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Comosusols A−D and Comosone A: Cytotoxic Compounds from the Brown Alga *Sporochnus comosus*

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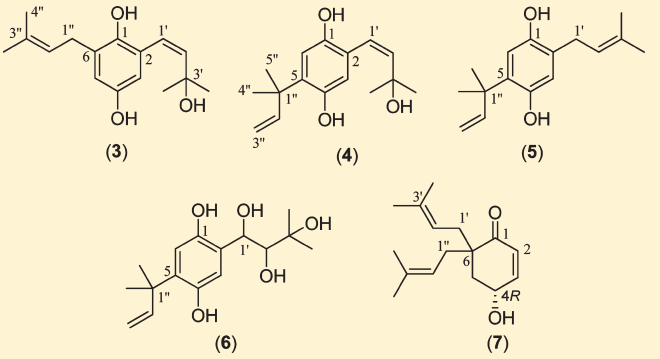
Supporting Information

ABSTRACT: Bioassay-guided fractionation of extracts of the brown alga *Sporochnus comosus* led to the isolation of five new compounds, comosusols A−D (**3**−**6**) and comosone A (**7**). The structures of all isolated compounds were elucidated using standard one- and two-dimensional NMR techniques, as well as comparison with literature values. The cytotoxic activity of all compounds was investigated against a panel of human tumor and mammalian cell lines. These assays found eight of the nine compounds had GI_{50} values in the 8−63 μM range.



Reports on the chemistry of the brown algae *Sporochnus* are rare, with only three found in the literature.[1−3] The first concerns the isolation of the prenylated phenols sporochnols A−C from a Caribbean specimen of *Sporochnus bolleanus*,[1] the second describes the isolation of the diprenylated phenol **1** from a Canary Island specimen of *Sporochnus pedunculatus*,[2] while the third details the presence of bromophenols in an east Australian sample of *Sporochnus comosus*.[3] Furthermore, there are only five additional reports of chemistry from the family Sporochnaceae.[4−8] All of these describe chemistry isolated from specimens of the genus *Perithalia*, including a report of **1** from a Tasmanian specimen of *P. caudata*[4] and **2** from a New Zealand specimen of *P. capillaries*.[5] This lack of reported chemistry makes algae of the family Sporochnaceae, and in particular, specimens of the genus *Sporochnus*, attractive potential targets for the isolation of novel bioactive secondary metabolites.

While screening extracts of marine flora and fauna for cytotoxic activity, a small-scale EtOH extract of *S. comosus*, collected off Shaw Island, Queensland, Australia, in October 1987, was found to have significant cytotoxic activity *in vitro* and a unique profile in the NCI 60 cell line COMPARE analysis.[9] Subsequent bioassay-guided fractionation of large-scale CH_2Cl_2, MeOH, and aqueous extracts of *S. comosus* led to the isolation of four new bis-prenylated quinols, comosusols A−D (**3**−**6**), and the new cyclohexenone comosone A (**7**). Also isolated were the known bis-prenylated phenol **1**,[2,4] the bis-prenylated quinone **2**,[5] fucoxanthin,[10] and galactitol.[11] The structures of the known compounds were readily identified through interpretation of NMR data and comparison with the literature. Discussed below in detail are the structure elucidations of **3**−**7** and the associated biological activities of all compounds.

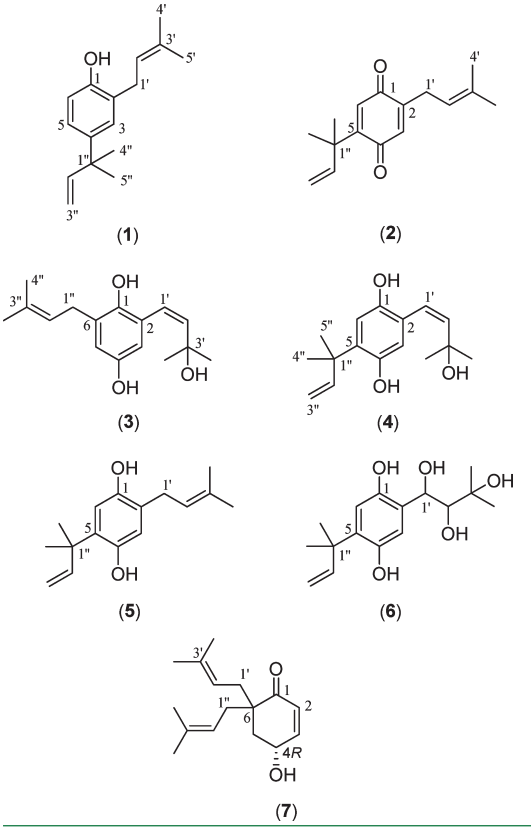


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Table 1. NMR Spectroscopic Data (600 MHz for ^{1}H, 150 MHz for ^{13}C, $CDCl_3$) for Comosusol A (3)

pos.	δ_C, mult	δ_H (J in Hz)	COSY	gHMBC[a]
1	144.3, C			
2	121.8, C			
3	110.3, CH	6.34, d (2.8)	H-5	1, 2, 4, 1′
4	148.9, C			
5	115.8, CH	6.48, d (2.8)	H-3	1, 4, 6, 1″
6	130.3, C			
1′	122.5, CH	6.23, d (9.7)		1, 2, 3, 2′, 3′
2′	131.7, CH	5.61, d (9.7)		2, 1′, 4′, 5′
3′	75.5, C			
4′	27.8, CH_3	1.39, s		2′, 3′, 5′
5′	27.8, CH_3	1.39, s		2′, 3′, 4′
1″	27.5, CH_2	3.22, d (7.4)		1, 5, 6, 3″
2″	122.4, CH	5.26, t (7.4)		6, 3″, 4″, 5″
3″	132.3, C			
4″	25.8, CH_3	1.73, s		1″, 2″, 3″, 5″
5″	17.8, CH_3	1.71, s		1″, 2″, 3″, 4″

[a] gHMBC correlations are from proton stated to the indicated carbons.

RESULTS AND DISCUSSION

Comosusol A (**3**) was isolated from the MeOH extract as a colorless oil with a molecular weight indicative of the molecular formula $C_{16}H_{22}O_3$, equating to six double-bond equivalents. Analysis of the one- and two-dimensional NMR data for **3** (Table 1) readily identified resonances consistent with 10 sp^2 carbons. This accounted for five of the six double-bond equivalents and, in the absence of any other sp or sp^2 carbons, indicated that **3** contained one ring. Also present within the molecule were resonances consistent with two olefinic (^{13}C: 25.8, 17.8 ppm; ^{1}H: δ 1.73, 1.71) and two aliphatic (^{13}C: 27.8 ppm; ^{1}H: δ 1.39) methyls, as well as a methylene (^{13}C: 27.5 ppm; ^{1}H: δ 3.22) and an oxygenated quaternary carbon (^{13}C: 75.5 ppm). This accounted for all ^{13}C and 19 of the ^{1}H resonances, indicating the three remaining protons were part of three hydroxy functions. Further analysis of the NMR data for **3** (Table 1) enabled several partial structures to be identified. The COSY correlation between δ 6.48 (H-3) and 6.34 (H-5), as well as the magnitude of the coupling constant between them (J = 2.8 Hz) determined them to be aromatic *meta*-coupled protons. Additionally, the ^{13}C chemical shifts of C-3 (115.8 ppm) and C-5 (110.3 ppm) were indicative of C-3 and C-5 being *ortho* to an aromatic hydroxy carbon (C-4). This conclusion was supported by the observation of common weak gHMBC correlations from H-3 and H-5 to C-4 (148.9 ppm). Additional gHMBC correlations from H-3 and H-5 to a second aromatic hydroxy carbon (C-1, 144.3 ppm) established the aromatic moiety of **3** to be a 2,6-disubstituted quinol. Continued analysis of the NMR data for **3** established connectivities consistent with a trisubstituted double bond that was part of an isoprene unit, and a 1,2-disubstituted double bond. The observation of gHMBC correlations from methyl resonances at δ 1.39 (H_3-4′/5′) to 75.5 ppm (C-3′), 131.7 (C-2′), and 122.5 (C-1′) established that the 1,2-disubstituted double-bond spin system was part of a 3-hydroxy-3-methylbut-1-enyl moiety.

The regiochemistry about the quinol moiety of **3** was readily elucidated from the gHMBC data. The observed gHMBC correlations from H-3 to C-1′ and from H-1′ to C-1, C-2, and C-3 established that the 3-hydroxy-3-methylbut-1-enyl moiety was attached at C-2. Additional gHMBC correlations from H-5 to C-1″, from H-2″ to C-6, and from H-1″ to C-1, C-5, and C-6 established that the isoprene unit was attached at C-6. Hence the gross structure of **3** is as shown. The geometry about the $\Delta^{1'-2'}$ double bond was identified as *Z* due to the value of the coupling constant ($J_{1'-2'}$ = 9.7 Hz).

Comosusol B (**4**) was isolated from the CH_2Cl_2 extract, with a molecular weight indicative of the molecular formula $C_{16}H_{22}O_3$ and equating to six double-bond equivalents. Analysis of the NMR data of **4** showed it contained the same 2-[(1*Z*)-3-hydroxy-3-methylbut-1-enyl]benzene-1,4-diol moiety as was present in **3**; however it was substituted at C-5, and not C-6, on the basis of the two aromatic protons (δ 6.72 and 6.51) being *para* to each other. The substituent at C-5 was identified as a reverse isoprene 2-methylbut-3-en-2-yl moiety on the basis of COSY correlations between H_2-3″ and H-2″, as well as the gHMBC correlations from H_3-4″ and H_3-5″ to C-1″, from H-6 to C-1″, and from H-2″ to C-1″ and C-5. Hence the structure of **4** is as shown.

Comosusol C (**5**) was isolated from the CH_2Cl_2 extract with a molecular weight indicative of the molecular formula $C_{16}H_{22}O_2$ and equating to six double-bond equivalents. The NMR data for **7** (Table 2) clearly showed that it was very similar in structure to **4**. Indeed, it was readily apparent that the only difference between **5** and **4** was the presence of an isoprene moiety at C-2 in **5**, as opposed to the 2-hydroxy-2-methyl-3-butene moiety present in **4**. Hence the structure of **5** is as shown and is the corresponding reduced form of the quinone **2**.

Comosusol D (**6**) was isolated from the CH_2Cl_2 extract with a molecular weight indicative of the molecular formula $C_{16}H_{24}O_5Na$ and equating to five double-bond equivalents. From analysis of the spectroscopic data of **6** it was evident that the same unsaturated side chain that was present at C-5 in **4** and **5** was also present at C-5 in **6**. However, further analysis of the NMR data for **6** suggested an alternative C-2-substituted side chain than those present in **3**−**5**. Analysis of the gHSQC data for **6** established the presence of two hydroxy methines [69.4 ppm, δ_H 4.52 (C-1′); 76.5 ppm, δ_H 3.60 (C-2′)], in addition to a quaternary hydroxy carbon at 77.8 ppm (C-3′). Furthermore, observed COSY correlations between H-1′ and H-2′, in addition to gHMBC correlations from the methyl protons H_3-4′ and H_3-5′ to C-2′, C-3′, C-4′, and C-5′, established the gross structure of **6** is as shown. The configurations at C-1′ and C-2′ for **6** remain unresolved at this time.

Comosone A (**7**) was isolated from the MeOH extract as a colorless oil with a molecular weight indicative of the molecular formula $C_{16}H_{24}O_2$ and equating to five double-bond equivalents. Analysis of the NMR data for **7** (Table 3) identified resonances consistent with an α,β-unsaturated ketone (^{13}C: 204.9, 154.3, 128.8 ppm, ^{1}H: δ 6.87, 5.83), two almost identical trisubstituted double bonds (^{13}C: 135.9, 135.4, 121.0, 119.9 ppm; ^{1}H δ 5.11, 5.00), as well as for four olefinic methyls (^{13}C: 26.2, 26.2, 18.1, 18.0 ppm; ^{1}H δ 1.73, 1.71, 1.59, 1.59), three methylenes (^{13}C: 41.4, 35.6, 35.0 ppm; ^{1}H δ 2.33, 2.22, 2.20, 2.13, 2.10, 1.88), a hydroxy methine (^{13}C: 65.5 ppm; ^{1}H δ 4.59), and a quaternary carbon (51.1 ppm). In the absence of any other sp or sp^2 carbons, **7** must be cyclic. The COSY NMR data for **7** (Table 3) established connectivities from H-2 (δ 5.83) through to H_2-5 (δ 2.10, 1.88). These correlations, in addition to observed gHMBC correlations (Table 3) from H-2 to C-4 and C-6 and from H-3 to C-1 and C-5, identified a 4-hydroxycyclohex-2-enone moiety. Further COSY correlations between H_2-1′ (δ 2.33, δ 2.13) and H-2′ (δ 5.00) and between H_2-1″ (δ 2.22,

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Table 2. NMR Spectroscopic Data (600 MHz for ^{1}H, 150 MHz for ^{13}C, $CDCl_3$) for Comosusols B (4), C (5), and D (6)

	4		5		6	
pos.	δ_C (m)	δ_H (J in Hz)	δ_C (m)	δ_H (J in Hz)	δ_C (m)	δ_H (J in Hz)
1	146.4, C		147.6, C		145.6, C	
2	120.6, C		126.3, C		122.5, C	
3	115.0, CH	6.51, s	118.3, CH	6.61, s	114.4, CH	6.72, s
4	148.4, C		147.9, C		148.0, C	
5	132.8, C		130.7, C		134.0, C	
6	113.9, CH	6.72, s	113.6, CH	6.74, s	114.4, CH	6.91, s
1′	121.7, CH	6.26, d (9.7)	29.9, CH_2	3.28, d (7.1)	69.4, CH	4.52, d (8.2)
2′	131.1, CH	5.60, d (9.7)	121.8, CH	5.32, t (7.1)	76.5, CH	3.60, d (8.2)
3′	75.8, C		134.6, C		77.8, C	
4′	27.8, CH_3	1.46, s	17.8, CH_3	1.77, s	18.7, CH_3	1.23, s
5′	27.8, CH_3	1.46, s	24.6, CH_3	1.77, s	26.5, CH_3	1.47, s
1″	40.0, C		40.0, C		40.4, C	
2″	147.6, CH	6.17, dd (17.7, 10.6)	147.0, CH	6.16, dd (17.5, 10.4)	147.2, CH	6.14, dd (17.9, 10.7)
3″	113.5, CH_2	5.34, d (17.7)	113.0, CH_2	5.32, d (17.5)	113.3, CH_2	5.31, d (10.7)
		5.29, d (10.6)		5.28, d (10.4)		5.28, d (17.9)
4″	26.9, CH_3	1.37, s	27.2, CH_3	1.41, s	26.5, CH_3	1.40, s
5″	26.9, CH_3	1.37, s	27.2, CH_3	1.41, s	26.5, CH_3	1.41, s

Table 3. NMR Spectroscopic Data (600 MHz for ^{1}H, 150 MHz for ^{13}C, CD_3OD) for Comosone A (7)

pos.	δ_C (m)	δ_H (J in Hz)	COSY	gHMBC[a]
1	204.9, C			
2	128.8, CH	5.83, d (10.2)	H-3	4, 6
3	154.3, CH	6.87, dd (10.2, 1.6)	H-2, H-4	1, 5
4	65.5, CH	4.59, m	H-3, H_2-5	2, 3, 5
5	41.4, CH_2	2.10, dd (13.6, 6.4)	H-4, H_b-5	1, 3, 4, 6, 1′, 1″
		1.88, dd (13.6, 10.5)	H-4, H_a-5	1, 3, 4, 6, 1′, 1″
6	51.1, C			
1′	35.0, CH_2	2.33, dd (14.6, 7.9)	H_b-1′, H-2′	1, 5, 6, 2′, 3′, 1″
		2.13, m	H_a-1′, H-2′	1, 5, 6, 2′, 3′, 1″
2′	121.0, CH	5.00, br t (7.9)	H_2-1′	6, 1′, 4′, 5′
3′	135.4, C			
4′	18.1, CH_3	1.59, s		2′, 3′, 5′
5′	26.2, CH_3	1.73, s		2′, 3′, 4′
1″	35.6, CH_2	2.22, m	H-2″	1, 5, 6, 1′, 2″ 3″
		2.20, m	H-2″	1, 5, 6, 1′, 2″, 3″
2″	119.9, CH	5.11, br t (6.7)	H_2-1″	6, 1″, 4″, 5″
3″	135.9, C			
4″	18.0, CH_3	1.59, s		2″, 3″, 5″
5″	26.2, CH_3	1.71, s		2″, 3″, 4″

[a] gHMBC correlations are from proton stated to the indicated carbons.

δ 2.20) and H-2″ (δ 5.11), as well as gHMBC correlations from H-2′ and H-2″ to C-6 and from H_2-1′ and H_2-1″ to C-1″ (35.6 ppm) and C-1′ (35.0 ppm), respectively, confirmed the presence of two isoprene units substituted at C-6. These data established the planar structure of 7 as shown.

The CD spectrum of 7 showed a large positive Cotton effect at 232 nm ($\Delta\varepsilon = 64.5$, $\pi\rightarrow\pi^*$) and a small negative Cotton effect at 343 nm ($\Delta\varepsilon = -11.6$, $n\rightarrow\pi^*$). Cyclohex-2-enones generally adopt either a half-chair or an inverted half-chair conformation, depending on the substituents.[12] The preferred conformation was determined through analysis of a 1D gCOSY spectrum. Selective irradiation of H-4 allowed an unambiguous analysis of the coupling constants associated with H_2-5 to be made. The large coupling for H_b-5 (δ 1.88, J = 10.5 Hz) with H-4 and the smaller coupling for H_a-5 (δ 2.10, J = 6.4 Hz) with H-4 were indicative of H-4 having an axial orientation. The minimum energy conformer of 7 with an axial H-4 was generated and minimized using MM2.[13] The cyclohex-2-enone ring is a very flattened half-chair and approximates well with an envelope conformation with five atoms (C-6−C-1−C-2−C-3−C-4) coplanar and one (C-5) out of the plane. Application of Snatzke's sector rules for planar enones[14] and the observed negative Cotton effect for the $n\rightarrow\pi^*$ transition yield a view of the molecule along the carbonyl bond with the double bond in the YZ plane and C-5 extending into the negative upper left quadrant; this observation is reconcilable with 7 having an *R* absolute configuration at C-4 (Figure 1).

All of the isolated compounds from this collection of *S. comosus* were screened for their cytotoxic activities against the four human tumor cell lines MCF-7 (breast), SF-268 (CNS), H460 (lung), and HT-29 (colon), as well as the normal mammalian cell line CHO-K1 (Chinese hamster ovary). The results of these assays are shown in Table 4. Of the newly reported compounds, **4** was found to be the most active, with GI_{50}'s against the panel of cell lines between 5 and 6 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. General experimental details are as previously reported.[15]

Plant Material. The specimens of the brown alga *S. comosus* (order Sporochnales, family Sporochnaceae) were collected at a depth of 5 m, 50 m off the coast of Shaw Island, Queensland, Australia, in October 1987. A voucher specimen (ascension number AQ642060) has been lodged with the Queensland Herbarium. Collection of this brown alga was conducted under the Queensland Fish or Marine Products Permit no. 1780 and the GBRMPA Permit no. 87/293.

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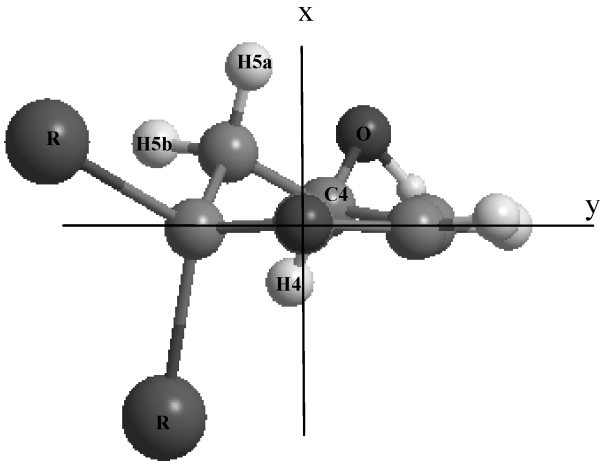

Figure 1. Minimum energy conformations of **7** with an axial H-4, viewed along the C═O bond toward C-4. R = $CH_2CHC{=}(CH_3)_2$ and applicaton of Snatzke's sector rules for planar enones.[14]

Table 4. GI_{50} (μM) Data for All Compounds against a Series of Human Tumour Cell Lines and a Normal Mammalian Cell Line

compound	SF-268[a]	MCF-7[b]	H460[c]	HT-29[d]	CHO-K1[e]
1	39	27	37	57	29
2	17	26	41	37	17
fucoxanthin	12	8	14	17	12
galactitol	>220	>220	>220	>220	>220
3	55	52	55	53	57
4	5	6	6	6	6
5	35	25	29	43	27
6	59	46	54	51	63
7	13	14	19	19	17

[a] SF-268 = Central nervous system-glioblastoma cells. [b] MCF-7 = Breast-pleural effusion adenocarcinoma cells. [c] H460 = Lung-large cell carcinoma cells. [d] HT-29 = Colon-recto-sigmoid colon adenocarcinoma cells. [e] CHO-K1 = Subclone of Chinese hamster ovary cells.

Extraction and Isolation. The freeze-dried plant material (76 g dry weight) was exhaustively extracted sequentially with CH_2Cl_2, MeOH, and H_2O, with activity being found in the CH_2Cl_2 and MeOH fractions. The MeOH fraction was subjected to reversed-phase C_{18} flash vacuum chromatography (0%, 10%, 25%, 50%, 70%, 90%, and 100% MeOH in H_2O), with activity found in the 90% MeOH fraction. This fraction was further purified using reversed-phase phenyl hexyl HPLC (4 mL/min, gradient elution from 20% CH_3CN/H_2O to 88% CH_3CN/H_2O over 17 min through a Phenomenex Luna 100 × 10 mm, 5 μm column) to yield the known compound galactitol[8] (6.0 mg, 0.008%), as well as the new compounds **3** (4.6 mg, 0.006%) and **7** (3.7 mg, 0.005%). The CH_2Cl_2 fraction was subjected to reversed-phase C_{18} HPLC (4 mL/min, gradient elution from 60% CH_3CN/H_2O to 100% CH_3CN over 10 min, then isocratic 100% ACN for 10 min through a 150 × 10 mm, 5 μm Phenomenex Luna C_{18} column) to yield galactitol[8] (18.7 mg, 0.02%), the known carotenoid fucoxanthin[4] (4.9 mg, 0.006%), the new compounds comosusol B (**4**, 16.4 mg, 0.02%), comosusol C (**5**, 8.4 mg, 0.011%), and comosusol D (**6**, 11.0 mg, 0.015%), and the known compound **2**[5] (0.8 mg, 0.001%). The H_2O fraction was found to contain exclusively the known compound **1**[2] (4.8 g, 6.3%).

Compound ***1****:* colorless oil. Spectroscopic data identical in all respects to literature values.[2]

Compound ***2****:* colorless oil. Spectroscopic data identical in all respects to literature values.[5]

Fucoxanthin: colorless oil. Spectroscopic data identical in all respects to literature values.[10]

Galactitol: colorless oil. Spectroscopic data identical in all respects to literature values.[11]

*Comosusol A (**3**):* optically inactive, colorless oil; UV (PDA, CH_3CN/H_2O) λ_{max} 216, 264, 273, 330 nm; IR (neat) ν_{max} 3436, 2974, 1653, 1462 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data see Table 1; HRESIMS *m/z* 267.1359 $[M - H_2O + Na]^+$ (calcd for $C_{16}H_{20}O_2Na$, 267.1356); *m/z* 285.1468 $[M + Na]^+$ (calcd for $C_{16}H_{22}O_3Na$, 285.1461).

*Comosusol B (**4**):* optically inactive, colorless oil; UV (PDA, CH_3CN/H_2O) λ_{max} 217, 289 nm; IR (neat) ν_{max} 3492, 2972, 1635, 1494 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data see Table 2; HRESIMS *m/z* 267.1355 $[M - H_2O + Na]^+$ (calcd for $C_{16}H_{20}O_2Na$, 267.1356); *m/z* 285.1464 $[M + Na]^+$ (calcd for $C_{16}H_{22}O_3Na$, 285.1461).

*Comosusol C (**5**):* optically inactive, colorless oil; UV (PDA, CH_3CN/H_2O) λ_{max} 251, 321 nm; IR (neat) ν_{max} 3442, 2971, 1664, 1429 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data see Table 2; HRESIMS *m/z* 269.1520 $[M + Na]^+$ (calcd for $C_{16}H_{22}O_2Na$, 269.1512).

*Comosusol D (**6**):* colorless oil; $[\alpha]_D$ +26.7 (*c* 0.015, CH_3OH); UV (PDA, CH_3CN/H_2O) λ_{max} 223, 297 nm; IR (neat) ν_{max} 3387, 2975, 1652, 1410 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data see Table 2; HRESIMS *m/z* 319.1520 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_5Na$, 319.1516).

*Comosone A (**7**):* colorless oil; $[\alpha]_D$ 0.0 (*c* 0.07, $CHCl_3$); UV (PDA, CH_3CN/H_2O) λ_{max} 225 nm; CD (4.0×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 232 (+64.5), 343 (−11.6) nm; IR (neat) ν_{max} 3434, 2931, 1664, 1500 cm^{-1}; ^1H (600 MHz, CD_3OD) and ^{13}C (150 MHz, CD_3OD) NMR data see Table 3; HRESIMS *m/z* 271.1668 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_2Na$, 271.1669).

Bioassay. MCF-7, SF-268, and H460 cells were grown in RPMI 1640 medium with L-glutamine supplemented with 5% fetal bovine serum and maintained in a humidified incubator at 37 °C with 5% CO_2. Conversely, CHO-K1 and HT-29 cells were grown in minimum essential medium (MEM) with Earle's salts, L-glutamine, nonessential amino acids, and sodium pyruvate.

Cells were plated in 96-well microtiter plates at a seeding density of 5000 cells/well in 100 μL of medium and allowed to attach for 24 h. Samples to be tested were solubilized in DMSO; then serial dilutions were prepared in medium and added to the cells so that the final doses ranged from 250 to 3 μg/mL. Pure compounds were tested in the dose range 66.7−0.01 μg/mL. Plates were returned to the incubator.

Total cellular protein was used as an indicator of cell number and was measured at 0 and 48 h after sample addition using the sulforhodamine B (SRB) assay.[16] Cells were fixed by addition of 30 μL of 50% TCA for 30 min at 4 °C, rinsed five times in running water, then air-dried before staining with 50 μL of 0.4% SRB in 1% acetic acid for 30 min at room temperature. Plates were washed in five changes of 1% acetic acid, then air-dried. SRB dye was solubilized in unbuffered 10 mM Tris base (100 μL), and plate absorbances were read on a Wallac Victor plate reader at 490 nm. Inhibition of growth by 50% (GI_{50}) was determined by comparing the sample treated values to those of vehicle only control and time 0 readings. Samples were tested in triplicate.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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