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SODWANONES G, H, AND I, NEW CYTOTOXIC TRITERPENES FROM A MARINE SPONGE

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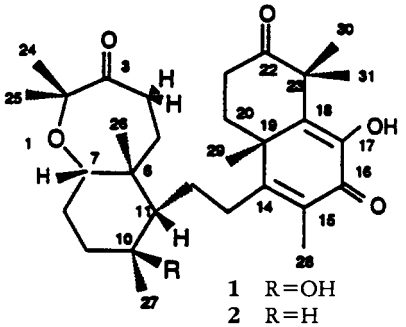
ABSTRACT.—Three cytotoxic triterpenes, sodwanones G [**5**], H [**6**], and I [**7**], have been isolated from a marine sponge. The three structures were determined by interpretation of nmr spectra, and in the case of **5**, also on the basis of the X-ray diffraction analysis. The X-ray diffraction analysis of two other earlier reported sodwanones, E [**3**] and F [**4**] is also discussed. The cytotoxic activity against several cancer cell lines, has been tested. IC_{50} values between 20 and 0.02 μM were obtained. Compounds **5** and **6** were 10- and 500-fold, respectively, more cytotoxic to A-549 cells than to any of the other cell lines tested (HT-29, MEL-28, P-388).

The isolation and structure elucidation of sodwanones A–F has recently been reported from the Indo-Pacific sponge *Axinella weltneri* (Axinellidae) (1,2). The sodwanones, e.g., A, B, E, and F [**1–4**], are new polyepoxysqualene-derived triterpenes. Similar secondary metabolites were isolated previously from the Red Sea sponge *Siphonochalina siphonella* (3) and from the Mediterranean sponge *Raspaciona aculeata* (4,5). The structure determinations of **1–4** were based on spectral data, including an X-ray diffraction analysis for **1** (1). This report describes the isolation and structure determination of three additional sodwanones, G [**5**], H [**6**], and I [**7**], as well as the X-ray diffraction analyses of sodwanones E [**3**] and F [**4**] which were carried out to ascertain some structural points (see below). In addition, the results of an experiment aimed at the determination of the absolute configuration of **1** based on the modified Mosher method (6,7) are also discussed.

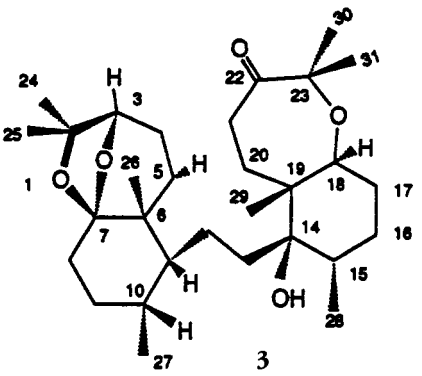
RESULTS AND DISCUSSION

A recollection of *A. weltneri* (March 1994) afforded sodwanones A–F in addition to the new sodwanone G [**5**] (0.01%, dry wt). Structure elucidation of compound **5** began by intensive study of its spectroscopic data. A molecular formula of $C_{30}H_{42}O_6$ $[M]^+$ was established by hreims and by its ^{13}C- and ^1H-nmr spectra. Comparison of the nmr data of **5** with those of **1** and **2** (Table 1) pointed clearly to the identity of fragments C-2 to C-9 and C-12 to C-31 (the left half), whereas differences were observed for fragment C-8 to C-11. Two ^{13}C-nmr resonances at δ 58.3 s and δ 50.3 t (δ_H 2.75 dd and 2.58 d, 1H each) suggested an exocyclic epoxide [on C-10(C-27)]. Indeed, **5** contained only seven methyl groups, with the eighth one being transformed into the epoxide via the C-10(C-27) methylene, a methylene that exists in sodwanone D (1,2). 2D-Nmr, COSY, TOCSY, and HMBC experiments (Table 1) confirmed the latter C-10(C-27) location of the epoxide.

Unequivocal proof of the complete structure and relative stereochemistry of **5** was established by an X-ray diffraction analysis. An ORTEP representation of the molecular structure is presented in Figure 1 (the right half of **5**, in the crystal, as shown in the figure,



1 R=OH
2 R=H
8 R=OH, 3-OH



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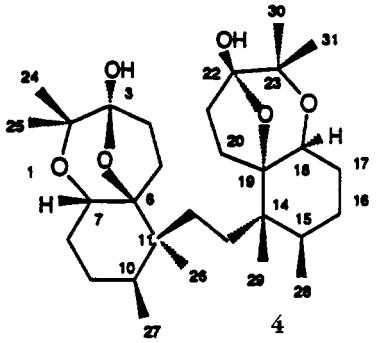

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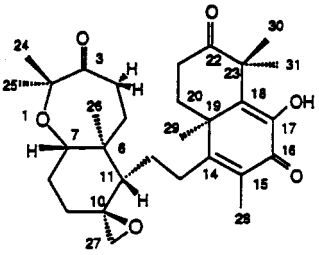
is rotated 180° in comparison to the drawing). Atomic coordinates are given in Table 2. The ORTEP illustration of **5** shows that the relative stereochemistry of the two halves of the molecule is identical to that of **1** (1), and that the left half has the same stereochemistry as sipholenol A (3) and raspacionin (4,5).

From yet another collection of the sponge (August 1994), three sodwanones of almost the same polarity were isolated. The first compound was determined to be the known sodwanone F [**4**] (2).

The second compound, sodwanone H [**6**], an amorphous powder, gave a molecular ion in its hreims at *m/z* 472.3537 $[M]^+$ corresponding to a molecular formula of



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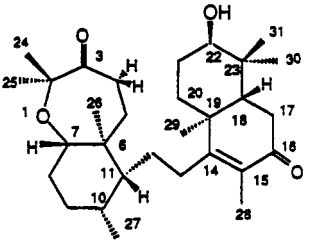

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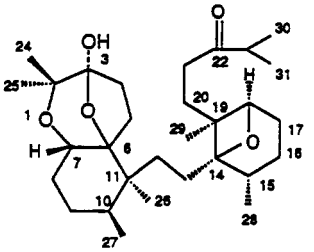

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TABLE 1. Nmr Data (125 and 500 MHz) Including CH-correlations of **2**, **5**, and **6**.[a–d]

Proton(s)	Compound						
	2[e]	**5**			**6**		
	δ_C	δ_C	δ_H[d]	HMBC (C to H)	δ_C	δ_H[d]	HMBC (C to H)
2	82.4 s	82.5 s		7,24,25	82.3 s		7,24,25
3	218.0 s	216.9 s		4a,4b,5a,24,25	217.8 s		4a,4b,5a,24,25
4	35.1 t	35.0 s	3.21 ddd		35.1 t	3.25 dd	
			2.18 m			2.10 tt	
5	40.5 t	39.3 t	1.92 m		40.4 t	1.90 m	7,26
			1.90 m			1.19 m	
6	41.5 s	43.1 s		26	40.3 s		26
7	81.9 d	79.8 d	3.10 dd	8a,8b,11,26	82.0 d	2.97 dd	24,25
8	31.1 t	29.8 t	1.80 m		31.1 t	1.65 m	
			1.75 m			1.48 m	
9	28.4 t	33.5 t	1.78 m		28.2 t	1.90 m	27
			1.78 m				
10	28.2 d	58.3 s		9a,9b,11,12a,12b 13a	28.6 d	1.92 m	27
11	51.3 d	50.5 d	1.49 m	26	51.6 d	1.13 m	27
12	25.5 t	21.9 t	1.40 m	4a,4b,7,26	25.8 t	1.60 m	
			0.80 m			1.45 m	
13	29.9 t	33.0 t	2.50 dt		28.6 t	2.28 dt	
			2.20 m			1.95 m	
14	164.5 s	164.7 s		13a,13b,28,29	168.5 s		13a,28,29
15	129.1 s	128.8 s		13a,13b,28	129.4 s		13a,28
16	181.5 s	181.5 s		OH-17,28	200.0 s		17a,17b,18,28
17	141.9 s	141.8 s		OH-17	34.5 t	2.38 dd	
						2.36 dd	
18	137.2 s	137.6 s		OH-17,29,30,31	43.3 d	2.20 dd	29
19	42.7 s	42.7 s		13a,29,30	41.3 s		21a,29
20	25.8 t	27.3 t	2.36 dd		27.3 t	1.62 m	18,29
			1.68 m			1.62 m	
21	32.5 t	32.6 t	2.74 d		25.2 t	2.15 ddd	
			2.62 dd			1.93 m	
22	215.0 s	214.8 s		20a,21a,21b,30,31	74.9 d	3.48 br s	30,31
23	48.0 s	48.0 s		30,31	37.3 s		30,31
24	20.5 q	20.2 q	1.34 s		20.4 q	1.27 s	25
25	26.4 q	26.4 q	1.27 s		26.0 q	1.21 s	24
26	13.4 q	11.9 q	0.96 s		13.4 q	0.97 s	7
27	14.7 q	50.3 t	2.75 dd	26	14.5 q	0.95 d	—
			2.58 d				
28	11.9 q	11.9 q	1.96 s		11.3 q	1.68 s	
29	21.6 q	21.6 q	1.09 s		17.8 q	0.94 s	
30	20.8 q	20.8 q	1.51 s		21.6 q	0.91 s	
31	24.4 q	24.3 q	1.47 s		26.4 q	0.93 s	

[a]In $CDCl_3$ on a Bruker ARX 500 instrument, chemical shifts refer to TMS (δ_H=0) and $CDCl_3$ (δ_C=77.0).
[b]Assignments aided by HMQC, HMBC, homo COSY, TOCSY, and NOESY experiments.
[c]Ha- is the lower-field proton in a geminal pair and Hb- is the higher-field proton.
[d]For *J* values, see Experimental.
[e]Data from Ref. (2) (nmr taken in C_6D_6); due to superior nmr analysis of the spectra of **6**, we have reassigned the signals of C-8, 9, 12, and 13 of compound **2**.

$C_{30}H_{48}O_4$. Comparison of the nmr data of **6** with those of the known sodwanones (1,2) determined the identity of the left part (moiety C-2 to C-13) of **6** and the same bicyclic system in sodwanone B [**2**] (Table 1). According to the nmr data, the second half of the molecule embodies a tetra-substituted α,β-unsaturated ketone (δ_C 200.0 s, 168.5 s, 129.4 s), a secondary hydroxyl group (δ_C 74.9 d, δ_H 3.48 br s), and four methyl groups. The two functionalities could further be expanded, from the nmr data, to the -C(17)H_2C(18)H- and -C(20)H_2C(21)H_2C(22)HOH- moieties. According to the seven degrees of unsaturation of **6** and the above functionalities, the right part of **6** must also

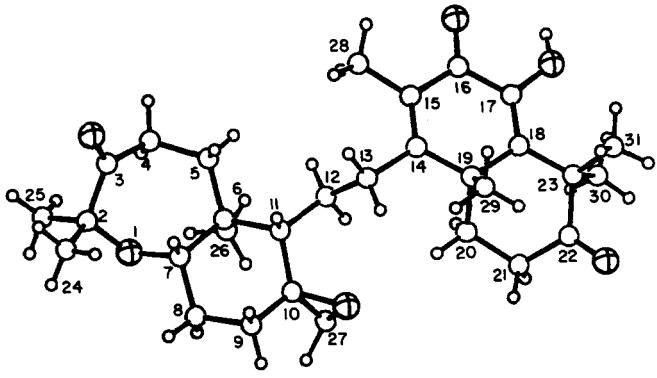


FIGURE 1. ORTEP representation of **5**.

TABLE 2. Atomic Coordinates and Equivalent Isotropic Thermal Parameters of **5**.

Atom	*x/a*	*y/b*	*z/c*	U_{eq}[a]
O-1	−0.4009 (6)	0.0348	0.4462 (3)	0.0503 (21)
C-2	−0.3617 (11)	0.1043 (10)	0.3787 (4)	0.0539 (33)
C-3	−0.2217 (10)	0.1967 (11)	0.4132 (5)	0.0556 (32)
C-4	−0.0988 (8)	0.1598 (11)	0.4935 (5)	0.0607 (33)
C-5	−0.1560 (9)	0.1942 (12)	0.5719 (4)	0.0609 (33)
C-6	−0.3001 (9)	0.1135 (9)	0.5897 (4)	0.0407 (25)
C-7	−0.4440 (8)	0.1107 (9)	0.5094 (4)	0.0462 (28)
C-8	−0.6050 (10)	0.0613 (11)	0.5230 (5)	0.0561 (33)
C-9	−0.6567 (9)	0.1309 (11)	0.5934 (5)	0.0562 (36)
C-10	−0.5207 (9)	0.1232 (10)	0.6707 (4)	0.0487 (34)
C-11	−0.3584 (8)	0.1814 (10)	0.6620 (4)	0.0437 (25)
C-12	−0.2278 (8)	0.1879 (10)	0.7457 (4)	0.0472 (29)
C-13	−0.2341 ()8)	0.3149 (10)	0.7928 (4)	0.0440 (25)
C-14	−0.1120 (7)	0.3204 (9)	0.8782 (3)	0.0358 (23)
C-15	0.0526 (9)	0.3306 (10)	0.8841 (4)	0.0433 (28)
C-16	0.1688 (9)	0.3366 (10)	0.9666 (4)	0.0429 (29)
C-17	0.1053 (7)	0.3259 (10)	1.0405 (4)	0.0403 (26)
C-18	−0.0577 (8)	0.3166 (10)	1.0371 (3)	0.0347 (23)
C-19	−0.1836 (7)	0.3160 (10)	0.9538 (4)	0.0383 (24)
C-20	−0.2844 (9)	0.1882 (10)	0.9499 (4)	0.0469 (29)
C-21	−0.3638 (9)	0.1744 (10)	1.0229 (4)	0.0476 (26)
C-22	−0.3050 (9)	0.2659 (9)	1.0949 (4)	0.0455 (30)
C-23	−0.1223 (8)	0.3032 (11)	1.1155 (4)	0.0471 (28)
C-24	−0.5129 (12)	0.1678 (13)	0.3247 (5)	0.0768 (42)
C-25	−0.2961 (16)	−0.0004 (13)	0.3287 (6)	0.0853 (47)
C-26	−0.2387 (12)	−0.0244 (10)	0.6148 (5)	0.0630 (38)
C-27	−0.5351 (10)	0.0238 (12)	0.7325 (5)	0.0621 (38)
C-28	0.1336 (10)	0.3377 (11)	0.8133 (5)	0.0571 (35)
C-29	−0.2970 (10)	0.4359 (10)	0.9485 (5)	0.0500 (32)
C-30	−0.0353 (11)	0.1945 (14)	1.1725 (6)	0.0767 (44)
C-31	−0.0974 (14)	0.4317 (13)	1.1639 (7)	0.0848 (50)
O-2	−0.2067 (8)	0.2996 (10)	0.3796 (4)	0.0893 (30)
O-3	−0.5694 (7)	0.1579 (9)	0.7459 (3)	0.0666 (27)
O-4	0.3182 (6)	0.3506 (8)	0.9753 (3)	0.0639 (24)
O-5	0.2254 (5)	0.3313 (9)	1.1137 (3)	0.0607 (21)
O-6	−0.3953 (6)	0.2978 (9)	1.1381 (3)	0.0735 (26)

[a] U_{eq} is one third of the trace of the orthogonalized U_{ij} tensor.

be bicyclic. Based on the nmr data, a similar decalin system to the one existing in sodwanones A [**1**], B [**2**], C, and G [**5**] was suggested. The latter suggestion was unequivocally confirmed by the HMBC correlations (Table 1). The relative stereochemistry of the right half of **6** was established from a NOESY experiment summarized in Figure 2.

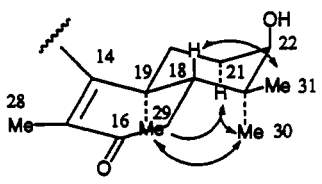
The third compound isolated from the sponge was sodwanone I [**7**], $C_{30}H_{50}O_5$, *m/z* 490.3672 $[M]^+$. The nmr data of **7** (Table 3) implied that the left half of the molecule (C-2 to C-13) is identical to the left half of sodwanone F [**4**] and that the other half differs from all other corresponding parts in sodwanones A–H. The chemical shifts, COSY, and HMBC experiments suggested for **7** a $-CH_2CH_2COCH(CH_3)_2$ moiety and an ethereal bridge (δ_C 86.4 s and 82.1 d; δ_H 3.96 d). Furthermore, the HMBC correlations set the quaternary ethereal *C*-atom between Me-29 and Me-28 of the $C(17)H_2C(16)H_2C(15)HCH_3(28)$ moiety (determined by a COSY experiment). The above data suggested an oxabicyclo[3,1,1]heptane system for **7** which was confirmed unequivocally by a NOESY experiment (see partial structure b, Figure 2).

Our earlier report (2) described the structure elucidation of compounds **3** and **4** on the basis of their nmr data. In each one of these structure determinations, there was a point which needed further clarification. In the case of **3** it was the doublet, rather than the expected double doublet of H-3 in the ^{1}H-nmr spectrum, requiring a deformation of the left half of the molecule. In the case of **4** it was the stereochemistry of the four C-26–C-29 methyl groups. In both cases, the relative configuration of the two halves of the molecules could not be established by NOEDS because of the ethylene bridge between the two halves.

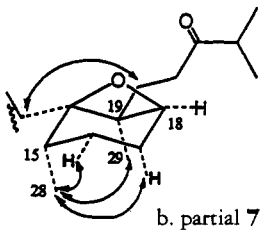
The earlier suggested structure and the relative stereochemistry of **3** were established unequivocally by an X-ray diffraction analysis. An ORTEP representation of the molecular structure is illustrated in Figure 3, and the atoms are given in Table 4. A slight twisting of the left half can be seen from the figure, consistent with that required by the nmr spectrum. The analysis also established the relative stereochemistry of the two halves of **3**.

In case of **4** it was very difficult to obtain good crystals. The crystals of **4**, from a variety of solvents diffracted poorly and yielded only low-resolution diffraction data. The relatively small number of observations allowed only a reliable determination of the overall molecular structure of **4**. The resulting covalent parameters (bond lengths and bond angles) are characterized, however, by low precision. An ORTEP representation of the molecule is shown in Figure 4. From this illustration it can be seen that two methyl groups on the left half of **4**, Me-26 and Me-27 are equatorial while the corresponding two methyls on the other half are equatorial (Me-29) and axial (Me-28). The X-ray structure also determined unequivocally the relative stereochemistry of the two halves of the molecule. The stereochemistry of the left half is the same as that of raspacionin A (4,5).

The last subject addressed in this study was the absolute configuration of one of the sodwanones by the modified Mosher method (6). Reduction with $NaBH_4$ of sodwanone A [**1**], for 30 min at room temperature, resulted in the selective reduction of the C-3 carbonyl only. Unfortunately, this reduction gave mainly (ca. 95%) the undesired axial hydroxyl isomer [**8**] and only trace amounts of its epimer with an equatorial hydroxyl group. The left half of **8**, carrying the axial C-3 hydroxyl, is identical with the same ring system in sipholenol-A (3), for which the absolute configuration was determined by the modified Mosher method (7). In the latter report (7) it was found that the positive and negative $\Delta\delta$ values are irregularly dispersed on the left and right sides of the MTPA plane, most likely because of steric hindrance of the ester group, which in the case of



a. partial **6**



b. partial **7**

FIGURE 2. Several key nOes measured for sodwanones H [**6**] and I [**7**].

TABLE 3. Nmr Data (125 and 500 MHz) Including CH-correlations of **4** and **7**.[a–c]

Protons	Compound			
	4[d]	**7**		
	δ_C	δ_C	δ_H	HMBC (C to H)
2	77.3 s	77.3 s		5b,24,25
3	105.5 s	105.4 s		5b,24,25
4	24.2 t	24.0 t	1.82 m	
			1.72 m	
5	32.3 t	32.3 t	2.32 m	
			1.29 m	
6	89.7 s	89.6 s		26
7	71.2 d	71.0 d	3.88 dd (J=11.2,5.2)	
8	28.5 t	28.2 t	1.65 m	
			1.25 m	
9	27.8 t	27.6 t	1.32 m	27
			1.32 m	
10	39.2 d	39.3 d	1.28 m	26,27
11	40.9 s	40.8 s		26
12	26.3 t	25.1 t	1.08 m	26
			1.05 m	
13	31.9 t	30.9 t	1.72 m	
			1.15 m	
14		86.4 s		16a,17a,17b,18,28,29
15		50.1 d	1.36 m	17a,17b,28
16		26.1 t	1.95 m	
			1.68 m	
17		38.1 t	1.45 m	29
			1.45 m	
18		82.1 d	3.96 d (J=5.1)	20a,20b
19		50.8 s		17a,17b,20a,20b,29
20		36.2 t	2.42 m	
			2.38 m	
21		24.5 t	1.60 m	
			1.60 m	
22		215.0 s		20a,20b,29,30,31
23		40.6 d	2.60 h (J=6.5)	30,31
24		24.4 q	1.26 s	25
25		18.9 q	1.34 s	
26		19.1 q	0.98 s	
27		15.4 q	0.81 d (J=7.0)	
28		13.5 q	0.88 d (J=7.0)	
29		18.1 q	1.28 s	
30		18.3 q	1.06 d (J=6.5)	
31		18.2 q	1.09 d (J=6.5)	23

[a]In $CDCl_3$ on a Bruker ARX 500 instrument; chemical shifts refer to TMS (δ_H=0) and $CDCl_3$ (δ_C=77.0).
[b]Assignments aided by HMQC, HMBC, homo-COSY, TOCSY, and NOESY experiments.
[c]Ha- is the lower-field proton in a geminal pair and Hb- is the higher-field proton.
[d]^{13}C-Nmr values from Rudi *et al.* (2) are given in C_6D_6.

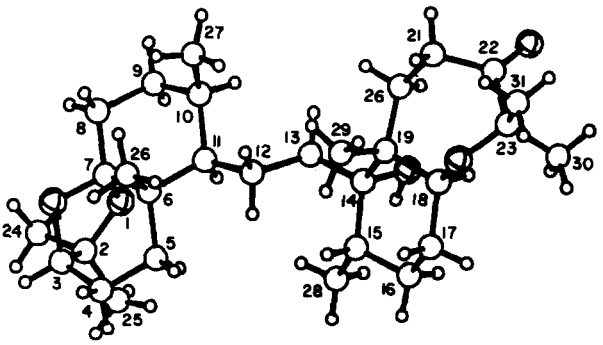


FIGURE 3. ORTEP representation of **3**.

TABLE 4. Atomic Coordinates and Equivalent Isotropic Thermal Parameters of **3**.

Atom	*x/a*	*y/b*	*z/c*	U_{eq}[a]
O-1	0.1930 (5)	0.2432	0.1578 (3)	0.0367 (13)
C-2	0.1538 (9)	0.2659 (3)	0.2841 (5)	0.0432 (21)
C-3	0.0134 (10)	0.3252 (3)	0.2566 (5)	0.0443 (20)
C-4	−0.1767 (9)	0.2998 (4)	0.2170 (6)	0.0493 (22)
C-5	−0.1786 (8)	0.2487 (3)	0.1036 (6)	0.0421 (20)
C-6	−0.0466 (8)	0.2741 (3)	0.0018 (5)	0.0343 (17)
C-7	0.1313 (8)	0.2994 (3)	0.0715 (5)	0.0349 (18)
C-8	0.2841 (9)	0.3179 (4)	−0.0133 (6)	0.0449 (21)
C-9	0.3291 (9)	0.2511 (4)	−0.0931 (6)	0.0523 (25)
C-10	0.1631 (9)	0.2231 (3)	−0.1718 (5)	0.0436 (23)
C-11	−0.0010 (8)	0.2095 (3)	−0.0873 (5)	0.0367 (18)
C-12	−0.1672 (8)	0.1786 (3)	−0.1609 (6)	0.0410 (22)
C-13	−0.1293 (9)	0.1045 (3)	−0.2214 (5)	0.0396 (20)
C-14	−0.2890 (9)	0.0545 (3)	−0.2589 (6)	0.0389 (20)
C-15	−0.4297 (10)	0.0511 (3)	−0.1552 (6)	0.0517 (24)
C-16	−0.5829 (12)	−0.0021 (5)	−0.1884 (8)	0.0755 (33)
C-17	−0.6725 (11)	0.0131 (5)	−0.3160 (8)	0.0686 (30)
C-18	−0.5373 (9)	0.0156 (4)	−0.4189 (6)	0.0429 (21)
C-19	−0.3810 (8)	0.0710 (3)	−0.3940 (5)	0.0350 (18)
C-20	−0.2386 (9)	0.0613 (4)	−0.4951 (6)	0.0499 (23)
C-21	−0.3106 (11)	0.0588 (4)	−0.6322 (7)	0.0620 (25)
C-22	−0.4043 (13)	−0.0099 (4)	−0.6677 (7)	0.0666 (31)
C-23	−0.6067 (11)	−0.0158 (4)	−0.6369 (7)	0.0614 (27)
C-24	0.0868 (11)	0.2019 (4)	0.3588 (7)	0.0587 (27)
C-25	0.3282 (10)	0.2970 (4)	0.3483 (6)	0.0566 (26)
C-26	−0.1357 (10)	0.3386 (3)	−0.0702 (7)	0.0497 (25)
C-27	0.1260 (12)	0.2707 (5)	−0.2882 (6)	0.0703 (33)
C-28	−0.3444 (14)	0.0332 (5)	−0.0252 (7)	0.0774 (37)
C-29	−0.4579 (10)	0.1483 (3)	−0.3998 (7)	0.0516 (24)
C-30	−0.7174 (14)	0.0146 (6)	−0.7479 (8)	0.0887 (43)
C-31	−0.6618 (17)	−0.0942 (5)	−0.6100 (10)	0.0978 (48)
O-2	0.0912 (6)	0.3598 (2)	0.1489 (3)	0.0409 (14)
O-3	−0.2115 (7)	−0.0172 (2)	−0.2761 (4)	0.0559 (19)
O-4	−0.3257 (10)	−0.0581 (4)	−0.7198 (6)	0.1028 (28)
O-5	−0.6389 (7)	0.0311 (3)	−0.5336 (4)	0.0587 (18)

[a] U_{eq} is one-third of the trace of the orthogonalized U_{ij} tensor.

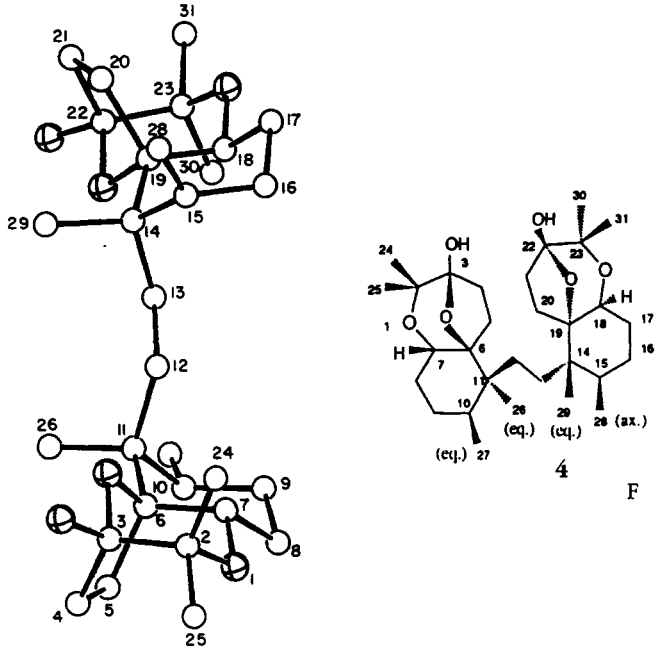


FIGURE 4. ORTEP representation of **4**.

sipholenol A is axial for both MTPA esters. In the case of **8**, the situation is even more complex as the *S*-ester is equatorial (J=6.5 Hz) and the *R* ester is axial (J=11.3 Hz), thus excluding the simple use of the empirical rule for the absolute configuration determination (6,7).

As the amounts obtained of the equatorial hydroxyl epimer were negligible, it was not possible to prepare the epimeric MTPA esters. Tentatively, it is suggested that the absolute stereochemistry of the sodwanones is the same as that of the sipholanes and raspacionins—the configuration given in the drawings of the compounds.

Sodwanones G [**5**], H [**6**], and I [**7**] have been found to have cytotoxic activity. The activity against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma are shown in Table 5. From this table it can be seen that **5–7** were active at 20 μM or less against the four test systems employed. The activity varied among the three compounds, with **6** being the least active. The potency of these sodwanones as cytotoxic agents varied among the different cell lines employed. Compounds **5** and **6** showed high specificity against the human lung carcinoma cell line A-549; the IC_{50} value obtained against this cell line was ten times less for **5** and 500 times less for **6** than against the other cell lines tested.

TABLE 5. Cytotoxicity of Compounds **5–7** (IC_{50} μM).

Cell Line	Compound		
	Sodwanone G [**5**]	Sodwanone H [**6**]	Sodwanone I [**7**]
P-388	2.0	10.5	20
A-549	0.2	0.02	20
HT-29	2.0	10.5	20
MEL-28	2.0	10.5	20

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet 205 Ft-ir spectrophotometer. Hrms were obtained on a VG Fisons autospec Q instrument. ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ_H=0). Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using a 1-cm microcell.

ANIMAL MATERIAL.—*Axinella weltneri* (Von Lendenfeld) (class Demospongiae, order Halichondria, family Axinellidae) (No. TASA-262) was collected in Sodwana Bay, South Africa by scuba diving during the winter of 1994. A voucher sample is deposited in the Department of Zoology at Tel Aviv University.

EXTRACTION AND ISOLATION.—The freshly collected sponge was immediately frozen at −25°. The freeze-dried sponge (50 g) was then extracted with EtOAc to yield a brown gum (2.0 g). The gum was chromatographed first on a Sephadex LH-20 column, eluted with MeOH-$CHCl_3$-hexane (1:1:2), and then several times on Si gel columns eluted with hexane/EtOAc mixtures to afford, in addition to sodwanones A–F, sodwanone G [**5**] [5 mg, 0.01%, R_f (EtOAc-hexane, 1:1) 0.62]. From another *Axinella* specimen, TASA 328 (10 g dry wt), sodwanones F [**4**], H [**6**], and I [**7**] were isolated: **4** (4 mg, 0.04%), **6** (3 mg, 0.03%), **7** (3 mg, 0.03%); R_f values (EtOAc-hexane, 1:1) 0.60, 0.57, 0.58, respectively.

Sodwanone G [**5**].—Mp 245° (MeOH); $[\alpha]_D$ −14.5° (*c*=0.9, $CHCl_3$); ir ν max (neat) 3510, 3400, 2970, 1715, 1613 cm^{-1}; ^{13}C-nmr data, see Table 1; ^{1}H nmr ($CDCl_3$) δ 3.21 (1H, ddd, *J*=16.4, 11.2, and 3.7 Hz, H-4a), 3.10 (1H, dd, *J*=10.7 and 5.1 Hz, H-7), 2.75 (1H, dd, *J*=4.2 and 2.1 Hz, H-27a), 2.74 (1H, d, *J*=9.1 Hz, H-21a), 2.62 (1H, dd, *J*=10.1 and 9.1 Hz, H-21b), 2.58 (1H, d, *J*=4.2 Hz, H-27b), 2.50 (1H, dt, *J*=5.1 and 13.0 Hz, H-13a), 2.36 (1H, dd, *J*=13.5 and 9.1 Hz, H-20a); COSY, in addition to the correlations observed for **1**, H-9a, H-9b/H-27a, H-27b; H-11/H-27a, H-27b; H-27a/H-27b; hreims *m/z* 498.2942 (M^+, $C_{30}H_{42}O_6$) calcd 498.2981).

Sodwanone H [**6**].—Amorphous powder; $[\alpha]_D$ −8° (*c*=0.1, $CHCl_3$), ir ν max (neat) 3400, 2950, 1700 cm^{-1}; ^{13}C-nmr data, see Table 1; ^{1}H nmr ($CDCl_3$) δ 3.25 (1H, ddd, *J*=13.0, 11.3, and 3.0 Hz, H-4a), 2.97 (1H, dd, *J*=11.8 and 4.3 Hz, H-7), 2.38 (1H, dd, *J*=16.8 and 6.5 Hz, H-17a), 2.36 (1H, dd, *J*=16.8 and 12.5 Hz, H-17b), 2.28 (1H, dt, *J*=12.5 and 6.2 Hz, H-13a), 2.20 (1H, dd, *J*=12.5 and 6.5 Hz, H-18), 2.15 (1H, ddd, *J*=13.0, 5.3, and 1.3 Hz, H-21a), 2.10 (1H, tt, *J*=14.2 and 3.0 Hz, H-4b); COSY, in addition to correlations observed for **1**, H-17a/H-17b, H-18; H-17b/H-18; H-22/H-21a, H-21b, H-20a, H-20b; hreims *m/z* 472.3537 (M^+, $C_{30}H_{48}O_4$) (calcd 472.3553).

Sodwanone I [**7**].—Oil; $[\alpha]_D$ +2° (*c*=0.2, $CHCl_3$); ^{13}C-nmr and ^{1}H-nmr data, see Table 2; hreims *m/z* 490.3672 (M^+, $C_{30}H_{50}O_5$) (calcd 490.3658).

REDUCTION OF SODWANONE A.—Preparation of sodwanol A [**8**]: **1** (20 mg) was dissolved in 10 ml of MeOH, $NaBH_4$ (10 mg) was added, and the mixture was stirred at room temperature for 30 min. HOAc (1:10; 0.1 ml) was added; the solvent was evaporated and the residue was dissolved in $CHCl_3$ (50 ml), washed with H_2O, dried, and evaporated to give after Si gel cc (EtOAc-petroleum ether, 8:2) 14 mg of **8** and only 0.5 mg of episodwanol A.

Compound **8**.—^{1}H nmr ($CDCl_3$, 500 MHz) δ 3.82 (1H, d, *J*=6.7 Hz, H-3), 3.65 (1H, m, H-7), 2.02 (3H, s, Me-28), 1.50 (3H, s, Me-30), 1.45 (3H, s, Me-31), 1.25 (3H, s, Me-24), 1.15 (3H, s, Me-27), 1.10 (3H, s, Me-25), 1.08 (3H, s, Me-29), 0.78 (3H, s, Me-26); ^{13}C nmr ($CDCl_3$) δ 77.5 (s, C-2), 76.5 (d, C-3), 26.3 (t, C-4), 34.9 (t, C-5), 42.7 (s, C-6), 75.5 (d, C-7), 28.8 (t, C-8), 41.8 (t, C-9), 74.0 (s, C-10), 57.8 (d, C-11), 24.8 (t, C-12), 34.3 (t, C-13), 165.5 (s, C-14), 129.0 (s, C-15), 182.5 (s, C-16), 141.0 (s, C-17), 137.2 (s, C-18), 42.9 (s, C-19), 27.6 (t, C-20), 32.8 (t, C-21), 216.0 (s, C-22), 48.0 (s, C-23), 21.4 (q, C-24), 28.9 (q, C-25), 12.6 (q, C-26), 23.4 (q, C-27), 12.1 (q, C-28), 21.5 (q, C-29), 20.8 (q, C-30), 24.3 (q, C-31).

PREPARATION OF THE (*R*) AND (*S*)-MTPA ESTERS OF **8**.—A solution of **8** (2.2 mg), dimethylaminopyridine (2.7 mg), and triethylamine (0.1 μl) in 0.3 ml of CH_2Cl_2 (distilled from P_2O_5) was treated with (−)-MTPA chloride (2.2 mg), and the mixture was allowed to stand at room temperature for 3 days. The solvent was evaporated and the residue was chromatographed on a Si gel column to afford the (*S*)-MTPA ester (1 mg), ^{1}H nmr δ 5.10 (1H, d, *J*=6.5 Hz, H-3); (*R*)-MTPA ester (1 mg), ^{1}H nmr δ 4.93 (1H, d, *J*=11.3 Hz, H-3).

BIOLOGICAL TESTING.—Cells were maintained, in logarithmic growth in EMEM/neaa, supplemented with 5% FCS, 2.0 mM L-glutamine, 10^{-2} M $NaHCO_3$, and 0.1 g/liter penicillin G+0.1 g/liter streptomycin sulfate. Cytotoxic activity was screened, using an adapted form of the method described by Bergeron *et al.* (8) against the following cell lines: P-388 (ATCC CCL 46), suspension culture of a lymphoid neoplasm from a DBA/2 mouse; A-549 (ATCC CCL 185), monolayer culture of a human lung carcinoma; HT-29 (ATCC HTB-38), monolayer culture of a human colon carcinoma; and MEL-28 (ATCC HTB-72), monolayer

culture of a human melanoma. P-388 cells were seeded into 16-mm wells at 1×10^4 cells/well in 1-ml aliquots of EMEM 5% FCS containing different concentrations of the corresponding sodwanones. A separate set of cultures without drug was seeded as growth control, to ensure that cells remained in the exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°, 10% CO_2 in a 98% humid atmosphere, an approximate IC_{50} value (drug concentration causing a 50% reduction in cell survival) was determined by comparison of the growth in wells with drugs to growth in control wells. A-549, HT-29, and MEL-28 cells were seeded into 16-mm wells at 2×10^4 cells/well in 1-ml aliquots of EMEM 5% FCS containing different concentrations of the corresponding sodwanones. A separate set of cultures without drug was seeded as a growth control to ensure that cells remained in the exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°, 10% CO_2 in a 98% humid atmosphere, cells were fixed with 0.4% formalin and stained with 0.1% crystal violet. An approximate IC_{50} value was determined by comparison of the growth in wells with drug to growth in control wells.

CRYSTAL STRUCTURE ANALYSIS OF SODWANONE E[1] [**3**].—The X-ray diffraction measurements were carried out at room temperature (ca 298° K) on an automated CAD4 diffractometer equipped with a graphite monochromator, using MoKα (λ=0.7107 Å) radiation. Intensity data were collected out to $2\theta=50°$ by the ω-2θ scan mode with a constant scan speed of 4° deg/min. Possible deterioration of the analyzed crystal was tested by detecting periodically the intensities of three standard reflections from different zones of the reciprocal space, and was found negligible during the experiment. A total of 2362 unique reflections with positive intensities was recorded. No corrections for absorption or secondary extinction were applied.

Crystal Data: $C_{30}H_{50}O_5$ formula wt 490.72, monoclinic, space group $P2_1$, $a=7.334(4)$, $b=18.456(3)$, $c=10.622(2)$ Å, $\beta=92.40(2)°$, $V=1436.5$ Å^3, $Z=2$, $D_{calcd}=1.135$ g/cm^{-3}, F(000)=540, μ(MoKα)=0.70 cm^{-1}.

The structure was solved by direct methods (SHELXS-86) (9), and refined by full-matrix least squares (SHELXL-93) (10), including the positional and anisotropic thermal parameters of the non-hydrogen atoms. The final refinement, based on F^2, converged at $R=0.064$ for 1670 observations having $I>2\sigma(I)$, and $R=0.090$ for all 2362 data. The hydrogen atoms attached to carbon were introduced in calculated positions, the methyls being treated as rigid groups. The H-atom attached to O was located in a Fourier map. At convergence, the peaks and troughs in the final difference density map did not exceed 0.17 and −0.21 e.A^{-3}, respectively.

Molecules related by the two-fold screw axis form intermolecular hydrogen bonds at OH(3)...O(2) 2.767(5) Å.

CRYSTAL STRUCTURE ANALYSIS OF SODWANONE F [**4**].—Data collection extended out to $2\theta=42°$. Crystal data: $C_{30}H_{50}O_6$, mol wt 506.72, orthorhombic space group $P2_12_12_1$, $a=10.861$ (8), $b=12.825$ (9), $c=20.557$ (9) Å, $V=2863.4$Å^3, $Z=4$, $D_{calcd}=1.175$ g/cm^{-3}, F(000)=1112, $\mu(M_0K_\alpha)=0.746$ cm^{-1}; $R=0.097$ for 700 observations above the threshold of 4σ (F).

CRYSTAL STRUCTURE ANALYSIS OF SODWANONE G [**5**].—A total of 1878 unique reflections with positive intensities was recorded ($2\theta=46°$). No corrections for absorption or secondary extinction effects were applied.

Crystal data: $C_{30}H_{42}O_6$ formula wt 498.66, monoclinic, space group $P2_1$, $a=8.317(1)$, $b=10.314(4)$, $c=16.519(2)$ Å, $\beta=104.46(1)°$, $V=1372.1$ Å^3, $Z=2$, $D_{calcd}=1.207$ g/cm^{-3}, F(000)=540, μ(MoKα)=0.77 cm^{-2}. The final refinement, based on F^2, converged at $R=0.057$ for 1386 observations having $I>2\sigma(I)$, and $R=0.081$ for all 1878 data. The hydrogen atoms attached to carbon were introduced in calculated positions, (the two hydrogens of C-27 were first identified, however, in electron difference density maps) with the methyls being treated as rigid groups. The hydrogen attached to the oxygen on C-17 was located by difference-Fourier. Exchange between C-27 and O-3 of the epoxide in the refinement calculations led to R=0.067, thus confirming that the structural model was correct. At convergence, the peaks and troughs of the final difference density map did not exceed 0.23 and −0.19 e.Å^{-3}, respectively.

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[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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