Tetrahedron Vol. 49, No. 20, pp. 4275-4282, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Toxicols A-C and Toxiusol - New Bioactive Hexaprenoid Hydroquinones from *TOXICLONA TOXIUS*

SARA ISAACS[a], AMNON HIZI[b] AND YOEL KASHMAN[a]*

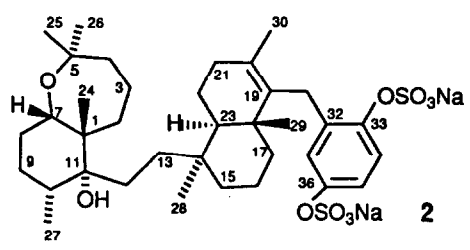
[a]School of Chemistry, [b]School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

(*Received in UK 25 January* 1993)

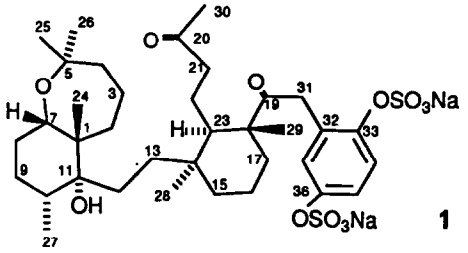
Abstract:
Four new compounds of two unprecedented types, toxicols A-C (3-5) and toxiusol (8) have been isolated from the Red Sea sponge *Toxiclona toxius*. The four compounds are hexaprenoid hydroquinone sulfates consisting of two different polycyclic halves. The structure of all new compounds was determined mainly by 1D and 2D NMR measurements as well as some chemical transformations. Several of the new compounds were found in a preliminary study to inhibit the reverse transcriptase of human immuno deficiency virus (HIV) and to possess activity against *Candida albicans*.

In the course of screening for novel antifungal and HIV - reverse transcriptase inhibitors the organic extract of the Red Sea sponge *Toxiclona toxius* was found to be active. Most recently we have reported the structure elucidation of two new hexaprenoid hydroquinone sulfates, shaagrockol B (1) and C (2), from this sponge[1]. Herewith we wish to report the isolation from the same sponge, of four new C_{36}-compounds belonging to two unprecedented hexaprenoid hydroquinone skeletons.

Compounds of mixed biogenesis, originating partly from mevalonate and partly from benzoic precursors, were earlier isolated from a variety of sponges. An example is disidein[2,3] which was isolated from the sponge *Disidea pallescens* and consists of a pentacyclic sesterterpene condensed with a sulfated hydroquinone. Another example are the sulfated hydroquinone sesqiterpenes: Siphonodictyal[4] C, G and H isolated from *Siphonodictyon coralliphagum*.



2



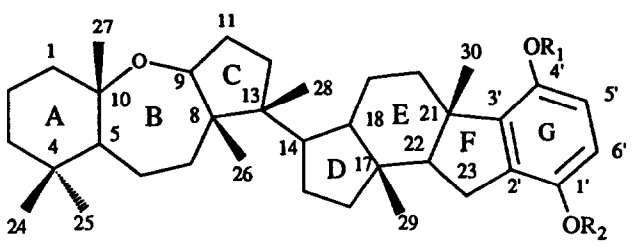
1

The aq. MeOH portion (partitioned with petrolether and carbon tetrachloride) of the MeOH-CH_2Cl_2 1:1 extract of the sponge was fractionated by repeated reverse-phase VLC and HPLC to yield shaagrockol B (1) and C (2)[1], compounds 3-5, and 8 which were named toxicols A-C, and toxiusol, respectively.

The molecular formula of toxicol A (3), $C_{36}H_{52}O_9S_2Na_2$, was determined by its positive and negative FAB-MS taken in the presence of K^+ or Na^+ ions; 809.2 $[M(K_2) + K]^+$, 731.1 $[M(K_2)-K]^-$, 761.2 $[M(Na_2) + Na]^+$ and 715 $[M(Na_2) - Na]^-$. The 36 carbon atoms of 3 were confirmed by the ^{13}C NMR spectrum (7 x Me's, 12 x CH_2's, 7 x CH's and 10 x C's). Furthermore the proton and carbon NMR spectra suggested a 1,

2, 3, 4-tetra-substituted benzene ring (δ_C 118.9d, 119.5d, 137.4s, 144.6s, 145.0s, 146.2s; δ_H 6.95d and 7.14d, J = 9 Hz, two ortho-protons) and an ethereal bridge (δ_C 78.0s and 73.6d and δ_H 3.36dd J=5, 11.5 which does not change upon acetylation). The carbon chemical shifts of the benzene ring[5] proposed two sulfate bearing carbon atoms in the *para* position to each other. Mild acid or basic hydrolysis of the two sulfates of **3** afforded the corresponding p-hydroquinone derivative - **4**, which following oxidation with Ag_2O gave the corresponding p-quinone **6** and by acetylation the 1′, 4′-diacetate **7**. Compound **4** was found to be identical with the second isolated natural product which was named toxicol B. Compound **3** according to its 10 degrees of unsaturation, one benzene ring and an ethereal bridge has to possess five additional carbocyclic rings. The complete planar structure of toxicol A (**3**) was established by careful analysis of its and mainly compound's **4** 2D NMR spectra. The ^{13}C and ^{1}H chemical shifts and the homo HH- and hetero CH- correlations of **4**, based on COSY, TOCSY, HMQC and HMBC experiments, are given in Table 1. From the spectral data it was concluded that compounds **3-5** consist of two, a tri and a tetracyclic part.

The starting point for the structure elucidation of the tetracyclic half of **4** (rings D-G) were HMBC correlations of the benzyl geminal 23-proton pair with the aromatic carbons on one side and C22 on the other. CH-correlations of methyl-30, located on the second benzylic position, with C22 and C3′ as well as C21 and C20 established the pentacyclic ring F and were the continuity to rings E and D. Most important, because of the high overlapping of the methylene proton signals, were also the correlations of the second angular methyl C29 (Table 1). The various one and multi bond CH-correlations (HMQC and HMBC) together with the HH-correlations (COSY and TOCSY experiments) enabled the complete proton and carbon line assignments and thence the structure determination of the tetracyclic half of **4**. In a similar way starting from the ethereal α, α′ (H-9 and Me27) groups the tricyclic structure of rings A-C were established. A crucial contribution to the structure elucidation of these rings (A-C) were the HMBC correlations of the five methyls (Me24-Me28, Table 1). Next, the linkage between rings C and D was determined from the correlation between $\underline{CH}_3$-28 and C14 as well as from NOE's between hydrogen atoms on both parts of **4**. The measured d-NOE's in the NMR spectrum of **4** (where in contrast to **3** protons 23a and 23b separate) also established the all trans A-C and D-F stereochemistry of the toxicols.



3 $R_1 = R_2 = SO_3^-Na^+$
4 $R_1 = R_2 = H$
5 $R_1 = SO_3^-Na^+$, $R_2 = H$
6 $R_1 = R_2 = O=$
7 $R_1 = R_2 = COCH_3$

The proposed all trans stereochemistry of rings A-C is based on the following NOE's: Me24 to Me27; Me25 to H-5; H-9 to H-5, H-11a and H-7a and Me26 to Me27, as well as the absence of NOE's of the angular methyls 26 and 27 to H-9 and H-5, respectively.

NOE's between Me29 and Me30 and H-23b on one side of the tetracyclic half of the molecule and between H-22 and H23a on the other side, together with the absence of NOE's between Me29 and H18 and 22

and between Me30 and H-22 defined the all trans stereochemistry of rings D-F.

Furthermore, NOE's between Me26 and Me28 and between Me28 and Me29 (and the absence of an effect between Me28 and H-14) suggested the stereochemistry of C13 and C14 (see figure), however, this was not enough to establish unequivocally the relative stereochemistry of the two halves of the toxicols. High overlapping of proton signals prevented measurements of NOE's between other protons of the two parts of **4**.

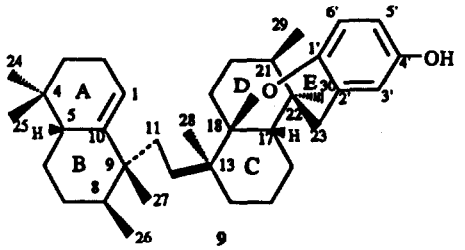
Another related compound which was isolated together with compounds **1-4**, was the mono sulfate derivative of **4** compound **5** which was designated toxicol C. The FABMS of **5** determined it to be the mono sulfate analog of **3** (Experimental) and the site of the sulfate was determined by comparison of its NMR data with those of toxicols A and B, and taking into consideration the substituent effects, i.e., the OH versus the SO_3Na group[5]. Furthermore, HMBC corellations between C3′ and Me30 and between C1′ and C2′ and H23a,b confirmed the $R_1 = SO_3Na$, $R_2 = H$ structure. Hydrolysis of compound **5** in acidic media gave compound **4**.

The fourth compound which was isolated form the sponge was compound **8**, designated toxiusol. HRFABMS provided m/z 723.2948 $[MH]^+$ for a molecular formula of $C_{36}H_{52}O_8S_2Na_2$. The 36 carbon atoms of **8** were confirmed by the ^{13}C NMR spectrum in d_6-DMSO at 70° and in $CDCl_3$ + d_4-MeOH (1:10) (experimental; 7 CH_3's, 11 CH_2's, 9 CH's and 9 C's, a total of $C_{36}H_{52}$). Furthermore, the ^{13}C-NMR spectrum pointed clearly to a high similarity between two decalin systems (*vide infra*).

Toxiusol (**8**) possesses the same hydroquinone disulfate moiety as shaagrockols B and C (**1** and **2**) and in addition it embodies two tri-substituted double bonds (δ_c d_6-DMSO, 70°, 145.7s, 143.0s, 115.5d, 115.0d and δ_H 5.33 brs (2H)). According to its 10 degrees of unsaturation toxiusol has to have 4 additional carbocyclic rings. Under acid hydrolysis conditions compound **8** furnished compound **9**, with minute amounts of the expected hydroquinone derivative. Due to the NMR line broadening in case of **8**, and high overlapping of proton signals even at 70° the structure elucidation of toxiusol was deduced from the structure determination of compound **9**.

Compound **9** was obtained as a colorless, optically active glass. HREIMS provided m/z 518.4149 $[M^+]$ for a molecular formula of $C_{36}H_{54}O_2$. The ^{13}C NMR spectrum (Table 1) displayed 36 resonances; 7 x Me's, 12 CH_2's, 8 x CH's and 9 C's - a total of $C_{36}H_{53}$. Furthermore, the NMR spectra suggested two functionalities; a 2,5-dihydroxy benzyl (δ_c 150.4s (x 2), 135.1s, 123.1d, 116.0d, 113.0d; δ_H 6.43d, J=3 Hz, 6.49dd, J=8, 3 and 6.72d, J=8) and a trisubstituted double bond (Table 1). The only single free OH group (the 54th proton of **9**) determined one of the two phenol oxygens to be attached to the sp^3 oxygen bearing C-atom (δ_c 85.3s). The latter deduction was affirmed by the dominant m/z 395.3698 ($C_{29}H_{47}^+$; M-$C_7H_7O_2$) fragment (100%). A second immense fragment m/z 191.1777 ($C_{14}H_{23}^+$, 77%) characteristic for tetramethyl bicyclodecane segments of terpenoids, suggested such a moiety to be part of **9**, thus requiring two additional carbocyclic rings to fulfill the 10 degrees of unsaturation of **9**.

Homo HH- correlations (Table 1) established five aliphatic segments of **9** (C1-C4, with Me24 and Me25; C5-C8, with Me26; Me27, C11-C12, Me28; C14-C17 and C19-C21, with Me29). Most important for the structure determination were again the HMBC correlations of the various methyl groups to their neighbour C-atoms (Table 1). The latter correlations together with the crucial correlations of H5 and H17 with their neighbour carbon atoms (Table 1) brought to the planar structure determination of compound **9**, a hexaprenyl hydroquinone derivative consisting of two decalins and a benzoxepine moiety.



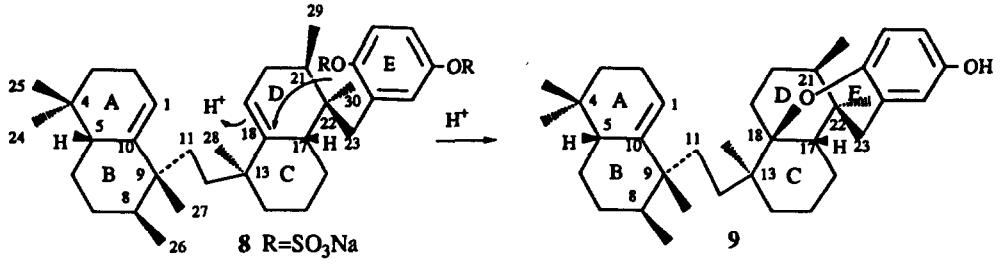
9

Similar $\Delta^{1(10)}$ decalin moieties to the ones found in **9** (and **8**) exist in the marine metabolites macfarlandin C[6] and agelasine A[7] and a benzoxepine-decalin system similar to the one in **9**, was suggested by Schmitz for an acid catalyzed rearrangement product of arenarol[8].

The stereochemistry of **9** was determined from d-NOE measurements; NOE's between Me25 and H2b and H5 require H2b and Me25 to be in a 1, 3-diaxial configuration and both on the same side of rings AB as H5. In addition, a NOE between Me26 and H5, suggested a twisted boat conformation for ring B. Based on this twisted conformation, Me27, because of its strong NOE's with H1 and Me26, has to be on the same side of **9**, as H5. Furthermore, based on NOE's a cis configuration was suggested for rings CD. Namely, NOE's between H23a and H3′ and H17 are only possible for a cis CD ring junction. A cis configuration is also in good agreement with the stronger NOE between Me30 and H23a than with H23b. A strong NOE between the latter proton (H23b) and Me29 determined this methyl to be on the same side as the benzoxepine system. The above rationale requires the benzene ring to be almost perpendicular to ring D. At last, a NOE between Me28 and H3′ asks for Me28 to be equatorial and in the opposite direction to the oxepane system. Thence, the ethyl bridge (C11-C12) has to be axial and on the same side as rings EF. Of interest also was a weak NOE between Me27 and H-6′ suggesting a spatial proximity of the two, however, as this was the only observable effect between the two halves of **9**, their relative configuration could not be established.

After elucidation the structure of compound **9**, the structure of toxiusol (**8**) was self-explanatory. It is proposed that toxiusol possesses the same $\Delta^{1(10)}$ decalin moiety as **9** and instead of the decalin benzoxepine system of **9** it has a disulfated dihydroxybenzyl-$\Delta^{1(10)}$-decalin system.

Protonation of the 18 (19) double bond of toxiusol (**8**) followed by nucleophilic attack by the hydrolysed ortho-hydroxy group is suggested to give the benzoxepine moiety of **9**[9,10].



8 R=SO_3Na **9**

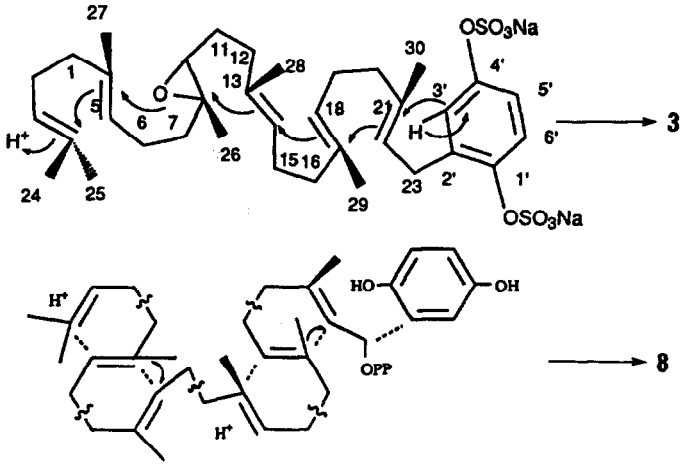
The suggested biogenesis of the two new skeletons of toxicol and toxiusol follows.

The bio-activity of the shaagrockols B and C and the new toxicols (3-5), toxiusol (**8**) and **9** will be published elsewhere.

Table 1: NMR data of toxicol B (4) and compound 9.

	Compound 4				Compound 9			
No.	^{13}C	^{1}H	COSY	HMBC	^{13}C	^{1}H	COSY	HMBC
1a	37.9t	1.22	Me27,1b,2b		116.5d	5.19[d]	H2a,H2b	
1b		1.34						
2a	20.9t	1.34	3a,3b		23.1t	1.30	H2b	
2b		1.40	3a,3b			1.88	H3a,H3b	
3a	40.7t	0.96	3b,Me24		30.9t	0.96	H3b	
3b		1.18				1.28	Me25	
4	35.3s				31.6s			
5	53.5d	1.28		C10,C6,C7,Me27	43.9d	1.42		C10,C6
6a	19.6t	1.24	7a,7b		30.4t	1.00	H6b,H7a,H7b	C5,C7
6b		1.28	7a,7b			1.71	H7a,H7b	
7a	45.0t	0.63	7b		31.4t	1.40	H7b,H8	C9,Me26
7b		1.58				1.46	H8	
8	41.3s				45.0d	1.22	Me26	
9	73.6d	3.38[c]	11a,11b	C11,C10,Me26,Me27	42.4s			
10	78.0s				146.1s			
11a	26.8t	1.23	11b,12a,12b		24.6t	0.81	H11b,H12a,H12b	
11b		1.50	12a,12b			1.45	H12a,H12b,Me27	
12a	38.7t	0.82	12b		26.2t	1.10	H12b,Me28	
12b		1.53				1.22		
13	36.8s				43.2s			
14	57.7d	0.63	15a,15b,18		33.4t	1.12	H14b,H15a	C13,C16,Me28
						1.39		
15a	17.7t	1.32	16a,16b		21.7t	1.22	H15b,H16b	C14
15b		1.38	16a,16b			1.43		
16a	41.8t	0.92	16b		25.2t	1.38	H16b,H17	
16b		1.48				1.68	H17	
17	36.8s				44.9d	1.89		C22,C18,C16,C21,C19
18	61.4d	0.72	19a,19b		85.3s			
19a	18.2t	1.36	19b,20b		26.9t	1.70	H19b,H20a,H20b	
19b		1.48	20b			1.86	H20a,H20b	
20a	37.6t	1.48	20b,Me30		27.9t	1.18	H20b,H21	C21,C19,Me29
20b		2.26				1.26	H21	
21	45.7s				33.6d	1.70	Me29	C22,C20,Me29,C23,Me30
22	64.2d	1.52[c]	Me29,23a,23b	C21,C23,Me30,Me29	35.5s			
23a	24.8t	2.28[c]		C22,C2',C1'	42.2t	2.20[d]	23b	C2',C22,C1',C17,C21,Me30
23b		2.45		C22,C2',C1',C3',C21		2.91[d]		C2',C22,C1',C17,C21,Me30
24	20.8q	0.63	Me25	C4,Me25,C3,C5	28.4q	0.78	Me25	C4,C5,Me25
25	32.7q	0.76		C4,C3,C5,Me24	27.3q	0.56		C4,C5,Me24
26	14.0q	0.64		C8,C9,C7	16.5q	0.83		C8,C9,C7
27	22.5q	0.99		C10,C5,C1	23.5q	1.00		C9,C10,C8,C11
28	15.9q	0.73		C13,C12,C14	20.5q	0.92		C13,C15,C14,C12
29	16.9q	0.87		C17,C16,C18,C22	15.4q	0.79		C21,C22,C20
30	19.9q	0.94		C20,C21,C22,C3'	29.0q	0.94		C22,C21,C23,C17
1'	146.0s				150.4s			
2'	129.4s				135.1s			
3'	140.6s				116.0d	6.43[d]		C2',C5',C23,C1'
4'	145.0s				150.4s			
5'	114.2d	6.26[c]			113.0d	6.49[d]		C4',C3'
6'	112.7d	6.26[c]			123.1d	6.72[d]		C1',C2'

a,b: a - the high field and b - the low field proton in a geminal pair, c: $J_{9,11a,11b}$ = 5, 11.5, $J_{23a,23b}$ = 14.5, $J_{23a,22}$ = 6, $J_{23b,22}$ = 13, d: $J_{1,2a}$ = 4, $J_{1,2b}$ = 4, $J_{23a,23b}$ = 14.5, $J_{Me26,8}$ = 7, $J_{Me29,21}$ = 6.5, $J_{3',5'}$ = 3.5, $J_{5',6'}$ = 8.



Suggested biogenesis of the toxicols and toxiusol

EXPERIMENTAL

Spectral Analysis - ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 500 spectrometer operating at 500 and 125 MHz for ^{1}H and ^{13}C respectively. IR spectra were recorded on a Nicolet 205 FT-IR spectrometer.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a 10 cm microcell. Mass spectra were measured with a Finnigan TSQ-70 spectrometer.

Collection, Extraction and Isolation - The sponge *Toxiclona toxius* was collected at depths of 15-20m near Shaag Rock, in the Gulf of Suez, the Red Sea. The specimens were frozen immediately after collection. The freeze dried organism was then extracted with MeOH: CH_2Cl_2 (1:1). The latter extract (1.2g) was partitioned between aq. MeOH and petrolether and CCl_4. The aq. MeOH fraction (0.5g) was chromatographed on RP-18, eluted with MeOH; H_2O 1:3 to 9:1 to afford compounds **1**, **2**, **3**, **8**, **5** (in order of polarity). Compound **4** came out with MeOH-$CHCl_3$ 9:1. Compounds **1-3** were purified on RP-18 HPLC (10 mg each), compound **4** on silica HPLC (petrolether-ethylacetate, 8:2, 3 mg), compound **5** on Diol (MeOH-ethylacetate, 2:8, 5 mg) and compound **8** on Sephadex LH-20 (MeOH-$CHCl_3$, 1:1, 80 mg).

Compound **3** (toxicol A). Colorless oil; $C_{36}H_{52}O_9S_2Na_2$; $[\alpha]_D$ + 35° (c 1, MeOH). ν_{max}^{neat} 2937, 1476, 1436, 1257, 1103, 958, 852 cm^{-1}; mass spectrum (FABMS, m/z) 809.2 $[M(K_2) + K]^+$, 90%), 731.1 ([$M(K_2)$ - K], 100%), 715.0 ([$M(Na_2)$ - Na]$^+$, 30%), 613.1 (100%), 431 (99%); ^{1}H NMR ($CDCl_3$+CD_3OD (1:10)): 3.36 (1H, dd J=5, 11.5, H9), 2.42 (1H, m, H20b), 2.48 (1H, t, J=14, H23a), 2.68 (1H, dd, J=6, 14, H23b), 0.64 (3H, s. Me24), 0.79 (3H, s, Me25), 0.65 (3H, s, Me26), 1.10 (3H, s, Me27), 0.74 (3H, s, Me28), 0.87 (3H, s, Me29), 0.99 (3H, s, Me30), 7.14 (1H, d, J=9, H5'), 6.95 (1H, d, J=9, H6'); ^{13}C NMR ($CDCl_3$-CD_3OD (1:10)): 38.0 (t, C1), 21.0 (t, C2), 40.7 (t, C3), 35.4 (s, C4), 53.5 (d, C5), 19.6 (t, C6), 45.0 (t, C7), 41.4 (s, C8), 73.6 (d, C9), 78.0 (s, C10), 26.8 (t, C11), 38.7 (t, C12), 36.8 (s, C13), 57.6 (d, C14), 17.7 (t, C15), 41.8 (t, C16), 37.0 (s, C17), 61.2 (d, C18), 18.2 (t, C19), 37.1 (t, C20), 47.3 (s, C21), 64.0 (d, C22), 25.7 (t, C23), 20.3 (q, C24), 32.7 (q, C25), 14.0 (q, C26), 22.5 (q, C27), 15.9 (q, C28), 16.9 (q, C29), 19.7 (q, C30), 146.2 (s, C1'), 137.4 (s, C2'), 144.6 (s, C3'), 145.0 (s, C4'), 118.9 (d, C5'), 119.5 (d, C6').

Compound **4** (1',4' dedisulfate derivative of toxicol A): white powder, $C_{36}H_{54}O_3$, $[\alpha]_D$ - 16° (c 0.04, MeOH: CH_2Cl_2 (2:1)); ν_{max}^{neat} 3428, 2949, 2857, 1450, 1383, 1071 cm^{-1}; mass spectrum (EI, m/z) 534.3 (M^+, 100%), 518.4 (44%), 336.9 (27%), 235.9 (13%), 190.9 (63%); ^{1}H and ^{13}C NMR ($CDCl_3$ + CD_3OD (10:1)) see Table 1.

Hydrolysis of toxicol A (3) under acidic conditions. A solution of toxicol A (2 mg) in 1% TFA in MeOH (2 ml) was stirred at 25°C for 5 hours, the solvent was then evaporated to give 1′, 4′-dedisulfate toxicol A which was purified on a silica-H column (petrolether - ethylacetate 4:1)). Compound 3 was found to be identical, by TLC analysis and ^{1}H NMR, with, the natural product, toxicol B.

Hydrolysis of toxicol A (3) under basic conditions. A solution of toxicol A (2 mg) in a mixture of dioxane (1 ml) pyridine (1 ml) was refluxed for 5 hours. The solvent was then evaporated to give 1′, 4′ dedisulfate toxicol A which was purified on a silica-H column (petrolether - ethylacetate, 1:4). The product was found to be identical with toxicol B.

Compound 5 (toxicol C). Colorless oil; $C_{36}H_{53}O_6SNa$; $[\alpha]_D$ + 21° (c 0.07 MeOH); ν_{max}^{neat} 2937, 2844, 1217, 1051, 805 cm^{-1}; mass spectrum (FABMS, m/z) 675.2 [(MK)$^+$, 20%], 659.3 [(MNa)$^+$, 100%], 557.4 [(MH$^+$ - SO_3), 40%]; ^{1}H NMR (CD_3OD): 3.52 (1H, dd, J=6.5, 11.5, H9), 2.42 (1H, dd, J=16.5, 18, H23a), 2.57 (1H, dd, J=6.5, 18, H23b), 0.76 (6H, s), 0.86 (3H, s), 0.91 (3H, s), 1.01 (3H, s), 1.12 (6H, s), 6.45 (1H, brs), 7.18 (1H, brs); ^{13}C NMR ($CDCl_3$ + CD_3OD): 14.8 (q), 16.7 (q), 17.7 (q), 18.4 (t), 19.0 (t), 20.5 (t), 21.1 (q), 21.4 (q), 21.8 (t), 23.1 (q), 25.6 (t), 27.7 (t), 30.3 (t), 33.3 (q), 36.2 (s), 37.7 (s), 37.8 (s), 38.1 (t), 38.8 (t), 39.5 (t), 41.5 (t), 42.3 (s), 42.8 (t), 45.9 (t), 54.4 (d), 58.6 (d), 62.2 (d), 65.1 (d), 74.7 (d), 77.1 (s), 113.5 (d), 120.2 (d), 130.0 (s), 141.7 (s), 146.8 (s), 150.6 (s).

Oxidation of toxicol B (4) to compound 5. To a solution of 5 mg of 4 in 50 ml of dry ether silver oxide (50 mg) was added and the heterogeneous mixture was stirred overnight at room temperature. The reaction mixture was then filtered and the solvent evaporated to give after chromatography on a silica-H column (petrolether, 3% ethylacetate) 4 mg of compound 6 (the p-quinone derivative of toxicol B); brown oil; $C_{36}H_{52}O_3$; $[\alpha]_D$ - 10° (c 0.2, CH_2Cl_2); ν_{max}^{neat} 2937, 2851, 1742, 1456, 1383, 1078, 739, 706 cm^{-1}; mass spectrum (ClMS, m/z) 533.4 [(MH)$^+$, 100%], 447.3 (10%), 391.3 (9%), 334.8 (12%), 279.1 (8%); ^{1}H NMR ($CDCl_3$): 3.45 (1H, dd, J=4.5, 11, H9), 2.31 (1H, dd, J=6, 17, H23a), 2.48 (1H, dd, J=12.5, 17, H23b), 0.76 (3H, s), 0.77 (3H, s), 0.85 (3H, s), 0.91 (3H, s), 0.97 (3H, s), 1.06 (3H, s), 1.12 (3H, s); ^{13}C NMR ($CDCl_3$): 38.1 (t, C1), 21.3 (t, C2), 41.0 (t, C3), 35.7 (t, C4), 53.8 (t, C5), 19.8 (t, C6), 45.2 (t, C7), 41.6 (s, C8), 73.4 (d, C9), 27.2 (t, C11), 38.2 (t, C12), 36.7 (s, C13), 57.8 (d, C14), 17.6 (t, C15), 41.8 (t, C16), 36.0 (s, C17), 61.3 (d, C18), 18.0 (t, C19), 37.0 (t, C20), 45.0 (s, C21), 62.5 (d, C22), 23.0 (t, C23), 21.0 (q, C24), 33.0 (q, C25), 14.3 (q, C26), 23.0 (q, C27), 16.2 (q, C28), 17.2 (q, C29), 19.3 (q, C30), 135.9 and 136.7 (d, C5′ and C6′).

Acetylation of toxicol B (4) to the diacetate 7. A solution of 10 mg of toxicol B (4) in a mixture of 1:1 dry pyridine and acetic anhydride (1 ml) was allowed to stand overnight at room temperature. The solvent was then evaporated to give 8 mg of compound 7 which was purified on a silica-H column (petrolether, 5% ethylacetate). Compound 7; white powder, $C_{40}H_{58}O_5$; $[\alpha]_D$ - 23° (c 0.2, CH_2Cl_2); ν_{max}^{neat} 2937, 2864, 1768, 1489, 1376, 1197, 1171, 1031, 746 cm^{-1}; mass spectrum (HREIMS, m/z) 618.4392 (M$^+$, 30%), LREIMS: 576.1 (M$^+$ - $COCH_3$, 30%), 534.5 (M$^+$ - $COCH_3$, 30%), 529.2 (40%), 421.2 (100%), 382.5 (30%), 367.4 (70%), 175.3 (80%); ^{1}H NMR ($CDCl_3$): 3.44 (1H, dd, J=5, 11.5, H9), 2.12 (1H, m, H20b), 2.44

(1H, brs, H23a), 2.46 (1H, d, J=3.5, H23b), 0.76 (3H, q, Me24), 0.92 (3H, q, Me25), 0.77 (3H, q, Me26), 1.11 (3H, q, Me27), 0.84 (3H, q, Me28), 0.98 (3H, q, Me29), 1.06 (3H, q, Me30), 6.81 (1H, d, J=9), 6.78 (1H, d, J=9), 2.25 (3H, s, $OCOCH_3$), 2.26 (3H, s, $OCOCH_3$); ^{13}C NMR ($CDCl_3$): 38.2 (t, C1), 21.3 (t, C2), 41.0 (t, C3), 35.7 (s, C4), 53.8 (d, C5), 19.8 (t, C6), 45.2 (t, C7), 41.7 (t, C8), 73.4 (d, C9), 77.6 (s, C10), 27.3 (t, C11), 38.9 (t, C12), 37.1 (s, C13), 57.8 (d, C14), 17.7 (t, C15), 42.0 (t, C16), 37.1 (s, C17), 61.2 (d, C18), 18.3 (t, C19), 37.0 (t, C20), 47.3 (s, C21), 63.9 (d, C22), 26.0 (t, C23), 21.0 (q, C24), 33.0 (q, C25), 14.4 (q, C26), 23.1 (q, C27), 16.2 (q, C28), 17.1 (q, C29), 21.1 (q, C30), 145.0 (s, C1′), 143.0 (s, C2′), 136.6 (s, C3′), 146.3 (s, C4′), 119.9 (d, C5′), 121.7 (d, C6′).

Compound 8 (toxiusol A); colorless foaming oil; $C_{36}H_{52}O_8S_2Na_2$, $[\alpha]_D$ + 9° (c 0.3, MeOH); ν_{max}^{neat} 2924, 2844, 1485, 1434, 1270, 1219, 1032, 1020, 947, 850 cm^{-1}, mass spectrum (HRFABMS) 723.2948 $(MH)^+$, LRFABMS: 745.1 $(MNa)^+$, 643.1 (MH^+-SO_3), 540.2 $(MH^+ - Na^+ - 2SO_3)$; ^1H NMR (d_6-DMSO, 340°K): 0.62 (3H, s), 0.79 (3H, d, J=7), 0.85 (3H, s), 0.91 (3H, s), 0.95 (3H, d, J=7), 0.96 (3H, s), 1.00 (3H, s), 5.33 (2H, brs), 6.89 (1H, dd, J=2.8, 8.7), 6.94 (1H, d, J=2.8), 7.22 (1H, d, J=8.7); ^{13}C NMR ($CDCl_3$ + CD_3OD (1:1)): 14.9 (q), 16.0 (q), 19.8 (q), 21.6 (t), 23.0 (t), 23.0 (q), 23.5 (t), 24.5 (t), 26.2 (q), 26.6 (t), 27.4 (q), 27.7 (q), 29.4 (t), 29.7 (t), 30.0 (t), 31.0 (t), 31.5 (t), 32.3 (s), 34.6 (t), 38.4 (s), 39.0 (s), 39.2 (d), 40.4 (t), 41.8 (s), 43.3 (d), 44.7 (d), 49.5 (d), 116.1 (d, 2C), 119.6 (d), 122.3 (d), 125.1 (d), 134.0 (s), 143.0 (s), 146.3 (s), 148.2 (s, 2C).

Hydrolysis of Toxiusol A (8) under acidic conditions. A solution of 1% TFA in MeOH (5 ml) was added to toxiusol A (20 mg). After being stirred at 25°C for 5 hours the solvent was evaporated to give the 1′, 4′ dedisulfate of toxiusol A and compound 9 which was purified on a silica-H column (petrolether, 2% ethylacetate). Compound 9: colorless oil; $C_{36}H_{54}O_2$; $[\alpha]_D$ + 34° (c 0.8, CH_2Cl_2); ν_{max}^{neat} 3394, 2958, 2912, 1485, 1451, 1366, 1270, 1219, 1202 cm^{-1}; mass spectrum (HREIMS m/z, %) 518.4149 (M^+, 14%), 395.3698 ($(M-C_7H_7O_2)^+$, 100%), 191.1799 ($C_{14}H_{23}^+$, 77%) 123.0463 ($C_7H_7O_2^+$, 25%); ^1H and ^{13}C NMR ($CDCl_3$)see Table 1.

Acknowledgement: This work was supported by the National Institute of Allergy and Infectious Diseases, grant number RO.1 31790. We are grateful to Professor A. Mandelbaum, the Mass Spectrometry Center, The Technion, Haifa, for the FABMS, and Dr. Y. Benayahu for the collection of the sponge.

REFERENCES

1. Kashman, Y.; Isaacs, S.; Tetrahedron Lett., 1992, 33, 2227-2230.
2. Cimino, G.; De Rosa, S.; De Stefano, S.; Puliti, R.; Strazzullo, G.; Mattia, C.A.; Mazzarella, L.; Tetrahedron, 1987, 43, 4777-4784.
3. Cimino, G.; De Luca, P.; De Stefano, S.; Minale, L.; Tetrahedron, 1975, 31, 271-275.
4. Faulkner, D.J.; Sullivan, B.W.; J. Org. Chem., 1986, 51, 4568-4573.
5. Ragan, M.A.; Can. J. Chem., 1978, 56, 2681-2685.
6. Faulkner, D.J.; Molinski, F.T.; Cun-heng, H.; Van-Duyne, G.D.; Clardy, J.; J. Org. Chem., 1986, 51, 4564-4567.
7. Nakamura, H.; Wu, H.; Ohizumi, Y.; Hirata, Y.; Tetrahedron Lett., 1984, 25, 2989-2992.
8. Schmitz, F.J.; Lakshmi, V.; Powell, D.R.; Van-der Helm, D.; J. Org. Chem., 1984, 49, 241-244.
9. Faulkner, D.J.; Salva, J.; J. Org. Chem., 1990, 55, 1941-1943.
10. Cimino, G.; De Stefano, S.; Minale, L.; Riccio, R.; Hirtosu, K.; Clardy, J.; Tetrahedron Lett.,