Notes

Triterpenes and Saponins from *Rudgea viburnioides*

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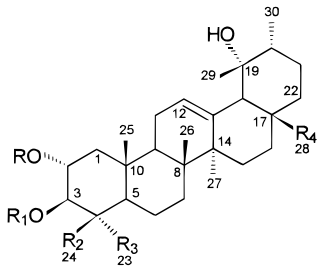
A novel triterpene, viburgenin (**1**), has been isolated from an extract of the ripe fruit rinds of *Rudgea viburnioides*, together with the known saponins, arjunglucoside I and trachelosperosides B-1 and E-1, and the triterpenes trachelosperogenin B (**2**) and arjungenin. Compound **2** was previously obtained as a product from enzymatic hydrolysis, and it is reported for the first time as a natural product. The structure of compound **1** was determined as 2α,3*β*,19α,23,24-pentahydroxyurs-12-ene by extensive use of 1D and 2D NMR spectroscopic methods. Compound **1** exhibited moderate antifungal activity against *Cladosporium cladosporioides*.

Rudgea viburnioides (Cham.) Benth. (Rubiaceae) is a small tree widely distributed over the "cerrado" in Brazil. The leaves and bark of this species are used traditionally in central Brazil as an antirheumatic and antisyphilitic, as well as in the treatment of dyspepsia.[1] A literature search has revealed the absence of any chemical work on this species. In our continuing chemical and biological investigations of plants from the family Rubiaceae, herein we report the isolation of new triterpenes **1** and **2**, together with the known triterpene arjungenin, the saponins trachelosperosides B-1 and E-1, and arjunglucoside I from the ripe fruit rinds of *R. viburnioides*. Compound **1** showed moderate antifungal activity against *Cladosporium cladosporioides* (Fres.) de Vries (Dematiaceae).



	R	R_1	R_2	R_3	R_4
1	H	H	CH_2OH	CH_2OH	CH_3
1a	Ac	Ac	CH_2OAc	CH_2OAc	CH_3
2	H	H	CH_2OH	CH_2OH	CO_2H

The EtOAc-soluble fraction of the aqueous ethanolic 40% extract obtained from the ripe fruit rinds of *R. viburnioides* yielded two new triterpenes **1** and **2** and four known compounds on repeated chromatographic purification on silica gel, Sephadex LH-20, and Diaion HP-20 columns. Several known saponins and triterpenes were identified by their spectral data and comparison with literature, respectively, as trachelosperoside B-1,[2] trachelosperoside E-1,[3] arjunglucoside I,[3] and arjungenin,[3,4] respectively. In addition, trachelosperogenin B (**2**), the aglycon of trachelosperoside B-1,[2] is reported for the first time as a natural product.

Viburgenin (**1**), obtained as a colorless powder, gave a positive Libermann−Buchard reaction. The eletrospray mass spectral analysis (ES-MS) of **1** showed the molecular ion peak $[M + Na]^+$ at *m*/*z* 513, corresponding to a molecular formula of $C_{30}H_{50}O_5$. Six units of unsaturation were evident from the molecular formula with five of them due to a pentacyclic ring system and one due to the presence of a double bond. The IR spectrum indicated the presence of hydroxyl groups (3600, 3450 cm^{-1}). The ^1H NMR spectrum (400 MHz, C_5D_5N) showed spectral features typical of a highly oxygenated triterpene. The acetylation of **1** yielded a tetraacetate **1a** (*δ* H 2.01, 2.00, 1.98, and 1.96, each 3H s) (Table 1), which had the molecular formula $C_{38}H_{67}O_9$, as deduced from its MS and ^{13}C NMR data (see the Experimental Section). The EIMS of **1** showed intense ion fragments at *m*/*z* 256 and *m*/*z* 234, due to the *retro* Diels−Alder fragmentation, characteristic for a Δ^{12} amyrin-type triterpenoid, which were attributed to the presence of four hydroxyl functions in the A/B and only one hydroxyl function in the D/E ring systems, respectively. The hydroxyl groups of **1** were located at C-2, C-3, C-19, C-23, and C-24 by comparison of the carbon and proton chemical shifts with the reported values for related triterpenes.[2−4] Complete assignments of the ^1H and ^{13}C NMR signals of **1** were carried out by $^1H-^1H$ COSY and HMQC for direct $^1J_{H-C}$ conectivities and HMBC for long-range $^2J_{H-C}$ and $^3J_{H-C}$ correlations

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Table 1. NMR Spectral Data of Viburgenin (**1**)[a]

position	δ ^{13}C[b]	δ ^{1}H[c]	HMBC (H to C)
1	48.0	2.08 dd (4.0, 9.0) H_a 1.65 dd (9.0, 12.0) H_b	
2	69.1	4.45 ddd (4.0, 9.0, 12.0)	C-4, C-10
3	79.8	3.66 d (9.0)	C-5, C-23, C-24
4	47.8		
5	48.3	1.34 dd (4.7, 10.7)	C-3, C-7, C-10
6	19.4	1.30−1.32 m	
7	33.5	1.43 m	C-5, C-9
8	40.6		
9	48.0	1.63 dd (12.5, 5.2)	C-7, C-10, C-12,
10	38.0		
11	24.0	1.94 br, m, H_a, 2.05 m H_b	
12	128.4	5.58 br s	C-9, C-14, C-18
13	139.0		
14	42.1		
15	29.3	1.76 m, H_a, 1.20 m, H_b	
16	25.2	1.75−2.10 m	
17	31.9		
18	53.0	3.18 s	C-12, C-19, C-20, C-28
19	73.0		
20	41.0	1.19 m	
21	25.3	1.45−1.89 m	
22	37.5	1.60 m	
23	64.3	3.99 d (11.0) H_a 4.62 d (11.0) H_b	C-3, C-4, C-5
24	62.9	4.28 d (11.0) H_a 4.88 d (11.0) H_b	C-3, C-4, C-5
25	16.7	1.02 s	C-1, C-5, C-10
26	16.9	0.65 s	C-7, C-8, C-14
27	24.5	1.22 s	C-8, C-13, C-14
28	27.3	1.17 s	C-17, C-18, C-22
29	27.0	1.26 s	C-18, C-19, C-20
30	16.9	0.92 d (6.0)	C-19, C-20, C-21

[a] Assignments of ^{1}H and ^{13}C NMR data were aided by DCCOSY, DEPT, HMQC, and HMBC experiments. [b] Spectrum recorded at 100 MHz. [c] Spectrum recorded at 400 MHz.

(Table 1). The ^{1}H NMR spectrum (Table 1) exhibited the presence of an olefinic proton (δ 5.58; br s) and six signals [δ 4.45 (ddd, J = 4.0, 9.0, 12.0 Hz), 3.66 (d, J = 9.0 Hz), 3.99 and 4.62 (d, J = 11.0 Hz), 4.28 and 4.88 (d, J = 11.0 Hz)] corresponding to hydroxymethine and hydroxymethylene protons. In addition, the ^{1}H NMR spectrum showed signals due to five tertiary methyl groups (δ 0.65, 1.02, 1.17, 1.22, and 1.26) and to a secondary methyl group (δ 0.92; d, J = 6.0 Hz). These values are quite similar to those observed for the aglycon of tracheloperoside B-1,[2,3] a known compound also isolated in this study. The ^{13}C NMR (Table 1) spectrum of **1** was almost superimposable with that of **2** (see the Experimental Section), with the only differences concerning the C-17 and C-28 resonances (Table 1). The chemical shifts for these carbons in compound **1** were δ 31.9 and 27.3, respectively, which differed significantly from those of compound **3** (δ 48.8 and 179.9) due the presence of carboxyl group at C-28.[2] Furthermore, the ^{13}C NMR spectrum, analyzed with the aid of DEPT and HETCOR spectra, showed the presence of two oxygenated methines (δ_C 69.1, 79.8) and methylenes (δ_C 62.9, 64.3). The foregoing suggested the presence of a urs-12-ene triterpene system hydroxylated at C-19. The relative configurations of the chiral carbons at C-2 and C-3 of **1** were assigned mainly on the basis of ^{1}H NMR coupling data and comparison with those reported for related triterpenes.[2−4] Ursane and oleanane triterpenes and their derived saponins are secondary metabolites encountered frequently in various species in the Rubiaceae.[5−7] However, the occurrence of polyoxygenated ursolic and oleanolic triterpene derivatives is being described now for the first time in plants of this family. The known compounds already were reported as the main secondary metabolites of *Trachelospermum asiaticum* (Apocynaceae), a traditional Japanese remedy known as "Teika-Kazura".[2] The presence of these compounds in species of the Apocynaceae and Rubiaceae could disclose possible evolutionary tendencies among these two families, considering the fact that Rubiaceae and Apocynaceae belong to the same morphological group of Gentianales.

Viburgenin (**1**) showed moderate antifungal activity against *C. cladosporidoides* in a TLC bioassay.[8] The amount of **1** required to inhibit fungal growth on a TLC plate was 50 μg. The inhibitory power displayed by **1** was considered moderate by comparison with the controls, nystatin (5 μg) and miconazole (1 μg).

Experimental Section

General Experimental Procedures. Optical activities were measured on a Polamat A (Carl Zeiss) polarimeter. IR spectra were recorded on Perkin-Elmer 1710 spectrometer. NMR spectra were recorded in C_5D_5N or $CDCl_3$ on a Varian Unity 400 NMR instrument at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using TMS as internal standard. The DEPT experiments were performed using polarization transfer pulses of 90 and 135°, respectively. The ES-MS spectra were obtained at 70 eV on a VG Platform II and EIMS spectra on a VG 70 70 E-HF instrument. For column chromatography, Si gel 60, 70−230 mesh, Sephadex LH-20 and Diaion HP-20, was used. TLC analysis was carried out on precoated silica gel 60 F_{254} and RF-18F_{254} plates. Spots were visualized by anisaldehyde 2% in H_2SO_4 after heating.

Plant Material. The ripe fruit rinds of *R. viburnioides* were collected in Campininha Farm, Mogi-Guaçú, SP, Brazil, by M. C. M. Young in December 1993. A voucher specimen (no. SP-196941) has been deposited in the herbarium of the Botanic Garden of São Paulo, Brazil.

Bioassay. The experimental method utilized in the bioautography assay has been described elsewhere.[8]

Extraction and Isolation. Dried and powdered ripe fruit rinds (2.0 kg) of *R. viburnioides* were extracted with aqueous EtOH (3×) at room temperature. The EtOH extract was evaporated in vacuo to give a crude extract (77 g), which was washed with EtOAc several times. The EtOAc-soluble part (2.9 g) was fractionated on silica gel column (120 g) using EtOAc−MeOH gradient elutions to give six fractions. Fraction 4 (1.0 g, EtOAc−MeOH 77:15) was further fractionated by silica gel flash chromatography using $CHCl_3$−MeOH (64:36) and $CHCl_3$−MeOH−H_2O (64:36:8). Altogether, 146 fractions were collected, and fractions 21−32 (240 mg) were fractionated on Sephadex LH-20 using MeOH−$CHCl_3$ (9:1) to give **1** (24 mg), trachelosperoside B-1 (15 mg), trachelosperogenin B (**2**, 51 mg), and trachelosperoside E-1 (36 mg). Fractions 95−117 (57 mg) were submitted to chromatography column on Diaion HP-20 using MeOH−H_2O (1:1). The combined fractions 7−9 from this column were fractionated further on silica gel (13 g) using $CHCl_3$−MeOH−H_2O (77:15:8) to give trachelosperoside B (37 mg) and arjungenin (12 mg). The residue (10 g) obtained from washing with EtOAc was extracted with *n*-BuOH (3×) at room temperature. The

n-BuOH extract (2.4 g) was fractionated on Si gel (140 g) using $CHCl_3$−MeOH (64:36) and then increasing the polarity to $CHCl_3$−MeOH−H_2O (64:36:8). Fractions 95−106 (382 mg) were further submitted to silica gel flash chromatography (3.5 g) using $CHCl_3$−MeOH−*n*-PrOH−H_2O (9:12:2:8, lower phase) to yield the saponins trachelosperoside E (30 mg) and arjungenin (39 mg).

Viburgenin (1): amorphous powder; $[\alpha]^{25}_D$ +20.5° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3450, 1640, 1422 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ES-MS $(M + Na)^+$ *m*/z 513; EIMS *m*/z 490 (20) $(M)^+$, 475 (55), 457 (60), 256 (100), 234 (26), 231 (10), 213 (5), 201 (70); HREIMS *m*/z 490.7225 (calcd for $C_{30}H_{50}O_5$, 490.3780).

Acetylation of 1. Compound **1** (20 mg) was treated with Ac_2O in pyridine at room temperature for 24 h. The reaction mixture was purified by preparative TLC using *n*-hexane−EtOAc (8.5:1.5) as eluent to obtain **1a** (8.2 mg).

Viburgenin tetraacetate (1a): gum; $[\alpha]^{25}_D$ +4.8° (*c* 0.50, $CHCl_3$); IR (KBr) ν_{max} 1720, 1640, 1420 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.26 (1H, br s, H-12), 5.09 (2H, br s, H-24), 4.22 (2H, br s, H-23), 4.22 (1H, br d, *J* = 8.0 Hz, H-2), 4.20 (1H, d, *J* = 8.0 Hz, H-3), 3.20 (1H, s, H-18), 2.01, 2.00, 1.98, 1.96 (12 H, s, each OAc), 1.22 (×2), 1.16, 1.05, 0.66, (15H, s, each CH_3), 0.90 (3H, d, *J* = 6.5 Hz, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4 (s, C_3OCOCH_3), 170.3 (s, $C_{23}OCOCH_3$), 170.2 (s, C_{24}-$OCOCH_3$), 170.1 (s, C_2OCOCH_3) 138.0 (s, C-13), 128.5 (d, C-12), 74.3 (d, C-3), 73.0 (s, C-19), 69.3 (d, C-2), 63.4 (t, C-24), 62.5 (t, C-23), 52.8 (d, C-18), 48.0 (d, C-5), 47.4 (d, C-9), 45.2 (s, C-4), 43.8 (t, C-1), 41.1 (s, C-14), 41.0 (d, C-20), 39.9 (s, C-8), 37.6 (s, C-10), 37.3 (t, C-22), 32.7 (t, C-7), 31.9 (s, C-17), 29.3 (t, C-15), 27.4 (q, C-29), 27.3 (q, C-28), 25.4 (t, C-16), 25.3 (t, C-21), 24.2 (q, C-27), 23.8 (t, C-11), 16.3 (q, C-26), 16.2 (q, C-30), 16.0 (q, C-25), 20.9, 20.8, 20.7, 20.5,(s, $OCOCH_3$ x 4), 19.2 (t, C-6); ES-MS $[M + Na]^+$ *m*/*z* 680.

Trachelosperogenin B (2): crystals; mp 248−252 °C (MeOH) (lit.[5] mp 245−255 °C); $[\alpha]^{25}_D$ +25° (*c* 0.5, MeOH) (lit.[5] $[\alpha]^{18}_D$ +25.2°); ^1H NMR (400 MHz C_5D_5N) δ 5.61 (1H, d, *J* = 8.0 Hz, H-1′), 5.50 (1H, br s, H-12), 4.90, 4.28 (2H, d, *J* = 12 Hz, H-24), 4.65, 4.05 (2H, d, *J* = 12.0 Hz, H-23), 4.50 (1H, d t, *J* = 3.9; 10.0 Hz, H-2β), 4.40 (1H, d, *J* = 10.0 Hz, H-3α), 2.90 (1H, s, H-18), 1.60, 1.30, 1.15, 0.89, (12H, s, each CH_3), 1.00 (3H, d, *J* = 6.5 Hz, H-30); ES-MS $[M + Na]^+$ *m*/*z* 543.

Methylation of 2. Compound **2** was methyleted with CH_2N_2, affording a monomethyl ester **2a**: mp 172−173 °C (MeOH) (lit.[2] mp 171−173 °C); $[\alpha]^{20}_D$ +23.0° (*c* 0.55, MeOH) (lit.[2] $[\alpha]^{18}_D$ +23.0°).

Trachelosperoside B-1: colorless crystalline powder; mp 226−229 °C (MeOH) (lit.[2] mp 226−230 °C); $[\alpha]^{20}_D$ +9.6° (*c* 0.05, MeOH) (lit.[2] $[\alpha]^{18}_D$ +9.8).

Trachelosperoside E-1: white powder; mp 228−230 °C (MeOH); $[\alpha]^{30}_D$ +15.0° (*c* 0.55, MeOH) (lit.[3] $[\alpha]^{31}_D$ +15.5°).

Arjunglucoside I: amorphous powder; $[\alpha]^{25}_D$ +2.0° (*c* 0.35, MeOH) (lit.[4] $[\alpha]^{31}_D$ +4.6°, MeOH)).

Arjungenin: crystal; mp 292−294 °C from MeOH (lit.[4] mp 285−295 °C); $[\alpha]^{25}_D$ +30° (*c* 0.50, MeOH) (lit.[4] $[\alpha]^{31}_D$ +26.2°).

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