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Sesterterpenes from the Tropical Sponge *Coscinoderma* sp.

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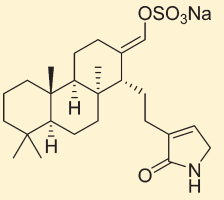
Supporting Information

ABSTRACT: Eight new sesterterpenes (**2**, **5**, and **10**−**15**), including structurally related pentaprenyl hydroquinones (**2** and **5**), and seven known ones of the same structural classes were isolated from the sponge *Coscinoderma* sp., collected from Chuuk Island, Micronesia. On the basis of the results of combined spectroscopic analyses, the new compounds were determined to be derivatives of the halisulfates and suvanine. These compounds exhibited moderate cytotoxicity against the K562 cell line and inhibitory activities against isocitrate lyase, sortase A, and Na^+/K^+-ATPase; significant structure−activity relationships were evident.



The structural variety and wide phyletic distribution of sesterterpenes is one of the most conspicuous features of sponge-derived terpenoids, and their presence distinguishes sponges from other marine or terrestrial organisms.[1−3] These compounds are particularly abundant among sponges of the orders Dictyoceratida and Dendroceratida, even serving as their taxonomical biomarkers.[4] Several of the sponge-derived sesterterpenes and related pentaprenyl hydroquinones, represented by the halisulfates and suvanine, possess sulfate groups and exhibit diverse bioactivities, such as cytotoxic, antimicrobial, and anti-inflammatory properties, as well as inhibitory effects on isocitrate lyase, phospholipase A_2, serine protease, phosphatase, and PMA-induced inflammation.[5−11]

During the course of our search for bioactive metabolites from tropical marine organisms, we encountered the sponge *Coscinoderma* sp., the organic extract of which exhibited significant cytotoxicity (LC_{50} 48.7 μg/mL) against the K562 leukemia cell line. Bioassay-guided separation of the organic extract yielded several sesterterpenes. Here we report the structures of eight new derivatives of coscinoquinol, the halisulfates, and suvanine along with seven known sesterterpenes (**1**, **3**, **4**, and **6**−**9**). The new compounds are geometric isomers (**2** and **5**), oxidized furan (**10**−**14**), and lactam (**15**) analogues of previously reported metabolites.[5,6,12] These compounds exhibit moderate to significant cytotoxicity and inhibitory activities against sortase A, isocitrate lyase, and Na^+/K^+-ATPase.

RESULTS AND DISCUSSION

The sponge was lyophilized, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined extracts were separated with solvent partitioning followed by reversed-phase vacuum flash chromatography. On the basis of the results of bioassay and ^{1}H NMR analyses, the moderately polar fractions were separated by reversed-phase HPLC, affording 15 compounds as amorphous solids.

The major constituents **3**, **7**, and **8** were readily identified to be halisulfate 1,[5] suvanine,[12] and suvanine's sodium salt,[12] respectively, on the basis of combined spectroscopic analyses and comparison of spectroscopic data with those in the literature. Similarly, compounds **1** and **6** were identified as coscinoquinol (**1**)[6] and halisulfate 2 (**6**).[5] Compound **4** is the sodium salt of a coscinosulfate isomer that originally had *N*,*N*-dimethylguanidium as its counterion.[8] Additionally, compound **9**, a previously reported synthetic analogue, was isolated for the first time as a natural product.[13] The absolute configuration of the γ-hydroxybutenolide moiety of this compound, originally undetermined, was assigned to be 25*S* by CD measurements [extrema at 202.0 nm ($\Delta\varepsilon$ −0.70), 230.0 nm (+0.38)] and application of an empirical rule for lactone systems.[14]

The molecular formula of compound **2** was deduced to be $C_{31}H_{46}O_2$ from HRFABMS data. The presence of 31 carbon signals, combined with two oxo-aromatic carbon signals at δ_C 151.1 and 148.9 in the ^{13}C NMR spectra (Table 1), suggested this compound to be a sesterterpene hydroquinone, such as coscinoquinol (**1**)[6] or halisulfate 1 (**3**).[5] The presence of the hydroquinone moiety was supported by the characteristic aromatic proton signals at δ_H 6.57 (1H, d, J = 8.5 Hz), 6.52 (1H, d, J = 2.9 Hz), and 6.42 (1H, dd, J = 8.5, 2.9 Hz) in the ^{1}H NMR spectrum. A combination of ^{1}H COSY, gHSQC, and gHMBC

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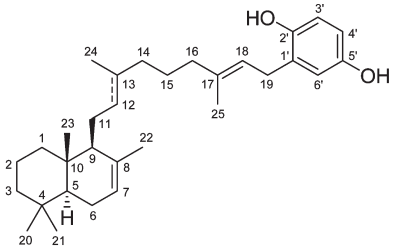
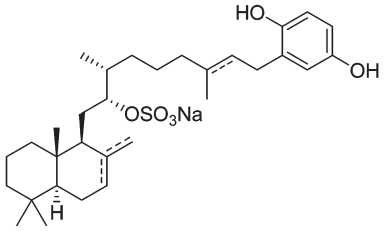
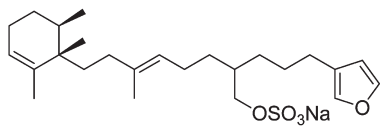
Chart 1



1 : 12*Z*
2 : 12*E*



3 : 17*E*, Δ^7
4 : 17*E*, $\Delta^{8,22}$
5 : 17*Z*, Δ^7



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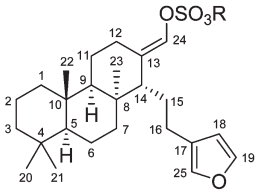
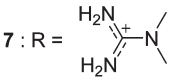
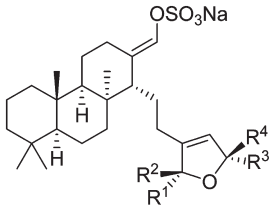


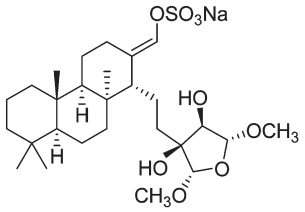


7 : R =

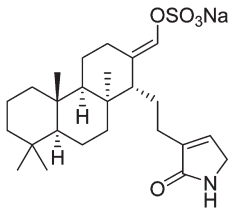
8 : R = Na^+



9 : R^1=OH, R^2=H, R^3R^4=O
10 : R^1=H, R^2=H, R^3R^4=O
11 : R^1R^2=O, R^3=H, R^4=OCH_3
12 : R^1R^2=O, R^3=OCH_3, R^4=H
13 : R^1=OCH_3, R^2=H, R^3=OCH_3, R^4=H



14



15

experiments confirmed this interpretation and further revealed this compound to possess the same carbon framework and functionalities as **1**.[6] However, signals of protons and carbons at the C-12 double bond and in the vicinity were shifted noticeably in both ^{1}H and ^{13}C NMR data. The marked shifts of the C-14 and C-24 carbons (δ_C 32.6 and 23.7 in **1** compared to δ_C 43.2 and 16.2 in **2**) revealed that the C-12 double bond of **2** possesses the *E* configuration. This interpretation was further supported by NOESY experiments in which cross-peaks were observed at H-11/H-24 and H-12/H-14. Thus, compound **2** was structurally defined as a Δ^{12} geometric isomer of coscinoquinol (**1**).

A similar relationship was found between halisulfate 1 (**3**) and **5**. Having the molecular formula $C_{31}H_{47}O_6SNa$, the same as **3**, the spectroscopic data of **5** were also very similar to those of **3**.[5] The significant shifts of C-16 and C-25 (δ_C 41.0 and 16.3 for **3**, δ_C 33.0 and 23.8 for **5**) in the ^{13}C NMR data, as observed for **1** and **2**, suggested the *Z* orientation at the C-17 double bond of **5**, which was confirmed by the NOESY cross-peaks at H-16/H-19 and H-18/H-25 for **5**.

Compound **10** analyzed for $C_{25}H_{37}O_6SNa$ by HRFABMS. The NMR data of this compound were highly reminiscent of those obtained for the suvanine salts (**7** and **8**).[12] However, characteristic carbon signals of the furan moiety at δ_C 145−110 of the suvanines are significantly different in both chemical shift and multiplicity, δ_C 177.2 (C), 174.9 (C), 115.3 (CH), and 75.1 (CH_2) (Table 1), suggesting oxidation of the furan ring. Corresponding differences were also observed in the ^{1}H NMR data, in which proton signals from the terminal furan moiety at δ_H 7.4−6.3 were replaced by those of a methine and a methylene at δ_H 5.92 (1 H, br s), 4.90 (1 H, d, *J* = 18.0 Hz), and 4.84 (1 H, d, *J* = 18.0 Hz). These changes were readily accommodated by a structure in which the furan ring has been oxidized to a butenolide. This was confirmed by 2D NMR analyses including long-range correlations between these protons and neighboring carbons in gHMBC data: H-18/C-16, C-17, C-19, C-25 and H-25/C-16, C-17, C-18. This was consistent with the strong absorption bands at 1744 and 1260 cm^{-1} in the IR spectrum and an absorption maximum at 213 nm in the UV spectrum.

The remaining portion of the molecule was found to be identical to that of suvanine on the basis of combined 2D NMR analyses. That is, the gHSQC data precisely matched all of the proton-bearing carbons and their attached protons. The ^{1}H COSY and TOCSY data revealed several proton spin systems insulated by quaternary carbons. Connection of these was accomplished by the long-range proton−carbon correlations, in particular those of the upfield methyl protons at δ_H 1.04, 0.89, 0.87, and 0.86 with neighboring carbons, which formed a tricyclic carbon framework. Similarly an enol-sulfate group was placed at C-13 (24) on the basis of the correlations between H-24 (δ_H 6.24) and C-12−C-14. The connectivity between the tricyclic system and the newly appearing butenolide was indicated by a series of correlations at H-14/C-7, C-16, C-23; H-15/C-14, C-16, C-17; H-16/C-14, C-15, C-18, C-25; H-18/C-16, C-17, C-19, C-25; and H-25/C-16, C-17, C-18 in the *g*HMBC data. Finally the relative configurations of the ring junctions of the tricyclic system were defined to be the same as suvanine by the NOESY cross-peaks at H-1β (δ_H 1.83)/H-22, H-5/H-9, H-5/H-20, H-6β (δ_H 1.38)/H-21, H-6β/H-22, H-7β (δ_H 1.40)/H-23, H-9/H-23, H-12β (δ_H 2.21)/H-22, H-14/H-22, and H-21/H-22. The *E* orientation of the C-13 enol-sulfate was also assigned by cross-peaks at H-15 (both)/H-24 and H-16 (both)/H-24.

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Table 1. ^{13}C NMR (ppm, mult) Assignments for Compound 2, 5, and 10−15[a]

position	2	5	10	11	12	13	14	15
1	40.4, CH_2	39.8, CH_2	43.4, CH_2	43.3, CH_2	43.4, CH_2	43.5, CH_2	43.5, CH_2	43.3, CH_2
2	19.9, CH_2	19.8, CH_2	19.9, CH_2	19.9, CH_2	19.9, CH_2	19.9, CH_2	19.9, CH_2	19.9, CH_2
3	43.5, CH_2	43.4, CH_2	43.3, CH_2	43.2, CH_2	43.2, CH_2	43.3, CH_2	43.3, CH_2	43.2, CH_2
4	33.9, C	33.9, C	34.3, C	34.3, C	34.3, C	34.3, C	34.4, C	34.3, C
5	51.6, CH	51.1, CH	53.9, CH	54.8, CH	54.3, CH	54.0, CH	54.1, CH	54.7, CH
6	24.9, CH_2	24.9, CH_2	19.6, CH_2	19.5, CH_2	19.6, CH_2	19.7, CH_2	19.6, CH_2	19.5, CH_2
7	123.0, CH	122.9, CH	36.0, CH_2	36.7, CH_2	36.3, CH_2	36.1, CH_2	36.2, CH_2	36.7, CH_2
8	136.6, C	137.2, C	39.8, C	39.9, C	39.8, C	39.8, C	39.9, C	39.9, C
9	55.6, CH	49.8, CH	58.7, CH	58.6, CH	58.6, CH	58.8, CH	58.8, CH	58.6, CH
10	37.9, C	37.6, C	40.9, C	40.3, C	40.2, C	40.1, C	40.2, C	40.3, C
11	27.6, CH_2	27.7, CH_2	21.1, CH_2	21.3, CH_2	21.2, CH_2	21.2, CH_2	21.2, CH_2	21.2, CH_2
12	125.8, CH	84.6, CH	25.4, CH_2	25.4, CH_2	25.5, CH_2	25.5, CH_2	25.5, CH_2	25.4, CH_2
13	136.6, C	38.4, CH	125.0, C	125.3, C	125.3, C	125.4, C	125.9, C	125.5, C
14	43.2, CH_2	34.8, CH_2	44.7, CH	43.5, CH	44.2, CH	44.8, CH	45.8, CH	43.7, CH
15	27.0, CH_2	27.4, CH_2	23.7, CH_2	23.2, CH_2	23.5, CH_2	24.0, CH_2	19.2, CH_2	23.7, CH_2
16	40.9, CH_2	33.0, CH_2	28.4, CH_2	25.0, CH_2	25.2, CH_2	26.7, CH_2	34.0, CH_2	25.5, CH_2
17	136.7, C	137.0, C	174.9, C	139.0, C	139.4, C	147.1, C	82.3, C	140.4, C
18	124.1, CH	124.5, CH	115.3, CH	145.5, CH	144.9, CH	125.1, CH	80.7, CH	140.9, CH
19	29.1, CH_2	29.0, CH_2	177.2, C	104.5, CH	104.5, CH	108.1, CH	111.5, CH	47.8, CH_2
20	33.7, CH_3	33.7, CH_3	34.0, CH_3	34.1, CH_3	34.0, CH_3	34.0, CH_3	34.0, CH_3	34.1, CH_3
21	22.3, CH_3	22.4, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3	22.3, CH_3
22	22.6, CH_3	23.0, CH_3	19.4, CH_3	18.9, CH_3	18.8, CH_3	18.6, CH_3	18.7, CH_3	18.9, CH_3
23	14.1, CH_3	14.1, CH_3	26.6, CH_3	26.8, CH_3	26.7, CH_3	26.7, CH_3	26.7, CH_3	26.8, CH_3
24	16.2, CH_3	13.6, CH_3	133.7, CH	133.6, CH	133.7, CH	133.8, CH	133.8, CH	133.6, CH
25	16.2, CH_3	23.8, CH_3	75.1, CH_2	173.5, C	173.5, C	109.4, CH	109.8, CH	176.2, C
1′	130.2, C	130.4, C						
2′	148.9, C	148.9, C						
3′	116.5, CH	116.7, CH						
4′	113.9, CH	113.9, CH						
5′	151.1, C	151.2, C						
6′	117.1, CH	117.2, CH						
19-OCH_3				57.2, CH_3	57.0, CH_3	53.9, CH_3	56.5, CH_3	
25-OCH_3						54.4, CH_3	55.4, CH_3	

[a] Data were obtained in MeOH-d_4 solutions. Data were measured at 150 MHz (**11**, **12**, and **15**), 125 MHz (**2**, **5**, and **13**), and 100 MHz (**10** and **14**), respectively.

Thus the structure of compound **10** was determined to be a butenolide-containing derivative of suvanine.

The molecular formula of the isomeric compounds **11** and **12** was found to be $C_{26}H_{39}O_7SNa$ by HRFABMS, in combination with ^{13}C NMR data. Apart from the appearance of signals for a methoxy group (δ_H 3.52 and δ_C 57.2 for **11**, δ_H 3.52 and δ_C 57.0 for **12**), the NMR data of these compounds were very similar to those of **10**, revealing the presence of a tricyclic system and an enol-sulfate group (Tables 1, 2). Thus, the newly appearing methoxy group was thought to be attached at the butenolide, possibly forming a γ-methoxy butenolide moiety. The gHMBC experiments confirmed this conclusion and located the carbonyl and methoxy-bearing methine carbons at C-25 and C-19, respectively, on the basis of long-range correlations at H-15/C-17; H-16/C-15, C-17, C-25; H-18/C-16, C-17, C-19, C-25; H-19/C-17, C-25, OCH_3; and OCH_3/C-19 for both **11** and **12**.

The spectroscopic data of **11** and **12** were very similar to each other. The 2D NMR experiments showed virtually the same proton−proton and proton−carbon correlations. However, their ^1H and ^{13}C NMR data showed noticeable differences for the signals at the butenolide and in its vicinity (Tables 1, 2), indicating their isomeric nature at the newly appearing C-19 methoxy-bearing center. The configuration at this remote center was approached by application of a CD empirical rule for a hydroxy-butenolide system.[14] The CD spectra of **11** exhibited extrema at 218.0 ($\Delta\varepsilon$ −1.21) and 248.0 nm (+1.76), while the opposite extrema at 220.0 (+0.74) and 251.0 nm (−1.13) were found for **12** (Figure 1). Thus, 19*S* and 19*R* configurations were assigned for **11** and **12**, respectively.

Compound **13** was isolated as an amorphous solid, with a composition of $C_{27}H_{43}O_7SNa$ on the basis of HRFABMS data. The NMR data for this compound were reminiscent of those of other suvanines, with the appearance of signals for two methoxy groups as the most noticeable difference (δ_H 3.37 and 3.33, δ_C 54.4 and 53.9). The characteristic signals (δ_H 5.52 and 5.41, δ_C 109.4 and 108.1) suggested the oxidative formation of methoxy acetals, possibly at the furan ring of suvanine (Tables 1, 2). This was confirmed by combined 2D NMR analyses, and methoxy

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Table 2. ^{1}H NMR (δ, mult (J in Hz)) Assignments for Compound 2, 5, and 10−15[a]

position	2	5	10	11	12	13	14	15
1	0.95, dt (3.5, 13.0)	1.22, m	0.87, m	0.84, m	0.84, m	0.85, m	0.84, m	0.84, m
	1.82, m	1.77, br d (12.9)	1.83, m	1.86, m	1.83, m	1.85, br d (12.8)	1.83, br d (12.4)	1.86, m
2	1.44, dt (13.0, 3.5)	1.39, m	1.39, br d (12.0)	1.38, br d (12.0)	1.38, br d (12.0)	1.46, m	1.38, m	1.39, br d (12.2)
	1.55, m	1.49, m	1.61, m	1.62, m	1.63, m	1.62, m	1.60, m	1.63, m
3	1.19, m	1.23, m	1.17, dt (3.6, 13.5)	1.14, dt (4.1, 14.0)	1.15, dt (4.1, 14.0)	1.15, dt (3.5, 13.0)	1.15, dt (3.1, 13.2)	1.15, dt (3.6, 13.8)
	1.39, br d (13.5)	1.37, m	1.41, br d (13.5)	1.39, br d (14.0)	1.40, br d (14.0)	1.40, br d (13.0)	1.41, br d (13.2)	1.40, br d (13.8)
5	1.19, m	1.29, dd (12.0, 4.8)	1.10, dd (11.4, 4.7)	1.03, dd (11.4, 4.8)	1.07, dd (11.4, 4.8)	1.09, dd (11.1, 4.4)	1.10, dd (11.2, 4.5)	1.05, dd (11.7, 4.8)
6	1.94, m	1.95, br d (13.4)	1.60, m	1.53, m	1.58, m	1.58, m	1.58, m	1.52, m
	1.86, m	1.85, br dd (13.4, 12.0)	1.38, m	1.33, br d (11.4)	1.34, br d (11.4)	1.44, m	1.40, m	1.29, m
7	5.35, br s	5.33, br s	1.40, m	1.29, m	1.32, m	1.35, dd (14.0, 4.1)	1.33, m	1.27, m
			1.88, m	1.88, m	1.86, m	1.87, m	1.85, m	1.90, m
9	1.60, m	2.28, m	0.93, t (7.1)	0.91, t (6.0)	0.91, t (6.0)	0.91, t (6.2)	0.88, m	0.90, t (5.8)
11	2.07, m	1.24, m	1.63, m	1.61, m	1.61, m	1.61, m	1.61, m	1.61, m
	2.15, m	1.61, m	1.69, dq (15.4, 6.3)	1.70, dq (14.2, 6.0)	1.70, dq (14.2, 6.0)	1.70, br dt (13.3, 6.2)	1.68, br dt (12.7, 6.1)	1.70, dq (18.1, 6.2)
12	5.13, t (6.7)	4.51, dd (11.0, 2.2)	2.55, dt (16.0, 6.3)	2.59, dt (15.8, 6.0)	2.57, dt (15.8, 6.0)	2.55, dt (15.8, 6.2)	2.52, dt (15.9, 6.1)	2.60, dt (16.0, 6.2)
			2.21, m	2.16, m	2.18, m	2.18, m	2.17, m	2.16, m
13		2.27, m						
14	1.93, m	1.15, m	2.34, br d (10.8)	2.30, br d (12.0)	2.33, br d (12.0)	2.33, br d (10.4)	2.24, br d (10.5)	2.34, br d (10.4)
	2.18, br dd (13.7, 4.3)	1.38, m						
15	1.22, m	1.44, m	1.58, m	1.48, m	1.50, m	1.48, m	1.44, m	1.45, m
	1.53, m	1.57, m	1.87, m	1.91, m	1.90, m	1.80, br dd (14.3, 7.6)	1.60, m	1.93, m
16	2.05, m	2.12, m	2.42, dt (16.4, 8.2)	2.28, m	2.25, dt (15.3, 8.0)	2.06, dd (15.3, 7.6)	1.43, m	2.24, dt (14.5, 7.5)
	2.13, br dd (13.5, 7.2)		2.69, ddd (16.4, 9.6, 3.8)	2.48, m	2.49, m	2.38, ddd (15.3, 10.0, 4.8)	1.87, m	2.46, m
18	5.31, br t (7.2)	5.29, br t (7.2)	5.92, br s	7.02, d (1.2)	7.02, d (1.2)	5.71, br s	3.78, d (4.5)	6.94, br s
19	3.23, d (7.2)	3.21, d (7.2)		5.82, br s	5.83, br s	5.52, br s	4.85, d (4.5)	3.94, br dd (10.8, 0.6)
								3.89, br dd (10.8, 0.6)
20	0.84, s	0.84, s	0.86, s	0.85, s	0.86, s	0.86, s	0.86, s	0.85, s
21	0.88, s	0.88, s	0.89, s	0.88, s	0.89, s	0.89, s	0.89, s	0.87, s
22	1.66, br s	1.68, s	1.04, s	1.04, s	1.04, s	1.04, s	1.06, s	1.04, s
23	0.75, s	0.74, s	0.87, s	0.82, s	0.83, s	0.84, s	0.84, s	0.82, s
24	1.60, s	0.92, d (6.9)	6.24, br s	6.29, br s	6.29, br s	6.27, br s	6.31, br s	6.32, br s
25	1.70, s	1.71, s	4.90, d (18.0)			5.41, s	4.68, br s	
			4.84, d (18.0)					
3′	6.57, d (8.5)	6.56, d (8.5)						
4′	6.42, dd (8.5, 2.9)	6.41, dd (8.5, 2.9)						
6′	6.52, d (2.9)	6.53, d (2.9)						
19-OCH_3				3.52, s	3.52, s	3.33, s	3.42, s	
25-OCH_3						3.37, s	3.37, s	

[a] Data were obtained in MeOH-d_4 solutions. Data were measured at 600 MHz (**11**, **12**, and **15**), 500 MHz (**2**, **5**, and **13**), and 400 MHz (**10** and **14**), respectively.

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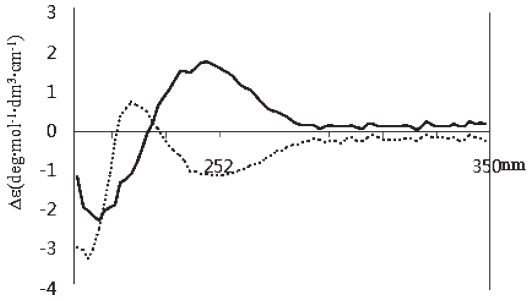

Figure 1. CD spectra of compounds **11** (solid line) and **12** (dotted line) in MeOH.

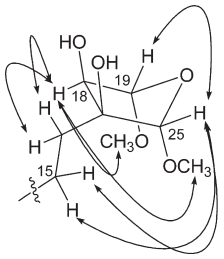

Figure 2. Key NOE correlations for compound **14**.

groups were placed at C-19 and C-25 by the correlations of the H-15, H-16, H-18, H-19, H-25, and methoxy protons with the neighboring carbons in the gHMBC data. The relative configurations were assigned as 19*R**, 25*S** by the significant NOESY cross-peak at H-19/H-25, supported by the relationship between configuration and chemical shifts of H-19 and H-25 in the 1,4-dihydro-1,4-dimethoxy furan ring revealed in the literature.[15] Thus, the structure of **13** was determined to be a dihydro-dimethoxy derivative of suvanine.

The molecular formula of compound **14**, another highly oxidized derivative of suvanine, was established as $C_{27}H_{45}O_9SNa$ by HRFABMS, assisted by ^{13}C NMR analyses. The presence of a tricyclic system with an attached enol-sulfate group was readily recognized by comparison of its ^{1}H and ^{13}C NMR data with those of other suvanines. In addition to the presence of two methoxy-acetal groups, as in **13**, the replacement of the C-17 double bond with oxygenated functionalities was evident from the NMR data of **14**: H-18 (δ_H 3.78) and C-17 (δ_C 82.3, C), C-18 (δ_C 80.7, CH) (Tables 1, 2). The chemical shifts of these signals, in conjunction with the molecular formula, revealed that hydroxy groups were attached at both C-17 and C-18, which was consistent with the combined 2D NMR data, including key correlations at H-18/C-16, C-17, C-19 and H-25/C-18, C-19 in the gHMBC data. The relative configurations were assigned as 17*S**, 18*R**, 19*R**, 25*S** by NOESY cross-peaks at H-15/H-25, H-16/H-18, H-18/19-OCH_3, H-18/25-OCH_3, H-19/H-25, and NOEDS experiments (Figure 2). Thus, the structure of **14** was determined to be a derivative of suvanine possessing a highly oxidized furan moiety.

In addition to the furan-oxidized derivatives, another derivative of suvanine, compound **15**, was isolated as an amorphous solid, determined to be $C_{25}H_{38}NO_5SNa$ by combined HRFABMS and ^{13}C NMR spectroscopy. The spectroscopic data of this compound were similar to those of suvanine, and the presence of a tricyclic system bearing an enol-sulfate was readily recognized by comparing the NMR data to those of other compounds. However, carbon signals at the furan ring were shifted significantly to δ_C 176.2 (C), 140.9 (CH), 140.4 (C), and 47.8 (CH_2) (Table 1). Correspondingly, proton signals were also shifted to δ_H 6.94 (1 H, br s), 3.94 (1 H, br dd, J = 10.8, 0.6 Hz), and 3.89 (1 H, br dd, J = 10.8, 0.6 Hz; Table 2). These spectroscopic features, combined with the presence of a nitrogen atom in the molecular formula, were accommodated by an unsaturated lactam moiety, which is also consistent with the IR absorption bands at 3500 and 1678 cm^{-1} and UV maxima at 205 and 232 nm. This interpretation was confirmed by combined 2D NMR analyses, and key evidence was provided by the gHMBC data, in which correlations were found at H-16/C-17, C-18, C-25; H-18/C-16, C-17, C-19, C-25; and H-19/C-17. Thus, the structure of **15** was determined to be a new derivative of suvanine with an α,β-unsaturated-γ-lactam with the carbonyl at C-25. This compound was structurally reminiscent of coscinolactam A recently isolated from the marine sponge *Coscinoderma mathewsi*.[11]

Among the compounds isolated in this work, compounds **9**−**14** possessed modified furan moieties with varying degrees of oxidation, indicating that some of these, if not all, might be the artifacts from suvanine formed during the storage and isolation processes. This was also consistent with the significantly lower amounts of these compounds compared to the suvanine salts (**7** and **8**) and the presence of methoxy groups in some of the compounds (**11**−**14**). In contrast, the isolation of **15**, a minor but nitrogen-bearing analogue, suggested that the oxygen-bearing ones might also be genuine natural products. However, this was not confirmed by the MS analysis of the organic extract, and this issue remains to be resolved.

Halisulfates, coscinoquinols, and suvanines have been reported to possess diverse bioactivities: cytotoxicity, antimicrobial activity, and inhibitory activities against isocitrate lyase, phospholipase A_2, serine protease, phosphatase, and PMA-induced inflammation.[5−11] In our extensive study of the bioactivities of these compounds and the new derivatives, several interesting phenomena were observed. First, compounds **1**, **3**, **4**, **6**, **7**, and **8** were active (MIC 1.56−25 μg/mL) against Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*) and/or -negative (*Proteus vulgaris* and *Salmonella typhimurium*) bacteria, while the others were inactive (MIC > 100 μg/mL; Supporting Information). Regarding the suvanine salts, compound **8** with a sodium counterion exhibited much more potent inhibition than **7**, which possesses a *N*,*N*-dimethylguanidium counterion, consistent with the literature.[7] Additionally, despite the significant inhibition shown by halisulfate 1 (**3**), the isomer **5** displayed no antibacterial activity at all. This same phenomenon was observed between coscinoquinol (**1**) and its isomer (**2**). Second, none of the isolated compounds showed inhibition against selected pathogenic fungi. Regarding cytotoxicity against the K562 cell line, coscinoquinols (**1** and **2**) showed more potent inhibition than doxorubicin, while the halisulfates with hydroquinone (**3**, **4**, and **5**) and furan (**6**) moieties were inactive (Table 3). Regarding the suvanine salts, **8** was again far more active than **7**. The modified furan-bearing derivatives (**9**−**15**) showed moderate to weak inhibition against K562 cells.

In enzyme-based assays, halisulfate 1 (**3**), a coscinosulfate analogue (**4**), and an isomer of halisulfate 1 (**5**) bearing hydroquinone and sulfate groups exhibited significant inhibition of sortase A, a key enzyme for the cell adhesion of Gram-positive bacteria, while other compounds lacking either of these functionalities were inactive. In an assay against *Candida albicans*-derived

Table 3. Results of Bioactivity Tests

	K562 LC_{50} (μM)	sortase A IC_{50} (μM)	ICL IC_{50} (μM)	Na^+/K^+-ATPase IC_{50} (μM)
1	8	>200	>200	44
2	8	>200	>200	117
3	>200	36	19	3
4	>200	98	45	4
5	>200	100	52	5
6	>200	>200	24	5
7	>200	>200	17	8
8	16	>200	5	9
9	59	>200	56	5
10	56	>200	150	19
11	16	>200	64	6
12	45	>200	165	17
13	49	>200	87	14
14	15	>200	75	6
15	22	>200	62	18
doxorubicin	13			
*p*HMB[a]		116		
3-NP[b]			9	
ouabain				6

[a] *para*-Hydroxymercuribenzoic acid. [b] 3-Nitropropionic acid.

isocitrate lyase (ICL), an enzyme of the glyoxylate cycle in microorganisms, the coscinoquinols were inactive, while all other sulfate-containing compounds displayed moderate to significant inhibition. Among them, the suvanine salts (**7** and **8**) were most active. A similar trend was observed for the Na^+/K^+-ATPase inhibitory assay, in which more potent activities were found for the sulfate-containing compounds (**3**−**15**) than the coscinoquinols (**1** and **2**). Overall, these results provide important insight regarding structure−activity relationships of sulfate-containing terpenoids.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. UV spectra were recorded on a Hitachi U-3010 spectrophotometer. CD spectra were obtained on a JASCO J-715 using a 0.2 mm cell, and IR spectra were recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded in MeOH-d_4 solution containing Me_4Si as an internal standard on Bruker Avance 400, 500, and 600 spectrometers. Proton and carbon NMR were measured at 600 and 150 MHz (**11**, **12**, and **15**), 500 and 125 MHz (**2**, **5**, and **13**), and 400 and 100 MHz (**10** and **14**), respectively. Mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer using *meta*-nitrobenzyl alcohol as a matrix for the FABMS. HPLC was performed on an Spectrasystem p2000 equipped with a refractive index detector, Spectrasystem RI-150. All solvents were spectroscopic grade or distilled from glass prior to use.

Animal Material. The specimens of *Coscinoderma* sp. (voucher collection number 06CH-115) were collected by hand using scuba off the shore of Weno Island, Chuuk state, Federated States of Micronesia, at a depth of 15 m on June 3, 2006. The sponge was massive in shape, and the texture was soft and compressible. The surface was invested with a sand armor and long conuleo of 5 mm height. The color in life was grayish-black, and brown inside the sponge. The skeleton was a network of trellised, primary fibers, which incorporate coring material, 70 μm in diameter, only at the near surface. Uncored secondary fibers were thin vermiform and intertwining, 15−20 μm in diameter. The specimen closely resembles *C. mathewsi* (Lendenfeld, 1886) in general morphology. However, the secondary fibers differ from those of *C. mathewsi*, which are 3−12 μm in diameter. A voucher specimen (registry no. spo. 60) is deposited at the Natural History Museum, Hannam University, Korea, under the curatorship of C.J.S.

Extraction and Isolation. Freshly collected specimens were immediately frozen and stored at −25 °C until use. Lyophilized specimens (222.1 g, dry wt) were macerated and repeatedly extracted with MeOH (2 L × 3) and CH_2Cl_2 (2 L × 3). The combined extracts (49.9 g) were successively partitioned between H_2O (31.1 g) and *n*-BuOH (17.8 g); the latter fraction was repartitioned between H_2O−MeOH (15:85) (13.76 g) and *n*-hexane (4.04 g). An aliquot of the former layer (4.93 g) was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as eluents (six fractions in gradient, H_2O−MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

On the basis of the results of ^{1}H NMR and cytotoxicity analyses, the fractions eluted with H_2O−MeOH (20:80) (0.59 g), H_2O−MeOH (10:90) (2.50 g), and 100% MeOH (0.54 g) were chosen for separation. The fraction eluted with H_2O−MeOH (20:80) was separated by semipreparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O−MeOH, 40:60), yielding five peaks rich in secondary metabolites. Further purification of the first to fourth peaks by reversed-phase HPLC (H_2O−MeOH, 65:35) afforded, in order of elution, compounds **9**, **14**, **11**, **12**, and **10**, respectively, as amorphous solids. Purification of the fifth peak was accomplished by reversed-phase HPLC (H_2O−MeOH, 70:30) to afford compound **15**.

The fifth fraction, eluted with H_2O−MeOH (10:90), was separated by reversed-phase HPLC (H_2O−MeOH, 25:75) to yield, in order of elution, compounds **3**, **7**, **5**, **4**, **6**, **13**, and **8**. These metabolites were then purified by reversed-phase HPLC (YMC-Pack CN column, 10 mm × 250 mm; H_2O−MeOH, 50:50, for **3** and **7**, H_2O−MeOH, 55:45, for **4**, **5**, and **6**, H_2O−MeOH, 60:40, for **13**, and H_2O−MeCN, 60:40, for **8**, respectively).

The sixth fraction, eluted with 100% MeOH, was separated and purified by reversed-phase HPLC (H_2O−MeOH, 5:95), yielding compounds **1** and **2**. The purified metabolites were isolated in the following amounts: 32.4, 46.5, 169.5, 13.4, 10.6, 7.7, 242.5, 208.0, 65.4, 14.0, 8.3, 10.4, 16.2, 24.4, and 8.2 mg of **1**−**15**, respectively.

Compound 2: white, amorphous solid; $[\alpha]_D^{25}$ +2.3 (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.99), 295 (3.17) nm; IR (ZnSe) ν_{max} 3370 (br), 2922, 1603 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 451.3571 $[M + H]^+$ (calcd for $C_{31}H_{47}O_2$, 451.3576).

Compound 5: white, amorphous solid; $[\alpha]_D^{25}$ −1.1 (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.96), 295 (3.29) nm; IR (ZnSe) ν_{max} 3427 (br), 2924, 1605, 1250 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 593.2886 $[M + Na]^+$ (calcd for $C_{31}H_{47}O_6SNa_2$, 593.2889).

Compound 9: CD (*c* 0.008, MeOH), λ_{max} ($\Delta\varepsilon$) 230.0 (+0.38), 202.0 (−0.70) nm.

Compound 10: white, amorphous solid; $[\alpha]_D^{25}$ +15.4 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.65) nm; IR (ZnSe) ν_{max} 2928, 1779, 1744, 1260 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 511.2103 $[M + Na]^+$ (calcd for $C_{25}H_{37}O_6SNa_2$, 511.2106).

Compound 11: white, amorphous solid; $[\alpha]_D^{25}$ +32.4 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.87) nm; CD (MeOH) 218.0 (−1.21), 248.0 nm (+1.76); IR (ZnSe) ν_{max} 2927, 2866, 1768, 1260 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 519.2394 $[M + H]^+$ (calcd for $C_{26}H_{40}O_7SNa$, 519.2392).

Compound 12: white, amorphous solid; $[\alpha]_D^{25}$ +0.4 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.79) nm; CD (MeOH) 220.0 (+0.74), 251.0 nm (−1.13); IR (ZnSe) ν_{max} 2928, 2867, 1767, 1260 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 541.2215 $[M + Na]^+$ (calcd for $C_{26}H_{39}O_7SNa_2$, 541.2212).

Compound 13: white, amorphous solid; $[\alpha]_D^{25}$ +4.4 (*c* 0.55, MeOH); IR (ZnSe) ν_{max} 2924, 2854, 1261 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 557.2527 $[M + Na]^+$ (calcd for $C_{27}H_{43}O_7SNa_2$, 557.2525).

Compound 14: white, amorphous solid; $[\alpha]_D^{25}$ +6.9 (*c* 0.60, MeOH); IR (ZnSe) ν_{max} 3427 (br), 2927, 2867, 1260 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 591.2576 $[M + Na]^+$ (calcd for $C_{27}H_{45}O_9SNa_2$, 591.2580).

Compound 15: white, amorphous solid; $[\alpha]_D^{25}$ −2.9 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.02), 232 (2.60) nm; IR (ZnSe) ν_{max} 3500, 2926, 1678, 1262 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 510.2264 $[M + Na]^+$ (calcd for $C_{25}H_{38}NO_5SNa_2$, 510.2266).

Biological Assays. The cytotoxicity assay was performed in accordance with literature protocol.[16] Sortase A,[17] isocitrate lyase,[9] Na^+/K^+−ATPase,[18] and antimicrobial inhibition assays[19] were performed according to literature methods.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^1H and ^{13}C NMR spectra of compounds **2**, **5**, and **10**−**15** are available free of charge via the Internet at http://pubs.acs.org.

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