Pergamon

Tetrahedron 55 (1999) 5555–5566

TETRAHEDRON

Several New Isoprenoids from Two Marine Sponges of the Family Axinellidae

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Received 2 October 1998; revised 23 February 1999; accepted 4 March 1999

Abstract:

Ten new isoprenoids have been isolated from two marine sponges *Ptilocaulis spiculifer* and *Axinella weltneri*. *P. spiculifer* afforded three triterpenes, dihydroyardenone (**1**), abudinol B (**2**), muzitone (**3**) and two C_{13} and C_{17} metabolites of abudinol, nakorone (**4**) and durgamone (**5**). *A. weltneri* gave five new sodwanones, N-R (**6** – **10**). The structures of all new compounds were elucidated by spectroscopic analyses, mainly NMR data, and by chemical transformations to known compounds and degradations. The absolute configuration of several compounds was determined by optical measurements and comparison of the CD-curves with those of several raspacionins. Several sodwanones were found to be cytotoxic.

Keywords: *Ptilocaulis; Axinella; Marine metabolites; stereochemistry*

Our interest in bioactive marine natural products brought us to the investigation of two sponges from the Axinellidae family, the extracts of which showed cytotoxicity – a property that was traced to several triterpenes. Although marine organisms are rich in isoprenoids, not many triterpenes have been isolated [1]. All known triterpenes, isolated from sponges and algae, are squalene-derived polyethers which, in almost all cases, consist of two separate ring systems. The first reported sponge triterpenes were the sipholanes isolated from the Red Sea sponge *Siphonochalina siphonella* (order Haploscleridae family Halichonidae) [2]. More recent ones were isolated from three other sponges, *Axinella weltneri* [3-5], *Raspaciona aculeata* [6], and *Ptilocaulis spiculifer* [7], all of the Axinellidae family, collected from the Indo Pacific, Mediterranean and Red Seas. From *A. weltneri* we have, thus far, isolated twelve compounds, namely sodwanones A-I and K-M. Representations showing the various carbon skeleta are shown in Figure 1 [8]. From the Red Sea *P. spiculifer*, we have isolated several triterpenes, i.e. sodwanones A-D, yardenone and abudinol (see Figure 1) [7].

Common to the above triterpenes are the condensed oxepane-cycloalkane systems. Within each class, additional oxidations result in alcohol and ketone functionalities which then form ethereal, ketal and lactol rings. In addition to the variations resulting from the oxidation state and number and size of the various carbocyclic and ethereal rings, the groups also differ as to whether they keep the squalene skeleton or possess skeleta in which one or two of the central squalene methyl groups migrated by a 1,2-shift. Changes also are observed for the stereochemistry of C-10 and 11 as seen e.g. in the cases of sodwanones A-C (and E) and sodwanone L (and F), (the structures of A, E and F were secured by X-ray crystallography). Outstanding is abudinol (Figure 1) in which one of the center methyl groups changed into a methylene becoming part of a cyclohexane ring.

A few of the sodwanones were found to be cytotoxic against several carcinoma cells. Sodwanone H was best with an IC_{50} value of 0.02 μM against A-549 human lung carcinoma [4].

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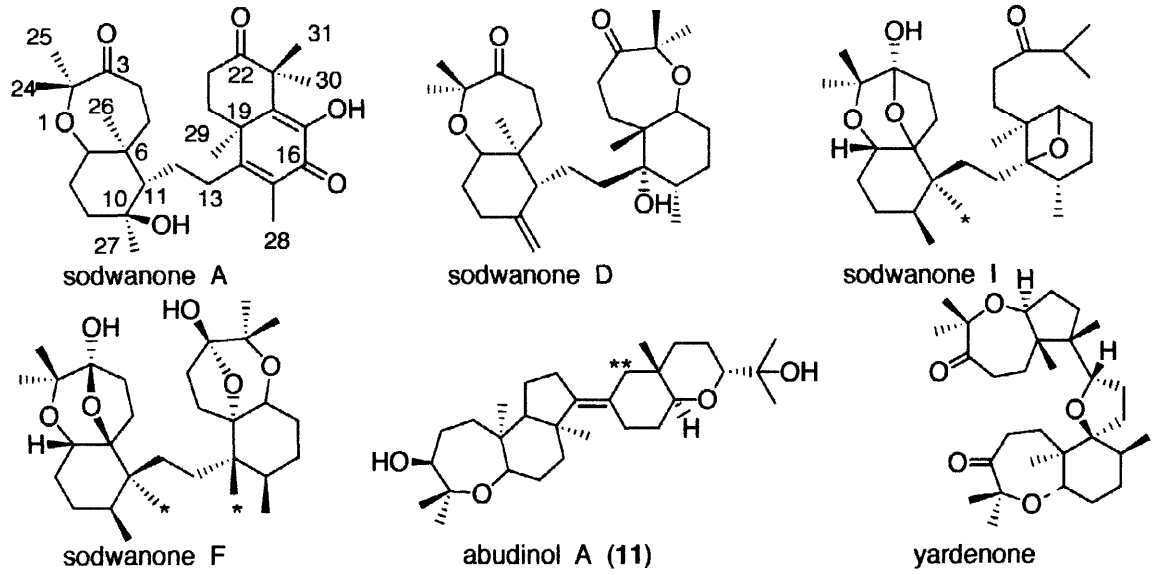
PII: S0040-4020(99)00221-5



* 1,2 Shifted Me Group, ** Methylene originating from a Me group.

Figure 1. Examples for Axinellidae metaboilites of different skeleta

The present report describes the isolation and structure elucidation of ten new isoprenoids from new collections of *P. spiculifer* and *A. weltneri* and discusses the absolute configuration of several of the sodwanones, abudinol and muzitone by comparison of their Cotton-effect with the CD-curves of other triterpenes.

From *Ptilocaulis spiculifer*, collected in the Dahlak archipelago, Eritrea, the Red Sea in 1996, we reported the isolation of six triterpenoids – four sodwanones (A-D), yardenone and abudinol (Figure 1), belonging to four groups of triterpenes with different carbon-skeleta [7]. The compounds isolated from a second collection of the sponge (February, 1998) are reported herewith. Remarkable changes in the composition of sodwanones have already been reported for *A. weltneri* [3-5]. The second present specimen of *P. spiculifer* was found to contain six sodwanones, sodwanones A-D (as in the first collection) and also sodwanones F and I, and an additional five new compounds which were isolated in minute amounts (Figure 2). Two of the latter compounds (**2** and **3**) possessing only seven, rather than the expected usual eight, methyl groups were suspected to be related to abudinol. Two other compounds, **4** and **5** were C_{17} and C_{13} isoprenoids, *vide infra*.

Compound **1**, the first of the new compounds, isolated from *P. spiculifer*, analyzed for $C_{30}H_{50}O_5$ from the HREIMS, *m/z* 490.3642 [M^+] and NMR data, with six degrees of unsaturation. As the only unsaturated functionality was a carbonyl (δ_C 218.0 s ppm) **1** had to be pentacyclic. A comparison of the NMR data of **1** with the data of the earlier reported triterpenes [2-7] suggested a close relationship to yardenone, obtained from the first collection of this sponge. Carbons 1 to 19, forming four rings, were identical to those in yardenone while the C-atoms of the fifth, a second oxepane ring, differed. The absence of a second carbonyl (C-22 of yardenone) and the appearance of a methinoxy group (δ_C 80.5 d, δ_H 3.51 d, J=7.2 Hz) suggested the replacement of the carbonyl by an OH-group. Indeed, Jones oxidation of **1** (aq.$Na_2Cr_2O_7$ / acetone) afforded yardenone (Figure 1), confirming the 22-dihydroyardenone structure of **1**. The stereochemistry of the 22-OH-group, being on the same side of the ring as H-18 was concluded from the chemical shift and multiplicity of H-22 (d, J = 7.2 Hz) which was very similar to H-3 of abudinol and similar protons in other sodwanones.

Compound **2** showed a molecular ion at *m/z* 474 in the EIMS, which indicates a formula of $C_{30}H_{50}O_4$ with six degrees of unsaturation. The only unsaturated moiety of **2**, according to the ^{13}C-NMR spectrum (δ_C 143.6 s and 125.6 s ppm), was a tetrasubstituted double bond; therefore **2** had to be pentacyclic. Comparison of the carbon resonances of **2** (Experimental) with those of the earlier reported triterpenes from this sponge suggested that **2** is a close isomer of abudinol and it was therefore named abudinol B (and the former abudinol A(**11**)).

Most characteristic for the structure elucidation of ring E of **2** was the $\Delta\delta_C$ value between the two methyls of the gem-dimethyl group. In abudinol A, the two methyls of the hydroxyisopropyl substituent (Me's 25, 26) resonate almost in the same chemical shift (δ 26.2 and 26.7) whereas the oxepane geminal pair (Me's 27, 28) resonate at 21.9 and 28.8 ppm. A similar $\Delta\delta_C$ value of 7 ppm was also found in yardenone and other

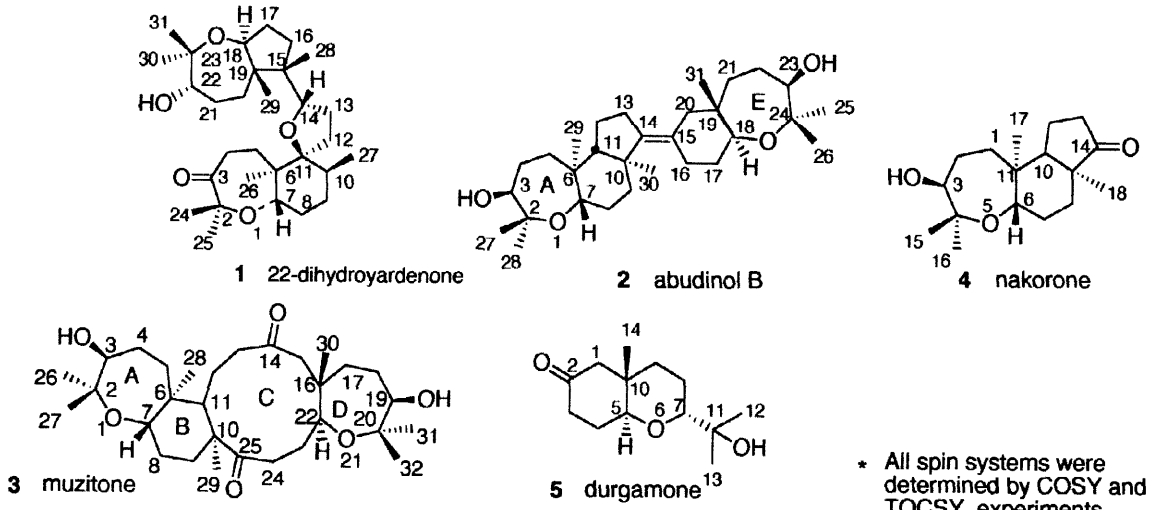


Figure 2. New metabolites of *P.spiculifer*

sodwanones possessing the 2,2-dimethyl oxepane moiety. Hence, from a difference of 8 ppm found for **2** (measured from the HMQC and HMBC experiments because of the small amount of available material), it was determined that ring E of abudinol B (**2**) is also an oxepane, rather than a THP as in abudinol A(**11**). The latter conclusion was confirmed by micro-acetylation of **2** (Ac_2O/pyridine, r.t., overnight) affording a diacetate (δ_H 2.15 s, 2.11 s) whereas abudinol A, possessing one secondary and one tertiary hydroxyl gave only a mono acetate (δ_H 2.15 s).

The third compound, designated muzitone (**3**), analyzed for $C_{30}H_{50}O_6$ from the HREIMS *m/z* 506.3586 and ^{13}C-NMR data (Table 1) with six degrees of unsaturation. Two losses of 18 a.m.u. in the mass spectrum (*m/z* 488 and 470) suggested two hydroxyl groups and the δ_C 207.0 s and 218.0 s ppm resonances in the ^{13}C-NMR suggested two ketones. Accordingly, and in the absence of double bonds, muzitone has to be tetracyclic. The 1D NMR data (Table 1), together with the COSY, HMQC and HMBC experiments (Figure 3) suggested two similar hydroxytrimethyl oxepane systems – a common heterocyclic system in the marine sipholanes, raspacionins and sodwanones [2-7].

The two- and three-bond CH-correlations, between the angular methyl groups and their adjacent C-atoms, were most helpful in the structure elucidation of the above mentioned triterpenes. This can also be seen in the

Table 1. NMR data of muzitone (**3**)[a]

#	δ_C	δ_H (*J* in Hz)[b]	HMBC (C to H)	#	δ_C	δ_H (*J* in Hz)	HMBC (C to H)
2	77.0 s		7, 26, 27	17	35.7 t	0.90 m, 1.76 m	15b, 19, 30
3	76.1 d	3.37 d (6.7)	26, 27	18	21.2 t	1.38 m, 1.45m	
4	25.5 t	1.38 m, 1.52 m	3	19	76.5 d	3.28 d (7.2)	31, 32
5	33.9 t	1.12 m, 1.58 m	3, 28	20	76.0 s		22, 31, 32
6	41.3 s		28	22	73.0 d	3.88 dd (11.3, 5.3)	15a,b, 30
7	76.9 d	3.56 dd (11.5, 5.0)	28	23	30.2 t	1.66 m, 1.74 m	
8	28.9 t	1.58 m, 1.65 m		24	38.9 t	2.10 m, 2.12 m	
9	30.6 t	1.65 m, 1.74 m	29	25	218.0 s		23a,b, 24a,b, 29
10	48.0 s		29	26	21.9 q	1.14 s	27
11	54.1 d	1.08 m	28, 29	27	28.8 q	1.02 s	26
12	18.1 t	1.33 m, 1.44 m		28	13.8 q	0.90 s	7
13	25.3 t	1.76 m, 1.95 m		29	15.7 q	0.80 s	
14	207.0 s		13a,b 15a,b	30	17.3 q	0.95 s	15a,b
15	53.2 t	1.95 m, 2.12 m	30	31	21.6 q	1.13 s	32
16	41.2 s		15b, 30	32	27.6 q	1.05 s	19, 31

[a]. Due to many overlapping proton resonances, especially of the methylene groups, the NMR experiments were performed in $CDCl_3$ and C_6D_6. The better overall results in C_6D_6 are given in Table 1. On the other hand, a better resolution of the six protons α to the two carbonyls was obtained in $CDCl_3$ in which a 1D-TOCSY experiment was performed for confirmation of the various spin-systems. [b] All $^1J_{CH}$ connectivities were established by an HMQC experiment.

HMBC experiment of **3** (Table 1 and Figure 3). The latter correlations, together with assignment of three spin systems (H-3–5, H-7–9 & H-17–19), established from COSY and TOCSY experiments, confirmed the suggested two oxepanes (rings A and D) and expanded ring A by an additional cyclohexane ring (B, C-6–11). Rings AB could further be extended by a CH_2CH_2CO moiety, according to the COSY experiment, attached to the identified H-11 (attached to the bridging C-atom between Me's 28 and 29). The low-field chemical shifts of H_2-13 (δ_H 2.40 and 2.02, in $CDCl_3$), placed them α to a carbonyl. Similarly, by the same argument, ring D could be extended by a CH_2CH_2CO unit attached to C-22. The above assignments left one methylene group to be determined (CH_2-15). HMBC correlations from the latter protons to C-14, 16, 17 and 30 established the linkage between rings B and D (C-11 to C-16). CH correlations from Me-29 to carbonyl-25 on one hand and the vicinity of the latter CO group to CH_2-24 (δ_H 2.30 & 2.20 in $CDCl_3$) *vide supra*, on the other hand, closed the fourth, a cycloundecanedione ring (C) completing the gross structure of muzitone (**3**). The 207.0 and 218.0 ppm chemical shifts of C-14 and C-25 respectively, are in full agreement with the adjacent non-substituted C-13 and C-15, in the α-positions to the former carbonyl and the tertiary C-10 adjacent to C-25.

Comparison of the NMR data of the oxepane rings (A and D) of muzitone with the corresponding ones in abudinol A, sipholenol A (Figure 6) and 4-episipholenol A, for which the structure with its stereochemistry was secured by an X-ray diffraction analysis, suggested the same stereochemistry for the three chiral centers in each of these rings. The latter rings (A and D) of **3** possess, on the basis of NOE's between one of the methyls, of each geminal pair, Me-27 and -31, and H-7 and H-22 respectively, a chair conformation as in sodwanone A (Figures 3 & 7, *vide infra*). The stereochemistry, of **3**, at C-10 and -11 is suggested to be the same as in abudinol B on the basis of the suggested biogenesis, *vide infra* (Scheme 1), and the NOE's shown in Figure 3.

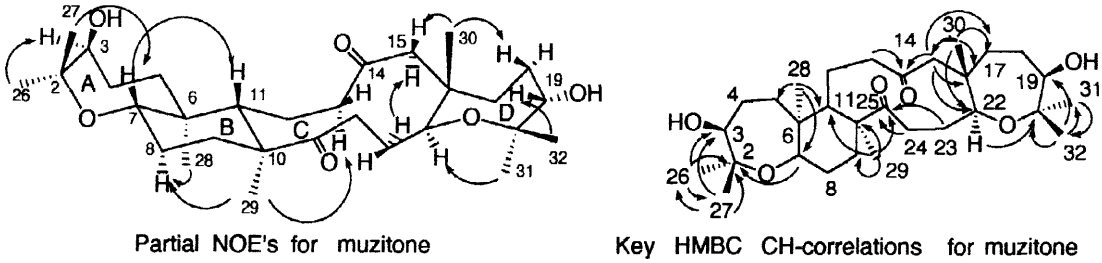


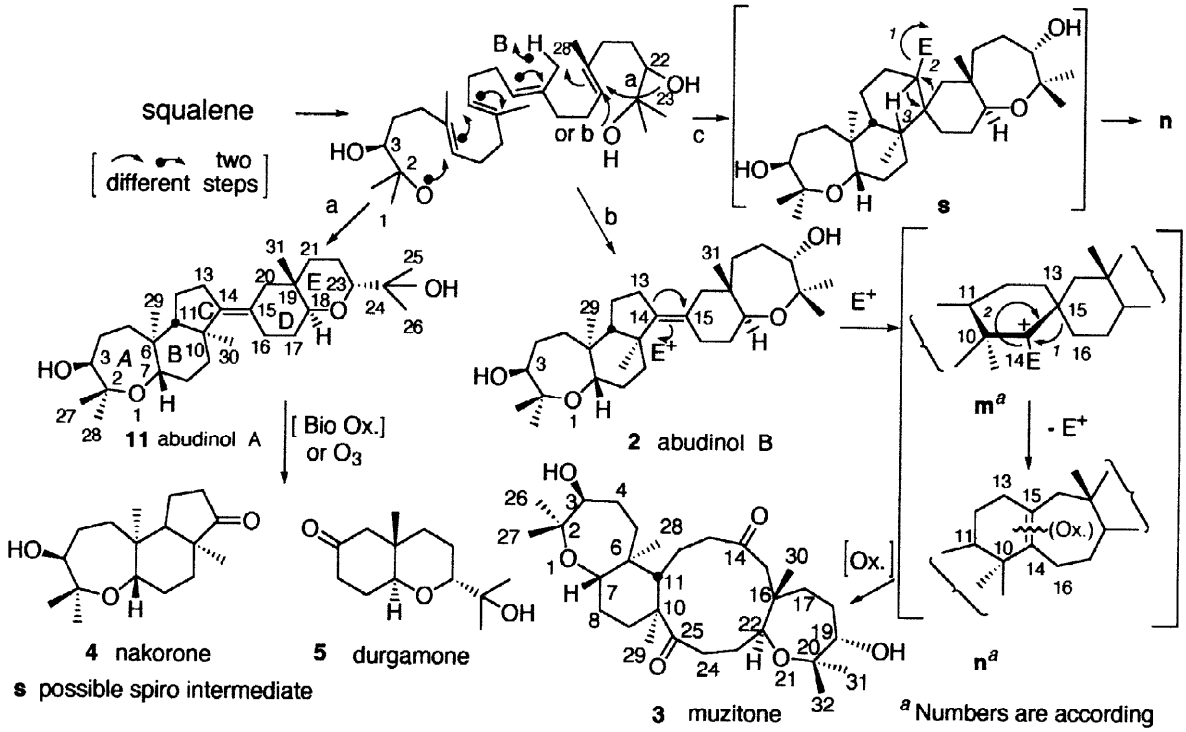
Figure 3 NOE's and HMBC correlations of **3**

Previously, a biogenesis, starting from di-or trioxidosqualene or mono- or dioxidohydroxylated squalene derivatives, was suggested for the sodwanones. A similar biogenesis can be suggested for the abudinols (Scheme 1). That is, 2,3,22,23-tetrahydroxysqualene can undergo a cyclisation after the functionalisation of Me-28 or, alternatively, two cyclisations, one of each half of the molecule, including at a certain step activation of Me-28, most likely by its oxidation to an alcohol as a precursor for a good leaving group. The latter change in Me-28 explains the disappearance of the 8th methyl group in the abudinols and muzitone. Routes a and b (Scheme 1) are two alternatives for the formation of either a THP or an oxepane ring, leading to abudinols A and B respectively. It is further suggested that abudinol B (**2**) [or an intermediate spiro compound (**s**)] is the precursor of muzitone (**3**) in a three step way (Scheme 1).

In the first step, an electrophile, E^+, attacks the center double bond of abudinol B to form a tertiary carbocation at C-15, which triggers a 1,2-shift of the 13,14-bond, and brings about the formation of a spiro intermediate **m** with the newly formed 13,15-bond. The second step implies the rearrangement of **m** to **n** in two stages: (1) formation of the 14,16-bond with the simultaneous formation of a 15-carbocation and (2) the exit of the electrophile, E^+, to afford a tetrasubstituted double bond. In the last step, oxidative cleavage of the latter double bond, of **n**, occurs resulting in the two carbonyls of **3**, alternatively **s** may be the precursor of **m**.

In addition to the above described triterpenes, *P. spiculifer*, also afforded two smaller compounds, designated nakorone (**4**) and durgamone (**5**) after the Nakora and Durgam islands in the Dahlak archipelago where the sponge was collected (Figure 2). Nakorone and durgamone analyzed for $C_{17}H_{28}O_3$ and $C_{13}H_{22}O_3$ from the HREIMS, *m/z* 280 and 226, and NMR data, respectively. From the ^{13}C-NMR, it became evident that each of the two compounds contains a carbonyl as the only unsaturated bond (δ 216.0 s and 210.5 s for **4** and **5**,

respectively). Therefore, **4**, with four degrees of unsaturation, has to be tricyclic and **5** bicyclic. According to the IR absorptions, **4** possesses a cyclopentanone (ν 1734 cm^{-1}) and **5** a cyclohexanone (ν 1707 cm^{-1}). Assignment of the NMR data of **4** and **5** (Experimental) with the assistance of COSY, HMQC and HMBC experiments together with the other spectral data, suggested that **4** and **5** are two halves of abudinol A obtained by oxidative cleavage of the 14,15-double bond to form the corresponding carbonyls (Scheme 1). Clear proof for the latter suggestion came from ozonolysis of abudinol A that, indeed, afforded nakorone and durgamone.



Scheme 1. Suggested biogenesis

Extraction and repeated chromatographies (Experimental) of a new collection (May, 1996) of the second investigated sponge *A. weltneri* resulted in the earlier reported sodwanones A-D, F, I, H and in five new sodwanones designated sodwanones N-R (**6-10**) (Figure 4) which are reported herewith.

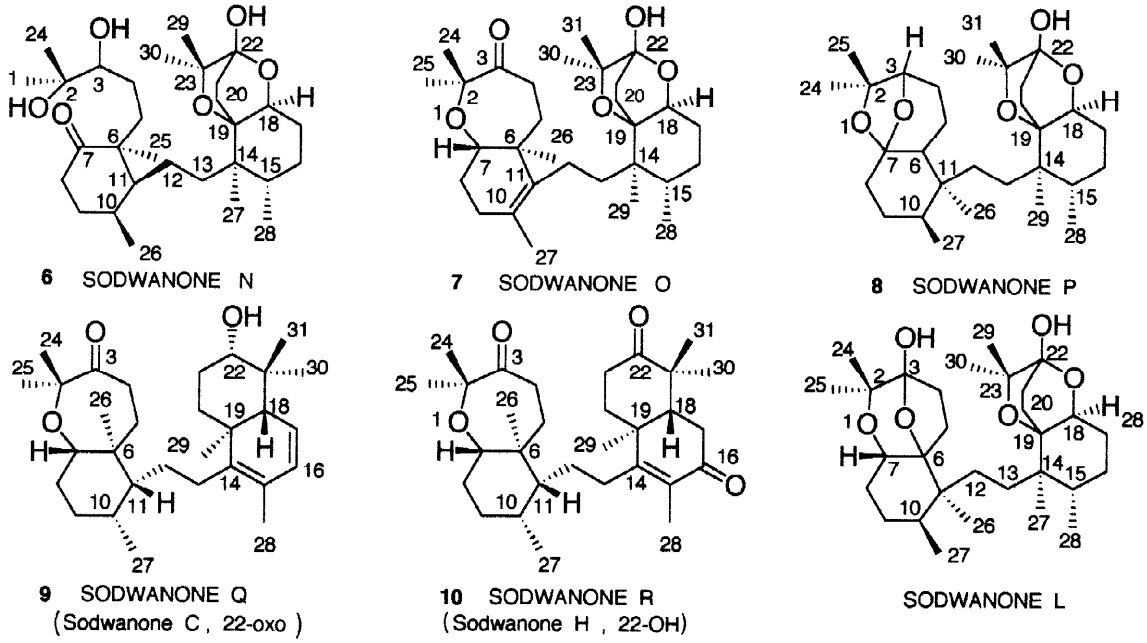


Figure 4. Sodwanone L and N - R from *A. weltneri*

Sodwanone N (**6**) analyzed for $C_{30}H_{52}O_6$ by HREIMS, giving a peak at *m/z* 508.3752 and by NMR spectral methods indicating five degrees of unsaturation. The ^{13}C-NMR and DEPT spectra (Table 2) are consistent with eight methyls (six singlets and two doublets), ten methylenes, five methines and seven non-protonated carbon atoms. Furthermore, the ^{13}C-NMR data suggested a ketone (δ_C 213.5 ppm) and six sp^3-oxygen bearing C-atoms, of which one, resonating at δ 95.4 s, has to carry two oxygen atoms. Moreover, comparison of the latter value with values of earlier reported sodwanones suggested a lactol as in sodwanone L (Figure 4). As mentioned above, most important for the structure elucidation were the two and three bond CH-correlations from the various methyl groups to establish their neighbor groups. The key HMBC correlations of sodwanone N are shown in Figure 5.

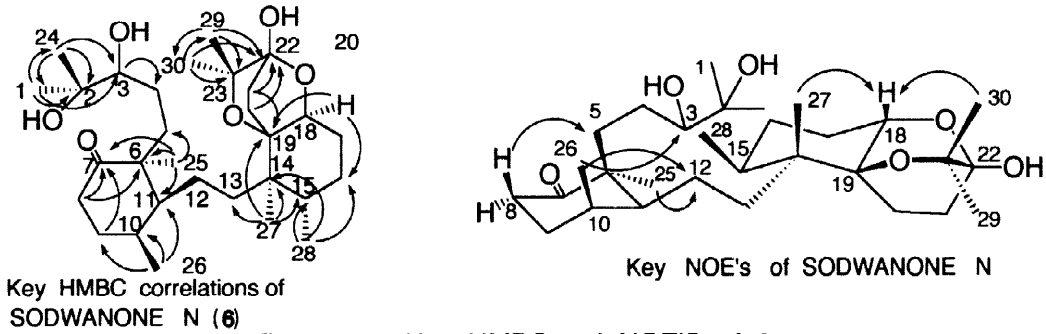


Figure 5 Key HMBC and NOE'S of **6**

Sodwanone N (**6**), as per the earlier reported sodwanones (e.g. see Figure 1), consists of two separate ring systems. One half, as established from two spin systems (Me-28, H-15–H_2-17 and H_2-20,21) seen in the COSY experiment and mainly from the HMBC correlations (Figure 5), is a dioxabicyclo[2,2,2]octane system fused to a cyclohexane as in sodwanone L. The second half, however, is new. Essentially, its gross structure is similar to the dimethyl-(2-methyl-2,3-dihydroxypentyl)cyclohexanone, which is the precursor of the "left" ketal half of sodwanone E. However, possibly due to changes in stereochemistry in **6**, there is no closure to a ketal. That one OH group was tertiary and the other secondary was deduced from a micro-acetylation under the usual conditions with Ac_2O-pyridine whereupon a shift of H-3 from δ 3.20 to δ 4.50 was observed. The suggested stereochemistry of **6** is based on coupling constants, whenever measurable, (Table 2) and mainly measured NOE's (Figure 5). The starting point for the tricyclic part was the double doublet at δ 4.38 of H-18 which, according to its *J*-values, 12.3 and 4.0 Hz, has to be axial. Two key NOE's between the latter proton and methyls 27 and 30, confirmed the bicyclo[2,2,2]octane system. Only in such a system, with cyclohexanes in a boat conformation, an NOE between groups in the 1,4-position becomes possible. The latter NOE's also determined the relative configuration of C-14, 18 and 19. As for the stereochemistry of C-15, a broad non-resolved resonance peak of H-15 made a decision difficult, however, a $\Delta W_{1/2}$ value of ~30 Hz (with one axial-axial coupling) agreed better with an equatorial Me-28 configuration.

The stereochemistry determination of the cyclohexanone begun with the readily observable axial H-8, α to the carbonyl group (δ 2.35 dt, J = 4.0 and 10.0 Hz). An NOE between the latter proton and H-5, suggested the β-configuration (as per H-8 axial) for the C_6-chain and hence the α-configuration for Me-25. An NOE between H-3 and Me-26 established the β-configuration of the latter methyl group. The relative chirality of the third asymmetric center of the cyclohexanone (C-11) was determined from the NOE between the axial Me-26 and H-12, which requires CH_2-12 to be in the β position. Additional NOE's (Figure 5) which agreed with the suggested structure, were measurable between H-12 and Me-28 and between H-10 and H-11.

The ^{1}H-NMR pattern of protons 10 and 11, which could further support the stereochemistry assignments, is not well defined – most likely due to the relatively easy ring-flip of the cyclohexanone ring (which possesses two axial and two equatorial substituents).

Table 2. NMR data of sodwanones N, O and P[a]

	Sodwanone N			Sodwanone O			Sodwanone P		
#	δ_C	δ_H (J in Hz)[b]	HMBC (C to H)	δ_C	δ_H (J in Hz)[b]	HMBC (C to H)	δ_C	δ_H (J in Hz)[b]	HMBC (C to H)
1.	23.0 q	1.16 s	24						
2.	72.5 s	-	1,24	82.6s	-	4b,7,24,25	78.6s	-	3,24,25
3	78.7 d	3.20 ddd (10.5, 4.5, 2.0)	1,4a,4b,24	218.0s	-		81.3d	3.95d (5.5)	4a,4b,5b, 24,25
4.	26.0 t	1.32, 1.10	-	35.7t	3.25t 2.20 (12.0)		23.5t	1.88 1.55	5a
5.	30.5 t	1.99, 1.74	25	37.8t	1.98 1.41		17.1t	1.92 1.51	3,4a,4b
6.	52.8 s	-	8a, 8b, 25	42.3s	-	26	42.0d	1.58	26
7.	213.5 s	-	8a, 8b, 9a, 9b, 25	79.9d	3.08dt (11.0, 4.9)		109.2s	-	3,5b,9b
8.	38.2 t	2.35, 2.25	-	26.9t	1.72 1.62		28.1t	1.55 1.45	
9.	35.4 t	1.58, 1.09	26	31.8t	2.15 1.95	27	26.2t	1.03 1.03	8a,27
10.	33.3 d	1.98	26	126.7s	-	8a,8b,9a,9b,27	35.0d	1.45	9b,26,27
11.	56.5 d	0.80	25,26	137.0s	-	8a,8b,9a,9b, 26,27	40.3s	-	26,27
12.	24.8 t	1.88, 1.20	-	23.1t	2.15 1.82	26	30.3t	1.25 1.15	26
13.	38.6 t	1.55, 1.17	27	34.6t	1.55 1.23	29	37.4t	1.74 1.74	
14.	42.4 s	-	27,28	42.4s	-	28,29	41.6s	-	28,29
15.	38.2 d	1.64	27,28	37.5d	1.61	27,28	36.6d	1.65	28,29
16.	26.5 t	1.68, 1.12	18,28	26.4t	1.82 1.38	28	26.4t	1.78 1.28	
17.	27.1 t	1.78, 1.52	-	27.1t	1.80 1.60		26.9t	1.75 1.55	
18.	78.2 d	4.38 dd (12.3, 4.0)	-	78.5d	4.35dd (11.9, 5.1)		78.5d	4.38ddd (12.5,4.5,1.5)	
19.	75.2 s	-	18,21a, 27	75.0s	-	18,29	75.2s	-	29
20.	25.2 t	1.92, 1.92	-	25.0t	2.10 2.05	18	24.8	2.00 2.00	18
21.	28.7 t	2.05, 1.67	-	28.4t	2.12 1.75		28.5	2.10 1.72	
22.	95.4 s	-	20a,21a, 21b,29,30	95.6s	-	20a,21b,30,31	95.7	-	20a,20b,30, 31,OH
23.	79.0 s	-	29,30	78,6s	-	21b,30,31	78.4	-	30,31
24.	26.3 q	1.38 s	1	20.7q	1.33	25	21.4	1.38s	25
25.	21.4 q	0.99 s	-	23,6q	1.28	24	29.1	1.26s	24
26.	20.2 q	0.92 d (6.5)	-	17.6q	1.17s		18.3	0.88s	
27.	18.4 q	0.98 s	-	19.9q	1.60s		15.7	0.82d (6.5)	
28.	16.4 q	0.91 d (6.5)	-	17.0q	0.98d (6.5)		16.9	0.91d (6.5)	
29.	23.8 q	1.46 s	30	21.09	1.10s		21.6	0.55s	
30.	26.3 q	1.49 s	29	26.1q	1.28	31	23.8	1.28s	31
31.				26.4q	1.24	30	26.2	1.25s	30

[a] $CDCl_3$, Bruker ARX 500 instrument, proton chemical shifts refer to TMS ($\delta_H = 0$) and carbons to $CDCl_3$ ($\delta_C = 77.0$)
[b] Assignments aided by HMQC, HMBC and COSY experiments.

The relative stereochemistry of the two halves of **6**, which due to conformational mobility around the 12,13-bond cannot be determined directly by NOE's, is suggested, with the same reservations, to be the same as that of sodwanone L with the "left" part as in sodwanone F. Whereas in sodwanone F, the two central methyl groups of the squalene precursor shifted to their neighboring C-atom, in **6** only one, Me-27, shifted.

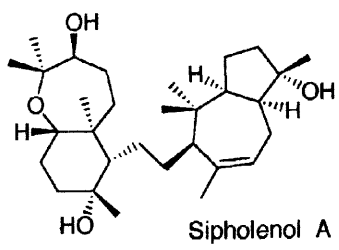
A second compound, isolated from *A. weltneri* in small amounts (1.5 mg., 0.003% dry wt) was sodwanone O (**7**), $C_{30}H_{48}O_5$, *m/z* 488. Comparison of the NMR data of **7** (Table 2) with those of the earlier reported sodwanones, suggested that one half of the molecule (C-14–C-23) had the same tricyclic structure as the "right" half of sodwanone N (**6**) and L. From the NMR data (Table 2), it became evident that the "left" half of **7** embodies the same substituted oxepanone ring as sodwanones A-D. In addition, this part incorporates a tetra-substituted double bond, most likely, between C-10 (carrying Me-27, δ_H 1.60 s ppm) and C-11. To fulfill the molecule's seven degrees of unsaturation and account for the remaining atoms, the oxepanone has to be condensed with a cyclohexene ring. Clear proof for the suggested 10(11)-double bond came from an HMBC experiment (Table 2), i.e. correlations (C to H) have been seen between: 11/9a,b,12a,b, 26 & 27 and 10/9a,b, 12a,b & 27.

Another sodwanone containing the "right" tricyclic half of sodwanones N (**6**) and O (**7**) was sodwanone P (**8**), $C_{30}H_{50}O_5$, *m/z* 490. Comparison of the NMR data of **8** (Table 2) with those of earlier reported sodwanones suggested that the "right" part of **8** is comparable with sodwanones L, N and P and the "left" part with sodwanone M. Interestingly, the lactol and ketal rings of **8** are highly stable. Treatment of **8** with methanolic-HCl didn't keep open the latter functionalities. That, at least, the dioxa[2.2.2]octane system opened during the reaction, became clear from the substitution of the 22-hydroxyl by a methoxyl group (δ_H 3.26 s, 3H, possessing a CH-correlation with C-22, δ_C 95.5, as seen in an HMBC experiment).

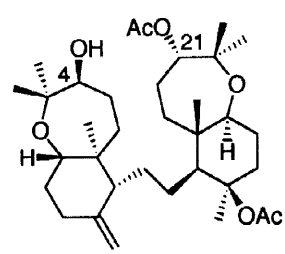
The last two sodwanones that were isolated from *A. weltneri* were sodwanones Q (**9**) and R (**10**), which analyzed for $C_{30}H_{48}O_3$, *m/z* 456 and $C_{30}H_{46}O_4$, *m/z* 470, respectively. Characteristic for **9** (Experimental) was a diene (δ = 144.5 s, 124.8 s, 125.7 d & 130.4 d and δ 5.80 dd (J = 9.3, 3.2 Hz) and 5.58 dd (J = 9.3, 2.7 Hz)) and for **10** (Experimental) were three carbonyls (δ 218.5 s, 216.0 s and 198.6 s – two saturated with α-substitutions and one unsaturated, respectively). Comparison of the NMR data of **9** and **10** with those of earlier reported sodwanones, suggested for **9** the 22-reduced sodwanone C and for **10** the 22-oxo sodwanone H structures. The position of the alcohol at C-22 of **9**, was established from a CH-correlation from the allylic H-18 (δ 2.33 d, J = 2 Hz) to C-22 (δ_C 74.9 ppm, δ_H 3.45 brs). As the 22-proton doesn't possess a large axial-axial coupling, it has to be equatorial and hence the OH-group is α-axial. The structures of both sodwanones, Q (**9**) and R (**10**), were confirmed by chemical transformations. That is, **9** on oxidation with Jones reagent afforded sodwanone C and similar oxidation of sodwanone H afforded compound **10**.

The last subject addressed in this study was the absolute configuration of several sodwanones (D, E, K, M, H and B), abudinol A (**11**) and muzitone (**3**). The relative stereochemistry of the chiral centers of the latter compounds was earlier suggested on the basis of NOE-experiments, in some cases also from biogenetic considerations and for sodwanones A, G, E and F, the assignments were secured by X-ray diffraction analysis [4]. Earlier, we reported the determination of the absolute configuration of sipholenol A by the modified Mosher method [9], which was also used by Cimino to determine the absolute configuration of raspacionin [6].

Using this method for sodwanone A by reducing its 3-CO group failed, as it resulted in the undesired epimer, and, moreover, during the MTPA-esterification, the conformation of the oxepane changed. Therefore, comparison of the CD-curves [10] of several sodwanones with the Cotton effect of the raspacionins [6], with



Sipholenol A



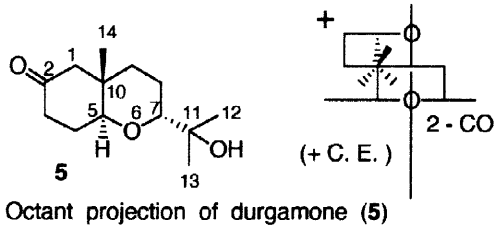
Raspacionin
(Raspacionin B, 4,21-dioxo)

Figure 6

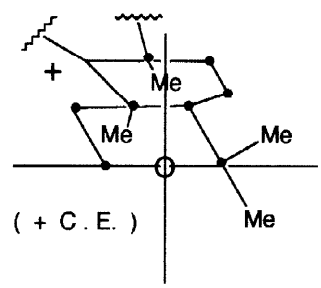
established absolute configuration, was undertaken. Raspacionin, as seen in Figure 6, is composed of two condensed oxepane-cyclohexane halves carrying on C-4 and 21 (3 and 22 according to the sodwanone numbering) an OH and an OAc group, respectively. Together with raspacionin, the 4-oxo and 21-oxo derivatives and the 4,21-dioxo derivative, raspacionin B, were also isolated. All three exhibit positive Cotton effects in the CD-curves while the effect of the latter is more than twice that of the mono oxo compounds. Hence, it was deduced that the two oxepane-cyclohexane halves are of the same absolute configuration i.e. C-7 and C-18 are both of the S-configuration. The positive Cotton effect in the former compounds agrees well with a conformation in which O-1 and C-4 to 7 are in the same plane – as seen in the X-ray analysis and deduced from the CH_3-24 to CH_3-26 NOE (and not the chair conformation with O-1 and C-2, 5 and –6 in one plane or the twisted conformer). On the basis of the positive Cotton effects measured for sodwanones K, E and M (θ_{296} +2500, θ_{290} +2400 and θ_{293} +2800, respectively), it can be suggested that they possess the same absolute configuration of the "right" oxepane half as the sipholenoles and raspacionins. (The absolute configurations are shown in Figures 1 and 6.)

Sodwanone D, possessing two carbonyl groups, like raspacionin B, shows a similar positive Cotton effect (θ_{289} +4500) (Figure 7), twice that of the mono carbonyl compounds. If the two chromophores were of opposite absolute configuration, the expected optical activity would be almost zero. Being enhanced, it establishes the same absolute configuration of the two halves as in the case of the raspacionins.

Sodwanone H also showed a positive Cotton effect (θ_{296} +4300), as did sodwanone D and the raspacionins, with an increased effect due to the two chromophores. However, here the two differ. One is a saturated and the other an αβ-unsaturated carbonyl [10]. As the conformation of the *transoid* enone in the ring is quite labile, it is difficult to predict the contribution of this group; one half-chair and the half-boat conformers are expected to give a positive Cotton effect while the second possible half-chair is expected to give a negative effect. In any case, according to the positive Cotton effect, the absolute configuration of the "left" half has to be identical to those of the above-discussed compounds. The situation with sodwanones A and B is even more complex than with sodwanone H. Sodwanones A and B possess three chromophores (two saturated CO groups and one cross-conjugated carbonyl). Moreover, one of the double bonds of the cross-conjugated dienone is also βγ-conjugated, to an unknown extent, to one of the ketones. The CD-curves of A and B, being double humped (θ_{275} +3050 and θ_{318} –1700), therefore serve mainly for identification and comparison purposes.



Octant projection of durgamone (**5**)



Octant projection of the oxepanone vicinity of abudinol and sodwanones

Figure 7.

The absolute configuration of the abudinols was also determined from CD measurements. For this purpose, we measured the optical activities of nakorone (**4**) (θ_{260} +3400) and of durgamone (**5**) (θ + 1800). The CD of **4** was compared with androstan-17-one possessing the same hydrindanone system [11]. Nakorone, as mentioned above, was obtained from ozonolysis of the abudinols and also as a natural compound. A positive Cotton effect obtained for **4** established the identity of its chirality to that of rings CD in the steroid. Furthermore, the positive Cotton effect of **5** determined its 5S, 7R, 10S configuration (Figure 7) in agreement with the 18S, 19S, 23R configuration of **11** suggested from the CD of nakorone. Thus the chirality of abudinol is the same as that found for the sodwanones, as discussed above.

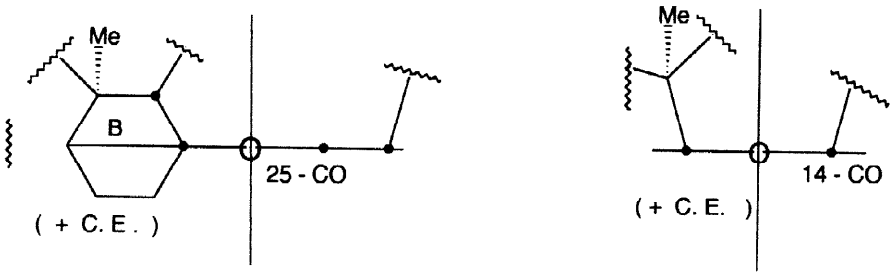


Figure 8 Octant projection of the 14 and 25 carbonyls vicinity of muzitone (**3**) in the prefered conformation according to the measured NOE's

The last optical measurement was of muzitone (3) which, according to the suggested biogenesis, has to be of the same absolute configuration as the abudinols and the sodwanones. Muzitone carries two carbonyls at C-14 and C-25 and it was found to have a positive Cotton effect (θ_{293} +9700). Although both carbonyls are not in a rigid ring, but on the eleven membered one, both are expected, according to the biogenetic consideration and applying the octant rule for these configurations, to give a positive effect (see Fig. 8). For both chromophores, the major perturbation of the $n\rightarrow\pi^*$ transition comes from the neighbor rings which are, clearly, in positive octants. The two sponges were identified by Prof. R.W.M. van Soest, Universsity of Amsterdam, the Netherlands.

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. ^{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) and $CDCl_3$ (δ_C = 77.0). All $^1J_{CH}$ connectivities were established by an HMQC experiment. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell and circular dichroism by using a Jasco 500C spectropolarimeter in methanol solutions. For details on the biological tests, see Ref. 4.

Extraction and Isolation of compounds 1 - 5. *Ptilocaulis spiculifer* (class Demospongiae, order Halichondria, family Axinellidae) (# ET-344) was collected at Dahlak archipelago, the Red Sea, Eritrea by SCUBA at a depth of 10m during February 1998. A voucher sample is deposited at Tel-Aviv University. The freeze-dried sponge (50g) was homogenized and extracted with EtOAc to give a brown gum (2.3g) 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 **1** (0.004%), **2** (0.004%), **3** (0.005%), **4** (0.002%) and **5** (0.001%) and the known compounds sodwanones A-D 0.6%, 0.1%, 0.2% and 0.05% respectively, sodwanone F 0.002%, sodwanone I 0.002%, yardenone 0.1% and abudinol 0.15%.

22-Dihydroyardenone (1): oil; IR ν_{max} (neat) 3410, 2950, 1715, 1466 cm^{-1} $[\alpha]_D$ + 3.3 (*c* 0.1, MeOH); ^{1}H-NMR (C_6D_6) δ_H 4.61 t (*J* = 9.0 Hz, H-18), 3.90 dd (*J* = 9.1 and 5.0 Hz, H-14), 3.66 dd (*J* = 11.5 and 5.3 Hz, H-7), 3.51 d (*J* = 7.2 Hz, H-22), 3.07 ddd (*J* = 2.3, 10.7 and 13.7 Hz, H-4a), 1.58 s (Me-24), 1.35 s (Me-30), 1.23 s (Me-25), 1.20 s (Me-31), 1.05 s (Me-29), 1.03 s (Me-26), 0.93 d (*J* = 6.5 Hz, Me-27), 0.70 s (Me-28). ^{13}C-NMR: all δ_C values are identical with those of yardenone except for 80.5 d (C-22), 76.5 s (C-23), 30.2 t (C-21). MS(EI) *m/z*: 490 (M^+, 50), 432 (15); HRMS (EI) M^+ found 490.3642. $C_{30}H_{50}O_5$ requires 490.3645.

Oxidation of 22-dihydroyardenone to yardenone: 22-Dihydroyardenone (**1**) (2 mg, 0.004 mmol) was dissolved in cold acetone (1 mL) and one drop of Jones reagent was added[12]. After 1hr at 10^0 two drops of MeOH were added, the acetone evaporated and the residue taken into $CHCl_3$ (5 mL). The organic phase was

washed with aq. $NaHCO_3$ and water, dried and evaporated. The residue was filtered through a small silica gel column to give yardenone (1 mg, 50%) identical in all respects with an authentic sample.

Abudinol B (2): oil; IR ν_{max} (neat) 3484, 2950, 1446, 1023 cm^{-1}; $[\alpha]_D$ - 5.0 (*c* 0.05, MeOH); ^{1}H-NMR ($CDCl_3$) δ_H 3.75 dd (*J* = 10.5 and 5.1 Hz, H-7), 3.66 dd (*J* = 11.9 and 4.6 Hz, H-18), 3.36 d (*J* = 6.8 Hz, H-3), 3.36 d (*J* = 6.8 Hz, H-23), 1.19 s (Me-27), 1.18 s (Me-25), 1.08 s (Me-26), 1.06 s (Me-28), 1.05 s (Me-31), 1.01 s (Me-29), 1.00 s (Me-30). ^{13}C-NMR (C_6D_6) δ_C 13.9 q (Me-29), 18.4 q (Me-31), 20.1 q (Me-30), 21.2 q (Me-25), 21.3 q (Me-27), 28.9 q (Me-28), 29.2 q (Me-26); eims *m/z* 474 (M^+, 20), 415 (100). HMBC C to H correlations C-2/27, 28; C-6/29; C-7/29; C-9/30; C-10/30; C-11/29,30; C-14/30; C-18/31; C-19/31; C-20/30; C-21/31; C-23/25,26; C-24/25,26; C-25/26; C-27/28; C-28/27. HRMS (EI) M^+ found 474.3702. $C_{30}H_{50}O_4$ requires 474.3696.

Acetylation of abudinol B (2): Abudinol B (1 mg) in a 1:1 mixture of Ac_2O/pyridine was left at r.t. overnight. Evaporation of the solution afforded the diacetate derivative of **2** (1 mg): oil; IR ν_{max} (neat) 2970, 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H 5.72 d (*J* = 6.8 Hz), 5.58 d (*J* = 6.8 Hz), 2.15 s (3H), 2.10 s (3H).

Muzitone (3): oil; IR ν_{max} (neat) 3410, 2950, 1715, 1450 cm^{-1} $[\alpha]_D$ - 14.2 (*c* 0.1, MeOH); For ^{1}H- and ^{13}C-NMR data, see Table 1. eims *m/z* 506 (M^+, 10), 488 (15), 470 (10), 427 (20); HRMS (EI) M^+ found 506.3586. $C_{30}H_{50}O_6$ requires 506.3594 .

Nakorone (4): oil; IR ν_{max} (neat) 2950, 2853, 1737, 1085, 1054 cm^{-1} $[\alpha]_D$ – 210.0 (*c* 0.2, MeOH); ^{1}H-NMR (C_6D_6) δ_H 3.47 dd (*J* = 11.3 and 5.0 Hz, H-7), 3.28 d (*J* = 4 Hz, H-3), 2.04 dd (*J* = 18.9 and 8.9 Hz, H-13), 1.82 t (*J* = 14.0 Hz, H-12), 1.75 dd (*J* = 18.9 and 17.8 Hz, H-13), 1.07 s (Me-15), 1.00 s (Me-16), 0.85 s (Me-17), 0.72 s (Me-18); ^{13}C-NMR (C_6D_6) δ_C 216.0 s (C-14), 79.5s(C-2), 76.8 d (C-7), 76.3 d (C-3), 54.3 d (C-11), 49.5 s (C-10), 42.0 s (C-6), 36.2 t (C-9), 33.8 t (C-5), 31.2 t (C-13), 29.5 q (C-16), 27.2 t (C-8), 25.2 t (C-12), 21.5 q (C-15), 18.2 t (C-4), 15.0 q (C-18), 13.3 q (C-17); MS(EI) *m/z* 280 (M^+, 80), 265 (25), 222 (90), 207 (60), 194 (50), 178 (100). HRMS (EI) M^+ found 280.2036. $C_{17}H_{28}O_3$ requires 280.2031.

Durgamone (5): oil; IR ν_{max} (neat) 2972, 2342, 1707 cm^{-1} $[\alpha]_D$ - 28.5 (*c* 0.1, MeOH); ^{1}H-NMR (C_6D_6) δ_H 3.74 dd (*J* = 11.5 and 4.5 Hz, H-7), 3.27 t (*J* = 5.5 Hz, H-5), 1.13 s (Me-13), 0.90 s (Me-12), 0.77 s (Me14). ^{13}C-NMR (C_6D_6) δ_C 210.5 s (C-2), 78.6 d (C-7), 74.9 s (C-11), 73.9 d (C-5), 53.4 t (C-1), 39.3 t (C-3), 35.7 s (C-10), 35.1 t (C-8), 27.3 t (C-4), 26.9 q (C-13), 19.3 t (C-9), 18.4 q (C-14). eims *m/z* 226 (M^+, 15), 168 (80), 125 (100). HRMS (EI) M^+ found 226.1558. $C_{13}H_{22}O_3$ requires 226.1563.

Ozonolysis of abudinol A (11) to compounds 4 and 5 : Through a solution of abudinol A (10 mg, 0.02 mmol) in CH_2Cl_2 (20 mL) at -70^0 was bubbled ozone for 10 min. Dimethyl sulfide (0.1 mL) was added and the solution kept at r.t. overnight. The residue after evaporation was chromatographed on silica gel to afford nakorone (**4**, 2 mg) and durgamone (**5**, 2 mg, 40%), identical in all respects with the natural compounds.

Extraction and Isolation of compounds 6 – 10. *Axinella weltneri* (Von Lendenfeld) (class Demospongia, order Halichondria, family Axinellidae) (# TASA 396) was collected in Sodwana Bay, South Africa by SCUBA during May, 1996. A voucher sample is deposited at Tel-Aviv University. the freeze dried sponge (8g) was treated in the same way as described above for ET 344 to afford **6** (0.08%), **7** (0.03%), **8** (0.03%), **9** (0.02%), **10** (0.02%).

Sodwanone N (6): oil; IR ν_{max} (neat) 3510, 3400, 2970, 1707, 680 cm^{-1}; MS(EI) *m/z* 508 (M^+, 15), 432 (15), 181 (100); HRMS (EI) M^+ found 508.3752. $C_{30}H_{52}O_6$ requires 508.3750.

Sodwanone O (7): oil; IR ν_{max} (neat) 3510, 3400, 2970, 1700, 980 cm^{-1} $[\alpha]_D$ + 18.0 (*c* 0.15, MeOH); MS(EI) *m/z* 488 (M^+, 50), 471 (100), 415 (60); HRMS (EI) M^+ found 488.3492. $C_{30}H_{48}O_5$ requires 488.3489.

Sodwanone P (8): oil; IR ν_{max} (neat) 3485, 2970, 1446, 1023 cm^{-1} $[\alpha]_D$ + 54.6 (*c* 0.70, MeOH); MS(EI) *m/z* 490 (M^+, 15). HRMS (EI) M^+ found 490.3649. $C_{30}H_{50}O_5$ requires 490.3645.

Methanolysis of sodwanone P (8) to its 22-methoxy derivative : Sodwanone P (1 mg, 0.002) was left overnight at r.t. in a solution of HCl in MeOH (1%, 2 mL). Evaporation of the solvent afforded the 22-methoxy derivative (0.8 mg, 80%); oil; ^{1}H-NMR (C_6D_6) δ_H 4.44 dd (*J* = 11.9 and 4.0), 3.68 d (*J* = 4.1), 3.26 (3H), 1.56 s (3H), 1.30 s (3H), 1.24 s (3H), 1.19 s (3H), 1.09 s (3H), 1.05 s (3H), 0.81 d (3H), 0.78 d (3H). MS (EI) M^+ *m/z* 504.

Sodwanone Q (9): oil; IR ν_{max} (neat) 3410, 2950, 1715 cm^{-1} $[\alpha]_D$ - 22.0 (*c* 0.15, MeOH); ^{1}H-NMR δ_H 5.80 dd (*J* = 9.3 and 3.2 Hz, H-17), 5.58 dd (*J* = 9.3 and 2.7 Hz, H-16), 3.45 bs (H-22), 3.10 t (*J* = 12.5 Hz, H-4a), 2.85 dd (*J* = 11.6 and 4.3 Hz, H-7), 1.68 s (Me-28), 1.30 s (Me-24), 1.24 s (Me-25), 1.01 s (Me-31), 1.00 s (Me-26), 0.98 s (Me-30), 0.97 d (*J* = 6.5 Hz, Me-27), 0.81 s (Me-29); ^{13}C-NMR δ_C 218.0 s (C-3), 144.5 s (C-14), 130.4 d (C-17), 125.7 d (C-16), 124.8 s (C-15), 82.3 d (C-7), 82.2 s (C-2), 74.9 d (C-22), 51.3 d (C-11), 46.5 d (C-18), 41.3 s (C-6), 40.5 t (C-5), 39.0 s (C-19), 37.3 s (C-23), 35.3 t (C-4), 32.3 t (C-9), 28.7 d (C-10), 27.9 t (C-20), 27.1 t (C-8), 27.0 q (C-31), 26.5 q (C-25), 26.4 t (C-12), 25.9 t (C-13), 25.7 t (C-21), 23.3 q (C-30), 20.5 q (C-24), 17.6 q (C-28), 15.1 q (C-29), 14.5 q (C-27), 13.5 q (C-26); MS(EI) *m/z* 456 (M^+, 30), 423 (35), 187 (100); HRMS (EI) M^+ found 456.3587. $C_{30}H_{48}O_3$ requires 456.3591.

Oxidation of sodwanone Q (9) to sodwanone C: Oxidation of sodwanone Q (**9**, 1 mg, 0.002 mmol) as described for compound **1**, *vida supra*, gave sodwanone C (0.5 mg, 50%) identical (NMR and MS data) with an authentic sample..

Sodwanone R (10): oil; IR ν_{max} (neat) 2950, 1715, 1625 cm^{-1} $[\alpha]_D$ - 18.0 (*c* 0.25, MeOH); ^{1}H-NMR δ_H 1.74 s (Me-28), 1.30 s (Me-24), 1.24 s (Me-25), 1.23 s (Me-29), 1.11 s (Me-30), 1.09 (Me-31), 0.98 s (Me-26), 0.94 d (*J* = 6.5 Hz, Me-27); ^{13}C-NMR δ_C 218.5 s (C-3), 216.1 s (C-22), 198.6 s (C-16), 165.9 s (C-14), 130.8 s (C-15), 82.4 s (C-2), 81.9 d (C-7), 51.5 d (C-11), 49.5 d (C-18), 47.0 s (C-23), 41.4 s (C-19), 40.5 d (C-5), 40.2 s (C-6), 35.7 t (C-7), 35.3 t (C-21), 35.1 t (C-20), 34.3 t (C-17), 31.1 t (C-8), 29.1 t (C-9), 28.3 d (C-10), 26.4 q (C-25), 26.2 t (C-13), 25.8 t (C-12), 25.4 q (C-31), 21.2 q (C-30), 20.5 q (C-24), 17.6 q (C-29), 14.6 q (C-27), 13.5 q (C-26), 11.5 q (C-28); MS(EI) *m/z* 471 (MH^+, 100). HRMS (EI) M^+ found 470.3389. $C_{30}H_{46}O_4$ requires 470.3384.

Oxidation of sodwanone H to sodwanone R (10) : Oxidation of sodwanone H (5 mg, 0.01 mmol) as described above for compound **1** gave sodwanone R (3 mg, 60%), identical (NMR and MS data) with the natural compound..

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