of *Raulinoa echinata*,[2,3] investigation of methanol extracts of stems and leaves has led to the isolation of the new 21α,25-dimethylmelianodiol (**1**) and 21*β*,25-dimethylmelianodiol (**2**) and also three new *N*-methylanthranilic acid esters of the glabretal-type compounds **3**−**5**. In this work, the isolated compounds were also assayed in vitro against trypomastigote forms of *Trypanosoma cruzi* and in the brine shrimp (*Artemia salina*) lethality test.

The hexane extract of the stems of *R. echinata* yielded the triterpenes melianone and melianodiol, which exhibited spectral data in agreement with the literature.[4,5] The hemiacetal ring of these compounds opens and closes in solution; thus, a mixture of epimers is always present.

From the methanol extract compounds of *R. echinata* stems **1** and **2** were obtained. As shown previously for melianone and melianodiol, these compounds exhibited resonances in their ^{1}H NMR spectra at ca. *δ* 5.30 (brd, *J* = 3.0), ascribable to H-7, and seven singlets for quaternary methyl groups between *δ* 0.82 and 1.25. Resonances at *δ* 216.7, 145.6/145.3, and 118.1/118.0 were assigned to C-3, C-8, and C-7, respectively, in the ^{13}C NMR spectra of **1** and **2**. In contrast to melianone or melianodiol, two methoxyl group signals were observed in the ^{1}H NMR spectra of **1** and **2**. The side chain in each case was found to be the 21,-25-dimethoxy analogue of melianodiol, as verified by heteronuclear correlations of the methoxyl groups with C-21 and C-25 in the HMBC spectra of **1** and **2**. The HSQC spectra of compounds **1** and **2** showed correlations of the C-21 group resonances at *δ* 109.7 and 104.7 with H-21 [at *δ* 4.79 and *δ* 4.68 d (*J* = 3.4 Hz) for **1** and **2**, respectively], characterizing them as C-21 epimers. The H-23 [4.16 ddd (*J* = 7.3, 5.4, 2.9 Hz)] and C-23 (*δ* 75.1) resonances, in addition to the H-24 (3.37 brd) and C-24 (*δ* 76.4) signals, showed correlations in the HSQC spectrum of compound **1**. The isomer **2** showed the same correlations but inverted chemical shifts for C-23 (*δ* 77.8) and C-24 (*δ* 76.7).



Additional information was obtained by HMBC and NOE difference NMR experiments. In the HMBC spectra, correlations for C-25 (*δ* 77.2) and H-26/27 (*δ* 1.15 and 1.25) for compound **1**, and at *δ* 77.7 with 1.18 and 1.25 for compound **2**, were observed. The H-18 (*δ* 0.82) signal in the HMBC spectrum of compound **1** correlated with C-12 (*δ* 31.1), C-13 (*δ* 43.6), C-14 (*δ* 51.0), and C-17 (*δ* 50.7). For compound **2**, H-18 showed the same correlations, but the chemical shift of C-17 was upfield (*δ* 44.9). This difference was attributed to a *γ*-gauche effect of the oxygenated substituent on C-21*β*. Nakanishi et al.[5] performed the X-ray crystallographic analysis of a melianone acetate analogue, comparing chemical shifts of α- and *β*-melianone acetates. According to their findings, C-21 exhibited downfield shifts and C-23 showed upfield shifts, in the C-21α epimer. These arguments were confirmed by NOE difference experiments for **1** and **2** (Table 3). In compound **1**, irradiation of the

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Table 1. ^{1}H NMR Spectral Data for **1**−**5**[a]

position	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[c]
2ax	2.75 ddd (14.4, 14.0, 5.6)	2.75 ddd (14.4, 14.0, 6.4)			
2eq	2.25 ddd (14.4, 3.6, 3.6)	2.25 ddd (14.4, 3.4, 3.2)			
3 α			4.70 dd (11.1, 5.0)	4.70 dd (11.1, 5.0)	4.70 dd (10.3, 5.8)
7	5.30 brd (3.0)	5.30 brd (2.9)	3.76 brs	3.77 brs	3.76 brs
18	0.85 s	0.82 s	0.49 d (5.0)	0.49 d (5.0)	0.48 d (4.7)
			0.74 d (5.0)	0.74 d (5.0)	0.67 d (4.7)
19	1.01 s	1.01 s	0.93 s	0.94 s	0.93 s
21	4.79 d (3.4)	4.68 d (3.4)	4.84 d (3.0)	4.77 d (3.4)	5.28 d (2.8)/5.30 *d* (3.7)
23	4.16 ddd (7.3, 5.4, 2.9)	4.45 ddd (7.3, 5.6, 2.5)	4.04 ddd (10.2, 5,0, 2.0)	4.31 m	4.40/4.23 m
24	3.37 m	3.27 m	3.51 d (5.0)	3.58 d (5.0)	3.58 d (3.0)/ 3.13 *d* (1.5)
26/27	1.15/1.25 s	1.18/1.25 s	1.29/1.21 s	1.27/1.19 s	1.17/1.22/1.23/1.25 s
28/29	1.04/1.11 s	1.04/1.12 s	1.00/0.92 s	1.00/0.93 s	0.99/0.91 s
30	1.00 s	1.02 s	1.03 s	1.05 s	1.04 s
OMe-21	3.34 s	3.35 s	3.36 s	3.39 s	
OMe-25	3.23 s	3.25 s			
HO-24	2.61 d (6,5)	2.90 brs			
4′			6.65 brd (8.0)	6.65 brd (8.1)	6.70 brd (7.0)
5′			7.31 ddd (8.0, 7.0, 1.6)	7.36 ddd (8,0, 7.0, 1.6)	7.37 ddd (8.0, 7.0, 1.6)
6′			6.58 ddd (8.0, 7.0, 1.6)	6.57 ddd (8,0, 7.0, 1.6)	6.60 ddd (8.0, 7.0, 1.6)
7′			7.90 dd (8.0, 1.6)	7.90 dd (7.0, 1.6)	7.88 dd (7.0, 1.6)
N-H			7.70 d (4.6)	7.69 brd (4.6)	7.63 brs
N-Me			2.90 d (4.6)	2.90 d (4.6)	2.90 d (4.7)

[a] Chemical shifts in δ (ppm) scale, with multiplicities and coupling constants in Hz in parentheses. [b] Recorded in $CDCl_3$ solution. [c] Recorded in $CDCl_3$ solution, with drops of $(CD_3)_2SO$.

Table 2. ^{13}C NMR Spectral Data (δ ppm) for **1**−**5**

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	38.5	38.5	38.1	38.3	38.1
2	35.1	35.0	25.4	25.4	25.4
3	216.7	216.7	80.4	80.5	80.3
4	47.9	47.9	36.1	37.0	36.1
5	52.3	52.3	46.1	46.1	45.8
6	24.5	24.5	23.6	23.6	23.5
7	118.1	118.0	74.1	74.3	74.0
8	145.6	145.3	37.6	37.6	37.6
9	48.3	48.3	43.8	44.0	44.8
10	34.2	34.9	37.1	37.2	37.1
11	18.0	17.8	16.2	16.4	16.2
12	31.1	31.0	24.0	24.1	24.0
13	43.6	43.6	28.4	28.4	28.5
14	51.0	51.0	38.8	38.8	39.5
15	34.9	34.9	25.7	27.5	26.0
16	27.9	29.7	32.5	28.8	27.7/30.1
17	50.7	44.9	48.5	44.8	48.6/43.9
18	22.8	23.2	13.5	13.6	13.6
19	12.7	12.7	15.7	15.9	15.8
20	47.8	49.3	49.5	49.5	49.8
21	109.7	104.7	108.5	104.8	96.5/97.0
22	31.8	32.5	26.3	26.2	27.2
23	75.1	77.8	77.6	79.8	79.1/78.0
24	76.4	76.7	77.9	78.0	77.7/75.8
25	77.2	77.7	72.0	71.4	71.2/72.5
26/27	20.3/22.0	22.3/20.0	26.5/25.0	27.6/25.2	26.9/26.5
					26.4/25.5
28/29	24.4/21.5	24.4/21.5	27.8/15.8	27.9/17.0	27.7/16.8
30	27.3	27.4	19.3	19.5	19.3
O*Me*-21	55.7	54.9	55.5	54.8	
O*Me*-25	49.4	49.2			
1′			168.2	168.3	167.2
2′			110.4	110.6	109.6
3′			152.0	152.1	151.1
4′			110.6	110.6	110.5
5′			134.3	134.4	134.2
6′			114.1	114.2	114.1
7′			131.3	131.4	131.1
N-*Me*			29.5	29.5	29.3

[a] Recorded in $CDCl_3$ solution. [b] Recorded in $CDCl_3$ solution, with drops of $(CD_3)_2SO$.

OMe-21 resonance at δ 3.34 gave an enhancement of the H-23 signal at δ 4.16. This enhancement was not observed for OMe-21 of compound **2**. Accordingly, the structures of **1** and **2** were assigned as 21α,25-dimethylmelianodiol-[(21*R*,23*R*)epoxy-24-hydroxy-21α,25-methoxy]tirucalla-7-en-3-one and 21*β*,25-dimethylmelianodiol[(21*S*,23*R*)epoxy-24-hydroxy-21*β*,25-dimethoxy]tirucalla-7-en-3-one, respectively.

Compound **3** exhibited a pair of doublets (one hydrogen each), at δ 0.49 (J = 5.0 Hz) and 0.74 (J = 5.0 Hz), in the COSY NMR spectrum. In the HSQC NMR spectrum, these hydrogens correlated with a methylene carbon at δ 13.4, indicating a 14,18-cycloapoeuphane skeleton. The side chain of compound **3** was assigned as a methylmelianodiol-type unit, as evidenced by resonances in the ^{1}H NMR spectrum at δ 4.84 [(d, J = 3.0 Hz), H-21], 4.04 [(ddd, J = 10.2, 4.8, 2.0 Hz), H-23], 3.51 [(d, J = 5.0 Hz), H-24], and 3.36 s (OMe-21).[6] The H-21 signal correlated in the HSQC spectrum with C-21 at δ 108.5, suggesting a C-21α-methoxy carbon, as in the case of **1**. In the HMBC spectrum, H-21 correlated with C-23 (δ 77.6) and C-20 (δ 49.5), while H-23 and H-24 correlated in the HSQC spectrum with oxymethine carbons at δ 77.6 and 77.9, respectively. A broad singlet at δ 3.76 indicated a H-7*β* substituent,[6] correlating in the HSQC spectrum with a carbon at δ 74.1. In the HMBC spectrum, the H-7*β* signal correlated with methine carbons at δ 43.8 (C-9) and 46.1 (C-5). The former showed correlations with singlets attributed to H-30 (δ 1.03) and H-19 (δ 0.93); the latter correlated with H-28 and H-29 (δ 1.00/0.92), respectively. The HREIMS of compounds **3**−**5** revealed an odd molecular weight, in each case indicating the presence of nitrogen in these molecules. A 3*β*-*N*-methylanthranilate group was indicated in the ^{1}H NMR spectra of compounds **3**−**5** by the resonances at δ 4.70 (dd, J = 11.1 and 5.0 Hz for **3** and **4**; J = 10.3 and 5.8 Hz for **5**) corresponding to H-3α, which correlated in their HSQC spectra with an oxymethine carbon (δ 80.4 ± 0.1). Compound **3** exhibited aromatic signals at δ 7.90 (dd, J = 8.0, 1.6 Hz), 7.31 (ddd, J = 8.0, 7.0, 1.6 Hz), 6.65 (brd, J = 8.0 Hz), and 6.58 (ddd, J = 8.0, 7.0, 1.6 Hz). In addition, doublets at δ 7.70 (br, J = 4.6 Hz, N-H) and 2.90 (J = 4.6 Hz, N-Me) were observed in the COSY spectra, consistent with a *N*-methylanthranilic acid ester. Similar features were observed for compounds **4** and **5** (Table 1).

Table 3. NOE Difference ^{1}H NMR Spectral Data for **1** and **2**

1		**2**	
irradiation (δ)	enhanced peaks (δ)	irradiation (δ)	enhanced peaks (δ)
4.78 (H-21)	3.34 (OMe-21)	4.67 (H-21)	3.35 (OMe-21)
4.16 (H-23)	3.37 (H-24), 1.15 (H-26/H-27)	4.45 (H-23)	
3.34 (OMe-21)	4.78 (H-21), 4.16 (H-23)	3.35 (OMe-21)	4.67 (H-21)
3.23 (OMe-25)	1.15 and 1.25 (H-26 and H-27)	3.25 (OMe-25)	4.45 (H-23), 1.25 and 1.18 (H-26/H-27)

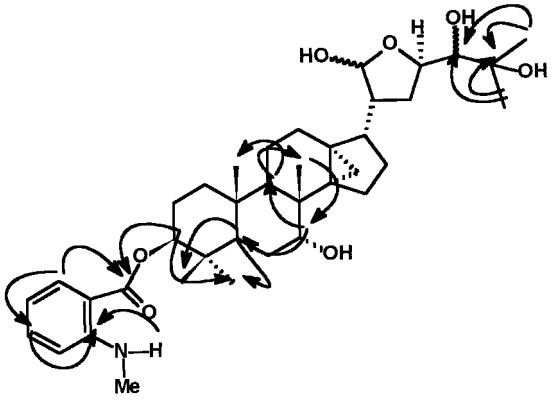

Figure 1. Main ^{1}H−^{13}C long-range correlation signals in the HMBC spectrum of compound **5**.

The N-Me hydrogens correlated in the HMBC spectra of **3** with the quaternary carbon at δ 152.0 (C-3′), which showed correlations with the signals at δ 7.90 (H-7′) and 7.31 (H-5′). The carbonyl C-1′ (δ 168.2) correlated with H-7′ (δ 131.3). In the COSY spectrum coupling between H-7′/H-6′ and H-5′/H-4′ was observed. Accordingly, compound **3** was assigned as (21*R*,23*S*)-epoxy-21α-methoxy-7α,24,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester.

Compound **4** differed from compound **3** only in the configuration of C-21, with chemical shift changes in the NMR spectra observed for the hydrogens of the side chain along with upfield changes for carbons C-21, C-17, and C-16, in agreement with a *β*-configuration, as discussed earlier for **2**. Compound **4** was assigned the structure (21*R*,-23*S*)-epoxy-21α-methoxy-7α,24,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester.

Compound **5** was also assigned an *N*-methylanthranilic acid ester with a 14,18-cycloapoeuphane skeleton, but did not exhibit a methoxyl singlet in the ^{1}H NMR spectrum. The H-21 resonance was observed downfield (δ 5.28) when compared to the methoxylated derivatives **3** and **4**. Additionally, the signals for the side chain of compound **5** were duplicated in the ^{1}H NMR spectrum. The H-21 resonance showed a second doublet (of minor intensity) at δ 5.32, multiplets at δ 4.40 and 4.23 attributed to H-23, and doublets at δ 3.58 and 3.13 assigned to H-24. These observations strongly suggested that **5** is a C-21 hydroxyl derivative, isolated as an epimeric mixture.[6,7] As for compounds **1**−**4**, compound **5** was finally elucidated by a series of 2D NMR experiments, including COSY, HSQC, and HMBC. NMR spectral data for compounds **1**−**5** are presented in Tables 1 and 2. Figure 1 shows the main H−C correlations in the long-range HMBC spectrum of **5**. Thus, the structure of **5** was assigned as (21,23*S*)-epoxy-7α,21,-24,25-tetrahydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester.

Methoxylated protolimonoids were described previously from *Turraea holstii* (Meliaceae).[8] It is possible that the methoxylated compounds **1**−**4** are artifacts of the extraction process. Protolimonoids of the melianone-type and compounds with the 14,18-cycloapoeuphane-type skeleton have been found mainly in species of the Meliaceae, but were also found in some Rutaceae species. The former group was encountered from *Phellodendron chinense*[9] and the latter from *Skimmia japonica*.[10] Benzoyl,[6] cinamoyl,[6] tigloyl,[7,11] acetyl,[11,12] and isobutyryl[13] ester acids of glabretal triterpenes at C-3 have been reported in the literature, with all exhibiting the α-configuration. However, 3*β*-*N*-methylanthranilic acid esters of glabretal-type triterpenes seem to be unprecedented in the literature.

Compounds **1**−**5** were assayed in vitro against the trypomastigote form of *Trypanosoma cruzi*. Compounds **1** and **2** displayed weak activity against the trypomastigote form (Y strain) of *T. cruzi* with IC_{50} values of 134.9 and 114.5 μg/mL, respectively, when compared with the reference compound crystal violet (IC_{50} 7.5 μg/mL). Compounds **3**−**5** were inactive in this assay, with IC_{50} values of > 1000 μg/mL. Compounds **1**−**5** were all inactive in the brine shrimp lethality test (LD_{50} > 100 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined on a Microquímica MQAPF-301 instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were determined with a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were taken on a Bomem Hartman & Braun MB-Series instrument. A Bruker DRX-400 NMR spectrometer, operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C, was used. All spectra were run in $CDCl_3$ with TMS as internal standard. For HREIMS an Autospec-Micromass-EBE was used (Universidade Estadual de Campinas).

Plant Material. Stems and leaves of *R. echinata* were collected in Ressacada, Ibirama, SC, Brazil, and identified by A. Reis (Universidade Federal de Santa Catarina) and J. R. Pirani (Universidade de São Paulo). A voucher specimen [A. Reis & M. Biavatti 2.570 (26/07/98)] has been deposited at the Herbário Barbosa Rodrigues (HBR), Itajaí, Santa Catarina, Brazil.

Extraction and Isolation. The dried and powdered stems (5 kg) and leaves (3 kg) were extracted with hexane and MeOH. Then, half (100 g) of the MeOH extract was fractionated over silica gel 60 (70−230 mesh) into three fractions: CH_2Cl_2, EtOAc, and MeOH. The latter fraction (89 g) was dissolved in H_2O−MeOH and successively partitioned with solvents of increasing polarity (hexane, CH_2Cl_2, and EtOAc). A portion of the ethyl acetate fraction (2 g) was chromatographed over silica gel (230−400 mesh) using as eluent hexane−acetone (75:25 and then a gradient of the same solvent of increasing polarities), furnishing fractions A to I. Fraction A (460 mg) was chromatographed over silica gel (230−400 mesh) affording several triterpenes. Among them, a mixture of compounds **1** and **2** (7 mg) was purified over a Lobar Lichroprep [(Merck) Si 60 (40−63 μm) B (310−25)] column, using hexane−acetone (95:5) as eluent, affording **1** (3.6 mg) and **2** (3.2 mg).

From the hexane extract of stems, significant amounts of melianone (350 mg) and melianodiol (150 mg) were obtained using column chromatography over silica gel (230−400 mesh) and using as eluent hexane−acetone in gradient.[3]

Half of the MeOH extract of the leaves (100 g) was submitted to liquid partition with hexane, CH_2Cl_2, EtOAc, and MeOH. The hexane (15 g) and CH_2Cl_2 fractions (10 g) obtained were chromatographed over silica gel (70−230 mesh) using solvents of increasing polarity: hexane, CH_2Cl_2, EtOAc, and MeOH. A fraction eluted by CH_2Cl_2 (1.7 g) from the hexane fraction yielded compounds **4** (39 mg) and **5** (44 mg) using column chromatography over silica gel (230−400 mesh) and hexane−acetone (9:1) for elution. The EtOAc (1.5 g) fraction from the CH_2Cl_2 fraction yielded compound **3** (50 mg) with the same solvent mixture above.

21α,25-Dimethylmelianodiol[(21*R*,23*R*)epoxy-24-hydroxy-21α,25-methoxy]tirucalla-7-en-3-one (1): colorless gum; $[\alpha]^{25}_D$ −65.2° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (2.95), 248 (2.42) nm; IR (film) ν_{max} 2920, 1707, 1458, 1375, 1083 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 389 (26), 359 (8), 307 (41), 239 (10), 199 (11), 161 (100), 135 (23), 131 (11) 119 (10); HREIMS *m*/*z* 516.38151 (calcd for $C_{32}H_{52}O_5$, 516.38147).

21*β*,25-Dimethylmelianodiol[(21*S*,23*R*)epoxy-24-hydroxy-21*β*,25-dimethoxy]tirucalla-7-en-3-one (2): colorless gum; $[\alpha]^{25}_D$ −103.0° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (3.37), 244 (2.89) nm; IR (film) ν_{max} 2920, 1707, 1458, 1375, 1083 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 389 (30), 359 (10), 307 (30), 239 (10), 199 (15), 161 (100), 135 (20), 131 (8), 119 (5); HREIMS *m*/*z* 516.38148 (calcd for $C_{32}H_{52}O_5$, 516.38147).

(21*R*,23*S*)-Epoxy-21α-methoxy-7α,24*S*,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13 α,-14 α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester (3): amorphous solid; mp 144−145 °C; $[\alpha]^{25}_D$ +15.5° (*c* 0.01, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 222 (4.16), 253 (3.78), 353 (3.64) nm; IR (film) ν_{max} 3439, 2926, 2864, 1671, 1635, 1384, 1243 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 152 (14), 151 (100), 134 (21), 105 (5); HREIMS *m*/*z* 653.42960 (calcd for $C_{39}H_{59}O_7N$, 653.42915).

(21*S*,23*S*)-Epoxy-21α-methoxy-7α,24*S*,25-trihydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13 α,-14 α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester (4): amorphous solid; mp 218−220 °C, $[\alpha]^{25}_D$ +25.5° (*c* 0.03, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 224 (4.01), 254 (3.60), 354 (3.44) nm; IR (film) ν_{max} 3435, 2928, 2860, 1673, 1631, 1384, 1243 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 152 (13), 151 (100), 134 (34), 105 (13), 59 (28); HREIMS *m*/*z* 653.42896 (calcd for $C_{39}H_{59}O_7N$, 653.42915).

(21,23*S*)-Epoxy-7α,21,24*S*,25-tetrahydroxy-4α,4*β*,8*β*,10*β*-tetramethyl-25-dimethyl-14,18-cyclo-5α,13α,14α,17α-cholestan-3*β*-*N*-methylanthranilic acid ester (5): amorphous solid; mp 176−178 °C; $[\alpha]^{25}_D$ +13.1° (*c* 0.01, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 222 (4.07), 254 (3.64), 354 (3.50) nm; IR (film) ν_{max} 3419, 2939, 2869, 1674, 1615, 1580, 1242 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* 523 (3), 368 (5), 236 (6), 84 (88), 66 (100); HREIMS *m*/*z* 639.41350 (calcd for $C_{38}H_{57}O_7N$, 639.41352).

Bioactivity Screening. In vitro trypomastigote forms of *Trypanosoma cruzi* (Y strain) and brine shrimp lethality (BSL) assays were performed according to established protocols.[14,15]

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References and Notes

(1) Kaastra, R. C. *Flora Neotropica* **1982**, *33*, 130−132.
(2) Biavatti, M. W.; Vieira, P. C.; Silva, M. F. G. F.; Fernandes, J. B.; Degani, A. L. G.; Cass, Q. B.; Schefer, A. B.; Ferreira, A. *Phytochem. Anal.* **2001**, *12*, 64−68.
(3) Biavatti, M. W.; Vieira, P. C.; Silva, M. F. G. F.; Fernandes, J. B.; Albuquerque, S.; Magalhães, C. M. I.; Pagnocca, F. C. *Phytomedicine* **2001**, *8*, 121−124.
(4) Merrien, A.; Polonsky, J. *Chem. Commun.* **1971**, 261−263.
(5) Nakanishi, T.; Inada, A.; Nishi, M.; Miki, T.; Hino, R.; Fujiwara, T. *Chem. Lett.* **1986**, 69−72.
(6) Mulholland, D. A.; Nair, J. J.; Taylor, D. A. H. *Phytochemistry* **1996**, *42*, 1667−1671.
(7) Miller, S. L.; Tinto, W. F.; Mclean, S.; Reynolds, W. F.; Yu, M.; Carter, C. A. G. *J. Nat. Prod.* **1995**, *58*, 1640−1642.
(8) Mulholland, D. A.; Monkhe, T. V.; Taylor, D. A. H.; Rajab, M. S. *Phytochemistry* **1999**, *52*, 123−126.
(9) Su, R.; Kim, M.; Kawaguchi, H.; Yamamoto, T.; Goto, K.; Taga, T.; Miwa, Y.; Kozuka, M.; Takahashi, S. *Chem. Pharm. Bull.* **1990**, *38*, 1616−1619.
(10) Ochi, M.; Tatsukawa, A.; Seki, N. I.; Kotsuki, H.; Shibata, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3225−3230.
(11) Mulholland, D. A.; Monkhe, T. V. *Phytochemistry* **1993**, *34*, 579−580.
(12) Kashiwada, Y.; Fujioka, T.; Mihashi, K.; Marubayashi, N.; Mizuki, K.; Chen, I. S.; Lee, K. H. *J. Nat. Prod.* **1995**, *58*, 495−503.
(13) Mulholland, D. A.; Taylor, D. A. H. *Phytochemistry* **1992**, *31*, 4163−4166.
(14) Alves, T. M. D.; Alves, R. D.; Romanha, A. J.; dos Santos, M. H.; Nagem, T. J.; Zani, C. L. *J. Nat. Prod.* **1999**, *62*, 369−371.
(15) Alkofahi, A.; Rupprecht, J. K.; Anderson, J. E.; McLaughlin, J. L.; Mikolajczak, K. L.; Scott, B. A. In *Insecticides of Plant Origin;* Arnason, J. T., Philogene, B. J. R., Morand, P., Eds.; American Chemical Society: Washington, 1989; pp 28−29.

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