New Cytotoxic 6,7-*cis* and 6,7-*trans* Configured Guaianolides from *Warionia saharae*

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Using cytotoxicity against the KB cancer cell line (ATCC CCL17) as a lead, bioactivity-guided fractionation of the MeOH-soluble part of the DCM extract of *Warionia saharae* leaves led to the isolation of six new cytotoxic guaianolide type sesquiterpene lactones (**1**−**6**). Besides two guaianolides showing the common 6,7-*trans* fused lactone ring (**3** and **4**), four compounds exhibiting the more rare 6,7-*cis* configuration (**1**,**2** and **5**,**6**) were also isolated. Compounds **1**, **2**, **5**, and **6** showed an unprecedented ether bridge between C-2 and C-4. The structures were deduced from extensive 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, DQF-COSY, HSQC, HMBC, ROESY), as well as mass spectrometry (EI and HR-MALDI). Cytotoxicity testing against the KB cancer cell line revealed IC_{50} values of 1.0 (**1**), 4.5 (**2**), 1.7 (**3**), 2.0 (**4**), 3.3 (**5**), and 5.5 (**6**) μg/mL.

The leaves of *Warionia saharae* Benth. & Coss. (Asteraceae), an endemic shrub from Morocco and South Oran in Algeria, are used for their anti-inflammatory and gastrointestinal properties.[1] An earlier phytochemical study on *W. saharae* focused on the main components of the essential oil and led to the isolation of eudesmol, linalool, and nerolidol.[2]

Results and Discussion

The MeOH-soluble part of the DCM extract afforded four new 6,7-*cis*- (**1**,**2** and **5**,**6**) and two new 6,7-*trans*-configured guaianolides (**3** and **4**). The ^{13}C NMR spectrum of compound **1** exhibited 20 carbon signals, which were sorted by DEPT 90 and 135 experiments into four CH_3, four CH_2, six CH, and six quaternary carbons (see Table 2). This corresponds to the molecular formula $C_{20}H_{26}O_5$, in agreement with a molecular peak at *m/z* 346 $[M]^+$ in low-resolution EIMS and a pseudomolecular ion at *m/z* 369.176 $[M + Na]^+$ in the HR-MALDI spectrum. The ^{13}C NMR spectrum indicated the presence of a α-methylene-γ-lactone moiety, which showed signals at δ_C 171.2 (s, C-12), δ_C 141.0 (s, C-11), δ_C 118.5 (t, C-13), δ_C 82.8 (d, C-6), and δ_C 55.0 (d, C-7). An additional carbonyl carbon resonating at δ_C 178.1 (s, C-1′) pointed to an ester moiety. Quaternary carbons at δ_C 143.3 (s, C-10) and δ_C 133.7 (s, C-1) confirmed the presence of another double bond, and carbons resonating at δ_C 76.0 (d, C-3), δ_C 65.5 (s, C-4), and δ_C 65.6 (d, C-2) indicated three further oxygen substitutions (see Table 2). The ^{1}H and ^{1}H,^{1}H-COSY spectra displayed the typical signals and correlations of an oxygen-substituted guaianolide type sesquiterpene lactone (see Table 1).[3,4] Two doublets at δ_H 6.07 (d, J = 2.9 Hz, H-13a) and 5.51 (d, J = 2.3 Hz, H-13b) coupled with a proton at δ_H 2.92 (m, H-7) and belong to the exomethylene group. H-7 showed additional couplings with methylene protons at δ_H 2.15 and 1.35 (each m, H-8a and b) and a methine proton at δ_H 3.78 (t, J = 10.3 Hz, H-6). HSQC and HMBC experiments were extensively utilized to complete the assignments of all ^{1}H and ^{13}C signals of the guaianolide skeleton, and especially HMBC correlations between H-2 (δ_H 3.65, br s) and C-4 (δ_C 65.5, s) as well as between C-2 (δ_C 65.6, d) and H_3-15 (δ_H 1.60, s) established the C-2−O−C-4 ether bridge. Remaining proton signals at δ_H 2.43 (dd, J = 7.0, 13.9 Hz, H-2′), 1.69 (m, H-3′a), 1.49 (m, H-3′b), 1.17 (d, J = 7.0, H_3-5′), and 0.93 (t, J = 7.4, H_3-4′) were indicative of a 2-methylbutyryl substituent, confirmed by the ^{13}C NMR and 2D NMR experiments. NMR data were supported by the EIMS spectrum showing intensive fragments at *m/z* 57 $[C_4H_9]^+$, 85 $[C_5H_9O]^+$, 261 $[M - C_5H_9O]^+$, and 289 $[M - C_4H_9]^+$. A strong cross-peak between C-1′ (δ_C 178.1, s) and H-3 (δ_H 5.71, br s) in HMBC confirmed the acylation with 2-methylbutyric acid at 3-OH. β-Orientation of the C-2−O−C-4 ether bridge and the acylated 3-hydroxy group as well as the *cis*-configuration of the lactone ring were deduced from a ROESY experiment. NOEs were observed between H-6 and H-7, H-5 and H-7, and H-3a and H-5, as well as between H_3-15 and H-6.

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**6** (δ ppm; m; J Hz)[a]

H	1	2	3	4	5	6
2a	3.65 (br s)	3.45 (d, 1.5)	2.51 (d, 15.0)	2.30 (br d, 15.2)	3.32 (d, 1.7)	3.46 (d, 3.1)
2b			1.90 (dd, 1.0, 15.0)	1.99 (d, 15.2)		
3a	5.71 (br s)	4.70 (br s)	3.55 (br s)	3.60 (br s)	2.38 (d, 15.8)	2.42 (dd 3.1, 15.5)
3b					2.21 (dd, 1.8, 15.8)	2.32 (m)[c]
5	3.30 (m)[b]	3.28 (br d, 10.7)	2.53 (d, 11.7)	2.31 (d, 10.0)	2.99 (d, 11.6)	2.38 (d, 10.6)
6	3.78 (t, 10.3)	3.69 (t, 10.7)	3.86 (dd, 8.8, 11.7)	3.94 (t, 10.0)	4.05 (dd, 8.8, 11.5)	4.19 (dd, 8.9, 10.6)
7	2.92 (m)	2.88 (m)	3.00 (m)	2.98 (m)	3.25 (m)	3.04 (m)
8a	2.15 (m)	2.13 (m)	2.63 (dddd, 1.1, 3.7, 7.0, 17.9)	2.24 (m)	2.72 (dddd, 1.4, 4.3, 5.8, 18.2)	2.30 (m)[c]
8b	1.35 (m)	1.35 (m)	2.02 (m)	1.49 (m)	2.05 (m)	1.46 (m)[c]
9a	2.31 (m)	2.26 (m)	5.49 (m)	2.48 (ddd, 4.9, 5.4, 14.0)	5.69 (m)	2.76 (dt, 5.7, 13.0)
9b	2.15 (m)	2.11 (m)		2.03 (m)		2.28 (m)[c]
13a	6.07 (d, 2.9)	6.05 (d, J = 3.3)	6.27 (d, 3.5)	6.22 (d, 3.6)	6.27 (d, 3.5)	6.15 (d, 3.5)
13b	5.51 (d, 2.3)	5.50 (d, J = 3.3)	5.54 (d, 3.1)	5.51 (d, 3.2)	5.52 (d, 3.2)	5.62 (d, 3.2)
14a				5.38 (br s)		5.18 (br s)
14b				5.05 (br s)		5.04 (br s)
H_3-14	1.70 (s)	1.89 (s)	1.91 (br s)		1.88 (d, 1.4)	
H_3-15	1.60 (s)	1.60 (s)	1.73 (s)	1.69 (s)	1.63 (s)	1.46 (s)
HO-1			4.25 (s)	4.02 (s)		
HO-2					8.05 (br s)	
H-2′	2.43 (dd, 7.0, 13.9)					
H-3′a	1.69 (m)					
H-3′b	1.49 (m)					
H_3-4′	0.93 (t, 7.4)					
H_3-5′	1.17 (d, 7.0)					

[a] The chemical shifts of compounds **1**, **2**, and **6** were determined in CD_3OD (295 K) at 500 MHz (**2** and **6**) and 600 MHz (**1**). Compounds **3**−**5** were measured in $CDCl_3$ (295 K) at 500 MHz. [b]In parts overlapped by the methanol signal. [c]Signals overlapped.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**6** (δ ppm, m)[a]

C	1	2	3	4	5	6
1	133.7, s	136.9, s	81.2, s	82.3, s	93.3, s	100.4, s
2	65.6, d	68.4, d	42.1, t	40.5, t	62.2, d	66.6, d
3	76.0, d	73.2, d	63.3, d	64.4, d	40.0, t	40.8, t
4	65.5, s	65.4, s	67.5, s	67.6, s	66.7, s	68.0, s
5	53.1, d	53.5, d	59.9, d	60.3, d	54.7, d	57.0, d
6	82.8, d	83.0, d	80.8, d	79.1, d	80.4, d	84.7, d
7	55.0, d	55.3, d	45.4, d	44.5, d	42.6, d	45.0, d
8	26.0, t	26.2, t	30.4, t	29.7, t	31.1, t	33.0, t
9	35.7, t	35.8, t	120.4, d	28.4, t	125.4, d	32.0, t
10	143.3, s	142.3, s	138.2, s	146.5, s	135.1, s	152.8, s
11	141.0, s	141.2, s	138.3, s	139.1, s	138.5, s	140.9, s
12	171.2, s	167.0, s	169.5, s	169.5, s	169.4, s	171.7, s
13	118.5, t	118.2, t	120.8, t	120.5, t	120.6, t	121.2, t
14	22.7, q	22.3, q	25.0, q	114.9, t	24.4, q	115.3, t
15	19.7, q	19.8, q	19.5, q	18.7, q	19.0, q	17.7, q
1′	178.1, s					
2′	42.8, d					
3′	27.8, t					
4′	12.0, q					
5′	17.3, q					

[a] The chemical shifts of compounds **1**, **2**, and **6** were determined in CD_3OD (295 K) at 75 MHz (**1**,**2**) and 125 MHz (**6**). Compounds **3**−**5** were measured in $CDCl_3$ (295 K) at 75 MHz (**3**,**4**) and 125 MHz (**5**).

Compound **2** showed ^{1}H, ^{13}C, and 2D NMR data identical with those observed for **1** (Tables 1 and 2), but signals of the 2-methylbutyryl moiety were missing and C-3 as well as H-3 showed a significant upfield shift from δ_C 76.0 to 73.2 and δ_H 5.71 to 4.70, respectively. This indicated a substance with identical guaianolide structure, but not acylated. This was supported by the LR-EIMS showing a molecular ion at *m*/*z* 262. Compounds **1** and **2** were named 3*β*-*O*-(2-methylbutyryl)moroccolide A and moroccolide A, respectively.

^{1}H and ^{13}C data obtained for compounds **3** and **4** were very similar to the shift values reported for 3α,4α-epoxyrupiculin-A and -B, isolated from *Achillea clypeolata*.[5] The most obvious distinction was due to the multiplicity and shift value of C-8, which appeared as a methylene carbon at δ_C 30.4 and 29.7 for **3** and **4** instead of a methine at δ_C 70.7 and 71.1, respectively. In accordance, the ^{1}H NMR showed two protons at δ_H 2.63 (dddd, J = 1.1, 3.7, 7.0, 17.9 Hz, H-8a) and 2.02 (m, H-8b) for compound **3** and δ_H 2.24 and 1.49 (m, H-8a and b) for compound **4** instead of one methine proton at δ_H 4.13 and 3.92, respectively. Therefore, **3** and **4** are the 8-desoxy derivatives of 3α,4α-epoxyrupiculin-A and -B. EIMS of both **3** and **4** showed a $[M]^+$ at *m*/*z* 262 and confirmed this assumption. Stereochemistry was again deduced from a ROESY experiment and revealed the same relative configurations as reported for the 3α,4α-epoxyrupiculins.[5] Therefore, we propose the name 8-desoxy-3α,4α-epoxyrupiculin-A for compound **3** and 8-desoxy-3α,4α-epoxyrupiculin-B for compound **4**.

Both the ^{1}H and ^{13}C NMR signals of saharanolides A (**5**) and B (**6**) indicated again the presence of a guaianolide. In contrast to 8-desoxy-3α,4α-epoxyrupiculin-A (**3**) and 8-desoxy-3α,4α-epoxyrupiculin-B (**4**), C-1 was downfield shifted from δ_C 81.2 and 82.3 to δ_C 93.3 and 100.4 (in $CDCl_3$ and CD_3OD), respectively, indicating a neighboring hydroxy group in both molecules. In accordance, C-1 showed a strong cross-peak to a methine proton (H-2) at δ_H 3.32 (**5**) and 3.46 (**6**) in the HMBC spectra. In C_6D_6 a deuterium shift was observed for the carbons at δ_C 93.3 and 99.5 (C-1), respectively, after adding one drop of D_2O, whereas signals of the methine carbons (C-2) at δ_C 62.2 (**5**) and 66.4 (**6**) remained unaffected (data not shown). Therefore, it was concluded that C-2 in both compounds is again part of an ether bridge. This was confirmed by a strong HMBC correlation between C-2 and H_3-15 and established the C-2−O−C-4 connection. A ROESY spectrum for compounds **5** and **6** showed strong correlations between H-6 and H-7 establishing the *cis*-configuration of the lactone ring as well as cross-peaks between H-3α, H-5 and H_3-15 indicating that these protons were on the same face of the molecules (see energy-optimized form of **6** in Figure 1). For compounds **5** and **6** we propose the names saharanolides A (**5**) and B (**6**).

The sesquiterpene lactone chemistry of *Warionia saharae* is characterized by the occurrence of oxygenated guaiano-

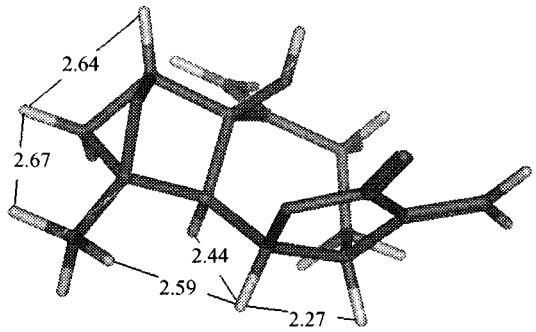

Figure 1. Energy-optimized model of compound **6** (lines: important NOEs).

lides including those with an unusual C-2−O−C-4 ether bridge. Besides the common 6,7-*trans* lactones,[3,4,6] guaianolides of the more rare 6,7-*cis* fused type were also isolated.[6−9]

All compounds showed significant cytotoxicity against the KB cancer cell line. The determined IC_{50} values were 1.0 (**1**), 4.5 (**2**), 1.7 (**3**), 2.0 (**4**), 3.3 (**5**), and 5.5 (**6**) mg/mL. No significant differences in cytotoxicity were observed between two compounds varying only in the position of the double bond (9,10 or 10,14). In contrast, acylation of one of the free hydroxy groups resulted in a clear increase of activity.

Experimental Section

General Experimental Procedures. ^{13}C NMR spectra of compounds **1**−**4** were measured on a Bruker AMX-300 spectrometer (operating at 300.13 MHz for ^1H and 75.47 for ^{13}C) at 295 K. All other NMR spectra were recorded on a Bruker DRX-500 spectrometer (operating at 500.13 MHz for ^1H and 125.77 for ^{13}C) and on a Bruker DRX-600 spectrometer (operating at 600.13 MHz for ^1H and 150.92 for ^{13}C) at 295 K. Spectra were measured in CD_3OD, $CDCl_3$, or C_6D_6 and referenced against residual CH_3OH and CD_3OD, or residual $CHCl_3$ and $CDCl_3$, or residual C_6H_6 and C_6D_6. DEI-MS spectra (at 70 eV) were measured on a micromass TRIBRID double-focusing mass spectrometer at 70 eV, and HR-MALDI were recorded for all compounds on an IonSpec HR-MALDI Fourier transform mass spectrometer. Only compound **1** expressed a detectable $[M + Na]^+$ pseudomolecular peak. For VLC and open column, silica gel ($60F_{254}$, 40−60 μm, Merck) was used. HPLC separations were performed on a Lichrosorb Si 60 column (250 × 16 mm, particle size 5 μm, Merck) and Spherisorb S 10 ODSII column (250 × 16 mm, particle size 5 μm, Merck), both from Knauer. A description of other instruments used in this study has been provided in previous reports.[10,11]

Plant Material. Leaves of *W. saharae* Benth. & Coss. were collected north of Agadir, Morocco, in May 1998. The plant was identified by Dr. A. Benchâabane, University Smlallia, Marrakech, Morocco, and Dr. F. Jacquemoud, Conservatoire et Jardin Botaniques de Genève, Geneva, Switzerland. A voucher specimen is deposited at the Conservatoire et Jardin Botaniques de Genève, Geneva, Switzerland, with the identification number 3A/98.

Extraction and Isolation. Air-dried and powdered leaves of *W. saharae* (1 kg) were percolated with dichloromethane at room temperature. The extract (157.2 g) was partitioned between *n*-hexane and methanol. The alcoholic phase (30.45 g) was subjected to VLC (silica gel) using a step gradient of hexane−ethyl acetate (9:1 to 1:1) and final washing with MeOH to give seven fractions (F1−F7). Fractions F2, F3, and F4 were further separated by VLC (silica gel) eluting with a gradient of hexane−ethyl acetate−MeOH (9:1:0 to 0:4:1) for F2, (9:1:0 to 0:1:1) for F3, and (9:1:0 to 0:9:1) for F4, to yield three subfractions (F2.1−F2.6, F3.1−F3.8, and F4.1−F4.9), respectively. The subfraction F2.3 was subjected to open column chromatography with silica gel, eluting with CH_2Cl_2−MeOH (99:1; 98:2; 90:10), followed by a Sephadex LH-20 open column with cyclohexane−CH_2Cl_2−MeOH (7:4:1). Separation by normal-phase HPLC using a step gradient of CH_2Cl_2−MeOH (100:0 to 9:1) gave a mixture (27 mg) of **3** and **4**. The final purification with HPLC (60% ACN/40% H_2O, RP-18 column) furnished **3** (3.8 mg) and **4** (8.3 mg). The subfraction F2.4 was chromatographed by repeated CC on silica gel and elution with a stepwise gradient mixture of chloroform−2-butanone−hexane and introduced to HPLC normal-phase using hexane−ethyl acetate (7:3) to yield compound **5** (3.5 mg). The subfraction F3.2 was separated by open column on Sephadex LH-20 with cyclohexane−CH_2Cl_2−MeOH (7:4:1) to give **1** (45.8 mg) and an impure compound, which was purified on normal-phase HPLC using hexane−ethyl acetate (3:2) to give **6** (5.1 mg). The subfraction F4.3 was fractionated by open column chromatography on Sephadex LH-20 eluting with cyclohexane−CH_2Cl_2−MeOH (7:4:1) followed by open column chromatography on silica gel using toluol−EtOAc (8:2; 4:6). Final purification of **2** (20.2 mg) was achieved by HPLC using MeOH−H_2O (4:3) and a RP-18 column.

Cytotoxic Activity. Cytotoxicity of the fractions and compounds was determined using KB-cells, a human cervix carcinoma cancer cell line (HeLa cells, ATCC CCL17). The test was performed in accordance with the literature.[12] Fractions were tested at 25 and 50 μg/mL. Pure compounds were tested at various concentrations between 0.1 and 10 μg/mL.

5αH-2*β*,4-Epoxy-3*β*-(2-methylbutyryloxy)guaia-1(10),-11(13)-dien-6*β*,12-olide (3*β*-*O*-(2-methylbutyryl)moroccolide A (1)): colorless gum (45.8 mg); $[\alpha]^{22}_D$ +102° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_3OH) *m*/*z* 346 $[M]^+$, 289 $[M - C_4H_9]^+$, 261 $[M - C_5H_9O]^+$ (6), 85 $[C_5H_9O]^+$ (8), 57 $[C_4H_9]^+$ (28); HR MALDI-MS (pos.) 369.167 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$ 369.178).

5αH-2*β*,4-Epoxy-3*β*-hydroxyguaia-1(10),11(13)-dien-6*β*,-12-olide (moroccolide A (2)): colorless gum (20.2 mg); $[\alpha]^{22}_D$ +62° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_3OH) *m*/*z* 262 $[M]^+$ (<1), 244 $[M - H_2O]^+$ (<1), 230 $[244 - CH_3 + H]^+$ (<1); HREIMS *m*/*z* 262.1194 (calcd for $C_{15}H_{18}O_4$, 262.1205).

8-Desoxy-3α,4α-epoxyrupiculin-A (3): colorless gum (3.8 mg); $[\alpha]^{22}_D$ −22° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_2Cl_2) *m*/*z* 262 $[M]^+$ (6), 244 $[M - H_2O]^+$ (70), 229 $[244 - CH_3]^+$ (19), 91 (39); HREIMS *m*/*z* 262.1193 (calcd for $C_{15}H_{18}O_4$, 262.1205).

8-Desoxy-3α,4α-epoxyrupiculin-B (4): colorless gum (8.3 mg); $[\alpha]^{22}_D$ +46° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_2Cl_2) *m*/*z* 262 $[M]^+$ (2), 247 $[M - CH_3]^+$ (5), 229 $[247 - H_2O]^+$ (4), 91 (17); HREIMS *m*/*z* 262.1201 (calcd for $C_{15}H_{18}O_4$, 262.1205).

5αH-2,4*β*-Epoxy-1-hydroxyguaia-9(10),11(13)-dien-6*β*,-12-olide (saharanolide A (5)): colorless gum (3.5 mg); $[\alpha]^{22}_D$ −86° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_3OH) *m*/*z* 262 $[M]^+$ (22), 244 $[M - H_2O]^+$ (100), 229 $[244 - CH_3]^+$ (14), 91 (8); HREIMS *m*/*z* 262.1201 (calcd for $C_{15}H_{18}O_4$, 262.1205).

5αH-2,4*β*-Epoxy-1-hydroxyguaia-10(14),11(13)-dien-6*β*,-12-olide (saharanolide B (6)): colorless gum (5.1 mg); $[\alpha]^{22}_D$ −86° (*c* 0.1, EtOH); ^1H NMR data, Table 1; ^{13}C NMR data, Table 2; EIMS (CH_3OH) *m*/*z* 262 $[M]^+$ (1), 244 $[M - H_2O]^+$ (1), 229 $[244 - CH_3]^+$ (1), 91 (5); HREIMS *m*/*z* 262.1200 (calcd for $C_{15}H_{18}O_4$, 262.1205).

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