New Sesquiterpene Lactones and Other Constituents from *Helianthus petiolaris*

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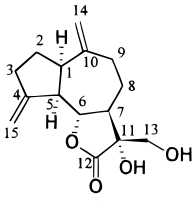
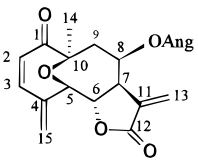
Three new sesquiterpene lactones, 11α,13-dihydroxydehidrocostuslactone (**1**), the unusual 5, 10-epoxygermacranolide 4,15-anhydrohelivypolide (**2**), and 3-methoxy-1,2-anhydridoniveusin A (**4**), together with the 5,10-epoxygermacranolide helivypolide, four known 3,10-furanoheliangolides, and five known kaurane- and trachylobane-type diterpenes, have been isolated from *Helianthus petiolaris*.

Helianthus petiolaris Nutt. (Asteraceae, Heliantheae), commonly known as "wild sunflower" or "mirasolcito", is an annual species native to North America that has been introduced to Argentina. The heliangolide budlein A as well as kauranoic and trachylobanic acids were previously isolated from the aerial parts of a sample collected in Eastern Kansas.[1] In the course of a chemotaxonomic investigation of species of the tribe Heliantheae we have examined the leaves and flower heads of a sample of *H. petiolaris* growing in Córdoba Province, Argentina.

The $CHCl_3$-soluble extract of the leaves yielded three new sesquiterpene lactones, the guaianolide, 11α,13-dihydroxydehidrocostuslactone (**1**), the unusual 5,10-epoxygermacranolide 4,15-anhydrohelivypolide **(2),** and the 3,10-furanoheliangolide, 3-methoxy-1,2-anhydridoniveusin A (**4**). The known 5,10-epoxygermacranolide helivypolide (**3**)[2] and the 3,10-furanoheliangolides, 1,2-anhydridoniveusin A (**5**),[3] niveusin B,[4] 3-*O*-methylniveusin A,[5] and 1-methoxy-4,5-dihydroniveusin A,[3] were also isolated, together with ciliaric acid.[6]



1



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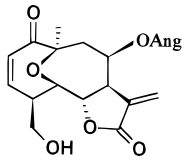
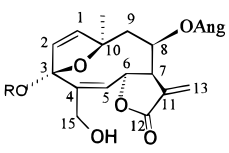


3



4 CH_3
5 H

The guaianolide **1** had a molecular formula of $C_{15}H_{20}O_4$ by HRMS. The IR spectrum showed an absorption at 1762 cm^{-1} due to a saturated γ-lactone carbonyl group, while in the ^1H NMR spectrum (Table 1) a typical pair of doublets for the exocyclic methylene protons of an unsaturated γ-lactone and methyl group signals were absent. The ^{13}C NMR spectrum (Table 2) showed signals due to two exocyclic double bonds at δ 149.6, 112.4 and 132.0, 109.8, and three signals of carbons bearing oxygen atoms at δ 85.0, 77.2, and 64.6. These data suggested a sesquiterpene lactone skeleton for **1**. The ^1H NMR assignments for **1** were determined on the basis of a COSY experiment. The configuration at C-11 was established on the basis of H-13 signal chemical shifts that were in agreement with those for solstitialin A and 3-*O*-acetylsolstitialin A, whose structures were confirmed by X-ray diffraction.[7]

A compound with the same structure as **1**, namely, 3-desoxysolstitialin A, was previously reported from *Centaurea imperialis*. However, its assigned ^1H NMR data differed considerably from ours in the chemical shifts of H-13, H-14, and H-15.[8] Our NMR data for the H-15 signals of **1** were in full agreement with those reported for related compounds without an oxygenated substituent in the ring A, such as annuolides C, D, and E,[9] two guaianolides from *Gochnatia smithii*,[10] and dehydrocostuslactone and two related guaianolides obtained from two species of *Saussurea*.[11,12] A similar situation was found for the chemical shifts of the H-14 signal of **1**, which correlated with those for the guaianolides of *Saussurea lappa*.[11] The chemical shifts for H-15 signals reported by Bohlmann *et al.* for 3-desoxysolstitialin A correlate with those of compounds with oxygenated groups attached to the A ring as for solstitialin A derivatives[7,13] and zaluzanin C.[14,15] Consequently, we propose that the structure **1** corresponds to the guaianolide obtained from *H. petiolaris* and should be named as 11α,13-dihydroxy-dehidrocostuslactone.

4,15-Anhydrohelivypolide (**2**) ($C_{20}H_{22}O_6$) possessed a conjugated γ-lactone group (IR band at 1776 cm^{-1}) and also contained an ester function (IR bands at 1716 cm^{-1}). The ^1H NMR spectrum (Table 1) showed typical signals due to an angeloyl moiety [δ 6.15 (1H, qq, J = 7.2, 1.5 Hz), 1.98 (3H, dq, J = 7.2, 1.5 Hz), and 1.80 (3H, q, J = 1.5 Hz)] and the typical doublets due to H-13 and H-13′ of an exocyclic methylene group conjugated with a γ-lactone at δ 6.33 (J = 1.3 Hz) and 5.62 (J =

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Table 1. ^{1}H NMR Data for Compounds **1, 2**, and **4** (200.13 MHz) in $CDCl_3$[a]

proton	1	2	4
H−1	2.90 m		5.65 d (5.7)
H−2	1.90 m	6.03 d (13.3)	6.40 d (5.7)
H−3	2.50 m	6.35 d (13.3)	
H−4			
H−5	2.75 m	4.58 d (9.8)	5.91 d (3.3)
H−6	4.31 t (9.4)	5.15 t (9.8)	5.91 overlapped
H−7	2.05 m	3.44 m	3.59 m
H−8	2.50 m	5.79 m	5.27 td (3.7, 1.6)
H−8′	1.80 m		
H−9	1.80 m	2.38 dd (15.2, 2.8)	2.40 dd (3.8, 3.7)[c]
H−9′	1.80 m	2.14 dd (15.2, 3.3)	
H−13	3.64 d (11.8)	6.33 d (1.3)	6.32 d (2.7)
H−13′	3.80 br d (11.8)	5.62 d (3.0)	5.69 d (2.4)
H−14	4.87 br s	1.43 s[b]	1.46 s[b]
H−14′	4.82 br dd (1.3, 1.1)		
H−15	5.07 q (1.9)	5.62 d (3.0)	4.19 br d (1.0)[c]
H−15′	5.20 dq (2.4, 1.9)	6.39 d (1.3)	
H−3′		6.15 qq (7.2, 1.5)	6.13 qq (7.2, 1.6)
H−4′		1.98 dq (7.2, 1.5)[b]	1.96 dq (7.2, 1.6)[b]
H−5′		1.80 q (1.5)[b]	1.82 q (1.6)[b]
OH	3.24 br		
OCH_3			3.41 s[b]

[a] Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz. [b] Intensity three protons. [c] Intensity two protons.

Table 2. ^{13}C NMR Data for Compounds **1**, **2**, and **4** (50.03 MHz) in $CDCl_3$[a,b]

carbon	1	2	4
C−1	47.3 d[c]	189.4 s	141.7 d
C−2	30.1 t	127.3 d	140.7 d
C−3	32.3 t	150.5 d	112.8 s
C−4	149.6 s	143.8 s	133.0 s
C−5	52.1 d	79.4 d	126.8 d
C−6	85.0 d	76.2 d	75.2 d
C−7	47.5 d[c]	47.2 d	48.0 d
C−8	25.2 t	65.5 d	74.1 d
C−9	36.0 t	46.9 t	44.1 t
C−10	132.0 s	80.5 s	87.8 s
C−11	77.2 s	134.5 s	138.0 s
C−12	180.0 s	166.1 s	169.3 s
C−13	64.6 t	122.6 t	124.1 t
C−14	109.8 t	32.9 q	27.2 q
C−15	112.4 t	128.4 t	66.8 t
C−1′		168.2 s	166.1 s
C−2′		126.4 s	126.8 s
C−3′		141.2 d	140.7 d
C−4′		15.9 q	15.8 q
C−5′		20.5 q	19.5 q
OCH_3			50.8 q

[a] Chemical shifts (relative to TMS) are in ppm. [b] Carbon multiplicities were established by DEPT experiment. [c] Assignments may be interchangeable.

3.0 Hz). The ^{13}C NMR spectrum (Table 2) showed signals for an α,*β*-*γ*,*δ*-unsaturated carbonyl group at *δ* 189.4, 127.3, 150.5, 143.8, and 128.4. These functionalities were confirmed by the ^{1}H NMR spectrum, which showed four doublets at *δ* 6.39 (J = 1.3 Hz), 6.35 (J = 13.3 Hz), 6.03 (J = 13.3 Hz), and 5.62 (J = 3.0 Hz). Spin-decoupling experiments established the location of the H-7 signal at *δ* 3.44 and a COSY experiment allowed the complete ^{1}H NMR assignments to be made. Careful analysis of the data obtained for **2** and comparison with those reported for the 5,10-epoxygermacranolide chapliatrin and its congeners[2−16,19] led us to conclude that **2** was 4,15-anhydrohelivypolide. This compound could be an artifact from helivypolide (**3**).[2]

The ^{1}H NMR spectrum of **4** (Table 1) was very similar to that of 1,2-anhydridoniveusin A (**5**)[3] but contained a singlet at *δ* 3.41 due to a methoxyl group, which was reflected in the ^{13}C NMR spectrum by a signal at *δ* 50.8 (Table 2). In the latter the only signals that were shifted significantly in comparison with **5** were C-3 and C-14, suggesting that the methoxyl group was located at C-3. All these data led us to conclude that compound **4** was the 3-methoxyderivative of 1,2-anhydridoniveusin A not isolated previously.

In contrast to the leaves, the profile of secondary metabolites in the flower heads of *H. petiolaris* differed considerably. The $CHCl_3$-soluble extract yielded only traces of the guaianolide **1** together with vanillin and the known diterpenoids ciliaric acid,[6] trachylobanic acid,[20] *ent*-kaurenic acid,[21] grandifloric acid,[21] and *ent*-17-hydroxykaur-15-en-19-oic acid.[22] These results may be contrasted with those reported by Spring, who concluded that in *H. petiolaris* sesquiterpene lactones are localized exclusively in the anther appendages.[23,24]

Previous results for *Helianthus niveus*,[4] *H. annuus*,[2,3−5−25,26] and *H. petiolaris*[1] as well as ours are in accordance with the Heiser's classification of the genus *Helianthus*, which places the three species in the section *Helianthus*.[27]

Sesquiterpene lactones with the chapliatrin skeletal-type have been found to exhibit activity in the P-388 lymphocytic leukemia and Lewis lung carcinoma in vitro test systems.[18]

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV-260 instrument. IR spectra were recorded in a Nicolet 5-SXC−FTIR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ with TMS as internal standard at 200 and 50 MHz, respectively, on a Bruker AC-200 NMR spectrometer. MS were recorded on a ZAB-SEQ4F instrument. TLC visualization was conducted by UV light and by heating the plates after spraying with 15% v/v H_2SO_4 in EtOH.

Plant Material. *H. petiolaris* Nutt. was collected near Toledo, Departamento Santa María, Córdoba Province, Argentina, in April 1994, and identified by Dr. Luis Ariza Espinar. A voucher specimen (LUIS ARIZA ESPINAR 2861) is deposited in the Museo Botánico, Córdoba (CORD).

Extraction and Isolation. The leaves (1024 g) and flower heads (231 g) of *H. petiolaris* were extracted separately. Thus, the leaves were air-dried and exhaustively extracted with $CHCl_3$. The residue obtained after evaporation of the solvent (65 g) was dissolved in hot EtOH, and a solution of 4% $Pb(AcO)_2$ was added. After standing overnight, the precipitate was filtered off, the organic solvent evaporated, and the aqueous solution extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent evaporated under reduced pressure, yielding a gummy residue (12.76 g). The residue was divided into 16 fractions by vacuum−liquid chromatography, eluting with hexane, C_6H_6, C_6H_6−$CHCl_3$, $CHCl_3$−EtOAc, EtOAc, and MeOH and combined according to their TLC profiles. Recrystallization of fractions 8−9 yielded ciliaric acid (23.4 mg). Methylation with CH_2N_2 afforded ciliaric acid methyl ester whose IR, NMR, and MS data were identical with those reported in the literature.[6] Fractions 10−12 were chromatographed on a Si gel column and eluted with

C_6H_6, $CHCl_3$, and EtOAc to give fractions A through D. Radial chromatography (hexane−Et_2O) of fraction A yielded **2** (28.4 mg) and **3** (36.6 mg). The same procedure was applied to fraction B followed by column chromatography on Si gel to give niveusin B[4] (3.5 mg). Fractions C and D were purified by column chromatography. Elution with Et_2O afforded **1** (3.7 mg) and **4** (2.1 mg) in the first case and 1,2-anhydridoniveusin A (**5**)[3] (8.3 mg) in the second case. Column chromatography on Si gel of fractions 13−15 using C_6H_6, $CHCl_3$, and MeOH mixtures as eluents, followed by radial chromatography afforded 3-*O*-methylniveusin A[5] (23.8 mg) and 1-methoxy-4,5-dihydroniveusin A[3] (1.4 mg).

The flower heads were extracted exhaustively with $CHCl_3$ yielding 87.11 g of a dark extract. This was processed as described above to give a residue (3.37 g) that after column chromatography (hexane and hexane−EtOAc mixtures of increasing polarity as eluent) yielded fractions A−E. Fraction A afforded trachylobanic acid (23.5 mg) after recrystallization, which was identified through its methyl derivative.[20] Vanillin was obtained from fraction B after column chromatography on Si gel eluting with Et_2O and petroleum ether and further purification by column chromatography on Sephadex LH-20 with MeOH. Column chromatography of fraction C on Si gel, using hexane−acetone as eluent, yielded kaurenoic acid (5.0 mg) and a mixture that was purified as their methyl ester derivatives by preparative TLC (Et_2O−petroleum ether) affording grandifloric acid methyl ester[21] (6.3 mg) and *ent*-17-hydroxykaur-15-en-19-oic acid methyl ester[22] (8.6 mg). Filtration on Sephadex LH-20 followed by preparative TLC (hexane−acetone) of fraction D yielded **1** (2.6 mg). Recrystallization of fraction E yielded ciliaric acid (13.6 mg), and methylation with CH_2N_2 afforded the corresponding methyl ester derivative that was identified as above.[6]

11α,13-Dihydroxydehidrocostuslactone (1): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 231 (4.03), 297 (sh, 3.33) nm; IR (AgCl) ν_{max} 3401, 2934, 1762, 1706, 1644 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* $[M + H]^+$ 265 (7), 109 (100); HREIMS *m/z* $[M]^+$ 264.1367, calcd for $C_{15}H_{20}O_4$ 264.1361.

4,15-Anhydrohelivypolide (2): colorless oil; UV (MeOH) λ_{max} (log ϵ) 213 (4.41), 254 (3.97) nm; IR (AgCl) ν_{max} 2934, 1776, 1716, 1664, 1611, 1236, 1150, 1130, 1019 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* $[M + H]^+$ 359 (56), 331 (90), 219 (55), 211 (45), 109 (100); HREIMS *m/z* $[M]^+$ 358.1410 calcd for $C_{20}H_{22}O_6$ 358.1416.

3-Methoxy-1,2-anhydridoniveusin A (4): colorless oil; IR (AgCl) ν_{max} 3401, 2934, 1762, 1705, 1644 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* $[M + H]^+$ 391 (12), 373 (27), 109 (100); HREIMS *m/z* $[M]^+$ 391.1657 calcd for $C_{21}H_{26}O_7$ 390.7386.

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