Notes

Sesquiterpene Lactones from *Staehelina fruticosa*

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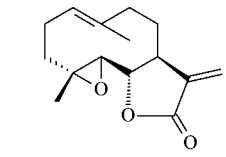
The phytochemical analysis of *Staehelina fruticosa* led to the isolation of four germacranolide-type sesquiterpene lactones (**1**–**4**), including two new glycosides. The structures of these sesquiterpene lactones were elucidated using spectroscopic techniques, and enzymatic hydrolysis was carried out to confirm the nature of the two glycoside derivatives. Molecular modeling was incorporated to substantiate their relative configuration.

The genus *Staehelina* (Asteraceae, tribe Cardueae) is extremely small and consists of only seven species worldwide.[1] *S. fruticosa* L. is endemic to Greece, occurring in Crete and the southern Aegean Islands. This study is a continuation of the ongoing phytochemical analyses of plants from Crete.[2] The Asteraceae family is characterized by the presence of mono- and diterpenes, sesquiterpene lactones,[3] and flavonoids.[4,5] These compounds impart medicinal activity to the family, and many of the Asteraceae species are used in folk medicine and herbal remedies.[6] Although the medicinal properties of the genus *Staehelina* have been scarcely documented, it has been reported that species of this genus were used in early folk medicine.[7,8]

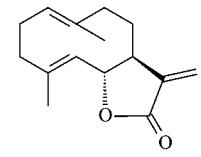
Two sesquiterpene lactones were isolated from the CH_2Cl_2 extract of the aerial parts of *S. fruticosa* and identified as parthenolide (**1**) and costunolide (**2**).[9] The MeOH extract subsequently yielded two glycosidic dihydro derivatives of **1** and **2**, which were identified as the new compounds 11β,13-dihydroparthenolide-14-*O*-β-D-glucopyranoside (**3**) and 11β,13-dihydrocostunolide-14-*O*-β-D-glucopyranoside (**4**). Structural elucidation of all compounds was determined by spectroscopic methods.

Compound **3** was isolated as a white, amorphous solid. The ^{1}H NMR spectrum (Table 1) indicated the presence of a parthenolide framework with an attached glycoside moiety. Of particular interest was the lack of the $\Delta^{11,(13)}$ exocyclic system resonances distinctive of the γ-lactone ring of such germacranolide compounds. Instead, a C-11 methyl group was evident, depicted by the resonance at 1.26 ppm (H-13, 3H, d, J = 7.0 Hz) in the ^{1}H NMR spectrum of **3**. The characteristic $\Delta^{1,(10)}$ system was indicated by the H-1 resonance at 5.49 ppm (dd, J = 12.1, 3.9 Hz). The HMBC correlations of H-1 with C-9 (δ 37.6) and an oxygenated methylene group at 67.9 ppm indicated that the typical C-14 methyl group had undergone oxygenation. These distinctions allowed for the sesquiterpene lactone nucleus to be identified as a 14-oxygenated 11,13-dihydro analogue of parthenolide.[10]

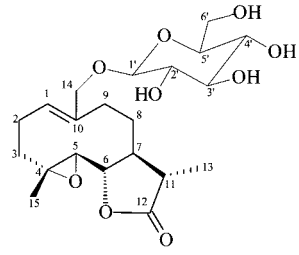
The C-14 geminal protons resonated at 4.73 and 4.07 ppm (d, J = 11.0 Hz) in the ^{1}H NMR spectrum of **3**, and the HMBC experiment indicated cross-coupling with C-1 (δ 130.8), C-9 (δ 37.6), and C-10 (δ 137.3), as well as with the anomeric C-1′ resonance at 105.0 ppm. This established that *O*-glycosylation had occurred at C-14. The anomeric resonance at 4.32 ppm (d, J = 7.8 Hz) was assigned to H-1′ of the sugar moiety, while the characteristic C-6′ methylene protons resonated at 3.91 (dd, J = 11.7, 1.6 Hz) and 3.68 ppm (1H, dd, J = 11.7, 5.5 Hz) and were assigned to H-6a′ and H-6b′, respectively. The COSY and HMQC spectra were used in the identification of the sugar moiety, which was established as β-glucose and confirmed as the D-sugar by subsequent enzymatic hydrolysis. Finally, MS analysis (APCI) of **3** showed a parent ion of m/z 429.3 $[M + 1]^+$ ($C_{21}H_{32}O_9$), indicating the presence of an oxygenated dihydroparthenolide nucleus with a linked hexosyl moiety. Compound **3** was thus elucidated as the new 11, 13-dihydroparthenolide-14-*O*-β-D-glucopyranoside.



1 Parthenolide



2 Costunolide



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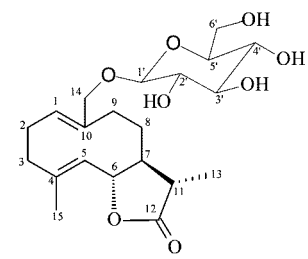

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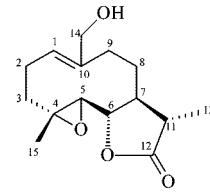
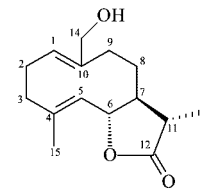


3a



4a

Compound **4** was isolated as a white, amorphous solid. The ^{1}H NMR spectrum indicated the presence of a glycosylated germacranolide. The $\Delta^{11,(13)}$ exocyclic system was replaced by a C-11 methyl group resonating at 1.23 ppm (H-13, 3H, d, J = 7.1 Hz). Use of ^{1}H and ^{13}C NMR spectra, as well as COSY, COSY-LR, HMQC, and HMBC experiments permitted the structural elucidation of **4** as the analogous costunolide derivative of **3**, where once again,

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Table 1. NMR Data of 11β,13-Dihydroparthenolide-14-*O*-β-D-glucopyranoside (**3**) and 11β,13-Dihydro-14-hydroxyparthenolide (**3a**) (CD_3OD)

C/H no.	**3** δ_H (H, m, *J* in Hz)	**3** δ_C	HMBC correlations	NOESY correlations	**3a** δ_H (H, m, *J* in Hz)	**3a** δ_C
1	5.49 (1H, dd, 12.1, 3.9)	130.8	C-14, C-9	H-2α, H-3α, H-5α, H-7α, H-9α	5.50 (1H, dd, 12.9, 3.8)	129.4
2α	2.20 (1H, m)	24.8		H-1, H-2β	2.21 (1H, br d, 13.0)	24.6
2β	2.59 (1H, dq, 12.9, 5.5)			H-2α, H-14a	2.56 (1H, dq, 13.0, 5.5)	
3α	1.30 (1H, m)	38.5		H-1, H-3β, H-5α	1.31 (1H, m)	38.1
3β	2.13 (1H, ddd, 12.5, 5.5, 1.6)			H-3α, H-15	2.13 (1H, ddd, 13.3, 5.5, 2.0)	
4		63.1				63.5
5α	2.89 (1H, d, 9.2)	67.9	C-3, C-6, C-7	H-1, H-3α, H-7α	2.88 (1H, d, 9.2)	67.8
6β	4.02 (1H, t, 9.2)	83.9	C-4	H-11β, H-15	3.98 (1H, t, 9.2)	83.7
7α	2.05 (1H, m)	52.9		H-1, H-5α	2.05 (1H, m)	52.4
8	1.90 (2H, m)	31.4	C-10, C-6, C-7	H-1, H-9β, H-11β	1.89 (1H, m) and 1.80 (1H, m)	30.9
9α	1.99 (1H, m)	37.6	C-10, C-1	H-1	1.97 (1H, br d, 13.3)	36.7
9β	2.78 (1H, br d, 12.9)			H-8α/β	2.75 (1H, br dd, 13.3, 5.8)	
10		137.3				140.1
11β	2.43 (1H, dq, 12.0, 7.0)	43.7		H-6β, H-13, H-8α/β	2.44 (1H, dq, 12.3, 6.8)	43.4
12		180.5				180.4
13	1.26 (3H, d, 7.0)	13.3	C-7, C-11, C-12	H-11β	1.27 (3H, d, 6.8)	13.0
14a	4.73 (1H, d, 11.0)	67.9	C-1, C-9, C-10	H-2β, H-14b, H-15	4.40 (1H, d, 12.0)	59.0
14b	4.07 (1H, d, 11.0)		C-1, C-10, C-1′	H-8α/β, H-14a, H-15, H-1′	4.03 (1H, d, 12.0)	
15	1.29 (3H, s)	17.7	C-3, C-4, C-5	H-3β, H-6β, H-14a, H-14b	1.25 (3H, s)	17.8
1′	4.32 (1H, d, 7.8)	105.0	C-14	H-14b		
2′	3.19 (1H, dd, 9.0, 7.8)	75.4				
3′	3.30 (1H, m)	78.5				
4′	3.35 (1H, m)	71.5				
5′	3.30 (1H, m)	78.4				
6a′	3.91 (1H, dd, 11.7, 1.6)	63.1				
6b′	3.68 (1H, dd, 11.7, 5.5)					

Table 2. NMR Data of 11β,13-Dihydrocostunolide-14-*O*-β-D-glucopyranoside (**4**) and 11β,13-Dihydro-14-hydroxycostunolide (**4a**) (CD_3OD)

C/H no.	**4** δ_H (H, m, *J* in Hz)	**4** δ_C	HMBC correlations	NOESY correlations	**4a** δ_H (H, m, *J* in Hz)	**4a** δ_C
1	5.05 (1H, dd, 12.6, 4.1)	132.3		H-2α, H-3α, H-5, H-7α, H-9α	5.00 (1H, dd, 12.6, 3.8)	131.4
2α	2.19 (1H, m)	26.7		H-1, H-2β	2.19 (1H, m)	26.5
2β	2.45 (1H, dq, 12.5, 5.1)			H-2α, H-14a	2.42 (1H, dq, 12.6, 5.5)	
3α	2.10 (1H, dq, 12.0, 5.1)	40.9		H-1, H-3β, H-5,	2.10 (1H, dq, 11.6, 5.5)	39.5
3β	2.32 (1H, ddd, 12.0, 4.7, 1.6)			H-3α, H-15	2.31 (1H, ddd, 11.6, 4.8, 1.6)	
4		142.0				141.5
5	4.75 (1H, m)	129.0	C-15	H-1, H-3α, H-7α	4.74 (1H, m)	129.3
6β	4.75 (1H, m)	83.4		H-11β, H-15	4.74 (1H, m)	83.5
7α	1.77 (1H, m)	56.6		H-1, H-5, H-11β	1.76 (1H, m)	55.8
8α	1.86 (1H, br dd, 12.6, 6.1)	30.6		H-9β, H-11β	1.88 (1H, m)	29.3
8β	1.80 (1H, br d, 12.6)			H-14b	1.76 (1H, m)	
9α	1.85 (1H, m)	38.2		H-1, H-9β	1.93 (1H, br d, 12.6)	37.2
9β	2.91 (1H, br dd, 12.5, 5.5)			H-8β, H-9α	2.85 (1H, br dd, 12.6, 5.8)	
10		140.0				142.5
11β	2.39 (1H, dq, 12.3, 7.0)	43.7		H-6β, H-7α, H-8α, H-13	2.39 (1H, dq, 11.9, 6.8)	41.1
12		182.2				181.8
13	1.23 (3H, d, 7.0)	13.7	C-7, C-11, C-12	H-8α, H-11β	1.23 (3H, d, 6.8)	13.6
14a	4.56 (1H, d, 11.0)	67.6	C-1, C-9	H-2β, H-14b, H-15	4.20 (1H, d, 11.9)	58.8
14b	3.78 (1H, d, 11.0)		C-1, C-10, C-1′	H-8β, H-14a, H-15, H-1′	3.78 (1H, d, 11.9)	
15	1.66 (3H, s)	17.6	C-3, C-4, C-5	H-3β, H-6β, H-14a, H-14b	1.63 (3H, s)	17.2
1′	4.27 (1H, d, 7.4)	104.9	C-14	H-14b, H-2′, H-6a′		
2′	3.18 (1H, dd, 9.0, 7.8)	74.4				
3′	3.36 (1H, t, 8.9)	78.9				
4′	3.33 (1H, m)	71.6				
5′	3.28 (1H, m)	78.4		H-6a′, H-6b′		
6a′	3.88 (1H, dd, 12.1, 2.0)	63.0		H-14a, H-1′, H-5′, H-6b′		
6b′	3.68 (1H, dd, 12.1, 5.1)			H-5′, H-6a′		

14-*O*-glucosylation was evident (Table 2) and confirmed by enzymatic hydrolysis. Furthermore, MS analysis (APCI) of **4** showed a parent ion, *m*/*z* 413.0 $[M + 1]^+$ ($C_{21}H_{32}O_8$), indicating the presence of an oxygenated dihydrocostunolide nucleus with a linked glycoside moiety. Compound **4** was thus elucidated as the new 11,13-dihydrocostunolide-14-*O*-β-glucopyranoside.

Enzymatic hydrolysis of **3** and **4** was undertaken to clarify the sugar moiety as β-D-glucopyranose and yielded the aglycones 11β,13-dihydro-14-hydroxyparthenolide (**3a**) and 11β,13-dihydro-14-hydroxycostunolide (**4a**). The ^{1}H NMR spectra (Tables 1 and 2) of the hydrolyzed products indicated loss of the sugar moiety resonances observed in **3** and **4**. Expected shielding of the H-14a proton was evident, while the remaining resonances remained generally unaffected. The HMBC and HMQC spectra allowed for the confirmation of the aglycone nuclei. Thus **3a** and **4a** were elucidated as 11,13-dihydro-14-hydroxyparthenolide and 11,13-dihydro-14-hydroxycostunolide, respectively, where the former was confirmed by comparison with literature data,[10] while the latter was found to be a new compound.

Assignment of the relative configuration of **3** and **4** was performed by analysis of the 2D NOESY experiments (Tables 1 and 2) as well as by consideration of the proton coupling constants exhibited in the ^{1}H NMR spectra and certain empirical ^{13}C NMR shifts. Deductions were drawn keeping in mind that molecular

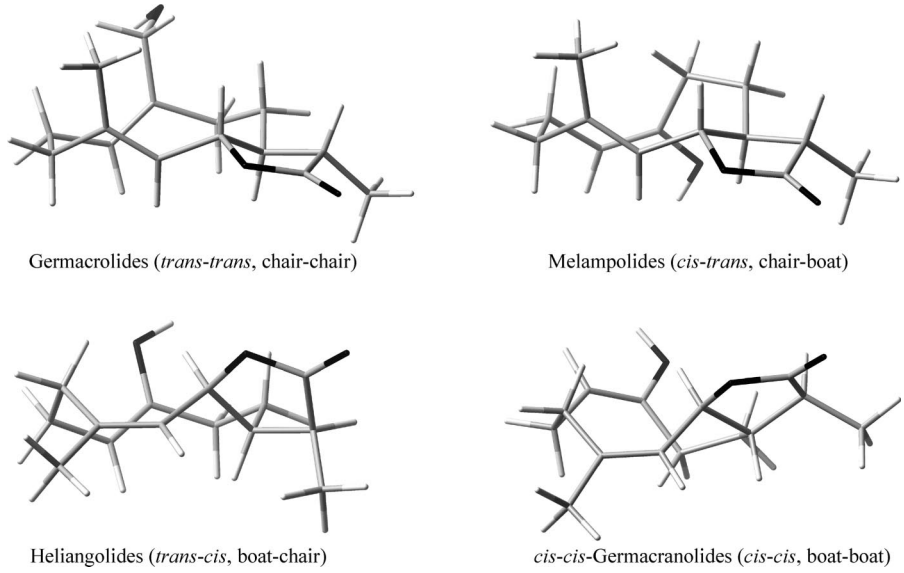

Figure 1. The four possible configurations for germacranolide **4a**.

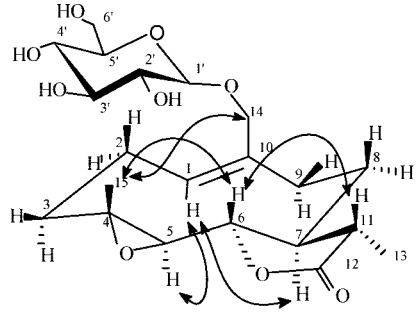

Figure 2. Decisive NOE correlations confirming the *trans*−*trans* chair−chair conformation of **3**.

modeling (MM) and X-ray structural data of the germacranolides (C_{10} ring) have indicated that four spatial isomers can be adopted depending on the geometry of $\Delta^{1,(10)}$ and Δ^4 (or the 4,5-epoxy system) of the cyclodecadiene ring. These systems can exist in *trans*−*trans* (chair−chair, germacrolides), *trans*−*cis* (boat−chair, helioangolides), *cis*−*trans* (chair−boat, melampolides), and *cis*−*cis* (boat−boat, *cis*−*cis* germacranolides) orientations, where all such configurations have been found to occur in nature. This has resulted in the necessary classification of the germacranolides into four subgroups.[11,12] Figure 1 illustrates the configurations that could exist for **4a**. It has been found that the *trans*−*trans* isomers are most common in nature, followed by the more strained *cis*−*trans* or melampolide isomers. These configurations can further be expressed according to the orientation of the C-14 and C-15 groups, where the adoption of what is referred to as a UU conformation[13] (the up–up arrangement of C-14 and C-15) is most common for *trans*−*trans* systems and results in the *syn*-orientation of these groups on the β-face of the ring,[12,14] as depicted in Figure 2 for **3**. In addition the up–down (UD), down−up (DU), and down–down (DD) arrangements of C-14 and C-15 are also possible.[13,15]

Examining compound **3**, certain ^{1}H NMR, ^{13}C NMR, and NOE observations proved crucial (Table 1, Figure 2). The C-1 double bond was assigned an *E*-configuration owing to the large coupling constants (δ 5.49, dd, J = 12.1, 3.9 Hz) exhibited due to the axial−axial orientation of H-1 and H-2β. Such $\Delta^{1,(10)}$ *cis*-isomers (melampolides) display distinctive downfield shifts of H-1 and H-15, the former to above 5.6 ppm with smaller coupling constants ($J \approx$ 8 Hz) and the latter to above 1.5 ppm, something that is not apparent in this case.[10,16−18] Furthermore, the δ_C shift of the hydroxylated C-14 methylene group of the hydrolyzed product **3a**, which

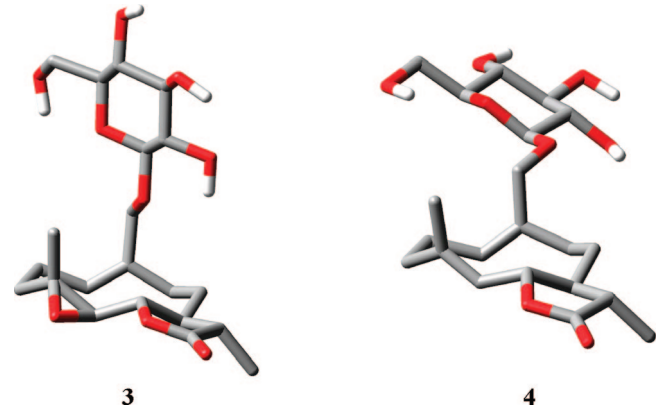

Figure 3. Molecular modelling results for compounds **3** and **4**.

resonated at approximately 60 ppm, was diagnostic of the germacrane *E*-$\Delta^{1,(10)}$ systems, where C-14 of such *Z*-$\Delta^{1,(10)}$ isomers appears at above 65 ppm.[10,15,19] This is an important distinguishing factor for these isomers and is also apparent in the more common cases involving a free C-10 methyl group, where C-14 appears at approximately 15 ppm in the germacrane systems and above 20 ppm for *Z*-$\Delta^{1,(10)}$ isomers.[20] The NOE correlations between H-14a and H-14b with H-15 indicated the existence of either a UU (portrayed in Figure 2) or a DD orientation thereof and thus a necessary *trans*-epoxy system across C-4 and C-5. In addition, NOE correlations of H-1 with both H-5α and H-7α indicated their homofacial axial alignment. The epoxy ring orientation is as depicted in Figure 2 given that the NOE experiment indicated correlations between H-1 and H-5α as well as between H-6β and H-15. Furthermore, the γ-lactone C-11 methyl group was assigned an α-orientation, as NOE correlations between H-11 and the β-oriented H-6 atom were observed, indicating their 1,3-diaxial relationship. The ^{13}C NMR resonance of C-13 (δ 13.3) was also indicative of the aforementioned orientation (for **3** and **3a**), where such β-oriented methyl groups resonate at ~11.0 ppm.[10,21] The stereochemistry of the dihydroparthenolide nucleus of **3** was thus equivalent to that of parthenolide (**1**).[22] The C-13 methyl group was assigned an α-orientation, allowing **3** to be identified as 11β,13-dihydroparthenolide-14-*O*-β-D-glucopyranoside and **3a** as11β,13-dihydro14-hydroxyparthenolide.

Similarly, the NOE results for **4** (Table 2) were in agreement with the stereochemistry of costunolide. Differentiation from the melampolide derivative thereof was directly discerned from the ^{1}H

Table 3. Experimental (NMR) and Theoretical (MM)[a] Coupling Constants Observed for **3** and **4**

C/H no.	**3** m, *J* in Hz	**MM-3 UU**[a] *J* in Hz	**MM-3 DD**[a] *J* in Hz	**4** m, *J* in Hz	**MM-4 UU**[a] *J* in Hz	**MM-4 DD**[a] *J* in Hz
1	dd, 12.1, 3.9	11.5, 3.8	11.5, 3.1	dd, 12.6, 4.1	11.0, 4.4	11.6, 3.6
2α	m	4.6, 3.8, 2.3	5.4, 3.1, 1.5	m	5.8, 4.4, 1.4	5.0, 3.6, 1.7
2*β*	dq, 12.9, 5.5	13.1, 11.5, 4.4	12.5, 11.5, 5.9	dq, 12.5, 5.1	12.0, 11.0, 5.8	12.5, 11.6, 5.3
3α	m	13.1, 4.6	12.5, 5.4	dq, 12.0, 5.1	12.0, 5.8	12.5, 5.0
3*β*	ddd, 12.5, 5.5, 1.6	4.4, 2.3	5.9, 1.5	ddd, 12.0, 4.7, 1.6	5.8, 1.4	5.3, 1.7
5α	d, 9.2	8.5	5.7	m	11.2	6.5
6*β*	t, 9.2	10.3, 8.5	11.0, 5.7	m	11.2, 10.4	10.3, 6.5
7α	m	13.3, 11.1, 10.3, 1.1	12.3, 11.0, 7.0, 2.4	m	11.7, 11.6, 10.4, 1.1	12.1, 11.6,10.3, 3.7
8α	m	5.8, 1.7, 1.1	4.3, 3.8, 2.4	br dd, 12.6, 6.1	6.4, 1.3, 1.1	6.3, 6.2, 2.4
8*β*	m	13.3, 12.2, 1.8	7.0, 12.9, 2.8	br d, 12.6	13.2, 11.6, 1.4	12.1, 11.4, 1.5
9α	m	12.2, 1.7	12.9, 4.3	m	13.2, 1.3	11.4, 6.3
9*β*	br d, 12.9	5.8, 1.8	3.8, 2.8	br dd, 12.5, 5.5	6.4, 1.4	6.2, 1.5
11*β*	dq, 12.0, 7.0	11.1, 6.5	12.3, 7.0	dq, 12.3, 7.0	11.7, 7.0	11.6, 7.0
13	d, 7.0	6.5	7.0	d, 7.0	7.0	7.0
14a	d, 11.0			d, 11.0		
14b	d, 11.0			d, 11.0		
15	s			s		
1′	d, 7.8	7.8	7.8	d, 7.4	7.7	7.6
2′	dd, 9.0, 7.8	8.9, 7.6	9.6, 7.8	dd, 9.0, 7.8	9.0, 7.7	8.9, 7.6
3′	m	8.9, 8.6	9.6, 9.3	t, 8.9	9.0, 8.6	8.9, 8.6
4′	m	9.6, 8.6	9.6, 9.3	m	9.6, 8.6	9.6, 8.6
5′	m	10.5, 9.6, 3.1	9.9, 9.6, 2.2	m	10.5, 9.6, 3.1	10.3, 9.6, 2.9
6a′	dd, 11.7, 1.6	3.1	2.2	dd, 12.1, 2.0	3.1	2.9
6b′	dd, 11.7, 5.5	10.5	9.9	dd, 12.1, 5.1	10.5	10.3

[a] Geminal proton coupling constants were not observed.

NMR spectrum of **4**, where melampolide isomers exhibit deshielded H-1 and H-15 resonances which appear above 5.5 and 1.8 ppm, respectively, due to the *cis*-orientation of the $\Delta^{1,(10)}$ system.[23,24] Furthermore, a *Z*-Δ^4 system is known to affect the downfield shift of the H-1, H-5, and H-15 resonances[25] as well as characteristically reducing the coupling constant $J_{6,7} \approx 3$ Hz.[20,26] Due to the overlap of the H-5 and H-6 resonances, their coupling constants could not be used to verify the presence of an *E*-Δ^4 system. Instead, the C-15 resonance proved diagnostic, where it has been shown that *Z*-Δ^4 isomers cause the deshielding of C-15 to above 20 ppm, something that is not evident in this case, as C-15 resonates at 17.6 and 17.2 ppm for **4** and **4a**, respectively.[20,27] Once again, the C-11 methyl group was assigned an α-orientation (C-13, δ 13.7), allowing **4** to be identified as 11*β*,13-dihydrocostunolide-14-*O*-*β*-D-glucopyranoside and **4a** as 11*β*,13-dihydro-14-hydroxycostunolide.

Finally, molecular modeling was carried out for compounds **3** and **4** using Macromodel v. 5[28] to calculate the possible low-energy conformations thereof, keeping in mind that only the UU and DD conformations are plausible arrangements (NOE results). Results of the UU conformers of **3** and **4** were consistent with the aforementioned experimental findings (Figure 3, Table 3), while those of the DD conformers were incompatible. The C_{10} nuclei are hence represented by a double chair conformation with crossed double bonds in the UU or $^{15}D_5$, $^{1}D_{14}$-conformation, as has been shown by X-ray diffraction.[13] The theoretical coupling constants for the UU and DD low-energy conformers of **3** and **4** were calculated by the NMR module[29] of Macromodel v. 5 and are shown in Table 3, where the former were in agreement with those obtained experimentally from the ^{1}H NMR spectra.

Four sesquiterpene lactones were identified in *S. fruticosa*, namely, parthenolide (**1**), costunolide (**2**), and the new 11*β*,13-dihydroparthenolide-14-*O*-*β*-D-glucopyranoside (**3**) and 11*β*,13-dihydrocostunolide-14-*O*-*β*-D-glucopyranoside (**4**). Enzymatic hydrolysis of the glycosidic constituents resulted in the aglycone derivatives **3a** and **4a**, where 11*β*,13-dihydro-14-hydroxycostunolide (**4a**) was identified as a new compound. Such dihydrogermacranolides are rare in nature and have been isolated mainly from species within the Asteraceae family. Examination of the Cardueae tribe, to which the *Staehelina* genus (subtribe Centaureinae) belongs, revealed the isolation of few dihydrogermacranes from subtribe Carduinae,[30] while the Centaureinae[11,31] subtribe has not yielded any such dihydro constituents.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. NMR spectra were obtained with a Bruker AC 200 and a Bruker DRX 400 spectrometer. Chemical shifts were given as δ values with TMS as the internal standard. The 2-D experiments (COSY, COSY LR, HMQC, HMBC, and NOESY) were performed using standard Bruker microprograms. The deuterated solvents (Aldrich) employed included C_6D_6, $CDCl_3$, and CD_3OD. MS spectra were recorded on a Nermag R 10 10C and an MSQ Surveyor, Finnigan apparatus. GC-MS analysis was carried out on a Hewlett-Packard 6890-5973 apparatus.

Plant Material. The aerial parts of *S. fruticosa* (220 g) were collected from eastern Crete in June 2004 when flowering had just begun. It was collected from hard limestone cliffs above the Limnakaros plateau of the Dikti mountain range in Lasithi of eastern Crete, 1200 m above sea level. A voucher specimen (KL 169) was deposited in the Herbarium of the Pharmacognosy Division in the University of Athens, Greece.

Extraction and Isolation. The dried and ground aerial parts of *S. fruticosa* were extracted with CH_2Cl_2, MeOH, and H_2O, respectively. Each solvent extraction was repeated three times, for 48 h per extraction. Isolates from the CH_2Cl_2 extract (14 g) were purified directly by normal-phase column chromatography, where costunolide (**2**) was obtained using a 9:0.5 CH_2Cl_2−EtOAc solvent system, while parthenolide (**1**) was eluted after increasing the polarity to 9:1. The MeOH extract (7.9 g) was further re-extracted (200 mL × 3) with *n*-BuOH (1.7 g), and 0.5 g of this extract was subjected to fast centrifugal partition chromatography (FCPC) incorporating the use of a CPC Kromaton with a 200 mL column, adjustable rotation of 200–2000 rpm, and a Laboratory Alliance pump with a pressure safety limit of 50 bar. The chosen biphasic system comprised EtOAc−*n*-BuOH−EtOH−H_2O, (3:0.6:1:5). The lower phase was used as the mobile phase (water based), while the upper phase was used as the stationary phase in a head-to-tail or descending mode. The effluent of the column was manually collected in 30 mL aliquots until the entire sample had been eluted. Fractions 2 and 3 yielded compounds **3** and **4**, respectively.

Parthenolide (1): white, amorphous solid (18.9 mg); Si gel TLC R_f 0.5 (9:1 CH_2Cl_2−EtOAc); $[\alpha]^{25}_D$ −80 (*c* 0.66, $CHCl_3$); EIMS *m*/*z* (rel int %) 248 (5), 230 (8), 190 (10), 105 (12), 91 (14), 81 (13), 58 (25).

Costunolide (2): white, amorphous compound (13.2 mg); Si gel TLC R_f 0.32 (8:2 cyclohexane−EtOAc); $[\alpha]^{25}_D$ +128 (*c* 0.34, $CHCl_3$); EIMS *m*/*z* (rel int %) 232 (53), 217 (33), 204 (5), 189 (10), 124 (46), 109 (66), 81 (100), 53 (76).

11*β*,13-Dihydroparthenolide-14-*O*-*β*-D-glucopyranoside (3): white, amorphous solid (11.7 mg), Si gel TLC R_f 0.78 (3:1 CH_2Cl_2−MeOH); $[\alpha]^{25}_D$ −36 (*c* 0.45, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C

NMR (CD_3OD, 50 MHz) see Table 1; +ve APCI *m/z* 429.3; HREIMS *m/z* 451.1950 (calcd for $C_{21}H_{32}O_9Na$, 451.1944).

11β,13-Dihydro-14-hydroxyparthenolide (3a): white, amorphous solid (1.9 mg, 0.0071 mmol, 88.1%) yielded by the treatment of **3** (3.5 mg, 0.0081 mmol) with β-glucosidase (10 mg) in deionized H_2O at 37 °C for 48 h; Si gel TLC R_f 0.65 (1:2 CH_2Cl_2−EtOAc); $[\alpha]^{25}_D$ −8 (*c* 0.25, MeOH); ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 50 MHz) see Table 1; HREIMS *m/z* 305.2504 (calcd for $C_{15}H_{22}O_4K$, 305.2501).

11β,13-Dihydrocostunolide-14-*O*-β-D-glucopyranoside (4): white, amorphous solid (8.2 mg); Si gel TLC R_f 0.84 (3:1 CH_2Cl_2−MeOH); $[\alpha]^{25}_D$ −15 (*c* 0.20, MeOH); ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 50 MHz) see Table 2; +ve APCI *m/z* (rel int %) 413 (100); HREIMS *m/z* 413.2102 (calcd for $C_{21}H_{33}O_8$, 413.2097).

11β,13-Dihydro-14-hydroxycostunolide (4a): white, amorphous solid (1.1 mg, 0.0044 mmol, 65.6%) yielded by the treatment of **4** (2.8 mg, 0.0067 mmol) with β-glucosidase (10 mg) in deionized H_2O at 37 °C for 48 h; Si gel TLC R_f 0. 49 (1:2 CH_2Cl_2−EtOAc); $[\alpha]^{25}_D$ +22 (*c* 0.45, MeOH); ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 50 MHz) see Table 2; HREIMS *m/z* 273.1469 (calcd for $C_{15}H_{22}O_3Na$, 273.1467).

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