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Yardenone and Abudinol two new triterpenes from the marine sponge *Ptilocaulis spiculifer*

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Abstract: Two novel triterpenes, yardenone (**5**) and abudinol (**6**) together with the known sodwanones A-D (**1 - 4**) have been isolated from the marine sponge *Ptilocaulis spiculifer*. The structures of compounds **5** and **6** were determined by interpretation of their 1D and 2D NMR spectra and were secured, including their relative stereochemistry, by X-ray diffraction analysis.

Marine organisms are a rich source of isoprenoids[1-3], however, only a few triterpenes all squalene-derived polyethers, have been reported[4-5]. In our continuing search for bioactive metabolites from marine invertebrates[4], we have examined several organisms collected in the Dahlak archipelago, Eritrea, the Red Sea. Among them was the sponge *Ptilocaulis spiculifer* (Demospongiae, order Halichondria, family Axinellidae) whose lipophilic extract was cytotoxic against P-388 cells. *P. spiculifer* from the Caribbean sea was earlier investigated and reported only to contain the alkaloids ptilocaulin[6] and ptilomycaline A[7]. None of these alkaloids were revealed in the Red Sea sponge, however, it was found to contain a variety of triterpenes. *P. spiculifer* belongs to the same Axinellidae family as *Axinella weltneri*, earlier investigated by us and found to be rich in the sodwanone triterpenes[4c-e]. The ethyl acetate extract of the sponge contained six triterpenes, the known sodwanones A,B,C and D (**1-4**,0.6%, 0.1%, 0.2%, and 0.05%, respectively) and two new compounds, yardenone (**5**,0.1%) and abudinol (**6**, 0.15%) (dry wt).

Yardenone (**5**), analyzed for $C_{30}H_{48}O_5$ from the FABMS, *m/z* 489 [MH^+] and NMR data, with seven degrees of unsaturation. As the only unsaturated funcionalities were two carbonyl groups (δ_c 216.0 s and 218.0 s) **5** had to be pentacyclic. A comparison of the NMR data of **5** (Table) with the data of the earlier reported sodwanones[4c] suggested one half of the molecule to be closely related to the cyclohexane-oxepane system of sodwanones B and C (C-2÷C-10). Most important for the structure elucidation of these triterpenes, because of the high degree of CH_2's overlapping in the proton NMR spectra, are the two and three bond $C\underline{H}_3$ to vicinal carbon atom correlations. Thus, correlation from $C\underline{H}_3$'s- 24÷27 to their vicinal carbon atoms, (Table , HMBC) supported the above suggested partial structure and in addition located a unique C-atom, resonating at 90.3s ppm, at C-11 -closing a cyclohexane ring. The low-field resonance of this carbon atom required not only its

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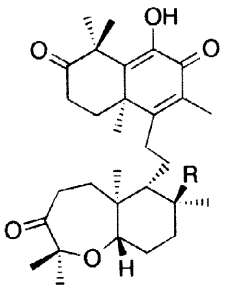
vicinity to an oxygen atom, but also for another structural feature to bring it to resonate that low.

A second bicyclic system, i.e a cyclopentane-oxepanone system (C-15÷C-23), together with four attached methyl groups (CH_3's 28÷31) and a connecting methinoxy group (C-14, δ_c 84.8, δ_H 3.75) were also suggested on the basis of 2D-NMR data (Table). Combining C-14 and C-11, in a spiro configuration, by a THF ring completed the structure of **5**. The suggested structure including the relative stereochemistry was unequivocally determined by an X-ray diffraction analysis[8]. The structure was solved by direct methods (SHELX-97)[9], no corrections for absorption and secondary extinction effects were applied. The final refinement , based on F^2, converged at R=0.046 for 1800 observations having $F_o > 4\sigma$ (F_o) and R=0.063 (wR2=0.138) for 2350 unique data. At convrgence, S=0.86 and $|\Delta\rho| \leq 0.17 e.Å^{-3}$.

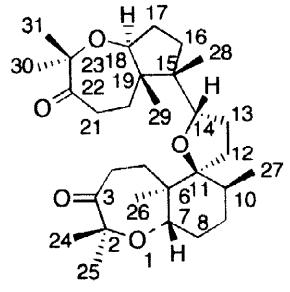
The second new compound designated abudinol (**6**) analyzed for $C_{30}H_{50}O_4$ from the FABMS, *m/z* 475 [MH^+], and NMR data (Table). Out of the six degrees of unsaturation, of **6**, one applied for a tetrasubstituted double bond (δ_c 120.0s and 143.6s) and the other five pointed to a pentacyclic structure. Contrary to compounds **1-5** abudinol possesses only seven, rather than eight , methyl groups. 2D-NMR experiments (Table) suggested a cyclohexane-oxepanol ring system (C-2÷C-11), which, based on H,H and C,H correlations (Table), could further be expanded by a cyclopentane ring (C-12 to C-14).

2D NMR data of the second half of the molecule established its cyclohexane-tetrahydropyrane structure as well as the C-23 dimethylcarbinol substituent and the C14=C15 attachment of the two parts. Most important for the structure elucidation were the Me-30 to C-14 and Me-31 to the allylic C-20 protons, *vide supra.*

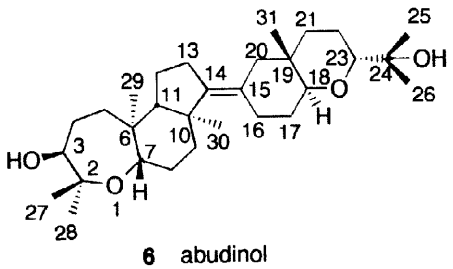
Further support for the structure of abudinol came from the ozonolysis of **6** which split the molecule into two halves, namely, a $C_{17}H_{28}O_3$ *m/z* 280 and a $C_{13}H_{22}O_3$ *m/z* 226 segment. Each half possessed the expected NMR data, reminiscent to the corresponding atoms in the two parts of **6** and characteristic IR absorptions at 1734 and 1707cm^{-1}, respectively, confirming unequivocally the five and six membered rings. As with **5** the high methylene-signals overlapping, in the NMR spectrum of **6**, made it difficult to determine the configuration of the double bond and the complete relative stereochemistry. The structure was solved by direct X-ray diffraction analysis methods[11], and refined by full matrix least-squares (SHELX-97)[9]. Refinement of the structural model,



1 sodwanone A, R=OH
2 sodwanone B, R=H



5 yardenone



6 abudinol

based on F^2, excluding the solvent atoms, converged at R=0.13. The *"Squeeze/Bypass"* procedure was then used to subtract the overall contribution of the disordered solvent to the diffraction pattern from the observed data[10]. Application of this technique allowed convergence of the refinement based on the Squeeze-modified data set down to R=0.078 for 1647 observations having $F_o > 4\sigma$ (F_o) and R=0.104 (wR2 = 0.219) for 2524 unique data. Non-hydrogen atoms were treated anisotropically. All hydrogen atoms were located in calculated positions, the methyls being treated as rigid groups; the hydroxyl H could not be positioned. The terminal i-Pr group appeared to be partly disordered as well, due to the possibility of unhindered rotation about a C-C bond which connects this substituent to the molecular framework; the three terminal C and O atoms were thus assigned an isotropic U in the final calculations.

NMR Data Of Yardenone (**5**, in $CDCl_3$) And Abudinol (**6**, in C_6D_6)

	5			6		
No	**^{13}C**	**HMQC**	**HMBC**	**^{13}C**	**HMQC**	**HMBC**
2	82.7s	-	7,24,25	73.7s	-	7,27,28
3	218.0s	-	4a,b,5a,24,25	76.7d	3.52d	5a
4	35.6t	3.08t,2.25dd		20.7t	1.65,1.45	
5	32.1t	1.90, 1.42		35.5t	1.75,1.32	
6	45.6s	-	26	41.2s	-	7,29
7	76.9d	3.25dd	26	76.8d	3.82dd	29
8	28.5t	1.62, 1.55		28.9t	1.90,1.70	7
9	28.4t	1.82, 1.82	27	37.3t	2.24,1.65	7,30
10	35.8d	1.74	27	44.8s	-	30
11	90.3s	-	26,27	59.6d	1.41	29,30
12	30.5t	1.60,1.60		25.7t	2.08, 1.62	
13	26.2t	1.75,1.35		30.5t	2.42, 2.35	
14	84.8d	3.75dd	28	143.6s	-	12b,13a,b,16a,b,17b,20a,b,30
15	48.8s	-	14,28,29	125.6s	-	13a,b,16,b,17a,b,20a,b
16	26.3t	1.78,1.38	18,28	27.6t	2.88 bd	
17	28.9t	1.70,1.50		29.1t	1.85, 1.62	
18	82.0d	4.02t	29	76.3d	3.78dd	31
19	48.3s	-	20a,21b,29	35.3s	-	31
20	31.5t	1.70,1.42	29	44.2t	2.21, 1.55	31
21	35.0t	3.12t		35.4t	1.64, 1.34	18,31
22	216.0s	-	20a,b,21a,b,30,31	20.0t	1.75, 1.65	
23	81.6s	-	18,30,31	78.1d	3.65dd	25,26
24	20.3q	1.24s	25	77.8s	-	25,26
25	27.0q	1.21s	24	26.7q	1.34s	26
26	15.2q	1.06s	7	26.2q	1.18s	25
27	17.8q	0.90d		21.9q	1.33s	28
28	20.9q	0.79s		28.8q	1.20s	27
29	15.7q	1.00s	18	13.9q	1.14s	7
30	21.9q	1.28s	31	20.1q	1.10s	
31	26.3q	1.24s	30	18.4q	1.09s	18

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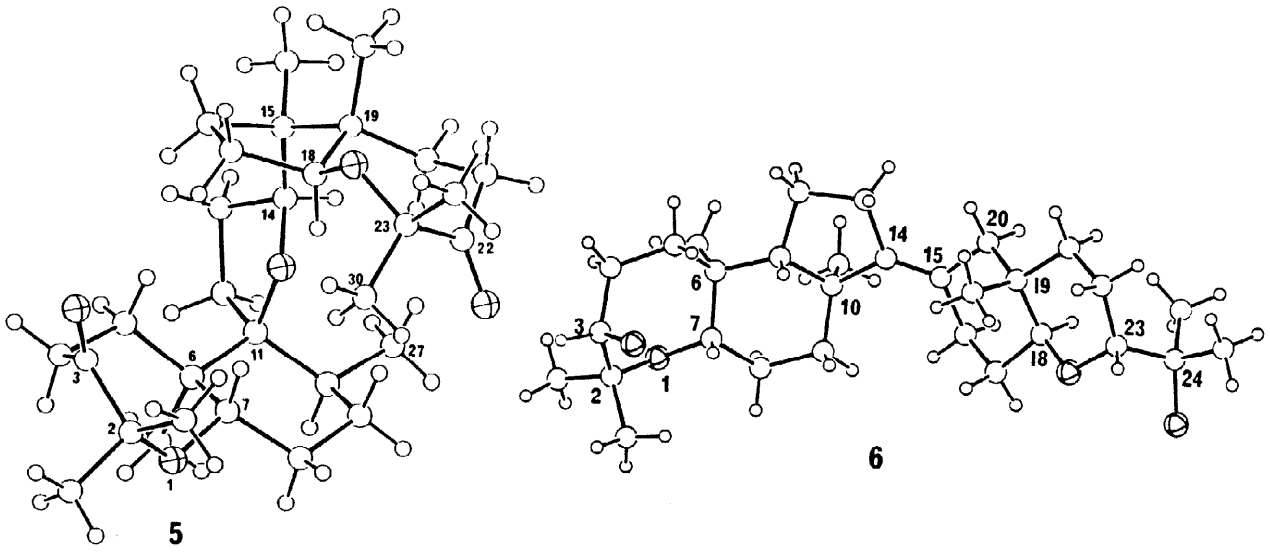

ORTEP representations of compounds **5** and **6**

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8. The X-ray diffraction measurements were carried out at ca. 295 K on an automated CAD4 diffractometer equipped with a graphite monochromator, using MoKα (λ =0.7107 A°) radiation. Intensity data were collected by the ω-2θ scan mode. The structure was solved by direct methods , and refined by full-matrix least-squared (SHELX-97)[9] Non-hydrogen atoms were treated anisotropically. All hydrogen atoms were located in calculated positions. Crystal data $C_{30}H_{48}O_5$, formula weight 488.71, monoclinic, space group $P2_1$, a = 10.296(3), b = 13.270(4), c =11.0.58(2) Å, β = 113.21(2)°, V=1388.55 Å^3, Z=2, D_{calc} = 1.169 g.cm^{-3}, F(000) = 536, μ(MoKα) = 0.77 cm^{-1}.
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11. The compound crystallized as 2:1 ethyl acetate solvate. X-ray diffracion measurements were carried out as described for **5**. Crystal data: $C_{30}H_{50}O_4 \cdot ½(C_4H_8O_2)$, formula weight 518.78, monoclinic, space group $P2_1$, a = 13.645(4), b = 7.515(2), c =14.776(5) Å, β = 93.02(2)°, V=1513.06 Å^3, Z=2, D_{calc}=1.134 g.cm^{-3}, F(000) = 568, μ(MoKα) = 0.74 cm^{-1}.