Two New C_{22} 1,2-Dioxane Polyketides from the Marine Sponge *Acarnus* cf. *bergquistae*

Tesfamariam Yosief,[†] Amira Rudi,[†] Yisak Wolde-ab,[‡] and Yoel Kashman*[,†]

School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel, Department of Chemistry, Asmara University, Asmara, Eritrea

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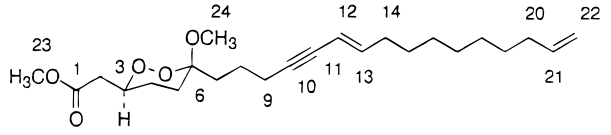
Two new cyclic peroxide-containing polyketide C_{22} methyl esters, peroxyacarnoic acid methyl esters A and B, **1** and **2**, have been isolated from the Red Sea marine sponge *Acarnus* cf. *bergquistae*. Both **1** and **2** are proposed to contain a single 1,2-dioxane ring, an eneyne functionality, and a terminal double bond or triple bond, respectively. The structures were elucidated mainly through 1D and 2D NMR spectral analysis.

In search of new biologically active substances from marine organisms,[1−3] we have isolated two new oxygen heterocycles, compounds **1** and **2**, from the Red Sea sponge *Acarnus* cf. *bergquistae* van Soest & Hooper, 1993 collected off Dahlak Island, Eritrea, from a depth of 2 m. Cyclic peroxides have been isolated from marine organisms and particularly from marine sponges.[1−6] Many of the peroxy compounds exhibit antimicrobial, ichthyotoxic, and cytotoxic activities.[1−8] The $CHCl_3$ extract of the present sponge exhibited cytotoxicity against P-388, A-549, and HT-29 tumor cells with an IC_{50} of 0.1 μg/mL.[9,10] Because of the instability of compounds **1** and **2**, however, the latter were not tested in the pure state.



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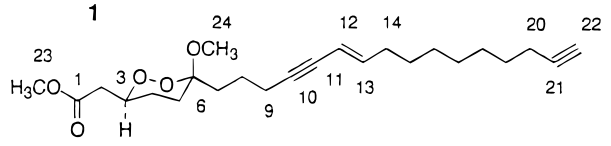

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The $CHCl_3$-soluble portion of the combined MeOH and MeOH−$CHCl_3$ (1:1) extracts of the sponge was subjected to repeated Sephadex LH-20 and Si gel chromatographies to provide pure peroxyacarnoic acid methyl esters A (**1**, 20 mg) and B (**2**, 15 mg) as colorless oils.

For compound **1** a molecular formula of $C_{24}H_{38}O_5$ could be established by EIMS and NMR data. Comprehensive 1D and 2D NMR studies, summarized in Table 1, enabled the determination of the complete structure of **1**. The NMR data established the following fragments (**a**−**d**):

a $H_3^{23}COCO^{1}CH_2^{3}CH(O-)-$ **b** $-CH_2^{8}CH_2C{\equiv}CCH{=}CHCH_2^{15}CH_2-$

c $-CH_2^{19}CH_2CH{=}CH_2^{22}$ **d** $-O-C^{6'}(OCH_3^{24})-O-$

The resonances attributed to the methyl ester moiety in **a** were readily recognizable [δ 3.70 s (3H), 170.5 s, and 51.9 q]. Furthermore, COSY and HMBC experiments established the vicinity of a $CH_2CH(O^-)$ unit (δ 2.51 dd, 2.38 dd, H-2,2′, and 4.49 dddd H-3). The structure of fragment **b** was based on the ^{13}C resonances of the conjugated eneyne moiety, δ 89.1 s, 78.9 s, 110.8 d, and 141.9 d, and the vicinal methylenes δ 19.3 and 32.8 t for CH_2-9 (next to the triple bond) and CH_2-14 (in the allylic position), respectively, and was also in full agreement with enyne UV absorptions (see Experimental Section). Extension of the latter six-carbon unit by an additional methylene on each side was deduced from the multiplicity of methylenes 9 and 15, a triplet and a double triplet, respectively. Characteristic chemical shifts and 2D NMR data also established the structures of functionalities **c** and **d** as a terminal allyl group and a methoxy lactol [δ 2.05 dt (2H), 5.82 ddt (1H), 4.93 dq (1H), and 5.00 br d (1H); and δ 102.5 s and 48.5 q]. The multiplicity of CH_2-20 (dt, J = 7, 7 Hz) identified the neighboring group as CH_2-19. The above four fragments (**a**−**d**) accounted for 18 of the 24 carbon atoms of **1**, leaving six methylenes to be identified. The presence of only two additional oxygen-bearing carbons (at δ 76.7 d and 102.5 s) to those of the CO_2CH_3 carbons, combined with the absence of exchangeable resonances in the ^1H NMR spectrum and lack of hydroxyl absorptions in the IR spectrum, required that the remaining two oxygen functionalities belong to a cyclic peroxide. Comparison of the spectral data of **1** with the NMR data of the peroxyplakoric acid methyl esters[5] confirmed unequivocally that **1** possesses the same cyclic peroxyketal moiety; C-3 of **a** must therefore be linked to C-6 of **d** through a peroxy linkage and a $-CH_2CH_2-$ group to form a 1,2-dioxane ring. The position of the last four CH_2 groups to be accounted for was established by a TOCSY experiment. This experiment correlated the following protons: H-3 to 2,2′, 4,4′, and 5,5′; H_2-9 to 7,7′

* To whom correspondence should be addressed. Tel: +972-3-6408419. Fax: +972-3-6409293. E-mail: kashman@ccsg.tau.ac.il.
† Tel Aviv Univeristy.
‡ Asmara University.

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Table 1. NMR Data for Compounds **1** and **2** (125 and 500 MHz)[a,b]

	1				2	
position	δ_C ($CDCl_3$)	δ_H[c] ($CDCl_3$)	COSY (H−H)	HMBC (H−C)	δ_C ($CDCl_3$)	δ_H[c] ($CDCl_3$)
1	170.5 s				170.5 s	
2	38.2 t	2.51 (dd, 16.0, 7.5)	2b, 3	1, 3, 4	38.2 t	2.50 (dd, 16, 7.5)
		2.38 (dd, 16.0, 5.5)	2a, 3	1, 3, 4		2.40 (dd, 16, 5.5)
3	76.7 d	4.49 (dddd, 11.2, 7.5, 5.5, 2.0).	2a, 2b, 4a	1, 2a, 4	76.7 d	4.50 (dddd J = 11.2, 7.5, 5.5, 2.0)
4	25.1 t b_{ax}	1.78 (m)	3, 5a, 5b	2, 3, 5, 6	25.1 t	1.80 (m)
	a_{eq}	1.66 (m)	3, 5a, 5b	3, 5		1.62 (m)
5	30.3 t a_{ax}	1.87 (m)	4a, 4b, 5b	3, 4, 6	30.4 t	1.86 (m)
	b_{eq}	1.63 (m)	4a, 4b, 5a	3, 4, 6		1.65 (m)
6	102.5 s				102.5 s	
7	28.75 t[d]	1.39 (m)	8	6, 8	28.6 t[d]	1.41 (m)
		1.32 (m)	8	6, 8		1.33 (m)
8	28.96 t[d]	1.52 (2H, quin, 7.0)	7, 9	7, 9, 10	28.7 t[d]	1.52 (2H, quin, 7.0)
9	19.3 t	2.28 (2H, t, 7.0)	8	8, 10−13	19.3 t	2.30 (2H, dt, 7.0, 2)
10	89.1 s				89.1 s	
11	78.9 s				78.9 s	
12	110.8 d	5.47 (br d 15.8)	9, 13, 14	10, 13, 14	110.8 d	5.50 (d quin, 15.8, 1.5)
13	141.9 d	6.00 (dt 15.8, 7.0)	9, 12, 14	11, 14, 15	141.9 d	6.00 (dt, 15.8, 7)
14	32.8 t	2.11 (2H, q, 7.0)	13, 15	11−13, 15, 16	32.8 t	2.11 (2H, q, 7.0)
15	22.2 t	1.42 (2H, m)		14, 16	22.2 t	1.42 (2H, m)
16	32.0 t	1.65 (m)		14, 15, 17	32.0 t	1.65 (2H, m)
		1.35 (m)		14, 15, 17		1.35 (2H, m)
17	28.82 t[d]	1.40 (2H, m)		16, 18	28.7 t[d]	1.40 (2H, m)
18	28.85 t[d]	1.40 (2H, m)		17, 19	28.6 t[d]	1.40 (2H, m)
19	28.96 t[d]	1.31 (2H, m)	18, 20	18, 20	28.4 t[d]	1.53 (2H, m)
20	33.75 t	2.05 (q, 7.0)	19, 21	18, 19, 21, 22a	18.3 t	2.18 (2H, td, 7.0, 2.7)
21	139.15 d	5.82 (ddt, 17, 10, 7)	20, 22a, 22b	19, 20	84.0 s	
22	114.1 t	4.93 (dq, 10,1)	20, 21	20, 21	68.1 d	1.94 t (2.7)
		5.00 (br d, 17)	21	20, 21		
23	51.9 q	3.70 (3H, s)		1	51.9 q	3.70 (3H, s)
24	48.5 q	3.25 (3H, s)		6	48.5 q	3.25 (3H, s)

[a] C/H correlations were established by interpretation of HMQC spectra. [b] Assignments were establishsed by interpretation of the COSY, TOCSY, and HMBC spectra. [c] J values are given in Hz; a, b are the geminal pair labels. [d] Interchangeable.

and 8; H_2-12 to 9, 14, 15, and 16; H-13 to 14, 15, and/or 17 and 16; H-22 to 18, 19, and 20; and H-21 to 19, 20, and 22—thereby establishing the locations of all six residual methylenes (CH_2-4,5,7,16,17, and 18) and completing the gross structure of compound **1**.

A coupling constant of 15.8 Hz between H-12 and H-13 established the *E* configuration of the 12,13 double bond and the coupling constants of H-3 (δ 4.50 dddd, $J_{3,2}$ = 7.5, $J_{3,2'}$ = 5.5, $J_{3,4b_{ax}}$ = 11.2, and $J_{3,4a_{eq}}$ = 2 Hz) determined the relative stereochemistry of C-3. This suggested configuration, with an axial H-3, is in full agreement with NOEs between H-2a and 2b and H-4b_{ax} and with the proton and carbon chemical shifts of C-4 and C-5 in comparison with the two 3-epimers of the peroxyplakoric acid methyl esters.[5] In peroxyplakoric acid A methyl ester, with an equatorial CH_3OCOCH_2 group, the H-4a and H-4b protons resonate at δ 1.52 and 1.85, as opposed to δ 1.42 and 2.10 in the 3-axial epimer. Similarly, C-4 resonates at δ 30.0 ppm, as in **1**, as opposed to 26.5 ppm in the axial epimer due to the γ-effect of the methyl ester. The suggested relative stereochemistry of the second chiral center of **1**, C-6, was based mainly on the ^{13}C resonance of C-6 at δ 102.5 ppm[5] and a weak NOE between the axial OCH_3 group and H-4ax.

The structure of compound **2**, $C_{24}H_{36}O_5$, is closely related to that of compound **1** (Table 1), with the only difference being the replacement of the terminal alkene unit of **1** by a terminal alkyne unit in **2**. Most significant for the structure elucidation of **2,** in addition to the disappearance of the 21,22-proton signals**,** was the replacement of the δ 33.75 triplet α to the terminal methylene in **1** by a resonance at δ 18.3 ppm, characteristic for a propargylic methylene. This methylene (CH_2-20) appeared as a double triplet at δ 2.18 with couplings of 7 and 2.7 Hz with CH_2-19 and H-22 (propargylic coupling), respectively. Also significant was the 3280 cm^{-1} absorption of **2** instead of 890 cm^{-1} of **1**, in the IR spectrum.

After the structures of **1** and **2** were elucidated, it became clear that the crude extract also contained the free acids, which, because of instability, could not be purified.

1,2-Dioxenes are well documented for marine natural products[6] and are assumed to be obtained by a cycloaddition of oxygen to conjugated dienes. 1,2-Dioxanes, on the other hand, are less abundant but not unknown, with muqubilin,[1] plakortis dioxanes,[2] peroxyplakoric acid methyl esters,[5] and sigmosceptrellin[4] as representative examples.

A second collection of the *Acarnus* sponge afforded, in addition to the methyl esters **1** and **2,** the corresponding free carboxylic acids. Due to their instability, they could not be purified; however, after methylation with CH_2N_2 they gave, after repeated chromatographies, the corresponding methyl esters (**1** and **2**).

Based on the negative α_D value, it is tentatively suggested that **1** and **2** possess the same absolute configuration (3*S*,6*R*) as the peroxyplakoric acid methyl esters.[5]

Experimental Section

General Experiment Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker ARX-500

spectrometer. Mass spectra were recorded on a Fisons Autospec Q instrument.

Biological Material. The sponges were collected in Dahlak Island, Eritrea, by scuba at a depth of 2 m during October 1995. A voucher sample is deposited at the Zoological Museum, Tel Aviv University (no. ET-93, SP 25100). The sponge is, or is closest to, *Acarnus* cf. *bergquistae* van Soest and Hooper, 1993 (Demospongiae, order Poecilosclerida, family Iophonidae). Although the spicules match, the habit and skeletal structure are more elaborate than authentic *A.* cf. *bergquistae.* Thus, it is also possible that this sponge is a new species.

Extraction and Isolation. The specimen of the sponge (200 g, wet wt) was immersed in MeOH in the field and then reextracted twice with $CHCl_3$−MeOH (1:1), affording a brown gum in the $CHCl_3$ fraction (400 mg). 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 eluting with hexane−EtOAc to afford compounds **1** and **2** (ca. 20 mg and 15 mg, respectively).

Compound 1: oil; $[\alpha]_D$ −26° (*c* 0.2, $CHCl_3$); λ_{max} 204 and 228 (log ϵ 4.0) (EtOH); IR υ_{max} (neat) 2150, 1710, 1460, 890 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS, *m*/*z* 375 (M−OCH_3, 1.3%), 374 (M−O_2), 1.2%), 358 (M−48, 3.5%), 343 (18%), 169 (100%), HREIMS, *m*/*z* 375.2486 [calcd for $C_{23}H_{35}O_4$ (M−OCH_3), 375.2490].

Compound 2: oil; $[\alpha]_D$ −26° (*c* 0.2, $CHCl_3$); λ_{max} 204 and 228 (log ϵ 4.0) (EtOH); IR υ_{max} (neat) 3280, 2150, 1410, 1460 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS, *m*/*z* 373(M−OCH_3, 1%), 372(1%), 356 (M-46, 3%).

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(10) Instability of **1** and **2** has prevented, so far, their further bioactivity evaluation.

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