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Unusual Fernane and Gammacerane Glycosides from the Aerial Parts of *Spergula fallax*

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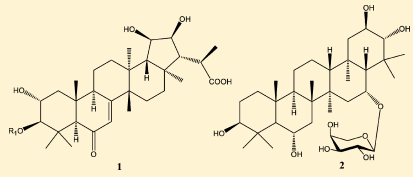
Supporting Information

ABSTRACT: The aerial parts of *Spergula fallax* afforded four glycosides (**1**−**4**) based on two new triterpene aglycones (**1a** and **2a**), along with the known hopane glycoside succulentoside A. Compound **1** was identified as belonging to the fernane class, unusual migrated hopane triterpenoids, mainly isolated from ferns and only rarely from higher plants. Compounds **2**−**4** were assigned as gammacerane glycosides, having as aglycone a hydroxylated derivative of tetrahymanol. The structures of the isolated compounds **1**−**4** and their aglycones **1a** and **2a** obtained by acid hydrolysis were elucidated by spectroscopic data interpretation. The growth inhibitory activity of the isolated compounds and their aglycones was evaluated against the HeLa and DLD-1 cancer cell lines.



As part of an ongoing investigations of wild medicinal plants growing in the Egyptian desert, a phytochemical study of *Spergula fallax* L. was carried out. The genus *Spergula* (Caryophyllaceae) comprises 14 species distributed in temperate regions (Central India, North Africa, Canary Islands, and South America).[1−3] A previous phytochemical investigation on the Peruvian *Spergularia* (syn. *Spergula*) *ramosa* led to the isolation of oleanane glycosides.[1]

Spergula fallax (syn. *Spergularia flaccida* and *Spergularia fallax*), is an annual herb distributed in the Egyptian desert, known by the Arabic name "Gileglaag".[4,5] The aerial parts of the plant are used as a diuretic, while the seeds are usually dried and ground into a meal and then used with flour for making bread.[6]

From the aerial parts of *S. fallax* four glycosides with new aglycones (**1**−**4**), along with the known hopane glycoside succulentoside A,[7] were isolated. Compound **1** was identified as a fernane derivative. Fernanes are unusual triterpenoids belonging to the migrated hopane class and mainly isolated previously from ferns.[8] Compounds **2**−**4** were assigned as gammacerane glycosides, possessing the same aglycone, a new hydroxylated derivative of tetrahymanol.[9]

RESULTS AND DISCUSSION

The structures of **1**−**4** were elucidated by spectroscopic methods including 1D- (^{1}H, ^{13}C, and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC, and ROESY) experiments as well as ESIMS analysis.

The HRMALDITOFMS of **1** (*m*/*z* 877.4192 [M + Na]$^+$, calcd for $C_{43}H_{66}O_{17}Na$, 877.4198) supported a molecular formula of $C_{43}H_{66}O_{17}$. The ESIMS showed a major ion peak at *m*/*z* 877 [M + Na]$^+$. Analysis of the ESIMS^2 data of **1** showed peaks at *m*/*z* 835 [M + Na − 42]$^+$, ascertaining the presence of an acetyl moiety, at *m*/*z* 673 [M + Na − 42 − 162]$^+$, and at *m*/*z* 541 [M + Na − 42 − 162 − 132]$^+$, ascribable to the loss of both a hexose and a pentose moiety.

The ^{13}C NMR spectrum of **1** showed 43 carbon signals, of which 30 were due to the aglycone moiety and 11 to a saccharide portion made up of two sugar units (Tables 1 and 2). The ^{1}H NMR spectrum displayed signals for six tertiary methyl groups at δ 0.87, 0.95, 1.07, 1.11, 1.17, and 1.34, a secondary methyl at δ 1.30 (d, J = 8.0 Hz), an olefinic proton at δ 5.83 (s), four oxygen-bearing methine protons at δ 3.09 (d, J = 10.8 Hz), 3.68 (m), 4.31 (dd, J = 11.0, 2.0 Hz), and 4.80 (dd, J = 7.0, 2.0 Hz), and one methyl signal at δ 2.16 (s), ascribable to an acetyl group (Tables 1 and 2). In the ^{13}C NMR spectrum, signals for methyl groups at δ 15.2, 16.9, 17.4, 17.6, 22.0, 22.4, and 27.9, two olefinic carbons at δ 124.0 and 171.7, two carboxyl carbons at δ 171.5 and 182.6, and a carbonyl group at δ 201.3 suggested the occurrence of a triterpene derivative. The structural units of rings A and B were established by HMBC correlations between the methyl signal at δ 1.07 (Me-24) and 1.34 (Me-23) with the carbon resonances at δ 94.0 (C-3) and 65.4 (C-5). A COSY correlation between the proton resonances at δ 3.09 (H-3) and 3.68 (H-2) revealed the presence of two hydroxy groups at C-2 and C-3, oriented as α and β, respectively, as deduced by the coupling constant of H-3 (d, J = 10.8 Hz) and by the ROESY correlations between H-2 and Me-25 and from H-3 to H-5 and Me-23. The HMBC

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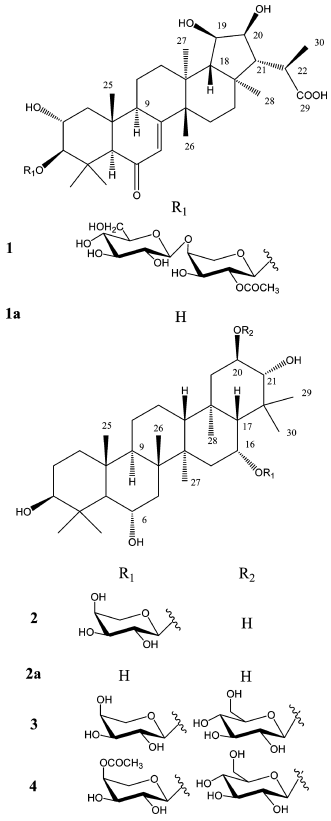

correlations between the olefinic proton at δ 5.83 (s) (H-7) and the carbon resonances at δ 65.4 (C-5) and 201.3 (C-6) allowed the double bond to be located at C-7 and the carbonyl group at C-6. Analysis of NMR data (HSQC, HMBC, COSY, and 1D-TOCSY) suggested that compound **1** is a triterpene derivative, belonging to either the arborane or fernane class.[9] The differences between arborane and fernane triterpenoids are the opposite configurations at C-8, C-13, C-14, C-17, C-18, and C-21. The relationship between Me-25 and H-8 is *syn*-1,3-diaxial in arboranes and *anti*-1,3-diaxial in fernanes.[10] The two skeletons can be distinguished based on a ROESY correlation between H-8 and Me-25, which is diagnostic for arborane derivatives, while not expected for fernane derivatives.

In the absence of a H-8 resonance, to establish the relative configuration of the triterpene skeleton of **1**, the ROESY correlations starting from H-9 were investigated. The correlations observed between H-9 (δ 3.09) and Me-27 (δ 1.11), as well as from H-18 (δ 1.80) to Me-26 (δ 1.17) and H-21 (δ 2.26), together with the absence of ROESY correlations between H-9 and Me-25 (δ 0.95) and from Me-28 (δ 0.87) to H-18 and H-21, indicated that **1** is a fernane-type triterpenoid. The long-range correlation between the secondary methyl proton at δ 1.30 (Me-30) and the carboxylic group at δ 182.6 revealed that one of the methyl groups of the isopropyl function connected to C-21 in a fernane-type skeleton is substituted by a carboxylic group. Moreover, the further secondary hydroxy group functions at δ 4.31 and 4.80 could be located at C-19 and C-20, respectively, on the basis of COSY correlations between the proton signal at δ 4.31 (H-19, dd, J = 11.0, 2.0 Hz) and the proton resonance at δ 4.80 (H-20, dd, J = 7.0, 2.0 Hz), which, in turn, correlated with a methine signal at δ 2.26 (H-21, d, J = 7.0 Hz). The β-orientation of the OH groups at C-19 and C-20 was deduced from the coupling constants of the protons of ring E and confirmed by a ROESY experiment, which showed a correlation between H-19 (δ 4.31) and Me-27 (δ 1.11). The ROESY correlation between H-20α (δ 4.80) and the methyl doublet at δ 1.30, along with the absence of ROESY correlations between this methyl doublet and H_2-16 (δ 1.61), supported the presence of this methyl group at Me-30.[8] On the basis of this evidence, the aglycone of **1** was established as 2α,3β,19β,20β-tetrahydroxyfern-7-en-6-oxo-29-oic acid.

The ^{1}H NMR spectrum in the saccharide portion of **1** exhibited signals ascribable to two anomeric protons at δ 4.54 (d, J = 5.2 Hz) and 4.83 (d, J = 8.0 Hz), which correlated in the HSQC spectrum to the corresponding carbon resonances at δ 103.4 and 103.0. The NMR data (HSQC, HMBC, COSY, 1D-TOCSY) indicated the presence of α-arabinopyranosyl (δ 4.54) and β-glucopyranosyl (δ 4.83) units. The configurations of the arabinose and glucose units were established as L and D, respectively, after hydrolysis of **1** with 1 N HCl, trimethylsilation, and GC analysis.[11] An unambiguous determination of the linkage site was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal of H-1′ (δ 4.54) and the ^{13}C NMR resonance of C-3 (δ 94.0) and between the proton signal of H-1″ (δ 4.83) and the ^{13}C NMR resonance of C-4′ (δ 77.2). Moreover, the downfield shift observed for H-2′ (δ 4.91), indicative of an esterification of the hydroxy group (Table 2), was observed. In the HMBC spectrum, correlations between the proton signal at δ 4.91 (H-2′) and the carbon resonance at δ 171.5 and between the proton signal at δ 2.16 and the same carbon revealed the presence of the acetyl group at C-2′. Therefore, compound **1** was determined as 3-*O*-[β-D-glucopyranoside-(1→4)-*O*-α-L-(2-*O*-acetyl)-arabinopyranosyl]-2α,3β,19β,20β-tetrahydroxyfern-7-en-6-oxo-29-oic acid.

The HRMALDITOFMS of **2** (m/z 647.4142 [M + Na]$^+$, calcd for $C_{35}H_{60}O_9Na$, 647.4135) supported a molecular formula of $C_{35}H_{60}O_9$. The ESIMS showed a major ion peak at m/z 647, which was assigned to [M + Na]$^+$. The MS/MS spectrum of this ion showed a peak at m/z 515 [M + Na − 132]$^+$, corresponding to the loss of a pentose unit.

The ^{13}C NMR spectrum of **2** showed 35 carbon signals, of which 30 were assigned to the aglycone moiety and five to a sugar unit (Tables 1 and 2). The ^{1}H NMR spectrum displayed signals for eight tertiary methyl groups at δ 0.93, 0.98, 1.02, 1.05, 1.12, 1.13, 1.31, and 1.40 and five oxygen-bearing methine protons at δ 2.88 (d, J = 9.7 Hz), 3.12 (dd, J = 11.6, 4.4 Hz), 3.62 (m), 4.00 (m), and 4.02 (ddd, J = 10.8, 9.5, 4.5 Hz) (Table 1). These signals, along with the carbon resonances in the ^{13}C NMR spectrum for the methyl groups at δ 15.6, 17.0, 17.2, 18.2 (3C), 31.2, and 31.7, suggested the presence of a triterpene derivative. In the HSQC spectrum, four methine correlations at δ_H 0.89/δ_C 61.3 (H-5), δ_H 1.19/δ_C 60.1 (H-17), δ_H 1.30/δ_C 50.3 (H-9), and δ_H 1.35/δ_C 50.4 (H-13) were observed. The chemical shifts of the A, B, and C rings were similar to those reported for an oleanane derivative,[12] but differences for the ^{13}C NMR resonances of the D and E rings were observed. In particular, in the HMBC spectrum, correlations between the methyl signal at δ 1.13 (Me-27) and the carbon resonance at δ 50.4 allowed a methine function to be assigned to C-13. A further correlation between the methyl signal at δ 1.02 and the latter ^{13}C NMR resonance allowed the tertiary methyl group to be located at C-18 (40.9). A long-range correlation between the methyl signal at δ 1.02 and the methine carbon at δ 60.1 permitted the location of a methine function at C-17. The remaining methyl groups at δ 1.05 (Me-29) and 1.40 (Me-30)

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Table 1. ^{13}C and ^{1}H NMR Data (J in Hz) of the Aglycone Moieties of Compounds 1, 1a, 2, 2a, and 3 (600 Mz, δ ppm, in CD_3OD)[a]

	1		1a		2		2a		3		4	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	44.4	2.00 dd (12.5, 4.2), 1.39, t (12.5)	45.5	1.95 dd (12.5, 4.2), 1.41, t (12.5)	39.9	1.72, 1.00, m	39.7	1.71, 1.00, m	39.9	1.72, 1.00, m	39.8	1.72, 1.00, m
2	66.3	3.68, m	68.5	3.65, m	27.5	1.69, 1.61, m	27.4	1.64, 1.60, m	27.5	1.69, 1.61, m	27.5	1.70, 1.61, m
3	94.0	3.09, d (10.8)	83.8	2.97, d (10.8)	79.4	3.12, dd (11.6, 4.4)	79.5	3.12, dd (11.6, 4.4)	79.7	3.13, dd (11.6, 4.4)	79.8	3.12, dd (11.6, 4.4)
4	40.1		39.9		40.0		40.2		40.5		40.5	
5	65.4	2.45, s	66.3	2.40, s	61.3	0.89, d (10.8)	61.5	0.88, d (10.8)	61.6	0.89, d (10.8)	61.4	0.89, d (10.8)
6	201.3		202.5		68.9	4.02, ddd (10.8, 9.5, 4.5)	68.9	4.00, ddd (10.8, 9.5, 4.5)	68.9	4.01, ddd (10.8, 9.5, 4.5)	68.8	4.00, ddd (10.8, 9.5, 4.5)
7	124.0	5.83, s	124.3	5.83, s	45.8	1.58 (2H), m	45.5	1.59 (2H), m	45.8	1.58 (2H), m	45.7	1.58 (2H), m
8	171.7		172.0		43.5		43.5		43.1		43.1	
9	50.0	3.09, m	50.3	3.10, m	50.3	1.30, m	50.4	1.32, m	50.7	1.31, m	50.7	1.31, m
10	45.2		45.7		50.0		50.2		50.4		50.1	
11	16.6	1.85, 1.61, m	16.4	1.87, 1.63, m	22.2	1.65, 1.35, m	22.0	1.65, 1.35, m	22.2	1.70, 1.36, m	22.4	1.70, 1.37, m
12	32.2	1.89, 1.79, m	32.5	1.90, 1.80, m	22.3	1.66, 1.34, m	23.4	1.66, 1.34, m	22.1	1.64, 1.34, m	22.1	1.65, 1.34, m
13	36.5		37.2		50.4	1.35, m	50.4	1.35, m	50.1	1.37, m	50.1	1.37, m
14	44.2		44.6		43.1		43.1		43.6		43.6	
15	29.6	1.72, 1.64, m	30.0	1.73, 1.65, m	44.1	2.00, 1.64, m	37.4	1.67, 1.32, m	44.1	2.01, dd (12.7, 3.5), 1.64, m	43.9	2.04, dd (12.7, 3.5), 1.65, m
16	34.9	1.61 (2H), m	35.2	1.62 (2H), m	80.8	4.00, m	69.3	4.00, m	80.5	4.03, m	80.5	4.02, m
17	42.1		42.4		60.1	1.19, d (10.8)	61.3	0.98, d (10.8)	60.5	1.21, d (10.8)	60.0	1.21, d (10.8)
18	57.0	1.80, d (11.0)	57.1	1.79, d (11.0)	40.9		40.2		41.2		41.2	
19	77.2	4.31, dd (11.0, 2.0)	77.5	4.31, dd (11.0, 2.0)	47.9	2.00, 0.90, m	47.7	1.98, 0.90, m	45.6	2.17, dd (4.4, 13.0), 1.00, m	45.6	2.17, dd (4.4, 13.0) 1.00, m
20	92.8	4.80, dd (7.0, 2.0)	93.3	4.78, dd (7.0, 2.0)	68.7	3.62, m	69.2	3.63, m	78.6	3.76, td (4.4, 9.7, 11.5)	78.6	3.76, td (4.4, 9.7, 11.5)
21	57.6	2.26, d (7.0)	58.5	2.25, d (7.0)	84.6	2.88, d (9.7)	84.5	2.88, d (9.7)	82.2	3.05, d (9.7)	82.2	3.05, d (9.7)
22	36.9	2.63, d (8.0)	37.4	2.63, d (8.0)	40.8		41.0		41.1		41.0	
23	27.9	1.34, s	29.1	1.35, s	31.2	1.31, s	31.8	1.31, s	31.8	1.31, s	31.6	1.31, s
24	16.9	1.07, s	16.5	1.12, s	15.6	0.98, s	16.1	0.98, s	15.5	0.98, s	15.4	0.98, s
25	15.2	0.95, s	15.4	0.95, s	17.2	0.93, s	17.0	0.93, s	17.4	0.93, s	17.3	0.93, s
26	22.0	1.17, s	22.5	1.19, s	18.2	1.12, s	18.2	1.13, s	18.3	1.12, s	18.3	1.12, s
27	22.4	1.11, s	22.6	1.12, s	18.2	1.13, s	18.2	1.13, s	18.5	1.14, s	18.4	1.13, s
28	17.6	0.87, s	17.3	0.88, s	18.2	1.02, s	18.3	0.99, s	18.2	1.01, s	18.2	1.01, s
29	182.6		183.3		17.0	1.05, s	17.0	1.02, s	17.5	1.08, s	17.6	1.08, s
30	17.4	1.30, d (8.0)	17.4	1.31, d (8.0)	31.7	1.40, s	31.8	1.35, s	32.1	1.43, s	32.3	1.44, s

[a]The chemical shift values of the aglycone moiety of **4** were superimposable on those of **3**.

Table 2. ^{13}C and ^{1}H NMR Data (J in Hz) of the Sugar Portions of Compounds 1−4 (600 Mz, δ ppm, in CD_3OD)

	1		2		3		4	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
		α-Ara (at C-3)		α-Ara (at C-16)		α-Ara (at C-16)		α-Ara (at C-16)
1′	103.4	4.54, d (5.2)	105.6	4.38, d (5.2)	105.8	4.38, d (5.2)	105.8	4.38, d (5.2)
2′	74.5	4.91, dd (8.0, 5.2)	72.2	3.64, dd (8.0, 5.2)	72.2	3.64, dd (8.0, 5.2)	72.8	3.58, dd (8.0, 5.2)
3′	73.7	3.70, dd (8.0, 3.0)	74.3	3.56, dd (8.0, 3.0)	74.4	3.56, dd (8.0, 3.0)	72.9	3.69, dd (8.0, 3.0)
4′	77.2	3.88, m	68.7	3.84, m	68.8	3.84, m	72.5	5.02, m
5′	64.5	4.18, dd (12.5, 3.0), 3.43, dd (12.5, 2.6)	65.6	3.83, dd (12.5, 3.0), 3.52, dd (12.5, 2.6)	65.7	3.83, dd (12.5, 3.0), 3.52, dd (12.5, 2.6)	64.0	3.88, dd (12.5, 3.0), 3.61, dd (12.5, 2.6)
$COCH_3$	171.5						172.2	
$COCH_3$	21.0	2.16, s					20.7	2.12, s
		β-Glc (at C-4_{Ara})				β-Glc (at C-20)		β-Glc (at C-20)
1″	103.0	4.83, d (8.0)			102.4	4.35, d (7.9)	102.4	4.35, d (7.9)
2″	74.1	3.24, dd (9.0, 8.0)			74.6	3.21, dd (9.0, 7.9)	74.7	3.21, dd (9.0, 7.9)
3″	77.5	3.38, dd (9.0, 9.0)			77.5	3.38, dd (9.0, 9.0)	77.5	3.38, dd (9.0, 9.0)
4″	71.1	3.29, dd (9.0, 9.0)			71.4	3.31, dd (9.0, 9.0)	71.3	3.31, dd (9.0, 9.0)
5″	77.5	3.38, m			77.5	3.32, m	77.7	3.31, m
6″	64.5	3.91, dd (12.0, 2.5), 3.66, dd (12.0, 4.5)			62.5	3.89, dd (11.0, 2.2), 3.68, dd (11.0, 5.0)	62.5	3.88, dd (11.0, 2.2), 3.67, dd (11.0, 5.0)

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were located at C-22 on the basis of the HMBC correlations between their proton resonances (δ 1.05 and 1.40) and the carbon resonance at δ 60.1 (C-17). Thus, compound **2** was identified as a gammacerane derivative.[9] The relative configuration was determined from the ROESY spectrum. The usual *trans* junction of rings A and B was assumed with H-5α and Me-25β. A ROESY correlation between Me-25 and Me-26 showed that these two methyl groups are on the same side (β) of the main plane of the molecule, whereas correlations of the methine protons H-5 and H-9 with Me-27 showed that these are all on the opposite side (α) of the molecule. The D and E ring junctions were established as *trans*, as determined by the presence of ROESY correlations between Me-28 (δ 1.02) and Me-27 (δ 1.13), Hβ-17 (δ 1.19), and Hβ-13 (δ 1.35). This relative configuration reported for compound **2** was in agreement with that reported for other gammacerane derivatives.[9] HMBC experiments allowed a secondary hydroxy group function to be located at C-3 on the basis of the correlations between the methyl groups at δ 0.98 (Me-24) and 1.31 (Me-23) and the ^{13}C NMR resonance at δ 79.4. Thus, the aglycone of **2** was identified as a tetrahymanol derivative.[9,13] The COSY correlation between the proton resonances at δ 0.89 (H-5) and 4.02 permitted a hydroxy group to be assigned at C-6. The coupling constant of H-6 (ddd, J = 10.8, 9.5, 4.5 Hz) suggested an α-orientation of this hydroxy group,[14] as confirmed by ROESY correlations between the proton resonances at δ 4.02 (H-6) and the methyl signals at δ 0.93 (Me-25) and 0.98 (Me-24). The COSY correlation between the oxygen-bearing methine proton at δ 4.00 (m) and the methine proton at δ 1.19 (H-17) allowed a hydroxy group to be proposed at C-16, with an α-orientation, as deduced by the coupling constant of H-17 (d, J = 10.8 Hz) and by the ROESY correlations between the proton resonances at δ 4.00 (H-16) and 1.40 (Me-30). A further secondary hydroxy group was assigned to C-21 on the basis of the HMBC correlations of the methyl protons at δ 1.05 (Me-29) and 1.40 (Me-30) with the carbon resonance at δ 84.6. In a COSY experiment the corresponding proton at δ 2.88 (H-21) correlated with the proton at δ 3.62 (H-20), allowing a final aglycone secondary hydroxy group to be located at C-20. The coupling constant of H-21 (d, J = 9.7 Hz) revealed a *trans*-diaxial orientation with the proton at δ 3.62 (H-20), and ROESY correlations between the proton at δ 2.88 (H-21) and the methyl signal at δ 1.40 (Me-30) and the protons at δ 3.62 (H-20) and the methyl signal at δ 1.05 (Me-29) allowed the proposal of a β- and an α-orientation for the hydroxy groups at C-20 and C-21, respectively. On the basis of the above information, the aglycone of **2** was determined as 6α,16α,20β,21α-tetrahydroxytetrahymanol.

The ^{1}H NMR spectrum of **2** displayed in the saccharide region an anomeric proton at δ 4.38 (d, J = 5.2 Hz), which was correlated by a HSQC experiment to the corresponding carbon resonance at δ 105.6. The 2D-NMR data showed the presence of an α-arabinopyranosyl unit (Table 2) that could be located at C-16 on the basis of the HMBC correlation between the proton signal of H-1′ (δ 4.38) and the carbon resonance of C-16 (δ 80.8). The L configuration of the arabinose unit was established after hydrolysis of **2** with 1 N HCl, trimethylsilation, and analysis by GC.[11] Hence, the structure of compound **2** was proposed as 16-*O*-α-L-arabinopyranosyl-6α,16α,20β,21α-tetrahydroxytetrahymanol.

The molecular formula of **3** was established as $C_{41}H_{70}O_{14}$ by HRMALDITOFMS (m/z 809.4669 $[M + Na]^+$, calcd for $C_{41}H_{70}O_{14}Na$, 809.4663). A $[M + Na]^+$ ion peak at m/z 809 could be observed in the ESIMS of **3**, and the MS/MS of this ion showed peaks at m/z 647 $[M + Na - 162]^+$ and at m/z 515 $[M + Na - 162 - 132]^+$, corresponding to the loss of a hexose and a pentose unit, respectively. The ^{1}H and ^{13}C NMR signals of the aglycone of **3** assigned from the 2D-NMR spectra were almost superimposable on those of **2**, except for the downfield shift of H-20 (δ_H 3.76/δ_C 78.6) (Table 1). The ^{1}H NMR spectrum of the sugar region, in comparison with that of **2**, displayed an additional anomeric signal at δ 4.35 (d, J = 7.9 Hz), which was assigned unambiguously from HSQC, COSY, HMBC, and 1D-TOCSY experiments to a β-glucopyranosyl unit (Table 2). The D configuration of the glucose unit and the L configuration of the arabinose unit were established after hydrolysis of **3** with 1 N HCl, trimethylsilation, and GC analysis.[11] The linkage site of the β-glucopyranosyl unit was obtained from the HMBC spectrum, which showed a key correlation peak between the proton signal of H-1″ (δ 4.35) and the carbon resonance of C-20 (δ 78.6). Therefore, the structure of compound **3** was determined as 16-*O*-α-L-arabinopyranosyl-20-*O*-β-D-glucopyranosyl-6α,16α,20β,21α-tetrahydroxytetrahymanol.

The HRMALDITOFMS of **4** (m/z 851.4774 $[M + Na]^+$, calcd for $C_{43}H_{72}O_{15}Na$, 851.4769) supported a molecular formula of $C_{43}H_{72}O_{15}$. The ESIMS showed a major ion peak at m/z 851 $[M + Na]^+$. The MS/MS of this ion showed peaks at m/z 809 $[M + Na - 42]^+$, corresponding to the loss of an acetyl unit, at m/z 647 $[M + Na - 42 - 162]^+$, corresponding to the loss of a hexose unit, and at m/z 515 $[M + Na - 42 - 162 - 132]^+$, due to further loss of a pentose unit. The ^{1}H NMR spectrum of **4** was almost superimposable on that of **3**, except for the presence of a signal at δ 2.12, corresponding to an acetyl function. The NMR data of the sugar moieties of **4** were superimposable on those of **3** except for the downfield shift observed for H-4′ (δ 5.02) indicative of the esterification of the hydroxy group (Table 2). In the HMBC spectrum, correlations between the proton signal at δ 5.02 (H-4′) and the carbon resonance at δ 172.2 and between the proton signal at δ 2.12 and the same carbon revealed the presence of an acetyl group at C-4′. Therefore, compound **4** was determined as 16-*O*-(4-*O*-acetyl)-α-L-arabinopyranosyl-20-*O*-β-D-glucopyranosyl-6α,16α,20β,21α-tetrahydroxytetrahymanol.

Additionally, succulentoside A was isolated and was identified by comparison of its ^{1}H and ^{13}C NMR data with values reported in the literature.[7] Since the aglycones of **1** and **2**–**4** have not been reported before, compounds **1** and **2** were submitted to acid hydrolysis to afford the corresponding aglycones, **1a** and **2a**, which were characterized by NMR spectroscopy (Table 1) and ESIMS analysis.

Due to their scarce occurrence in Nature, very little is known on the biological activity of fernane and gammacerane derivatives. Antitumor-promoting activity has been reported for fernanes and gammaceranes isolated from ferns,[15] and inhibitory effects on DNA topoisomerases in vitro have been reported for fernane-type triterpenoids isolated from the genus *Euphorbia*.[16] Therefore, the growth inhibitory effects of compounds **1**–**4**, succulentoside A, and the aglycones **1a** and **2a** were tested against the HeLa (human epitheloid cervix carcinoma) and DLD-1 (colorectal adenocarcinoma) cancer cell lines. Only compound **4** showed weak activity, with IC_{50} value of 52 μM in both HeLa cells and DLD-1 cells.

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, Sigma-Aldrich), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. Exact masses were measured using a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from the ACTH (fragment 18−39) at 2465.1989 Da, with α-cyano-4-hydroxycinnamic acid at 190.0504 Da as an internal standard.

Plant Material. The aerial parts of *Spergula fallax* were collected at Elba-Mountain, South-Eastern Desert, Aswan, Egypt, in April 2011, and identified by Prof. N. Kadry (Sohag University, Egypt) according to Boulos.[2] A representative voucher specimen (no. 10415) was deposited at the Botany Department Herbarium, Faculty of Science, Aswan University, Aswan, Egypt.

Extraction and Isolation. The aerial parts of *S. fallax* (190 g) were powdered and extracted exhaustively with 80% MeOH (3 × 1.5 L) for 20 days by maceration at room temperature. The crude extract was concentrated under reduced pressure to a syrupy consistency (23 g). A 14 g aliquot from the crude extract was dissolved in a small quantity of H_2O and loaded on a water-preconditioned short flash C_{18} column (6 × 10 cm, LiChroprep, RP-18, 40−60 μm, Merck). Six fractions (1000 mL) were collected: 100% H_2O (S-1, 550 mg), 20% MeOH (S-2, 624 mg), 40% MeOH (S-3, 226 mg), 60% MeOH (S-4, 349 mg), 80% MeOH (S-5, 500 mg), and 100% MeOH (1.411 g), respectively. Fraction S-3 was loaded on a small C_{18} column (3 × 30 cm, LiChroprep, RP-18, 25−40 μm, Merck) and eluted with a gradient of 20−25% MeOH to give compounds **2** (10 mg) and **3** (20 mg), respectively. Fraction S-4 was loaded on a small C_{18} column (3 × 30 cm, LiChroprep, RP-18, 25−40 μm, Merck) and eluted with a gradient of 30−35% MeOH to give compounds **4** (12 mg) and **1** (10 mg), respectively. Fraction S-5 was fractioned on an RP-18 column (50 × 2) eluted with 60−70% MeOH as mobile phase to yield succulentoside A (40 mg).

Compound ***1:*** amorphous, yellow solid; $[\alpha]^{25}_D$ −36.0 (*c* 0.13 MeOH); IR (KBr) ν_{max} 3430, 2930, 1665, 1650 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) data of the aglycone moiety and the sugar portion, see Tables 1 and 2, respectively; HRMALDITOFMS $[M + Na]^+$ *m/z* 877.4192 (calcd for $C_{43}H_{66}O_{17}Na$, 877.4198).

Compound ***2:*** amorphous, white solid; $[\alpha]^{25}_D$ +20.7 (*c* 0.20 MeOH); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) data of the aglycone moiety and the sugar portion, see Tables 1 and 2, respectively; HRMALDITOFMS $[M + Na]^+$ *m/z* 647.4142 (calcd for $C_{35}H_{60}O_9Na$, 647.4135).

Compound ***3:*** amorphous, white solid; $[\alpha]^{25}_D$ +32.0 (*c* 0.04 MeOH); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) data of the aglycone moiety and the sugar portion, see Tables 1 and 2, respectively; HRMALDITOFMS $[M + Na]^+$ *m/z* 809.4669 (calcd for $C_{41}H_{70}O_{14}Na$, 809.4663).

Compound ***4:*** amorphous, white solid; $[\alpha]^{25}_D$ +5.23 (*c* 0.19 MeOH); IR (KBr) ν_{max} 3450, 2945 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) data of the aglycone moiety and the sugar portion, see Tables 1 and 2, respectively; HRMALDITOFMS $[M + Na]^+$ *m/z* 851.4774 (calcd for $C_{43}H_{72}O_{15}Na$, 851.4769).

Acid Hydrolysis. Compounds **1** (5.3 mg) and **2** (5.0 mg) were treated with 2 M HCl in 1,4-dioxane−H_2O (1:1, v/v, 1.5 mL) at 80 °C for 3 h. On cooling, solvent was eliminated with a stream of N_2, each dry residue was suspended in water, and the aglycones were extracted with ethyl acetate (3 × 1.5 mL). After evaporating the solvent, aglycones were purified by silica gel column chromatography (1.5 cm i.d. × 5 cm), eluted with $CHCl_3$−MeOH (15:2), to obtain compounds **1a** and **2a**, respectively.

Compound ***1a:*** amorphous, yellow solid; $[\alpha]^{25}_D$ −8.5 (*c* 0.10 MeOH); IR (KBr) ν_{max} 3445, 2935, 1660, 1650 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 1; HRMALDITOFMS $[M + Na]^+$ *m/z* 541.3138 (calcd for $C_{30}H_{46}O_7Na$, 541.3131).

Compound ***2a:*** amorphous, white solid; $[\alpha]^{25}_D$ +10.2 (*c* 0.10 MeOH); IR (KBr) ν_{max} 3440, 2940 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 600 MHz) data, see Table 1; HRMALDITOFMS $[M + Na]^+$ *m/z* 515.3716 (calcd for $C_{30}H_{52}O_5Na$, 515.3712).

Determination of Sugar Configuration. The configurations of sugar units of compounds **1** and **2** were established after hydrolysis of **1**−**4** with 1 N HCl, trimethylsilation, and determination of the retention times by GC operating in the experimental conditions previously reported by De Marino et al.[10] The peaks of the hydrolysate of **1** were detected at 14.72 min (D-glucose) and at 8.94 and 9.81 min (L-arabinose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)imidazole in pyridine were detected at 14.71 min (D-glucose) and 8.92 and 9.80 (L-arabinose).

Cancer Cell Lines and Treatment. HeLa and DLD-1 cells, from American Type Culture Collection, were grown in RPMI-1640 medium and DMEM, respectively, as previously reported.[17]

HeLa and DLD-1 cells, growing as monolayers, were plated one day before the beginning of treatment at a density of 1.3 × 10^5/mL. After 72 h of incubation with different concentrations of each test compound, cell viability was determined by an MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]) assay.[18] Mitochondria in living cells transform MTT to formazan salts, which, upon solubilization with DMSO, can be read at 550 nm using a microplate reader (LabSystems, Vienna, VA, USA). Data were subtracted of the corresponding appropriate blank. The number of viable cells in treated samples was calculated as a percentage of control samples containing equal amounts of vehicle (DMSO). Etoposide was used as a positive control (IC_{50} values of 16 and 20 μM against HeLa and DLD-1 cells, respectively). To exclude any interference of test compounds with the tetrazolium salt-based assay, cell growth inhibition was randomly verified also by cytometric count (trypan blue exclusion test).

Statistical Analysis. IC_{50} data reported are the mean values ± SD of at least two experiments performed in duplicate.

ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR, HSQC, HMBC, COSY, and ROESY spectra of compounds **1**−**4** and their aglycones **1a** and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Prof. Dr. Otto Sticher, of ETH-Zurich, Zurich, Switzerland, for his pioneering work in pharmacognosy and phytochemistry.

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