New Antifungal Terpenoid Glycosides from *Alibertia edulis* (Rubiaceae)[1])

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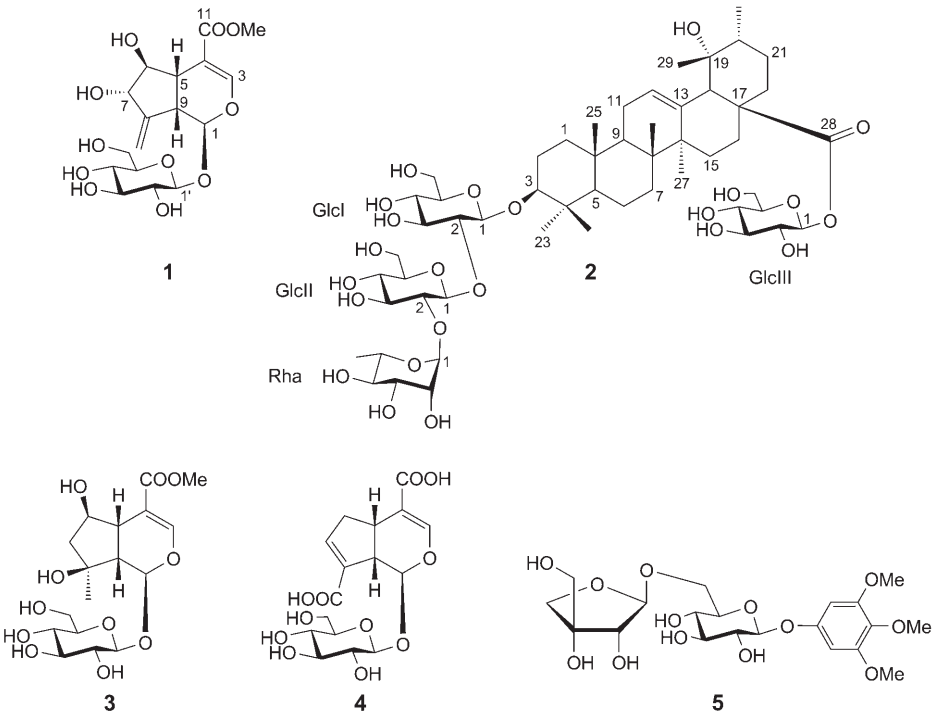
Phytochemical investigation from the stems of *Alibertia edulis* led to the isolation and identification of a new iridoid 6β-hydroxy-7-epigardoside methyl ester (**1**) and a new saponin 3β-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside pomolate (**2**), along with three known compounds, shanzhiside methyl ester (**3**), ixoside (**4**), and 3,4,5-trimethoxyphenyl 1-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**5**). The structures of **1** and **2** were established on the basis of their spectroscopic data. Iridoid **1** and saponin **2** exhibited moderate inhibitory activities against *Candida albicans* and *C. krusei* in a dilution assay.

Introduction. – *Alibertia edulis* (RICH.) A. RICH. ex DC, commonly known as 'marmelada-bola', is widely spread in the Brazilian 'Cerrado'. The *Alibertia* genus is known for the occurrence of iridoids, triterpenoids and phenolic derivatives [2–6]. Recent phytochemical investigations of stems of *A. macrophylla* led to the isolation of a diterpene, 2β,3α,16α-trihydroxy *ent*-kaurane [5]. Previous studies of the leaves of *A. edulis* resulted in the isolation of ten triterpenes [7]. In our continuing chemical and biological investigations of the Brazilian Rubiaceae plant species, we report the isolation and structure elucidation of a new iridoid, 6β-hydroxy-7-epigardoside methyl ester (**1**), and a new saponin 3β-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside pomolate (**2**), from *A. edulis* stems together with known iridoids: shanzhiside methyl ester (**3**) and ixoside (**4**), and phenolic derivative 3,4,5-trimethoxyphenyl 1-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**5**). We also report strong antifungal activity against pathogenic *Candida*-species yeast and *Cryptococcus neoformans* of the AcOEt extract of this species and, the moderate antifungal activity of compounds **1** and **2**.

[1]) Part of the results have been presented with a poster at the 1st Brazilian Conference of Natural Products on November 4th–7th, 2007 [1].



Results and Discussion. – The EtOH extract prepared from dried and powdered stems of *A. edulis* was submitted to partition in hexane, AcOEt, and BuOH. The preliminary biological screening indicated that the AcOEt extract showed antifungal activity (*cf. Table 4*). Fractionation and purification over silica *RP-18* led to the isolation of compounds **1**–**5**. The known compounds **3**–**5** were identified by comparing their spectroscopic data to those reported in literature [8–10].

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS of **1** showed a peak at *m/z* 403.1289 ($[M-H]^-$), determining the molecular formula as being $C_{17}H_{24}O_{11}$, and a peak at *m/z* 241.0738 ($[M-H-162]^-$), corresponding to the loss of a hexose moiety. The ^{1}H- and ^{13}C-NMR spectroscopic data were consistent with a β-glucopyranosyl unit and an iridoid aglycone. The ^{1}H-NMR spectrum showed signals at δ(H) 7.34 (*d*, $J=1.0$, H–C(3)) and 5.61 (*d*, $J=2.0$, H–C(1)) corresponding to an iridoid dihydropyran ring. The signal at δ(H) 3.64 (*s*, 3 H) corresponded to a MeO group, and the signal at δ(H) 5.21 (*d*, $J=2.5$, 2 H) corresponded to an exocyclic $=CH_2$ group. An anomeric H-atom signal at δ(H) 4.41 (*d*, $J=8.5$) confirmed the presence of a sugar residue with β-configuration. The ^{13}C-NMR spectrum of **1** showed 17 signals. The C-atom signals at δ(C) 35.5 (C), 41.8 (C), 95.0 (CH), 107.9 (C) and 152.2 (CH) were attributed to C-atoms C(5), C(9), C(1), C(4), and C(3), respectively of the dihydropyran ring of the iridoid aglycone. The remaining C-atoms signals, δ(C) 73.7 (CH) and 73.5 (CH) were assigned to C(6) and C(7), respectively, and the signals at δ(C) 110.0 (CH_2(10)) and 150.4 (C(8)) were attributed to an *exo*-methylene function present in the structure. The *g*HMBC spectrum confirmed the assignment of C(6) and

C(7) due to the ^3J-correlation observed between H–C(10) (δ(H) 5.21) and C(7) (δ(C) 73.5). The attachment of the β-D-glucopyranosyl moiety in **1** was established as in C(1) position in the aglycone, on the basis of the cross-peak correlations, in *g*HMBC, between H–C(1′) (δ(H) 4.41) and C(1) (δ(C) 95.0) (*Table 1*).

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**, *together with* g*HMBC* (H → C) *Correlations.* At 500/126 MHz, resp., in (D_6)DMSO; δ in ppm, J in Hz.

	δ(H)	δ(C)	*g*HMBC
H–C(1)	5.61 (*d*, J = 2.0)	95.0 (*d*)	
H–C(3)	7.34 (*d*, J = 1.0)	152.2 (*d*)	C(1), C(4), C(5), C(11)
C(4)	–	107.9 (*s*)	
H–C(5)	2.81 (br. *d*, J = 8.0)	35.5 (*d*)	C(3), C(4), C(6), C(7), C(8), C(9)
H–C(6)	4.08 (br. *s*)	73.7 (*d*)	
H–C(7)	4.02 (br. *s*)	73.5 (*d*)	
C(8)	–	150.4 (*s*)	
H–C(9)	3.12–3.15 (*m*)	41.8 (*d*)	
CH_2(10)	5.21 (*d*, J = 2.5)	110.0 (*t*)	C(7), C(9)
C(11)	–	166.3 (*s*)	
MeO	3.64 (*s*)	50.9 (*q*)	C(11)
H–C(1′)	4.41 (*d*, J = 8.5)	98.4 (*d*)	C(1)
H–C(2′)	2.94 (*d*, J = 8.5)	72.9 (*d*)	
H–C(3′)	3.12–3.15 (*m*)	76.6 (*d*)	C(2′), C(4′)
H–C(4′)	3.03 (*t*, J = 8.5)	70.0 (*d*)	
H–C(5′)	3.13–3.16 (*m*)	77.2 (*d*)	
CH_2(6′)	3.44 (*dd*, J = 11.5, 6.0), 3.68 (*dd*, J = 11.5, 5.0)	61.0 (*t*)	

The α-orientation of H–C(1) and β-orientation of H–C(5) and H–C(9) is in accordance with the biosynthetic origin of the iridoids [11]. The coupling constant between H–C(5) and H–C(6) (J < 1.0 Hz), together with the upfield shift of C(1) (δ(C) 95.0), and the calculation of the shift difference between C(3) and C(4) ($\Delta\delta$ = 44.3) indicated that the orientation of the OH group at C(6) was β [12]. The appearance of H–C(6) as a broad *singlet* (δ(H) 4.08) provided a small coupling constant between H–C(6) and H–C(7) (J < 1.0 Hz), corresponding to a dihedral angle close to 90°, necessitating a *trans*-relationship between these H-atoms, which suggested the α-orientation of the OH group at C(7) [13]. This configuration was confirmed on basis of NOESY interactions between H–C(5) (δ(H) 2.81) and H–C(9) (δ(H) 3.12–3.15, β-oriented), and no interactions between H–C(6) or H–C(7). Thus, the structure of compound **1** was elucidated as 6β-hydroxy-7-epigardoside methyl ester.

Compound **2** was obtained as a brown amorphous powder. The HR-ESI-MS of **2** showed a peak at m/z 1127.5641 ($[M + Na]^+$), corresponding to the molecular formula $C_{54}H_{88}O_{23}$. The ^{1}H-NMR spectrum showed signals of seven Me groups at δ(H) 0.81, 0.90, 0.99, 1.14, 1.23, 1.36 (*s*, each 3 H), and 0.96 (*d*, J = 6.7, 3 H), and an olefinic H-atom at δ(H) 5.33 (br. *s*). The ^{13}C-NMR data confirmed the presence of seven Me C-atom signals at δ(C) 15.8, 16.4, 16.7, 17.5, 24.6, 27.0, and 28.6, and a pair of olefinic C-atoms at δ(C) 129.6 and 139.5, suggesting that the aglycone possesses an urs-12-ene skeleton. In the *g*HSQC spectrum, the long-range correlations observed for Me groups at δ(H) 1.23

(*s*, Me(29)) and 0.96 (*d*, $J = 6.7$, Me(30)) with a quaternary C-atom at δ(C) 73.5, together with the multiplicity of H–C(18) at δ(H) 2.55 (*s*), observed in the ^{1}H-NMR spectrum, were consistent with the aglycone pomolic acid (*Table 2*) [14].

Table 2. *^{1}H- and ^{13}C-NMR Data of Aglycone Part of* **2**. At 600/150 MHz, resp., in CD_3OD; δ in ppm, J in Hz.

	δ(H)	δ(C)
CH_2(1)	1.68 (*m*), 1.05 (*m*)	39.7 (*t*)
CH_2(2)	2.00 (*m*), 1.77 (*m*)	26.9 (*t*)
H–C(3)	3.23 (*m*)	92.1 (*d*)
C(4)	–	40.3 (*s*)
H–C(5)	0.82 (*m*)	56.9 (*d*)
CH_2(6)	1.57 (*m*), 1.43 (*m*)	19.3 (*t*)
CH_2(7)	1.57 (*m*), 1.36 (*m*)	34.0 (*t*)
C(8)	–	41.1 (*s*)
H–C(9)	1.71 (*m*)	48.0 (*d*)
C(10)	–	37.7 (*s*)
CH_2(11)	2.00 (*m*)	24.5 (*t*)
H–C(12)	5.33 (br. *s*)	129.6 (*d*)
C(13)	–	139.5 (*s*)
C(14)	–	42.5 (*s*)
CH_2(15)	1.88 (*m*), 1.04 (*m*)	29.5 (*t*)
CH_2(16)	2.64 (*m*), 1.67 (*m*)	26.4 (*t*)
C(17)	–	49.0 (*s*)
H–C(18)	2.55 (*s*)	54.8 (*d*)
C(19)	–	73.5 (*s*)
H–C(20)	1.38 (*m*)	42.7 (*d*)
CH_2(21)	1.77 (*m*), 1.27 (*m*)	26.9 (*t*)
CH_2(22)	1.82 (*m*), 1.65 (*m*)	37.7 (*t*)
Me(23)	1.14 (*s*)	28.6 (*q*)
Me(24)	0.90 (*s*)	16.7 (*q*)
Me(25)	0.99 (*s*)	15.8 (*q*)
Me(26)	0.81 (*s*)	17.5 (*q*)
Me(27)	1.36 (*s*)	24.6 (*q*)
C(28)	–	178.5 (*s*)
Me(29)	1.23 (*s*)	27.0 (*q*)
Me(30)	0.96 (*d*, $J = 6.7$)	16.4 (*q*)

The presence of four sugar moieties was established from the signals of four anomeric H-atoms at δ(H) 4.44 (*d*, $J = 7.7$), 4.91 (*d*, $J = 7.6$), 5.22 (br. *s*), and 5.36 (*d*, $J = 8.1$), attached to the C-atoms at δ(C) 105.5, 101.8, 101.9, and 95.6, respectively, in the *g*HSQC spectrum. The C-atom signals observed at δ(C) 92.1 (C(3)) and 178.5 (C(28)) in the aglycone are consistent with a bidesmoside saponin moiety. Glycosylation of the alcoholic function at C(3) and esterification of the C(28)OOH group were indicated by the downfield shift (+13.9 ppm) and upfield shift (−2.1 ppm) observed, respectively, for the resonance of these C-atoms in **2**, relative to the corresponding signals in pomolic acid [14].

To confirm our assumption regarding the glycosidic composition of this compound, an ESI-IT-MS^n experiment was performed. In the ESI-IT-MS^n spectrum, the peak at

m/*z* 1103 was assigned to a deprotonated molecule ($[M-H]^-$) of compound **2**. An adduct at *m*/*z* 1139 was observed, and it was tentatively assigned to $[M-H+2\,H_2O]^-$, which could result from the use of H_2O to dissolve the sample [15]. The second order fragmentation of the adduct *m*/*z* 1139 led to a base peak at *m*/*z* 941 ($[M-162-H]^-$), indicating the loss of a hexose moiety. The MS^3 fragmentation from this ion (*m*/*z* 941) gave a product ion at *m*/*z* 795 ($[M-162-146-H]^-$), corresponding to the loss of a deoxyhexose moiety. MS^4 fragmentation from the ion *m*/*z* 795 yielded a peak at *m*/*z* 633 ($[M-(2\times162)-146-H]^-$), due to the loss of a second hexose moiety, and the MS^5 fragmentation of this precursor ion produced a product ion at *m*/*z* 471 ($[M-(3\times162)-146-H]^-$), corresponding to the loss of a third hexose moiety and is in agreement with the molecular weight of the aglycone pomolic acid.

The structures of the sugar moieties were deduced by 1D-TOCSY and 2D-NMR experiments (*Table 3*). The 1D-TOCSY method [16] allowed the subspectrum of a single monosaccharide unit to be extracted from the crowded overlapped region. Selected 1D-TOCSY data obtained by irradiation of each anomeric H-atom signal yielded the subspectrum of each sugar residue. Thus, the shifts of the sugar resonances were attributable to an α-L-rhamnopyranosyl (δ(H) 5.22 (H–C(1_{Rha})) and three β-D-glucopyranosyl (δ(H) 4.44 (H–C(1_{GlcI})), δ(H) 4.91 (H–C(1_{GlcII})), and δ(H) 5.36 (H–C(1_{GlcIII}))) units. The β-orientation of all three anomeric centers of the glucopyranosyl moieties was supported by the relatively large coupling constant values observed for the H–C(1_{GlcI}) (*d*, $J=7.7$), H–C(1_{GlcII}) (*d*, $J=7.6$), and H–C(1_{GlcIII}) (*d*, $J=8.1$). And the α-orientation of the anomeric H-atom of the rhamnopyranosyl moiety was based on the multiplicity of the H–C(1_{Rha}) signal (br. *s*). The absolute configuration of the sugar residues has been tentatively determined by biogenetic considerations.

The positions of the sugar moieties were unambiguously defined by the *g*HMBC experiment. A cross-peak due to long-range correlations between H–C(1_{GlcI}) (δ(H) 4.44) and C(3) (δ(C) 92.1) of the aglycone indicated that this sugar unit was linked at C(3) of the aglycone. Yet, a long-range connectivity between H–C(1_{GlcIII}) (δ(H) 5.36) and C(28) (δ(C) 178.5) indicated this sugar to be involved in an ester linkage with the C(28)OOH group. Similarly, cross-peaks were observed between H–C(1_{GlcII}) (δ(H) 4.91) and C(2_{GlcI}) (δ(C) 78.1), and between H–C(1_{Rha}) (δ(H) 5.22) and C(2_{GlcII}) (δ(C) 79.4). On the basis of these findings, compound **2** was established to be 3β-*O*-[α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside pomolate.

All isolated compounds were evaluated with respect to antifungal activity (*Table 4*), and all showed moderate antifungal activity against *Candida albicans* and *C. krusei* in a dilution assay [17]. On the other hand, none of the compounds showed inhibitory effects against *Candida parapsilosis* and *Cryptococcus neoformans* within the concentration range tested (250.0 – 0.4 μg/ml).

The stronger activity of the AcOEt extract compared to the isolated compounds may be due to the occurrence of tannins that were detected by $FeCl_3$ and gelatin/NaCl tests [18]. Toxicity of tannins towards microorganisms is well documented [19].

This is the first report of the isolation of a saponin from the *Alibertia* genus. Among the isolated compounds, only **3** had already been reported in this genus [5].

Table 3. *^{1}H- and ^{13}C-NMR Data of the Sugar Moieties of* **2**. At 600/150 MHz, resp., in CD_3OD; δ in ppm, J in Hz.

	δ(H)	δ(C)
GlcI		
H–C(1)	4.44 (*d*, *J* = 7.7)	105.5 (*d*)
H–C(2)	3.71 (*dd*, *J* = 8.9, 7.7)	78.1 (*d*)
H–C(3)	3.59 (*dd*, *J* = 8.9, 8.9)	78.7 (*d*)
H–C(4)	3.26 (*dd*, *J* = 8.9, 8.9)	72.0 (*d*)
H–C(5)	3.28–3.31 (*m*)	77.9 (*d*)
CH_2(6)	3.69 (*dd*, *J* = 11.0, 4.0), 3.91 (*dd*, *J* = 11.0, < 3.0)	62.8 (*t*)
GlcII		
H–C(1)	4.91 (*d*, *J* = 7.6)	101.8 (*d*)
H–C(2)	3.40 (*dd*, *J* = 9.3, 7.6)	79.4 (*d*)
H–C(3)	3.47 (*dd*, *J* = 9.3, 9.3)	79.1 (*d*)
H–C(4)	3.07 (*dd*, *J* = 9.3, 9.3)	72.5 (*d*)
H–C(5)	3.24–3.27 (*m*)	77.6 (*d*)
CH_2(6)	3.56 (*dd*, *J* = 11.3, 6.7), 3.87 (*dd*, *J* = 11.3, < 3.0)	63.5 (*t*)
Rha		
H–C(1)	5.22 (br. *s*)	101.9 (*d*)
H–C(2)	3.94 (*d*, *J* = 3.2)	72.1 (*d*)
H–C(3)	3.76 (*dd*, *J* = 9.5, 3.2)	72.0 (*d*)
H–C(4)	3.43 (br. *t*, *J* = 9.5)	73.7 (*d*)
H–C(5)	4.17–4.20 (*m*)	69.4 (*d*)
Me(6)	1.28 (*d*, *J* = 6.1)	18.1 (*q*)
GlcIII		
H–C(1)	5.36 (*d*, *J* = 8.1)	95.6 (*d*)
H–C(2)	3.35 (*dd*, *J* = 8.9, 8.1)	73.5 (*d*)
H–C(3)	3.36 (*dd*, *J* = 9.1, 8.9)	78.3 (*d*)
H–C(4)	3.39 (*dd*, *J* = 9.1, 9.1)	70.7 (*d*)
H–C(5)	3.40–3.43 (*m*)	78.1 (*d*)
CH_2(6)	3.72 (*dd*, *J* = 11.7, 3.5), 3.84 (*dd*, *J* = 11.7, 2.0)	62.3 (*t*)

Table 4. *Antifungal Activity of Compounds* **1**–**5** (MIC in μg/ml)

Extract or Compound	*Candida albicans*	*Candida krusei*	*Candida parapsilosis*	*Cryptococcus neoformans*
AcOEt extract	125	31.2	62.5	15.6
1	125	125	> 250	> 250
3	250	125	> 250	> 250
4	250	250	> 250	> 250
5	250	125	> 250	> 250
2	125	62.5	> 250	> 250

Amphotericine B (3 μg/ml) was used as positive control.

The species of the Rubiaceae family contains a great diversity of secondary metabolites: iridoids, indole alkaloids, anthraquinones, flavonoids, phenolic derivatives, diterpenes, triterpenes, and other types of alkaloids. The *Alibertia* genus is known for the occurrence of iridoids, triterpenoids and phenolic derivatives [2–6]. The presence

of iridoids (**1**, **3**, and **4**) and the phenolic derivative **5** in *A. edulis* is in agreement with chemosystematic correlations and botanical positioning of this genus in the Ixoroideae subfamily [2][20].

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Experimental Part

General Procedures. Column chromatography (CC): silica *RP18* (15–25 μm; *Merck*). TLC: silica gel *60 GF_{254}* plates (*Merck*); visualization under UV light ($\lambda = 254$ nm), I_2 vapor, and by spraying 0.5% anisaldehyde (in H_2SO_4), followed by heating. HPLC Separations were performed on a *Varian PrepStar Dynamax SD-1* system having a UV-VIS detector and a *RP18* column (*Phenomenex Luna RP-18* (2) column (250 × 21.20 mm i.d.; 10 μm; flow rate 10 ml/min, detection at 235 nm), eluting with MeOH/H_2O mixtures. Optical rotations: *Polamat A Carl Zeiss Jena* polarimeter. UV Spectra: *Varian ProStar 330* diode array spectrometer; λ_{max} in nm. IR Spectra (KBr): *Nicolet-730 FT-IR* spectrometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Varian INOVA 500* and *Bruker DRX 600* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Bruker Daltonics UltrOTOF-Q*; in *m*/*z*. MS^n: *Thermo Finnigan LCQ Deca* ion-trap mass spectrometer, with an electrospray interface. Negative ion mass spectra were recorded in the range of *m*/*z* 50–2000, with a collision energy of 25% and activation time of 30 ms. Data acquisition and processing were performed using the *Xcalibur* software.

Plant Material. Alibertia edulis (RICH.) A. RICH. ex DC was collected in the surrounding of Aporé – GO, Brazil, in July 2004 by Dr. *C. C. da Silva* and identified by Dr. *I. Cordeiro*. A voucher specimen (SP 370.913) was deposited at the Herbarium of Instituto de Botânica, São Paulo – SP, Brazil.

Extraction and Isolation. The dried and powdered stems (600.0 g) of *A. edulis* were extracted with EtOH (2 l, r.t., 3 d each time) to give a crude material (43.2 g), which was solubilized with MeOH/H_2O (80:20, *v*/*v*) and partitioned between hexane, AcOEt, and BuOH (3 × 800 ml each one). These extracts were examined for the occurrence of tannins using $FeCl_3$ and gelatin/NaCl tests, and evaluated for their antifungal activity. After evaporation, a part of the active AcOEt extract (0.50 g) was subjected to CC (*RP18*; gradient of MeOH/H_2O 05:95 to 100:0, *v*/*v*). A total of nine fractions were collected and combined into seven pools (1–7) on the basis of similar TLC profiles. *Fr. 3* (MeOH/H_2O 20:80, *v*/*v*; 84.4 mg) was submitted to a prep. *RP18* HPLC (MeOH/H_2O 18:82, *v*/*v*) to give four subfractions. *Subfr. 3.2* (16.0 mg) was purified by prep. *RP18* HPLC (MeOH/H_2O 20:80, *v*/*v*) to afford compounds **1** (t_R = 19.5 min, 5.7 mg) and **4** (t_R = 24.0 min, 2.4 mg). *Subfr. 3.3* (30.0 mg) was purified by prep. *RP18* HPLC (MeOH/H_2O 26:74, *v*/*v*) to afford compounds **3** (t_R = 24.0 min, 8.4 mg), **4** (t_R = 15.5 min, 2.9 mg), and **5** (t_R = 20.5 min, 8.7 mg). *Fr. 6* (MeOH/H_2O 80:20, *v*/*v*) yielded compound **2** (31.1 mg) with no further purification step.

6β-Hydroxy-7-epigardoside Methyl Ester (= *Methyl (1S,4aS,5S,6S,7aS)-1-(β-D-Glucopyranosyloxy)-1,4a,5,6,7,7a-hexahydro-5,6-dihydroxy-7-methylidenecyclopenta[c]pyran-4-carboxylate*; **1**). White amorphous powder. $[\alpha]_D^{28} = -17.49$ (*c* = 0.032, MeOH). UV (MeOH): 236 (3.01). IR (KBr): 3400, 2925, 1692, 1638, 1235, 1156, 947, 840. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 403.1289 ($[M-H]^-$, $C_{17}H_{24}O_{11}^-$; calc. 403.1246).

3β-O-[α-L-Rhamnopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl]-28-O-β-D-glucopyranoside Pomolate (= *1-O-[(3β)-3-[[6-Deoxy-α-L-mannopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oxy]-19-hydroxy-28-oxours-12-en-28-yl]-β-D-glucopyranose*; **2**). Brown amorphous powder. $[\alpha]_D^{26} = +2.43$ (*c* = 0.126, MeOH). ^{1}H- and ^{13}C-NMR: *Table* 2. HR-ESI-MS: 1127.5641 ($[M+Na]^+$, $C_{54}H_{88}NaO_{23}^+$; calc. 1127.5614). ESI-IT-MS^n: MS^2 (1139, $[M-H+2\,H_2O]^-$): 941; MS^3 (941, $[M-H-162]^-$): 795; MS^4 (795, $[M-H-162-146]^-$): 633; MS^5 (633, $[M-H-(2\times 162)-146]^-$): 471 ($[M-H-(3\times 162)-146]^-$).

Antifungal Assay. Microorganisms Used, and Growth Conditions. The test organisms included *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 22019), and *Cryptococcus neoformans* (ATCC 90012). The microorganisms were originally obtained from the

Mycology Laboratory of the Department of Clinical Analysis at São Paulo State University (UNESP). The yeasts were grown and maintained on Sabouraud-dextrose agar for 24 h at r.t.

Antimicrobial Susceptibility Testing. The antifungal activity tests were performed using the broth microdilution method as described in the M27-A2 document of the Clinical and Laboratory Standards Institute (CLSI) with modifications. The medium used was *RPMI 1640*, with L-glutamine buffered to pH 7.0 with 0.165M morpholinepropanesulfonic acid (MOPS), supplemented with 2% glucose. The test samples were dissolved in DMSO. The cell suspension was prepared in 0.85% saline soln. with an optical density equivalent to McFarland 0.5 and diluted to 1:100 in RPMI for the final concentration to be $1 \cdot 10^5$ to $5 \cdot 10^5$ CFU/ml. This suspension was inoculated onto a microdilution plate previously prepared with the test samples diluted to concentrations from 250 μg/ml to 0.4 μg/ml. The plates were incubated under agitation at 37° for 24 h for the *Candida* species, and 48 h for *Cryptococcus neoformans*. Fungal growth was evaluated visually and spectrophotometrically. The MIC was defined as the lowest concentration at which the optical density (OD) was reduced to 90% of the OD of the growth control well. Amphotericin B (3 μg/ml) was used as positive control and DMSO as a negative control. MIC values were determined from three independent experiments.

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