Sodwanone S, a Triterpene from the Marine Sponge *Axinella weltneri*

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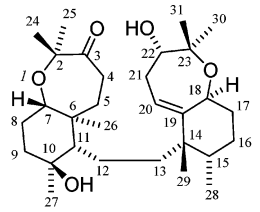
The bioassay-guided fractionation of the crude extract of the marine sponge *Axinella weltneri* led to the isolation and the identification of a new triterpene named sodwanone S (**1**), with an uncommon oxepane-cyclohexane system, along with the known sodwanones A and G. The structure was elucidated using spectroscopic data, and the biological activity was evaluated against 13 human tumor cell lines. A biogenetic pathway of this new compound is also proposed.

Marine sponges are known to produce triterpenoids that are squalene-derived polyethers constituted of two separate polycyclic systems connected with different types of linkers. The first of these, the sipholanes, siphenellinol, sipholenol, and sipholenone, were isolated from the Red Sea sponge *Siphonochalina siphonella*.[1−3] Other structurally related triterpenes were later identified as sodwanones from *Axinella weltneri* (Indian ocean),[4−8] yardenones and sodwanones from *Axinella* cf. *bidderi* (Indian ocean),[9,10] raspacionins from *Raspaciona aculeata* (Mediterranean sea),[11−15] and sodwanones, yardenone, and abudinols from *Ptilocaulis spiculifer* (Red Sea).[8,16] Some of these were found to be cytotoxic against human tumor cell lines.

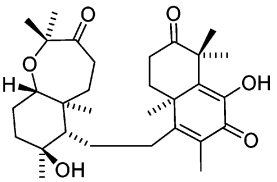
In the course of our search for antitumor metabolites from marine sponges, the Indian Ocean sponge *Axinella weltneri* (Lendenfeld, 1897), whose extract exhibited antitumor activity, was investigated, and a new triterpene named sodwanone S (**1**), along with the known sodwanones A and G, was isolated. The structural elucidation of the new metabolite and its cytotoxicity against several human tumor cell lines are presented below, together with a proposed biogenetic pathway.

A. weltneri (order Halichondrida, family Axinellidae) was collected using scuba off the southwest reef of Mayotte Island, Comoros Islands, at a depth of 25 m. The CH_2Cl_2−MeOH (1:1) extract of the marine sponge *A. weltneri* was subjected to a bioassay-guided fractionation using three human tumor cell lines: A549, HT29, and LOVO-DOX. After an H_2O−CH_2Cl_2 partition, the bioactive CH_2Cl_2 layer was successively purified by silica gel chromatography and HPLC to afford three purified compounds: sodwanone A (**2**), a new triterpene (**1**), and sodwanone G. Compound **1** was isolated as a white powder. Its molecular formula was established as $C_{30}H_{50}O_5$ by HRESIMS and required six degrees of unsaturation. The ^{13}C NMR data (Table 1) indicated the presence of one carbonyl group at δ_C 217.8 ppm and one trisubstituted double bond at δ_C 148.8 and 115.8 ppm. The structure of **1** therefore had to include four cycles. The ^{13}C and DEPT NMR spectra were consistent with eight methyls, nine methylenes, six methines, and seven quaternary carbons.

Comparison of NMR data of **1** with those of reported sodwanones[4] showed that compound **1**'s left side (C-2 to C-11 and C-24 to C-27) was similar to the perhydrobenzoxepine system (oxepane-cyclohexane) of sodwanone A (**2**). Furthermore, examination of NMR spectra indicated that the right side of the molecule was constituted by an uncommon unsaturated oxepane-cyclohexane system linked to the left side by an ethylene bridge. This condensed oxepane-cyclohexane system being typical of the sodwanones A−I and K−R isolated by Rudi et al.,[4−8] the new compound **1** was consequently named sodwanone S.



Sodwanone S (**1**)



Sodwanone A (**2**)

The key features presented below allowed the determination of the uncommon right half of the molecule. Aside from the resonances corresponding to the left side, the ^1H NMR spectrum indicated the presence of two oxygen-bearing methines at δ_H 3.77 (d, J = 6.2 Hz, H-22) and 4.34 (m, H-18) along with one vinylic proton at δ_H 5.23 (d, J = 9.1 Hz, H-20). The HSQC spectrum showed that these protons were borne by the carbons resonating at δ_C 78.8 (CH, C-22), 71.1 (CH, C-18), and 115.8 (CH, C-20), respectively. In the HMBC experiment, the ethylenic proton H-20 correlated with the carbons at δ_C 78.8 (C-22), 30.2 (C-21), 71.1 (C-18), and 43.9 (C-14). Furthermore, the protons H-13b, H-17a,b, H-21a,b, and H-29 correlated with the quaternary ethylenic carbon at δ_C 148.8 (C-19). Consequently, the double bond had to be located on C-19/C-20 in accordance with the COSY correlations H-20/H-18 and H-20/H-21a,b. All the HMBC correlations observed were consistent with the proposed structure and were especially useful to assign the methyl signals. The *gem*-dimethyl protons at δ_H 1.17 (s, H-31) and 1.26 (s, H-30) showed

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Table 1. ^{1}H, ^{13}C, COSY, and HMBC NMR Data of Sodwanone S (**1**)[a]

C#	δ_C		δ_H mult. (J in Hz)	COSY H−H	HMBC C−H
2	82.7	qC			4a, 7, 24, 25
3	217.8	qC			4a, 4b, 5a, 5b, 24, 25
4	35.3	CH_2	3.17 ddd (13.7, 11.0, 2.6)	4b, 5b, 5b	5a
			2.15 ddd (11.0, 6.6, 1.8)	4a, 5a, 5b	
5	40.5	CH_2	1.73 ddd (13.7, 6.4, 2.8)	4a, 4b, 5b	4a, 4b, 7, 26
			1.23 m	4a, 4b, 5a	
6	42.1	qC			4b, 5a, 5b, 26
7	81.4	CH	2.92 dd (10.6, 5.4)	8	5a, 8, 26
8	28.8	CH_2	1.60 m	7, 9a, 9b	7, 9a
9	40.1	CH_2	1.69 dt (12.6, 3.3)	8, 9b	8, 27
			1.42 m	8, 9a	
10	73.2	qC			8, 9b, 11, 27
11	57.7	CH	1.00 m		26, 27
12	20.1	CH_2	1.25 m	12b	13a
			1.06 m	12a, 13a	
13	34.4	CH_2	1.41 m	12b, 13b	29
			1.22 m	13a	
14	43.9	qC			13a, 20, 28, 29
15	42.1	CH	1.39 m	28	28, 29
16	28.6	CH_2	1.43 m		15, 28
17	36.2	CH_2	1.84 m	17b, 18	
			1.40 m	17a, 18	
18	71.1	CH	4.34 m	17a, 17b, 20, 21a	16, 17a, 17b, 20
19	148.8	qC			13b, 17a, 17b, 21a, 21b, 29
20	115.8	CH	5.23 br d (9.1)	18, 21a, 21b	21a, 21b, 22, 29
21	30.2	CH_2	2.98 br d (15.6)	18, 20, 21b, 22	20
			2.33 ddd (15.6, 9.1, 6.2)	20, 21a, 22	
22	78.8	CH	3.77 d (6.2)	21a, 21b, 31	20, 21b, 30, 31
23	77.6	qC			21b, 22, 30, 31
24	20.7	CH_3	1.31 s		25
25	26.7	CH_3	1.25 s		24
26	12.6	CH_3	0.95 s		5a, 5b, 7, 12
27	23.7	CH_3	1.18 s		
28	16.7	CH_3	0.86 d (6.7)	15	
29	22.7	CH_3	0.99 s		
30	19.9	CH_3	1.26 s		22, 23, 31
31	29.9	CH_3	1.17 s		22, 23, 30

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).

correlations with the C-22 hydroxy-bearing methine at δ_C 78.8 and with the C-23 quaternary carbon at δ_C 77.6. The position of the CH_3-28 methyl group (δ_H 0.86, d, J = 6.7 Hz) on the C-15 methine was supported by the HMBC correlations H-28/C-14, H-28/C-15, and H-28/C-16. In addition, the location of the CH_3-29 methyl group (δ_H 0.99, s) on the C-14 quaternary carbon was supported by the HMBC correlations H-29/C-14, H-29/C-15, and H-29/C-19.

The key chemical shifts and coupling constants of the left half of sodwanone S were similar to those described for sodwanone A, whose stereochemistry was determined by X-ray diffraction studies.[4] Consequently, the relative configuration was the same as sodwanone A: Me-25, Me-26, and Me-27 were α-oriented and H-7, H-11, and Me-24 were β-oriented. These conclusions were consistent with the key NOEs observed in this system.

Because the relative stereochemistry of the sodwanone S right half could not be deduced from the coupling constants or the NOESY spectrum in $CDCl_3$, additional NMR experiments were performed in DMSO-d_6 (Table 2). The key NOE cross-peaks H-18/H-13a, H-18/H-30, and H-13b/H-28 placed H-18, H-13, Me-28, and Me-30 on the same face of the bicyclic system. Because no clear conclusion could be deduced from H-22 NOEs, a detailed examination of the coupling constants was undertaken to determine the relative stereochemistry at C-22. The two epimers at this position were examined through molecular modeling (Chem 3D, global energy minimization after MM2 molecular dynamics, RMS = 0.005). The model of the epimer with H-18 and H-22 in a *trans* relative configuration showed dihedral angles of −78° between H-22 and H-21a, and 39° between H-22 and H-21b. Using the Karplus equation these values were consistent with coupling constants of 0.3 and 6.9 Hz, respectively.[17] In the other epimer (H-18 and H-22 in a *cis* configuration) dihedral angles were found to be −79° and 163°, inducing coupling constants of 0.2 and 11.2 Hz, respectively. Because in the ^{1}H NMR spectrum the H-22 signal appeared as a doublet with a coupling constant of 6.2 Hz, we concluded that H-18 and

Table 2. ^{1}H, ^{13}C, and NOESY NMR Data of the Right Side of Sodwanone S (**1**) in DMSO-d_6[a]

C#	δ_C		δ_H mult. (J in Hz)	NOESY H−H
13	34.3	CH_2	1.55 m	13b, 18
			0.98 m	13a, 28
14	42.9	qC		
15	41.6	CH	1.21 m	29
16	28.4	CH_2	1.45 m	17a, 18, 28
			1.23 m	
17	35.6	CH_2	1.70 m	16a, 18
			1.26 m	
18	70.5	CH	4.23 m	13a, 16a, 17a, 30
19	146.6	qC		
20	115.8	CH	5.10 br d (7.1)	21b, 29
21	30.5	CH_2	2.70 br dd (15.7, 1.3)	21b, 22, 31
			2.12 ddd (15.7, 7.1, 6.5)	20, 21a, 22, 30, OH_{22}
22	76.4	CH	3.58 ddd (6.5, 5.5, 1.3)	21a,b, 30, 31(st), OH_{22}
23	77.0	qC		
28	16.4	CH_3	0.81 d (6.8)	16a, 29
29	22.8	CH_3	0.93 s	13b, 15, 20, 28
30	19.5	CH_3	1.15 s	18, 21a, 22, OH_{22}
31	30.0	CH_3	1.05 s	18, 22, OH_{22}
OH_{22}			4.56 d (5.5)	21b, 22, 30 (st),[b] 31

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b] st: strong.

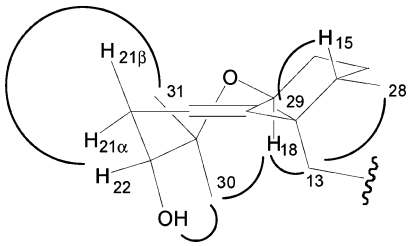
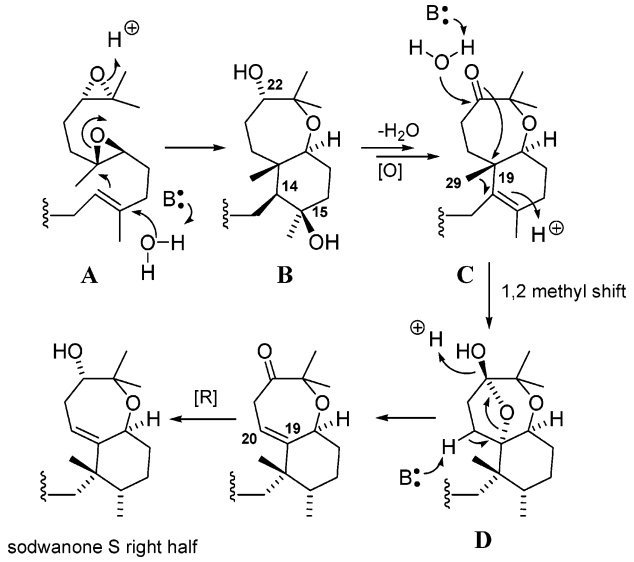


Figure 1. Key NOESY correlations of the right half of sodwanone S.

Scheme 1. Proposed Biogenetic Pathway of the Right Half of Sodwanone S



H-22 had a *trans* relative configuration. This assumption was confirmed by the strong NOE correlations H-22/H-31 and OH-22/H-30 (Figure 1). Due to conformational mobility around the C-12/C-13 bond, the relative stereochemistry between the two halves of **1** could not be determined directly by NMR studies. Nevertheless, the known absolute stereochemistry of the squalene precursor of the sodwanones led us to propose the relative stereochemistry of the two halves of sodwanone S, as presented above.[18]

We then turned our attention to the biosynthesis of the new right half of sodwanone S. As proposed in previous studies, the common precursor for the oxepane-cyclohexane half of the sodwanones could be the diepoxy fragment **A** (Scheme 1).[18] After an acid-catalyzed opening of the terminal epoxide a cascade cyclization would occur and lead to the dihydroxylated oxepane-cyclohexane **B**, this double ring system being identical with the right half of siphonelol.[1] A C-14/C-15 dehydration step and an oxidation of the C-22 hydroxyl group would lead to the intermediate **C**, similar to the left side of sodwanone O.[8] A 1,2-shift of the C-29 methyl group from C-19 to C-14 would be induced by the hemiketal formation at C-22. The same mechanism would afford one-half of raspacionin A, sodwanones F, I, and L, which all possess a dioxabicyclo[3.2.1]octane system with the same relative stereochemistry.[5−7,12] Considering **D** as a precursor of the right half of sodwanone S, the two following stages were assumed: first an acid-catalyzed dehydration step, which would lead to the unsaturation at C-19/C-20, then a reduction of the resulting ketone at C-22. To be in accordance with the observed C-22 stereochemistry of sodwanone S, the hydride attack during the final reduction process would occur from the less buried β-face. Interestingly, the same face was attacked by a molecule of water during the hemiketal formation of the intermediate **D**. Among the sodwanones family,[4−8] it is noteworthy that sodwanone S is the first report of a structure including an unsaturation in its oxepane ring.

Table 3. IC_{50} Values in μg/mL for Sodwanones A, G, and S against Human Tumor Cell Lines

	IC_{50} (μg/mL)		
cell lines	sodwanone A (**2**)	sodwanone G	sodwanone S (**1**)
DU-145	1.8	>10	>10
LN-CaP	3.8	4.3	5.0
IGROV	1.7	5.0	>10
IGROV-ET	0.068	2.7	4.7
SK-BR3	>10	>10	3.8
SK-MEL-28	7.5	>10	6.5
A549	3.5	>10	>10
PANC1	0.7	3.1	4.1
HT29	>10	>10	>10
LOVO	8.5	>10	6.9
LOVO-DOX	0.3	2.7	3.3
HeLa	2.1	5.4	>10
HeLa-APL	2.9	4.2	>10

The cytotoxic activities of sodwanones A, G, and S were evaluated against 13 human tumor cell lines. The results, presented in Table 3, indicated that sodwanone A was the most active against IGROV-ET (IC_{50} 0.068 μg/mL), while sodwanones S and G showed similar moderate activities against several cell lines (IC_{50} mean 5 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotation was determined on an Optical Activity Ltd AA-5 polarimeter. NMR experiments were performed on a Bruker DRX 500, using a standard Bruker program. Chemical shifts were reported in ppm using residual $CDCl_3$ (δ 7.26 ppm for ^1H and 77.16 ppm for ^{13}C) as internal reference. HPLC was performed with a Waters model 600 pump and diode array and light-scattering detectors. HRMS was realized at Service Central d'Analyses du CNRS (Solaize, France).

Animal Material. The sponge *Axinella weltneri* (order Halichondrida, family Axinellidae) was collected using scuba off the southwest reef of Mayotte Island, Comoros Islands, northwest of Madagascar, at a depth of 25 m, on April 27, 2002. The sample (210 g, wet weight) was kept frozen until required. The material was identified by Dr. Iosune Uriz (Blanes, Spain), and a voucher specimen (ORMA005468) has been deposited at the company PharmaMar SA.

Extraction and Isolation. The sponge was exhaustively extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The combined extracts were filtered, evaporated in vacuo, and dried. The resulting brown extract (8.2 g) was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 residue (2.0 g), which showed antitumor activity, was subjected to Si 60 gel (35−70 μm) column chromatography, eluting with solvent mixtures of increasing polarity from 100% *n*-hexane to 100% EtOAc. The final purification of the two most active fractions was accomplished. The first one was subjected to reversed-phase HPLC (C18 column, 150 × 4.6 mm, 3.5 μm), eluting with a gradient of water and acetonitrile (95:5 to 0:100) in 30 min, and led to the pure known sodwanone A. The second one was purified by normal-phase HPLC (Diol column, 250 × 10 mm, 5 μm) eluting with *n*-hexane−CH_2Cl_2 (7:3) during 25 min, then *n*-hexane−CH_2Cl_2 (7:3) to CH_2Cl_2 (100%) in 25 min, to afford sodwanone S (**1**) (1.5 mg, 7.1×10^{-4}% wet wt, t_R = 9.2 min) and the known sodwanone G (2.2 mg, 1.0×10^{-3}% wet wt, t_R = 12.1 min).

Sodwanone S (1): white powder; $[\alpha]^{25}_D$ +187° (*c* 0.033, CH_2Cl_2); ^1H NMR in $CDCl_3$, see Table 1; ^{13}C NMR in $CDCl_3$, see Table 1; HRESIMS *m/z* 513.3544 $[M + Na]^+$, calcd for $C_{30}H_{50}O_5Na$ 513.3556.

Biological Activity. A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability, follow-

ing the technique described in the literature.[19] The in vitro activity of the compounds was evaluated against 13 human tumor cell lines including prostate (DU-145 and LN-CaP), ovary (IGROV and IGROV-ET), breast (SK-BR3), melanoma (SK-MEL-28), NSCL (A549), pancreas (PANC1), colon (HT29, LOVO, and LOVO-DOX), and cervix (HeLa and HeLa-APL).

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