Sodwanones K, L, and M; New Triterpenes from the Marine Sponge *Axinella weltneri*

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Three new triterpenes, sodwanones M, K, and L (**4**, **5**, **6**), together with three known sodwanones (A, B, and D) have been isolated from the sponge *Axinella weltneri* from the Comoros Islands, in the Indian Ocean. The three new structures were determined by interpretation of NMR spectra and, in the case of sodwanone K, also by oxidation to the known sodwanone D. Sodwanone M, the major isolated compound, was found to be cytotoxic to P-388 murine leukemia cells at a concentration of 1 μg/mL.

In our continuing search for bioactive metabolites from marine invertebrates,[1−3] we have examined several organisms collected around the Comoros Islands, northwest of Madagascar, in the Indian Ocean. We investigated, among others, the sponge *Axinella weltneri* whose lipophilic extract was cytotoxic against P-388 cells. South African *Axinella weltneri*, from the Indian Ocean in the vicinity of Durban, was previously tested by us and found to be rich in sodwanone-triterpenes.[2,3] The nine sodwanones (A−I) isolated so far possess three different carbon skeletons and are among the few known marine triterpenes. Similar polyepoxysqualene-derived triterpenes were isolated from the Red Sea sponge *Siphonochalina siphonella*[4] and from the Mediterranean sponge *Raspaciona aculeata*.[5,6] This paper presents the structure determination of three new sodwanones (K, L, and M) isolated from *A. weltneri* from the Comoros islands.

A specimen of *A. weltneri* was collected at a depth of 10−15 m at Prevoyante Reef in the lagoon of Mayotte, Comoros Islands, northwest of Madagascar. The $CHCl_3$−MeOH (2:1) extract of the sponge was found to contain six triterpenes, the known sodwanones A, B, and D (**1**) (0.01%, 0.08%, and 0.005%, respectively) and three new compounds K (**5**, 0.005%), L (**6**, 0.005%), and M (**4**, 0.05) (dry wt).

The major triterpene, sodwanone M (**4**), showed a molecular ion at *m/z* 491 in the FABMS, which indicated a formula of $C_{30}H_{50}O_5$ with six degrees of unsaturation. The only unsaturated moiety of **4**, according to the ^{13}C-NMR spectrum (δ_c 218.6 ppm), was a ketone; therefore, sodwanone M had to be pentacyclic.

Comparison of the carbon resonances of **4** (Table 2) with those of the earlier reported sodwanones[2,3] suggested that sodwanone M had the same perhydrobenzoxepine right half as sodwanone E (**2**). This was unequivocally confirmed by COSY, HMBC (Table 2), and NOE experiments. For instance, relatively strong NOEs were measured between Me-29 and the axial H-15 and H-21 protons (δ_H 1.78 and 2.11; 3.5 and 4.0%, respectively) and between Me-30 and the axial H-18 (δ_H 3.45 dd, J = 10.2, 7.1 Hz) (4.4%). The second half of **4**, although showing similarities to the left part of E (**2**), was clearly different. Long-range CH-correlations, observed in an HMBC experiment, showed, among other things (Table 2), correlations between Me-26 and carbons 6, 10, 11, and 12 (5, 6, 7, and 11 in the case of **2**), thus suggesting the migration of the methyl group (26) from C-6 (in **2**) to C11 (in **4**). A similar 1,2-shift was previously observed for sodwanone F (**3**)[2] and raspacionin.[5] Further support for the new location of Me-26 came from an upfield shift of C-5 by 16 ppm in **4** as compared to **2**, due to one less downfield β-effect and an additional upfield γ-effect.

The relative configuration assignment of the five chiral centers of the left half of **4** began with C-10. Irradiation of Me-27 in a 1D TOCSY experiment showed that H-10 is equatorial (a narrow signal at 1.54, $\Delta w_{1/2}$ = 20 Hz, without larger axial−axial couplings). Additionally, an NOE cross peak between Me-27 and H-6 determined the axial configuration of the latter proton, whose multiplicity could not be seen due to overlap with several other proton signals (Table 1).

A weak NOE between the axial Me-27 (α-oriented) and Me-26 indicated that the latter methyl group was in the α orientation, too. The stereochemistry of the acetal was also established by NOEs; for this purpose the distinction between the two C-5 protons was essential. The spatial proximity of one of the acetal oxygens to what has to be the axial H-5 caused the latter's downfield shift to δ_H 2.00, distinguishing it from its equatorial geminal counterpart. An NOE between one of the geminal C-2 methyls (Me-24) and the latter axial H-5 (3.5%) differentiated between this methyl group and Me-25, and determined the β-orientation of the O1−C-2(Me 24, 25) bridge. Me-25 exhibited a stronger (12.5%) NOE with the equatorial H-3 than Me-24 (0.6%), in accordance with their closer proximity as measured on a Dreiding model. An NOE between the α-equatorial H-5 and Me-26 (2.0%) further supported the α-orientation of the latter methyl group. Lastly, the chemical shift difference between the two C-12 geminal-protons, and their relatively lowfield (δ_H 1.95 and 1.35 ppm) position in comparison to 1.22 (2H) in **2**, where C-12 is equatorial, determined the axial position of CH_2(12) in **4** in agreement with the above-discussed α-equatorial orientation of Me-26.

Sodwanone K (**5**) with the formula $C_{30}H_{50}O_5$ (M^+ 490.3648, Δ mmu 3) also has six degrees of unsaturation, as does **4**. Of the six, two were assigned to a

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Table 1. ^{1}H-NMR Data (500 MHz) of Sodwanones M(**4**), K(**5**), and L(**6**)[a,b]

	compound					
position	**4**		**5**		**6**	
3	3.92 d (4.6)		3.82 d (6.8)			
4	1.83 m	1.55m	2.00 m	1.80 m	1.85 m	1.75 m
5	2.00 m	1.48 m	1.70 m	1.50 m	2.30 m	1.30 m
6	1.46 m					
7			3.65 dd (11.8, 4.9)		4.00 dd (11.2,5.8)	
8	2.08 m	2.01 m	1.65 m	1.40 m		
9	1.95 m	1.35 m	2.25 m	1.95 m	1.40 m	1.30 m
10	1.54 m				1.30 m	
11			1.56 m			
12	1.95 m	1.52 m	1.55 m	1.45 m	2.05 m	1.25 m
13	2.08 m	2.01 m	1.90 m	1.15 m	1.70 m	1.35 m
15	1.78 m		1.82 m		1.45 m	
16	1.45 m	1.45 m	1.35 m	1.35 m	1.42 m	1.32 m
17	1.55 m	1.55 m	1.52 m	1.52 m	1.85 m	1.42 m
18	3.45 dd (10.2,7.1)		3.45 dd (10.4, 5.2)		4.14 dd (12.1,5.0)	
20	2.05 m	1.55 m	2.00 m	1.60 m	2.20 m	2.08 m
21	3.12 m	2.11 m	3.25 m	2.15 m	2.15 m	1.75 m
24	1.38 s		1.26 s		1.32 s	
25	1.26 s		1.15 s		1.16 s	
26	1.15 s		0.82 s		0.86 s	
27	0.94 d (6.5)		4.90 s	4.55 s	0.78 d (6.5)	
28	0.89 d (6.5)		0.90 d (6.5)		0.75 d (6.5)	
29	1.11 s		1.15 s		0.77 s	
30	1.31 s		1.31 s		1.21 s	
31	1.25 s		1.25 s		1.23 s	

[a] $CDCl_3$, Bruker ARX 500 instrument, chemical shifts refer to TMS ($\delta_H = 0$). [b] Assignments aided by HMQC, HMBC, and COSY experiments.

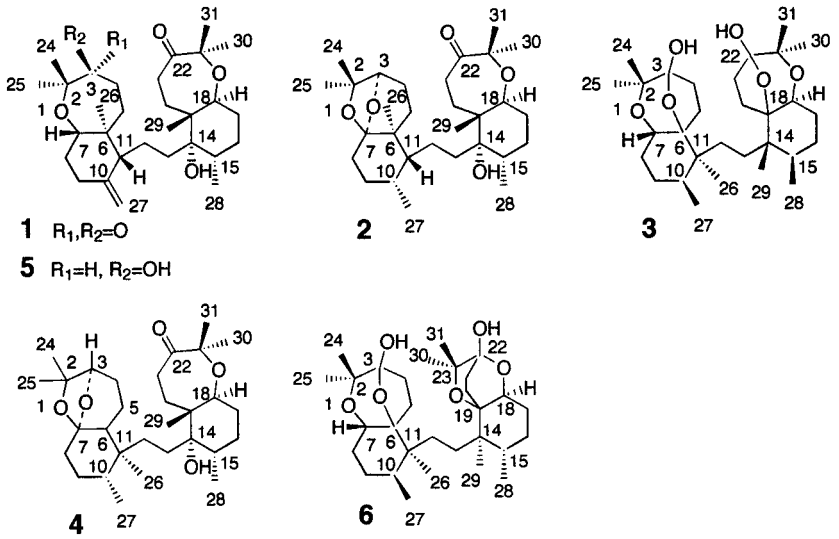
Table 2. ^{13}C-NMR Data (125 MHz) Including CH-Correlations of Sodwanones M(**4**), E(**2**), K(**5**), D(**1**), L(**6**), and F(**3**)[a,b]

	compounds							
position	**4**	**2**	**4** HMBC (C to H)	**5**	**1**	**6**	**3**	**6** HMBC (C to H)
2	78.6 s	78.9 s	Me-24, -25	81.0 s	81.8 s	77.0 s	76.7 s	Me-24, -25
3	81.2 d	80.6 d	Me-24, -25	77.3 d	218.0 s	105.5 s	105.7 s	4b, 5b, Me-24, -25
4	23.4 t	29.9 t		26.0 t	35.8 t	24.2 t	24.2 t	
5	16.6 t	32.1 t		33.8 t	39.3 t	32.3 t	32.3 t	
6	41.2 d	41.3 s		43.6 s	42.5 s	89.9 s	89.3 s	5a, 7, Me-26
7	108.7 s	110.1 s	H-3	75.9 d	80.3 d	71.3 d	70.6 d	
8	28.1 t	22.7 t		32.7 t	32.5 t	27.8 t	26.7 t	Me-27
9	26.2 t	28.0 t	Me-27	35.7 t	35.3 t	28.8 t	28.5 t	
10	34.4 d	29.7 d	Me-26, -27	145.0 s	145.3 s	39.5 d	39.2 d	Me-26, -27
11	40.1 s	48.1 d	Me-26, -27	54.7 d	54.2 d	41.0 s	40.9 s	Me-26, -27
12	31.3 t	20.5 t	Me-26	20.0 t	20.1 t	25.3 t	29.7 t	Me-26
13	32.8 t	35.1 t		36.2 t	36.0 t	33.1 t	29.9 t	Me-29
14	77.4 s	77.6 s	Me-28, -29	78.0 s	78.0 s	42.0 s	42.0 s	Me-28, -29
15	36.0 d	33.3 d	Me-28	33.8 d	33.6 d	38.2 d		Me-29
16	28.5 t	28.7 t	Me-28	28.3 t	28.2 t	28.4 t		
17	30.2 t	30.5 t		30.2 t	30.0 t	31.1 t		
18	76.3 d	76.1 t	Me-29	76.3 d	76.1 d	78.9 d		
19	46.1 s	45.5 s	Me-29	45.5 s	45.3 s	75.8 s		18, 21a, Me-29
20	32.5 t	32.9 t		32.9 t	32.7 t	22.8 t		
21	35.2 t	35.4 t		35.3 t	35.7 t	28.2 t		
22	218.6 s	215.8 s	20a, 20b, 21a, 21b, Me-30, -31	218.0 s	217.6 s	95.4 s		20b, 21a, Me-30, -31
23	81.9 s	81.6 s	Me-30, -31	82.2 s	82.2 s	78.4 s		21b, Me-24, -25
24	21.2 q	20.7 q	Me-25	21.3 q	20.3 q	18.9 q		Me-25
25	29.2 q	28.2 q	Me-24	28.3 q	26.9 q	24.4 q		Me-24
26	21.4 q	16.8 q		12.2 q	11.2 q	18.7 q		
27	14.4 q	14.7 q		107.4 t	108.3 t	15.5 q		
28	16.2 q	15.5 q		15.8 q	15.3 q	16.4 q		
29	13.9 q	14.4 q		14.3 q	14.0 q	11.9 q		
30	20.4 q	20.4 q	Me-31	20.4 q	20.4 q	23.5 q		Me-31
31	26.9 q	26.7 q	Me-30	28.3 q	26.6 q	26.2 q		Me-30

[a] $CDCl_3$, Bruker ARX 500 instrument, chemical shifts refer to $CDCl_3$ ($\delta_C = 77.0$). [b] Assignments aided by HMQC, HMBC, and COSY experiments.

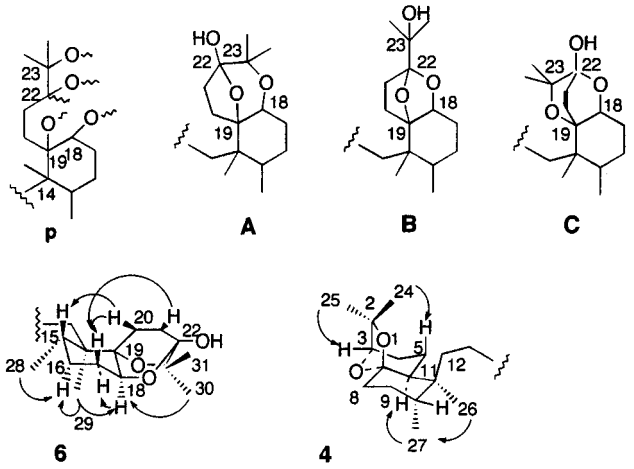
terminal double bond (δ_c 107.4 t and 145.0 s) and a ketone (δ_c 218.0 s), hence **5** has to be tetracyclic. A comparison of the NMR data of **5** (Tables 1, 2) with the data of the earlier reported sodwanones unequivocally established the right half of **5** as identical to the right part of sodwanone D (**1**).[2] The left half of **5** was found to carry a secondary alcohol [δ_H 3.82 d (J = 6.8 Hz), δ_c 77.3 d] in addition to the terminal double bond and an ethereal bridge (Tables 1, 2). 2D NMR experiments and especially CH-correlations from Me-24 and -25 to the 2° alcohol indicated it was on C-3 and axial [δ_H 3.82 d (J = 6.8)] as in sipholenol A.[4] Oxidation of **5** gave sodwanone D (**1**) readily confirming the structure of **5**.

The third new compound, sodwanone L (**6**), $C_{30}H_{50}O_6$, M^+ 506.3591, Δmmu 2) had six degrees of unsaturation and was isolated in minute amounts only (0.005%, dry



wt). In the absence of unsaturations compound **6** had to be hexacyclic. Comparison of the NMR data of **6** (Tables 1, 2) with those of the earlier-reported sodwanones[2] showed the left half of **6** to be identical to the left part of sodwanone F (**3**). 1D and 2D NMR measurements suggested partial structure **p** for the right half of **6**. Outstanding in the ^{13}C resonances was the 95 ppm singlet (all other double oxygenated carbons in the other sodwanones resonate around 105−110 ppm). Moiety **p** must be tricyclic and possess one OH group.

Three possible structures, **A**−**C**, possessing ethereal bridges, 18, 23 and 19, 22; 18, 22 and 19, 22; and 18, 22 and 19, 23, respectively, are



Structure **A** is identical to the right half of sodwanone F and was disqualified because of dissimilar δ_c-values and especially the 95 ppm value of C-22, see above. An NOE cross peak between Me-30 and the axial H-18 (δ_H 4.14 dd, J = 12.1, 5.0 Hz) (4.5%) discounted the dioxabicyclo[2,2,1]octane structure (**B**) as the latter groups are too distant. This left structure **C**, the dioxabicyclo[2,2,2]octane, in which Me-30 and H-18 have the appropriate stereochemistry for a mutual NOE. Proton 18, as mentioned above, is axial, and Me-30 pseudoaxial. H-18 also showed NOEs with the equatorial H-17α (δ 1.42) and with Me-29 (seen from a 2D NOESY experiment). Hence, Me-29 has to be axial and α-oriented in a 1,3-diaxial arrangement with H-18. Me-29 also showed an NOE with the axial 16α-proton (5.0%) in the second 1,3-diaxial position. The relative configuration of C-14 and -15 was also established by NOE measurements. An NOE between the axial 16α-proton and Me-28 determined the α-configuration of C-28, which was further confirmed by an NOE between the axial H-15β and the bridge H-20 proton. The relative highfield resonance of C-22 is explained by the γ-effects of its surrounding atoms[7]. Sodwanone L represents a new class of sodwanones possessing the unprecedented dioxabicyclo [2,2,2]octane system.

The suggested stereochemistry is relative, and either half could be its enantiomer. The relationship between the two halves, which are too far away for NOEs, is based on X-ray structures of several sodwanones.[3] Efforts to determine the absolute configuration are underway.[3]

Several of the sodwanones have been found to be cytotoxic. Among them sodwanones G and H were most interesting because of selectivity against different tumor cells.[3] Sodwanone M was also found to be cytotoxic to P-388 murine leukemia cell at a concentration of 1 μg/mL, but, in contrast to sodwanones G and H, was not more active against the A-549, MT-29, and MEL-28 human tumor cells.[3]

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. LRMS and HRMS were recorded on a Fisons, Autospec Q instrument. ^1H- and ^{13}C-NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) and $CDCl_3$ ($\delta_C = 77.0$). Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell. For details on the biological tests see Rudi *et al.*[3]

Biological Material. *A. weltneri* (Von Lendenfeld) (class Demospongiae, order Halichondrida, family Axinellidae) (no AM 43) was collected at Preroyante Reef, Lagoon of Mayotte, Comoros Islands, northwest of Madagascar by scuba at a depth of 10−15 m during April 1996. A voucher sample is deposited at the Marine Station at d'Endoume, Marseille, France. The freeze-dried sponge (120 g) was homogenized and extracted with MeOH−$CHCl_3$ 1:2 (200 mL × 4) to give a brown gum (11 g) after evaporation.

This gum was chromatographed first on a Sephadex LH-20 column, eluting with MeOH−$CHCl_3$−hexane (1:

1:2) and then several times on Si gel columns eluting with hexane−EtOAc mixtures to afford sodwanone A (0.01%), sodwanone B (0.08%), sodwanone D (**1**, 0.005%), sodwanone K (**5** 0.005), sodwanone L (**6**, 0.005%), and sodwanone M (**4**, 0.05%).

R_f values (EtOAc−hexane, 1:3) for the new compounds are 0.06 **4**, 0.1 **5**, 0.02 **6**.

Sodwanone M (4): oil; $[\alpha]_D$ +18 (*c* 0.1, $CHCl_3$); IR ν_{max} (neat) 3400, 2950, 1700 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; COSY in addition to the usual, H-3/-4a, -4b, H-4a/4b, 5a, 5b; H-5a/5b, 6; FABMS, 491 (MH, 20), 473 (M − OH, 40), 455 (M − OH − H_2O, 60), 223 (100); HREIMS *m/z* 490.3641 (calcd for $C_{30}H_{50}O_5$ 490.3645).

Sodwanone K(5): oil; $[\alpha]_D$ +6.5 (*c* 0.15, $CHCl_3$); IR ν_{max} (neat) 3410, 2950, 1700 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; COSY in addition to the usual, H-3/4a, 4b; FABMS, 491 (MH^+, 10); HREIMS *m/z* 490.3648 (calcd for $C_{30}H_{50}O_5$ 490.3645).

Sodwanone L(6): oil; $[\alpha]_D$ +19.1 (*c* 0.15, $CHCl_3$); IR ν_{max} (neat) 3510, 3400, 2970, 680 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; CIMS (CH_4) 507 (MH^+, 10), 489 (M − OH, 100), 471 (50), 453 (10); HREIMS *m/z* 506. 3591 (calcd for $C_{30}H_{50}O_6$ 506.3594).

Oxidation of Sodwanone K to D. Sodwanone K (2 mg) was dissolved in Me_2CO (3 mL), and one drop of Jones's reagent was added, at 10 °C. After 30 min the excess of the oxidant was destroyed by a drop of MeOH, the solvent removed under vacuum, and the residue filtered through silica to afford sodwanone D.[2]

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