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SHAAGROCKOL B AND C; TWO HEXAPRENYLHYDROQUINONE DISULFATES FROM THE RED SEA SPONGE TOXICLONA TOXIUS

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Abstract: Two hexaprenylhydroquinone derived disulfates, shaagrockol B and C (**1** and **2**) were isolated from the Red Sea sponge *Toxiclona toxius* (Levi, 1958). The structure of these two new hexaprenoid, antifungal metabolites was determined by spectroscopic methods, mainly 2D-NMR measurements as well as chemical modifications. Ozonolysis of compound **2** afforded compound **1** and the acid catalysed rearrangement of **1**, leading to compounds **3** and **4**, has been elucidated.

In our continuing search for physiologically active marine metabolites[1] we have isolated from a Red Sea sponge *Toxiclona toxius*, which was collected near Shaag rock in the entrance to the Gulf of Suez, a series of sulfates with antifungal activity. Solvent partitioning of the CH_2Cl_2-MeOH extract of the sponge concentrated the antifungal activity against *C. albicans* in the aq. MeOH fraction. The subject of this report is the structure of two of these sulfates: Shaagrockol B (**1**) and Shaagrockol C (**2**), named after the place of collection.

Compound **2**, $[\alpha]_D$ + 8° (c = 0.7, MeOH) ν_{max} 3500, 2966, 2948, 1264, 1239, 1039, 864 cm^{-1}, had a molecular formula of $C_{36}H_{54}O_{10}S_2Na_2$ which was established by positive and negative FABMS (m/z 779.4 $[M(Na_2) + Na]^+$, 733.1 $[M(Na_2)-Na]^-$ confirmed by the addition of K^+ to give 827.2 $[M(K_2)+K]^+$ and 749.1 $[M(K_2)-K]^-$) and the ^{13}C-NMR spectrum. The carbon spectrum of **2** showed well resolved resonances for all 36 carbon atoms in the molecule (see Table 1) and a DEPT experiment indicated that 53 hydrogen atoms were bonded to carbons ($7xCH_3$, $13xCH_2$, 6xCH).

Furthermore, the ^{13}C-NMR spectrum suggested a three substituted phenyl group, a tetrasubstituted double bond bearing one of the methyls (δ_H 1.49), a tert. alcohol which could be eliminated, *vide infra* (δ_C 79.8), and an ethereal bridge (δ_C 76.1 & 74.4). According to the 9 degrees of unsaturation of **2** it required in addition to the above functionalities three carbocyclic rings. The acid sensitivity of the two sulfates (1% TFA in MeOH), and the ^{1}H and ^{13}C chemical shifts of the aromatic ring[2] (Table 1) proposed for **2** a 2-alkylated hydroquinone disulfate moiety. This moiety was confirmed and expanded by a series of COSY and TOCSY experiments to a $CHCH_2CH_2C(CH_3)=CCH_2$-$C_6H_3(OSO_3Na)_2$ unit. The latter experiments together with a HMQC experiment (which established all thirteen geminal methylene pairs) also proposed a -$OCHCH_2CH_2CH(CH_3)$- and two -$(CH_2)_3$- units. Most of the information, which ultimately led to the total planar structure of shaagrockol-C, came from two HMBC experiments (J_{CH}=8 and 4 Hz) summarized in Table 1. It was established that the alicyclic portions of compound **2** consist of two bicyclic ring systems; a *trans*-decalin and a *cis*-fused cyclohexane-oxepane system which are linked via an ethylene bridge. The stereochemistry of the chiral centers (Figure) was determined mainly by NOE's[3] and, for the decalin portion, also by comparison with a model compound[4].

The relative stereochemistry of the two halves of the molecule, which are separated by a flexible ethylene bridge, has to be established. The decalin of **2** resembles siphonodictyol H[4], while the second bicyclic part resembles in part sipholenol[5].

Compound **1**, $[\alpha]_D$ + 4° (c = 0.5, MeOH), ν_{max} 3500, 2958, 2929, 1714, 1263, 1234, 1043, 852 cm^{-1}, was obtained as an amorphous powder of molecular formula $C_{36}H_{54}O_{12}S_2Na_2$ (FABMS, m/z 811.3 $[M(Na_2)+Na]^+$ 789.0 $[M(Na_2)+H]^+$, 767.0 $[M(NaH)+H]^+$ and 765.0 $[M(Na_2)-Na]^+$). On the basis of careful analysis of the ^1H and ^{13}C NMR spectra, (Table 1) shaagrockol B has been determined to have the same hydroquinone disulfate and *cis*-cyclohexane-oxepane units as in **2** and to differ from the latter compound in the decalin part. That is, the absence of the 19(20) double bond, the existence of two carbonyls (δ_C 215.0s & 212.0s), one of which is a part of a methyl ketone (δ_H 2.06s), and in the down-field shift of the benzyl protons (H_2-31). This shift of the two 31-protons, from δ 3.45d, 3.57d in **2** to δ 4.06d, 4.16d in **1**, suggested this pair to be adjacent to one of the two carbony groups.

Mild acid hydrolysis, 1% TFA in abs. MeOH at rt., aimed to remove the two sulfate groups, surprisingly resulted, not only in the disappearance of these two groups, but also of the two carbonyls. In both compounds **3** and **4**[6], that were obtained during the hydrolysis of **1**, the carbonyl-carbon signals disappeared and the $C\underline{H}_3CO$ singlet at δ_H 2.06s was replaced by a signal at δ_H 1.66s. Significant changes were also observed in the aromatic region of the NMR spectra[6]. The difference between compounds **3** and **4** was in the cyclohexane-oxepane unit: whereas in **3** this site remained intact, in **4** the 11-OH group was eliminated bringing about a 1,2-shift of Me-24 to C-11 and the formation of a 1(2) double bond. Eventually, the total planar structure determination of **1** was achieved from two HMBC experiments (with the emphasis on CH-coupling constants of 8 and 4 Hz).

Comparison of shaagrockol B and C pointed clearly to the close relationship between the two, namely, that shaagrockol B is the oxidative cleavage product of the 19(20) double bond of shaagrockol C. And, indeed, reductive ozonolysis of **2** (-20°C, MeOH, Me_2S) afforded compound **1**.

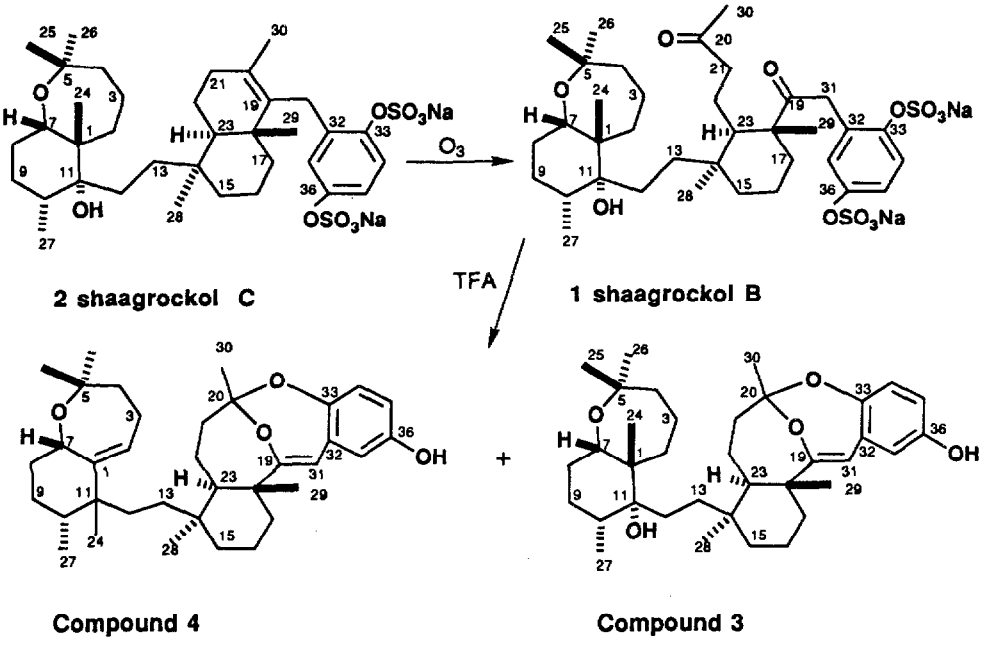

Table 1: NMR Data of Compounds **1** *and* **2** *(CD_3OD, 125 MHz and 500 MHz)*

	Compound **2**							Compound **1**					
C#	^{13}C	^{1}H	COSY	HMBC J_2	J_3	J_4		^{13}C	^{1}H	COSY	HMBC ^2J	^3J	^4J
1	42.1s	-						42.3s					
2a	39.7t	1.97m	3a,3b	1,3	4			39.5t	1.94m	2b,3a,3b		11	
2b		1.54m	2a,3a,3b		4,7,11				1.55m	3a,3b	3	5,11	
3a	20.0t	1.78m	3b,4a, 4b					19.9t	1.70m	2a,2b, 4a,4b	2	4	
3b		1.52m							1.50m		2	4	26
4a	44.6t	1.62m	4b					44.4t	1.66m	4b	5	25,2	
4b		1.49m		5,3	26,2				1.45m		5,3	25,26,2	
5	76.1s	-						76.0s	-				
7	74.4d	3.71d	8a,8b	1	5,11	25		74.25	3.74d	8a,8b	1	11,5	25
8a	25.5t	2.06m	8b,9a,9b	7				25.5t	2.03m	8b,9b			
8b		1.26m	9a,9b						1.28m				
9a	24.5t	2.02m	9b,10,27					24.7t	1.98m	9b			
9b		1.14m	10						1.15m		10		1
10	37.6d	1.58m	27	11		7		37.7d	1.60m	27			
11	79.8s	-						79.7s	-				
12a	28.0t	1.61m	12b,13a,13b					27.4t	1.63m	12b			
12b		1.14m	13a,13b						1.16m	13a	10		
13a	25.1t	1.50m	13b					27.6t	1.32m	13b			
13b		1.12m	28						1.12m				
14	37.9s	-						38.3s	-				
15a	37.4t	1.65m	15b,17b		23,17			37.4t	1.65m	15b			
15b		1.02m	16a,28	16					1.02m				
16a	20.0t	1.52m	16a,17a, 17b					19.4t	1.52m		18		
16b		1.28m							1.26m				
17a	38.2t	1.74m	17b,15b,	18				37.4t	1.74m				
17b		0.91m							1.02m				
18	40.2s	-						54.0s	-				
19	138.4s	-						215.0s	-				
20	130.5s	-						212.0s	-				
21a	34.8t	2.21m	21b,30 22a,22b 31a,31b	20,22	19,23			46.1t	2.59m	21b,22a, 22b	20		
21b		2.08m	31a,31b	20,22	19,23				2.44m	22a,22b	20		
22a	20.1t	1.76m	22b,23	21,23	18,20			21.8t	1.76m	22a			
22b		1.62m	23	21	18				1.36m		18		
23	55.6d	1.36dd	29	18	21,13 29,15			49.9d	1.96	22a,22b	22,14	18	
*24	24.3q	1.18s		1	11	10,12		24.6q	1.18s		1	11	3
*25	24.5q	1.17s		5	4,26			24.1q	1.20s			26	
26	32.2q	1.14s		5	4,25			32.2q	1.15s		5	25,4	
27	17.2q	1.06d		10	9	1		17.2q	1.09d		10	9	12
28	29.4q	0.91s		14	23,13, 15			28.4q	0.91s		14	23,15	18,16
29	21.3q	1.06s		18	19,23			20.4q	1.30s		18	19,23,	17
30	20.4q	1.49s		20	19,21			30.0q	2.06s		20	21	
31a	28.5t	3.57d	31b	19,32	18,20,33			40.1t	4.16d	31b	19,32	33	37,29
31b		3.45d		19,32	18,20				4.06d		19,32	33	37,29
32	136.5s	-						131.1s	-				
33	148.8s	-						149.0s	-				
34	123.0d	7.34d	35	33	36,32			123.4d	7.38d	35			
35	119.7d	7.09dd	37					121.5d	7.17dd	37			
36	150.9s	-						150.3s	-				
37	123.4d	7.03d		36	31,33,35			125.3d	7.12d				

a,b a-the low field and b-the high field protons in a geminal pair, * interchangeable signals

c $J_{7/8a8b}$ = 3, $J_{23/22a,22b}$ = 1.5, 12.5, $J_{27/10}$= 7.5 $J_{31a/31b}$= 18, $J_{34/35}$= 9, $J_{35/34,37}$= 9, 2.5, $J_{37/35}$=2.5

d $J_{7/8a,8b}$ = 3, $J_{27/10}$ = 7.5, $J_{31a/31b}$ = 18, $J_{34/35}$ = 9, $J_{35/34,37}$ = 9, 2.5, $J_{37/35}$ = 2.5

The structure of **1** readily explained the route leading to **3** and **4** by the TFA-acid, that is, formation of a lactol between one of the free hydrolysed phenols and C(20)=O, followed by a second lactol formation between the intermediate 20-OH and C(19)=O and finally elimination of the 19-OH to afford the conjugated 19(31) enol ether, (in **4**, in addition, as explained above, the other half of the molecule changed also).

Interestingly, from the biogenetic point of view, was the isolation of the earlier reported 2-tetraprenylhydroquinone[7] (from the less polar fractions of the sponge), as the higher homologue the 2-hexaprenylhydroquinone[8] is assumed to be the precursor of the shaagrockols.

Cyclisations of tetra and pentaprenylhydroquinones, in sponges, to give in a single cyclisation process of the entire aliphatic chain, penta or hexacyclic molecules have earlier been reported[9,10]. In the shaagrockols, however, two cyclisation reactions are involved, one leading to the benzyl decalin unit which resembles siphonodictyol H[4], and the other to the cyclohexane-oxepane system which is similar (although *cis* and not *trans* fused) to this part in the sipholanes[5].

Shaagrockols B and C were found to be responsible for the antifungal activity (IC_{50}=6μg/ml), the structure of additional sulfates is on going.

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References and Notes

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2. Ragan, M.A. Can. J. Chem., **56**, 2681 (1978) and Petsch et al, "Table of Spectral Data for Structure Determination of Organic Compounds", Springer-Verlag, Berlin, 1983.
3. NOE's were observed between H-7 and H-3b, 8a, 8b and either Me-24 or 25 (overlapping signals). The latter NOE's together with the equatorial configuration of H-7 (t, J=3 Hz) agree with the suggested *cis* ring juction. In addition, the absence of a NOE between Me-24 (axial) and 27 proposed an equatorial Me-27 group. Although a NOE between Me-24 and H-10ax could also be observed it was not unequivocal because of overlapping of H-10ax with H-4a.
The tentative stereochemistry at C-11 (see Figure) is proposed on the basis of the preferred equatorial position of the ethylene bridge and the clean rearrangement leading to **4** (elimination of the axial H-11 and a 1,2-shift of the axial Me-24). The following NOE's were measured for the decalin portion: Me-28 to 22a, 23; Me-30 to H-31b; and Me-29 to H-16a, 21 and 22b.
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6. **4**, $C_{36}H_{52}O_4$ (HREIMS 548.3894, mmu - 2.8) ν_{max} 3050, 2984, 1417, 1261, 900 cm^{-1}, ^1H NMR ($CDCl_3$) δ_H 4.11 (t, J=3, H-7), 0.96 (s, Me-24), 1.17 (s, Me-25), 1.25 (s, Me-26), 0.84 (d, J=7.5, Me-27) 0.88 (s, Me-28), 1.35 (s, Me-29), 1.66 (s, Me-30), 6.30 (s, H-31), 7.26 (d, J=8, H-34), 6.67 (dd, J=8, 2.5, H-35), 6.81 (d, J=2.5, H-37); ^{13}C NMR, δc 146.9s (C-1), 123.6d (C-2), 74.0s (C-5), 70.8d (C-7), 42.5s (C-11), 168.2s (C-19), 101.6d (C-31), 129.6s (C-32). 149.0s (C-33), 111.2d (C-34), 111.8d (C-35), 151.3s (C-36), 105.5d (C-37). The following NOE's fully supported the suggested structure, NOE's between H-23 and Me-28, between H-31 and H-37 and Me-29 and between Me-30 and H-34.
Compound **3**, has the formula $C_{36}H_{54}O_5$ (m/z 566.3, M^+), its NMR data were, as for the cyclohexane-oxepane unit, identical with those of compounds **1** and **2**, while for the rest of the molecule they were the same as for this part in compound **4**.
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