Oleanane Saponins and Glycerogalactolipids from the Leaves of *Guapira graciliflora*

by **Juliana A. Severi**[a][b], **Orlando Fertig**[a], **Inken Plitzko**[a], **Wagner Vilegas**[c], **Matthias Hamburger**[a], and **Olivier Potterat***[a]

[a]) University of Basel, Division of Pharmaceutical Biology, Klingelbergstrasse 50, CH-4056 Basel (phone: +41 61 267 15 34; fax: +41 61 267 14 74; e-mail: olivier.potterat@unibas.ch)

[b]) School of Pharmaceutical Sciences, UNESP – São Paulo State University, Araraquara 14800-900, Brazil

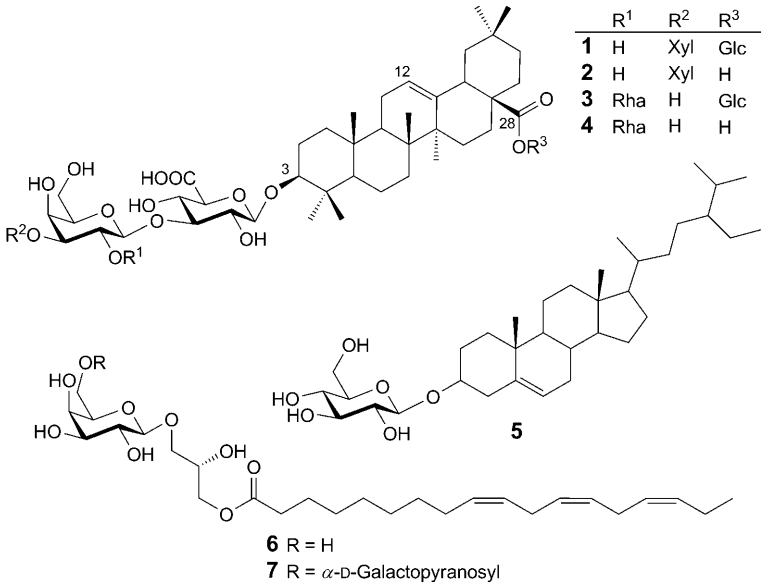
[c]) Institute of Chemistry, UNESP – São Paulo State University, Araraquara 14800-900, Brazil

Two new saponins, 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**1**) and the corresponding monodesmoside, 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic acid (**2**), have been isolated from the leaves of *Guapira graciliflora* (Nyctaginaceae), together with two further oleanane saponins, **3** and **4**, daucosterol (**5**), and two known glycerogalactolipids, **6** and **7**. The structures of the new compounds were established by extensive NMR and MS experiments, in conjunction with acid hydrolysis and sugar analysis. This is the first report on the phytochemistry of plants of the genus *Guapira*.

Introduction. – *Guapira graciliflora* (MART. ex J. A. SCHMIDT) LUNDEL belongs to the family Nyctaginaceae which includes 30 genera, and 390 herbaceous and shrub species distributed in tropical and subtropical regions [1]. It is a small tree endemic of Brazilian Atlantic forest and Cerrado. Infusions of the bark are used in Brazilian folk medicine for cicatrisation [2]. While there have been several phytochemical studies on the family Nyctaginaceae revealing a variety of secondary metabolites such as flavonoids [3][4], rotenoids [5][6], betacyanins [7][8], alkaloids [9], and saponins [10][11], no information is available on the chemical constituents of plants from the genus *Guapira*. This prompted us to undertake the phytochemical study of *G. graciliflora* as part of our investigations on Brazilian medicinal plants. We report here on the isolation and characterization of four oleanane saponins, including the new glycosides **1** and **2**, together with β-sitosterol 3-*O*-β-glucopyranoside (daucosterol) and two glycerogalactolipids from the leaves.

Results and Discussion. – The dry leaves of *G. graciliflora* were defatted with $CHCl_3$ and then extracted with MeOH. After removal of the solvent under reduced pressure, the MeOH extract was partitioned between BuOH and H_2O. Fractionation of the BuOH-soluble portion by a combination of *Sephadex LH-20* CC and semipreparative HPLC on *C-18* afforded compounds **1**–**7**.

Compound **1** was obtained as a colorless gum and gave a purple spot on TLC plate after spraying with anisaldehyde/H_2SO_4 reagent [12]. The molecular formula was



established as $C_{53}H_{84}O_{23}$ from the pseudo-molecular $[M-H]^-$ ion at m/z 1087.5339 (calc. 1087.5325) in the HR-ESI-MS spectrum, and from the NMR data. The ESI-MS spectrum showed a $[M-H]^-$ pseudo-molecular-ion peak at m/z 1087. Subsequent fragmentation by MS^2 and MS^3 experiments gave fragment ions at m/z 925 ($[M-H-162]^-$), 793 ($[M-H-162-132]^-$), 631 ($[M-H-162-132-162]^-$), and 455 ($[M-H-162-132-162-176]^-$), suggesting the presence of two hexose, one pentose, and one uronic acid residues. Acid hydrolysis afforded oleanolic acid as aglycone. The sugar residues were identified by TLC and GC analyses after derivatization with L-cysteine methyl ester as D-glucose, D-galactose, D-xylose, and glucuronic acid.

The ^{1}H-NMR spectrum exhibited signals corresponding to seven tertiary Me groups at δ(H) 0.70, 0.75, 0.86, 0.87 (2×), 0.99, 1.08; one trisubstituted olefinic moiety at δ(H) 5.16 (*Table 1*), and four anomeric H-atoms at δ(H) 4.38, 4.40, 4.44, and 5.28 (*Table 2*).

The ^{13}C-NMR spectrum presented 53 signals, from which 30 were attributed to the aglycone (*Table 1*) and 23 to the saccharidic portion (*Table 2*). With signals evidencing an olefinic bond (C(12)=C(13); δ(C) 121.6 and 143.3), a CO group (δ(C) 175.1), and seven Me groups (δ(C) 32.7 (C(29)), 27.3 (C(23)), 25.4 (C(27)), 23.3 (C(30)), 16.6 (C(26)), 16.4 (C(24)), and 15.1 (C(25))), the NMR data of the aglycone were in full agreement with those of oleanolic acid [13]. The presence of four sugar residues was confirmed by the anomeric C-atom signals at δ(C) 94.0, 102.1, 104.1 and 105.9, and characteristic signals in the region of δ(C) 60–80 ppm.

Detailed analysis of the HSQC, HSQC-TOCSY, and ^{1}H,^{1}H-COSY experiments allowed the assignment of all H-atoms to their corresponding C-atoms, and confirmed the identification of the sugars as glucuronic acid, glucose, galactose, and xylose. Vicinal coupling constants, J(1,2), of the anomeric H-atoms (δ(H) 5.28 (J = 8.0 Hz), 4.44 (J =

Table 1. *^{13}C- and ^{1}H-NMR Data of the Aglycone Portion of Compounds* **1** *and* **2** *in (D_6)DMSO.* δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	37.9	0.87–0.91 (*m*, H_a), 1.47–1.53 (*m*, H_b)	37.9	0.88–0.91 (*m*, H_a), 1.48–1.54 (*m*, H_b)
CH_2(2)	25.4	1.53–1.58 (*m*, H_a), 1.72–1.77 (*m*, H_b)	25.3	1.50–1.55 (*m*, H_a), 1.73–1.74 (*m*, H_b)
H–C(3)	87.9	3.08–3.13 (*m*)	87.9	3.11–3.18 (*m*)
C(4)	38.6		38.6	
H–C(5)	54.8	0.68–0.70 (*m*)	54.8	0.72–0.74 (*m*)
CH_2(6)	17.7	1.28–1.31 (*m*, H_a), 1.46–1.50 (*m*, H_b)	17.8	1.23–1.28 (*m*, H_a), 1.41–1.44 (*m*, H_b)
CH_2(7)	32.1[a])	1.22–1.28 (*m*, H_a), 1.33–1.42 (*m*, H_b)	32.3[a])	1.23–1.30 (*m*, H_a), 1.40–1.47 (*m*, H_b)
C(8)	38.7[b])		38.7[b])	
H–C(9)	46.9	1.45–1.53 (*m*)	47.0	1.47–1.53 (*m*)
C(10)	36.2		36.2	
CH_2(11)	22.4[c])	1.59–1.63 (*m*, H_a), 1.90–1.97 (*m*, H_b)	22.5[c])	1.58–1.61 (*m*, H_a), 1.89–1.92 (*m*, H_b)
H–C(12)	121.6	5.16 (br. *s*)	121.5	5.16 (br. *s*)
C(13)	143.3		143.8	
C(14)	41.2		41.2	
CH_2(15)	27.1	0.95–0.98 (*m*, H_a), 1.72–1.77 (*m*, H_b)	27.1	0.94–0.98 (*m*, H_a), 1.68–1.74 (*m*, H_b)
CH_2(16)	22.8[c])	1.77–1.86 (*m*, 2 H)	22.8[c])	1.77–1.84 (*m*, 2 H)
C(17)	45.8		45.4	
H–C(18)	40.6	2.75–2.83 (*m*)	40.7	2.79–2.84 (*m*)
CH_2(19)	45.4	1.07–1.14 (*m*, H_a), 1.60–1.67 (*m*, H_b)	45.6	1.03–1.10 (*m*, H_a), 1.56–1.64 (*m*, H_b)
C(20)	30.2		30.4	
CH_2(21)	33.1	1.16–1.20 (*m*, H_a), 1.30–1.36 (*m*, H_b)	33.3	1.20–1.24 (*m*, H_a), 1.38–1.42 (*m*, H_b)
CH_2(22)	31.5[a])	1.48–1.54 (*m*, H_a), 1.57–1.62 (*m*, H_b)	32.0[a])	1.41–1.50 (*m*, H_a), 1.60–1.64 (*m*, H_b)
Me(23)	27.3	0.99 (*s*)	27.4	0.99 (*s*)
Me(24)	16.4	0.75 (*s*)	16.5	0.75 (*s*)
Me(25)	15.1	0.86 (*s*)	15.1	0.87 (*s*)
Me(26)	16.6	0.70 (*s*)	16.8	0.70 (*s*)
Me(27)	25.4	1.08 (*s*)	25.5	1.08 (*s*)
C(28)	175.1		178.6	
Me(29)	32.7	0.87 (*s*)	32.8	0.87 (*s*)
Me(30)	23.3	0.87 (*s*)	23.3	0.87 (*s*)

[a]) Assignments with the same superscript in each column may be interchanged. [b]) Overlapped by the solvent signal. δ derived from HMBC J^3 correlation Me(26)/C(8). [c]) Assignments with the same superscript in each column may be interchanged.

7.0 Hz), 4.41 ($J = 8.0$ Hz), and 4.38 ($J = 8.0$ Hz)) indicated diaxial coupling for all sugars, indicative of β-configuration. The sites of glycosylation and the interglycosidic linkages were determined on the basis of the HMBC spectrum. Long-range correlations (^3J) were observed between the anomeric H-atom of glucose (δ(H) 5.28) and the CO C-atom (δ(C) 175.1 (C(28))) as well as between the anomeric H-atom of glucuronic acid (δ(H) 4.38) and C(3) of the aglycone (δ(C) 87.9), thus revealing the bidesmosidic nature of the saponin. Two further key correlations were observed between the H–C(3) of glucuronic acid (δ(H) 3.33–3.39) and the anomeric C-atom of galactose (δ(C) 102.1), and between the anomeric H-atom of xylose (δ(H) 4.41) and C(3) of the galactose residue (δ(C) 82.2; *Fig.*). This indicated that a sugar chain [β-

Table 2. *^{13}C- and ^{1}H-NMR Data of the Glycosidic Portion of Compounds* **1** *and* **2** *in (D_6)DMSO.* δ in ppm, J in Hz.

	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
3-*O*-GlcAc[a])				
H–C(1′)	104.1	4.38 (*d*, *J* = 8.0)	104.1	4.34 (*d*, *J* = 7.5)
H–C(2′)	72.3	3.22–3.27 (*m*)	72.6	3.21–3.26 (*m*)
H–C(3′)	88.3	3.33–3.39 (*m*)	88.1	3.32–3.38 (*m*)
H–C(4′)	70.1	3.43–3.48 (*m*)	70.3	3.42–3.47 (*m*)
H–C(5′)	75.1	3.60–3.64 (*m*)	75.0	3.58–3.64 (*m*)
C(6′)	170.1		171.1	
Gal[b])				
H–C(1″)	102.1	4.44 (*d*, *J* = 7.0)	102.1	4.46 (*d*, *J* = 7.0)
H–C(2″)	72.8	3.59–3.62 (*m*)	72.9	3.59–3.65 (*m*)
H–C(3″)	82.2	3.58–3.64 (*m*)	82.1	3.60–3.66 (*m*)
H–C(4″)	67.5	3.69–3.71 (*m*)	67.5	3.70–3.74 (*m*)
H–C(5″)	75.4	3.48–3.53 (*m*)	75.3	3.52–3.57 (*m*)
CH_2(6″)	60.4	3.47–3.52 (*m*), 3.54–3.59 (*m*)	60.3	3.49–3.62 (*m*, 2 H)
Xyl[c])				
H–C(1‴)	105.9	4.41 (*d*, *J* = 8.0)	105.8	4.40 (*d*, *J* = 7.5)
H–C(2‴)	74.4	3.08–3.12 (*m*)	74.4	3.08–3.14 (*m*)
H–C(3‴)	75.8	3.18–3.23 (*m*)	75.8	3.19–3.24 (*m*)
H–C(4‴)	69.0	3.30–3.35 (*m*)	69.1	3.30–3.37 (*m*)
CH_2(5‴)	65.9	3.15–3.20 (*m*), 3.66–3.72 (*m*)	65.9	3.15–3.21 (*m*), 3.67–3.72 (*m*)
28-*O*-Glc[d])				
H–C(1⁗)	94.0	5.28 (*d*, *J* = 8.0)		
H–C(2⁗)	72.2	3.13–3.19 (*m*)		
H–C(3⁗)	76.5[e])	3.24–3.29 (*m*)		
H–C(4⁗)	69.4	3.14–3.20 (*m*)		
H–C(5⁗)	77.6[e])	3.17–3.22 (*m*)		
CH_2(6⁗)	60.5	3.46–3.51 (*m*), 3.61–3.68 (*m*)		

[a]) GlcAc = β-Glucopyranosiduronic acid. [b]) Gal = β-D-Galactopyranosyl. [c]) Xyl = β-D-Xylopyranosyl. [d]) Glc = β-D-Glucopyranosyl. [e]) Assignments may be interchanged.

xylopyranosyl-(1 → 3)-β-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl] [14] was attached at C(3), and the C(28) position was esterified by a β-glucopyranosyl moiety.

On the basis of these evidences, the structure of **1** was determined to be the new saponin 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

The molecular formula of compound **2** was established as $C_{53}H_{72}O_{17}$ from the $[M-H]^-$ pseudo-molecular-ion peak at m/z 925.4820 (calc. 925.4797) in the HR-ESI-MS spectrum, and from the NMR data. MS^2 of the $[M-H]^-$ ion gave an intense fragment at m/z 793 which indicated the presence of a terminal pentosyl residue. Acid hydrolysis afforded D-xylose, D-galactose, and glucuronic acid. The ^{1}H- and ^{13}C-NMR data assigned to the aglycone and the 3-*O*-glycosidic chain were closely similar to those of **1** (*Tables 1* and *2*). On the other hand, no β-glucopyranosyl unit was detected in the spectra, and the resonance of C(28) at δ(C) 178.6 was shifted downfield compared to

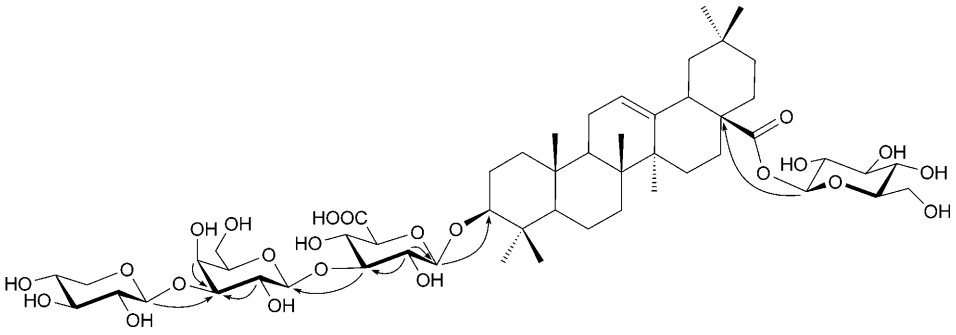


Figure. *Key HMBCs* (H → C) *of compound* **1**

that of **1**, suggesting the presence of a free carboxy group. HMBC Correlations confirmed the interglycosidic linkages and definitively established the structure of **2** as 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]-oleanolic acid.

Compounds **3** (ESI-MS: *m*/*z* 1101 $[M-H]^-$) and **4** (ESI-MS: *m*/*z* 939 $[M-H]^-$; ESI-MS^2 (939): *m*/*z* 793) were identified by extensive analysis of their ^{1}H- and ^{13}C-NMR data, and comparison with literature values [13–15] as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester and 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic acid, respectively. The sites of glycosylation and the interglycosidic linkages in **3** are confirmed by 2D-NMR analysis including HSQC-TOCSY and HMBC experiments. Alkaline hydrolysis of **3** gave **4**, which was identified by comparison of the ^{1}H-NMR and HSQC data of the hydrolysis product with those of the isolated compound as well as co-chromatography on HPLC/ESI-MS, thus confirming the structural relationship between both compounds. The absolute configuration of the L-rhamnose, D-galactose, and D-glucose residues was established by GC analysis after acid hydrolysis. The structure of **3** was previously assigned to a saponin isolated from *Ximenia americana* (Olacaceae) [16], while saponin **4** is described here for the first time as a natural product. The latter was reported as a hydrolysis product of **3** [16]. Since ^{1}H-NMR data reported in [16] are fragmentary, and δ(C) assignments not supported by any 2D-NMR experiments, NMR data of the glycosidic portion of saponins **3** and **4** are presented in *Table 3*. Compound **5** was identified as daucosterol (= β-sitosterol 3-*O*-β-D-glucopyranoside) [17]. The ^{1}H- and ^{13}C-NMR data of compounds **6** and **7** revealed, in both compounds, the presence of a fatty acid residue, a carbohydrate moiety, and a glycerol unit. The compounds were identified as 3-*O*-β-D-galactopyranosyl-1-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-*sn*-glycerol [18][19] and (2*S*)-3-*O*-[α-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl]-1-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-*sn*-glycerol (gingerglycolipid A) [20], respectively, by comparison of their spectroscopic data with those in the literature. The connectivity in both molecules was confirmed by 2D-NMR experiments. The optical rotation of **7** ($[\alpha]_D^{25} = +30$ ($c = 0.5$, MeOH); [20]: $[\alpha]_D^{20} = +37.7$ ($c = 10.0$, MeOH)) was consistent with the reported absolute configuration. The weak optical activity of **6** was not conclusive.

Table 3. *^{13}C- and ^{1}H-NMR Data of the Glycosidic Portion of Compounds* **3** *and* **4** *in (D_6)DMSO.* δ in ppm, J in Hz.

	3		**4**	
	δ(C)	δ(H)	δ(C)[a])	δ(H)
3-*O*-GlcAc[b])				
H–C(1′)	105.4	4.22 (*d*, *J* = 7.5)	104.8	4.18 (*d*, *J* = 8.0)
H–C(2′)	72.7[c])	3.09–3.21 (*m*)	73.2[c])	3.18–3.24 (*m*)
H–C(3′)	85.4[d])	3.37–3.48 (*m*)	83.5	3.45–3.50 (*m*)
H–C(4′)	70.6	3.28–3.34 (*m*)	70.1	3.27–3.33 (*m*)
H–C(5′)	75.4	3.37–3.44 (*m*)	74.6[e])	3.35–3.40 (*m*)
C(6′)	170.5		n.o.[f])	
Gal[g])				
H–C(1″)	102.3	4.61 (*d*, *J* = 7.5)	101.2	4.65 (*d*, *J* = 7.5)
H–C(2″)	73.8	3.53–3.59 (*m*)	74.1	3.52–3.58 (*m*)
H–C(3″)	74.3	3.45–3.50 (*m*)	73.9[e])	3.43–3.49 (*m*)
H–C(4″)	68.9	3.60–3.66 (*m*)	68.4	3.61–3.66 (*m*)
H–C(5″)	75.2	3.37–3.44 (*m*)	74.6[e])	3.35–3.40 (*m*)
CH_2(6″)	60.6	3.49–3.58 (*m*)	60.0	3.51–3.59 (*m*)
Rha[h])				
H–C(1‴)	100.0	5.04 (br. *s*)	99.7	5.04 (br. *s*)
H–C(2‴)	70.4	3.68–3.72 (*m*)	69.9	3.69–3.75 (*m*)
H–C(3‴)	70.0	3.57–3.63 (*m*)	70.0	3.59–3.66 (*m*)
H–C(4‴)	72.5[c])	3.18–3.21 (*m*)	72.2[c])	3.16–3.22 (*m*)
H–C(5‴)	67.5	4.01–4.07 (*m*)	67.4	4.01–4.08 (*m*)
CH_3(6‴)	17.9	1.11 (*d*, *J* = 6.0)	17.4	1.10 (*d*, *J* = 5.5)
28-*O*-Glc[i])				
H–C(1⁗)	94.2	5.26 (*d*, *J* = 8.0)		
H–C(2⁗)	72.6[c])	3.09–3.21 (*m*)		
H–C(3⁗)	76.8[e])	3.21–3.27 (*m*)		
H–C(4⁗)	69.6	3.11–3.18 (*m*)		
H–C(5⁗)	77.9[e])	3.15–3.19 (*m*)		
CH_2(6⁗)	60.8	3.61–3.66 (*m*), 3.44–3.49 (*m*)		

[a]) ^{13}C-NMR Data of **4** are derived from HSQCs. [b]) GlcAc = β-Glucopyranosiduronic acid. [c]) Assignments with the same superscript in each column may be interchanged. [d]) Signal observed at δ(C) 84.0 in HSQC spectrum. [e]) Assignments with the same superscript in each column may be interchanged. [f]) n.o. = Not observed. [g]) Gal = β-D-Galactopyranosyl. [h]) Rha = α-L-Rhamnopyranosyl. [i]) Glc = β-D-Glucopyranosyl.

Plants from the Nyctaginaceae family are mainly represented in Brazil by the genera *Neea*, *Boerhaavia*, *Bougainvillea*, *Mirabilis*, *Pisonia*, and *Guapira* [21]. Within the family, the taxonomic classification based on morphological characters is difficult [22]. Hence, additional informations such as chemotaxonomic data are desirable. Our study is the first phytochemical report on the genus *Guapira*. Oleanane saponins related to the isolated compounds have been already isolated from various plants belonging to the Nyctaginaceae family. Bidesmosidic saponins containing 2–5 sugar units were found in *Pisonia umbellifera* [10], *Colignonia scandens* [11], and *Bougainvillea spectabilis* [23]. The report of oleanane saponins in the genus *Guapira* represents, therefore, a useful contribution to the chemotaxonomy of the Nyctaginaceae.

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Amersham Biosciences*), *C-605* pump, *C-660* collector (*Büchi Labortechnik*). TLC: precoated SiO_2 Al sheets (F_{254}, 0.22-μm thickness, *Merck*). Anal. HPLC: *Alliance 2695* separation module (*Waters*) equipped with a *996 DAD* detector and a *2000ES ELSD* detector (*Alltech*, N_2 as carrier gas, 2.9 l/min, 100° dry tube, impactor mode off); *SunFire C18* column (3 × 150 mm i.d., 5 μm; *Waters*). Semi-prep. HPLC: *Agilent 1100 Series* instrument with a DAD detector; *SunFire C18* column (10 × 150-mm i.d., 5 μm; *Waters*) equipped with a precolumn (10 × 10 mm); flow-rate 4 ml/min; UV detection at 209 nm. GC: *HP 5890 Series II* gas chromatograph equipped with a *HP 5971* mass-selective detector (*Hewlett Packard*); injector temp. 180°; detector temp. 260°; He as carrier gas. Optical rotation: *Jasco P-1020* (for **1** and **7**) and *Perkin-Elmer 341* (for **2**) polarimeters. IR Spectra: *Jasco FT/IR-4100* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR: *Avance III* spectrometer (*Bruker*) equipped with a 5-mm BBI probe (^{13}C-NMR) or a 1-mm TXI microprobe (^{1}H- and 2D-NMR); at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Esquire 3000 plus* ion-trap mass spectrometer (*Bruker*). HR-ESI-MS: *MicrOTOF* (*Bruker*).

Plant Material. The leaves of *Guapira graciliflora* were collected in May 2007 at Itapetininga City, São Paulo State, Brazil, and identified by Prof. Dr. *Jorge Yoshio Tamashiro* from the Instituto de Biologia, Unicamp, São Paulo. A voucher specimen (HUEC 1441) has been deposited with the Herbarium of the Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo State, Brazil.

Extraction and Isolation. The leaves were dried (40°, 4 d under air circulation) and powdered. The powdered dried leaves (500 g) were defatted by maceration with $CHCl_3$ (2 l) and subsequently extracted by percolation with MeOH (5 l) at r.t. The solvents were evaporated at 35° under reduced pressure to afford $CHCl_3$ (8 g) and MeOH (49 g) extracts. A portion (15 g) of the MeOH extract was partitioned between BuOH (2 × 500 ml) and H_2O (500 ml). The BuOH layer was concentrated under reduced pressure to give a dark brown residue (7 g), which was chromatographed on a *Sephadex LH-20* column (85 × 5 cm i.d.) with MeOH. A total of 153 fractions (10 ml) were collected and analyzed by TLC ($CHCl_3$/MeOH/PrOH/H_2O 5:6:1:4 (org. phase)); detection with anisaldehyde/H_2SO_4 reagent and HPLC/ELSD for the presence of saponins. Selected fractions were combined and subsequently separated by reversed-phase (RP) semi-prep. HPLC-UV with MeCN/H_2O mixtures. Compound **1** (7 mg; t_R 12 min) was obtained from *Fr. 20* (150 mg) with MeCN/H_2O 33:67; additional 8 mg of **1** were obtained from *Fr. 24* (101 mg) with MeCN/H_2O 33:67 (t_R 12 min). Compound **3** (5 mg; t_R 13 min), was purified from *Fr. 19* (180 mg) with MeCN/H_2O 29:71. Compounds **2** (2.0 mg; t_R 21 min), **4** (2.5 mg; t_R 13 min), and **6** (2.5 mg; t_R 26 min) were isolated from *Fr. 37* (470 mg) with a gradient of MeCN/H_2O (25:75 → 40:60 in 10 min, 40:60 → 55:45 in 7 min, 55:45 → 65:35 in 6 min, 65:35 → 85:15 in 7 min). Compounds **5** (5 mg; t_R 18 min) and **7** (2 mg; t_R 20 min) were obtained from *Fr. 38* (420 mg) and *Fr. 24* (101 mg), resp., with MeCN/H_2O (33:67 for 20 min, 33:67 → 80:20 in 25 min).

3-O-[β-D-Xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic Acid 28-O-β-D-Glucopyranosyl Ester (=*1-O-[(3β)-28-Oxo-3-{[β-D-xylopyranosyl-(1 → 3)-β-D-galacto-pyranosyl-(1 → 3)-β-D-glucopyranuronosyl]oxy}olean-12-en-28-yl]-β-D-glucopyranose*; **1**). Colorless gum. $[\alpha]_D^{25} = +17$ ($c = 0.4$, MeOH). IR (KBr): 3425, 2929, 1737, 1718, 1635, 1460, 1074. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Tables 1* and *2*. ESI-MS: 1087 ($[M-H]^-$). ESI-MS^2 (1087): 925 ($[M-H-162]^-$). ESI-MS^3 (925): 793 ($[M-H-162-132]^-$), 731, 631 ($[M-H-162-132-162]^-$), 613, 551, 483, 455 ($[M-H-162-132-162-176]^-$). HR-ESI-MS: 1087.5339 ($[M-H]^-$, $C_{53}H_{83}O_{23}^-$; calc. 1087.5325).

3-O-[β-D-Xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-glucuronopyranosyl]oleanolic Acid (=*(3β)-28-Hydroxy-28-oxoolean-12-en-3-yl-β-D-xylopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-β-D-glucopyranosiduronic Acid*; **2**). Colorless gum. $[\alpha]_D^{20} = 22$ ($c = 0.25$, MeOH). ^{1}H- and ^{13}C-NMR

((D$_6$)DMSO): *Tables 1* and *2*. ESI-MS: 925 ([M – H]$^-$). ESI-MS^2 (925): 793 ([M – H – 132]$^-$). HR-ESI-MS: 925.4820 ([M – H]$^-$; $C_{47}H_{73}O_{18}^-$; calc. 925.4797).

Acid Hydrolysis. Compound **1** or **2** (1 mg) was heated at 105° for 1 h in 1 ml of 2M CF_3COOH (TFA). The mixture was extracted with AcOEt (2 × 0.5 ml). Oleanolic acid was detected in the org. layer by TLC comparison (hexane/AcOEt 4:6) with an authentic sample. The aq. phase was freeze-dried, and the sugar residues were analyzed by TLC co-chromatography with authentic samples using $CHCl_3$/MeOH/AcOH/H_2O 60:32:12:8 and i-PrOH/AcOEt/H_2O 7:2:1 as developing solvents. Detection by spraying with *p*-anisidinephtalate reagent: xylose, galactose, and glucuronic acid (weak spot) from **1** and **2**; glucose from **2**.

For the determination of their absolute configuration, the sugars re-dissolved in anh. pyridine were derivatized with L-cysteine methyl ester hydrochloride (200 µl, 60°, 1 h) and subsequently silylated with hexamethyldisilazane and Me_3SiH (*Fluka*) in pyridine (2:1:10; 300 µl; 60°, 30 min) [24]. GC Analysis on a cap. *DB-225MS* column (30 m × 0,25 mm i.d., 0,25 µm; *Agilent*; column temp. 150° for 2 min, then 5°/min. to 210°, then 10°/min to 240°): D-xylose (t_R 16.3 min) and D-galactose (t_R 18.2 min) from **1** and **2**; D-glucose (t_R 17.8 min) from **1**.

Compounds **3** or **4** (*ca.* 0.1 mg) were hydrolyzed following the procedure described above. GC Analysis: D-galactose and L-rhamnose (t_R 16.8 min) from **3** and **4**; D-glucose from **3**. The absolute configuration of glucuronic acid in **1**–**4** has not been established.

Alkaline Hydrolysis of **3**. A soln. of **3** (0.5 mg) in 0.5N KOH (0.5 ml) was heated at 100° for 2 h. After cooling, the mixture was poured into H_2O (10 ml), neutralized with 2N HCl, and extracted with BuOH (2 × 10 ml) to give 0.4 mg of **4**. Compound identity was established by ^{1}H- and HSQC-NMR data as well as co-chromatography on HPLC/ESI-MS with the isolated compound.

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