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Hanishenols A-B, Novel Linear or Methyl-Branched Glycerol Enol Ethers of the Axinellid Sponge *Acanthella carteri* (= *Acanthella aurantiaca*) from the Hanish Islands, Southern Red Sea

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Abstract: The axinellid sponge *Acanthella carteri* Dendy, 1889 (= *Acanthella aurantiaca* Keller, 1889) from the Hanish Islands, Yemen, on EtOH extraction followed by FC and HPLC purification gave the first example of branched glycerol enol ether, hanishenol B (**3**) alongside a major unbranched analogue, hanishenol A (**1**). Their structures were elucidated from NMR and MS spectra and through the ozonolysis product of **1**, while the absolute configuration was assigned from exciton coupling with the dibenzoate derivative **2**.

In the sole examples of glycerol enol ethers from terrestrial organisms the enol ether functionality is part of a polyolefinic system, which makes them highly reactive; they were isolated from the faeces of humans liable of colon cancer.[1] In the sea glycerol enol ethers ending in a long, saturated alkyl chain were found in anthozoans,[2] sponges (either free[3a] or in the phospholipid fraction[3b]) and brachiopods (unusually odd chain).[4] Sponges gave also glycerol enol ethers ending in an enyne[5a] or dienyne[5b] long chain; unusually for enol ethers, the first showed resistance to acid hydrolysis and singlet oxygen attack while proving sensitive to triplet oxygen.[5a]

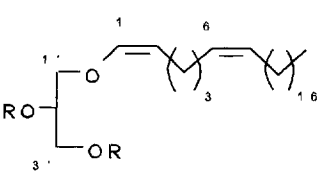
We report here on a novel type of glycerol enol ether, hanishenol B (**3**), and its congener, hanishenol A (**1**), isolated from the axinellid sponge *Acanthella carteri* Dendy, 1889 (= *Acanthella aurantiaca* Keller, 1889) from the Hanish Islands, in the southern Red Sea.

Results and Discussion

The glyceryl portion of hanishenol A (**1**) found evidence in NMR spectra: the H_2-1' m and the H_2-3' dd are correlated to δ_C 73.22 and 63.61 t's, respectively, while the H-2' *pseudo* quintet is coupled to both above systems and is correlated with a δ_C 70.55 d (Table). The enol ether double bond system found evidence in δ_H 5.93 and 4.40 dt's, these two protons having $J_{1,2}$ = 6.3 Hz, typical for *Z* configuration, and being correlated to δ_C 144.45 and 108.37 d's, respectively. The length of the linear chain was deduced from the composition $C_{27}H_{52}O_3$ for $M^{+\cdot}$ (HR-EI-MS on *m z* 424); the position of the isolated olefinic bond (δ_H 5.34 m coupled to δ_C 129.89 d) resulted from ozonolysis of **1** to give 1-octadecanal,[6] while its *Z* configuration was based on upfield resonance, δ_C 27.20 t, for C-5 and C-8.[7]

HR-EI-MS on *m z* 438 molecular ion of composition $C_{28}H_{54}O_3$ for minor hanishenol B (**3**) suggested the presence of an additional methyl group. That this must be at an olefinic carbon was indicated by long-range coupling of the methyl group (Table), while its location at just C-2 was deduced from (a) the absence of both the δ_H 4.40 dt and δ_C 108.37 d of **1**, replaced by δ_C 115.83 s and (b) lack of 6.3 Hz vicinal coupling for the δ_H 5.82

signal. *E*-configuration at the enol ether double bond was proven by NOE enhancement between H-1 and H_2-3, lack of NOE between H-1 and Me-2, and upfield resonance,[8] δ_C 12.93, of Me-2.



1 R = H

2 R = C(O)C_6H_5

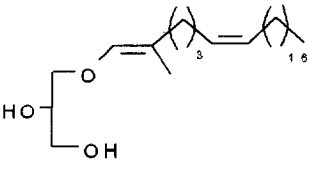


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Table ^{1}H-and ^{13}C-NMR Data ($CDCl_3$, δ in ppm, *J* in Hz) of Hanishenol A (**1**) and Hanishenol B(**3**)

Atom	**1**		**3**	
	δ_H	δ_C	δ_H	δ_C
1	5.93 (dt, 6.3, 1.5)	144.45 d	5.82 (q, 1.2) [a]	139.98 d
2	4.40 (dt, 6.3, 7.5)	108.37 d	--	115.83 s
3	2.04 (qd, 7.3, 1.6)	23.95 t	1.84 (br. t, 6.9)	33.78 t
4	1.30 (quint, 6.9)	28.98 t	1.31 (quint, 6.9))	27.93 t
5	2.02 (m)	27.20 t	2.02 (br. t; 6.9) [b]	27.20 t
6 and 7	5.34 (m)	129.89 d	5.34 (m)	129.89 d
8	2.02 (m)	27.20 t	1.98 (br. t; 6.9) [b]	27.20 t
9-21	1.24 (br. s)	29.69 -29.00 (series of t)	1.24 (br. s)	29.68 -29.00 (series of t)
22	1.24 (br. s)	31.78 t	1.24 (br. s)	31.78 t
23	1.24 (br. s)	22.65 t	1.24 (br. s)	22.65 t
24	0.87 (t; 7.1)	14.11 q	0.87 (t, 7.1)	14.11 q
1'	3.74 (m)	73.22 t	3.73 (m)	72.96 t
2'	3.91(*pseudo* quint, 4.8)	70.55 d	3.91 (m)	70.54 d
3'	3.65(dd, 11.4, 5.4), 3.74 (m)	63.61 t	3.65 (dd, 11.5, 5.5), 3.74 (dd, 11.5, 4.6)	63.73 t
CH_3-2	--	--	1.57 (d, 1.2)	12.93 q
HO-2'	2.41 (br. d, 4.8)	--	2.45 (br. d, 4.8)	--

[a] NOE enhancement observed with 1'-H at δ_H 3.73 and H_2-3 at δ_H 1.84. [b] Interchangeable data in the same column.

Dibenzoylation of the more abundant **1** gave **2** showing negative exciton-coupled dichroism. This[9] suggests 2'-(*R*) absolute configuration for **2** and therefore 2'-(*S*) for both **1** and co-occurring **3**. Except a sponge product,[10] this is the typical configuration for marine ethers and enol ethers of glycerol .

To the best of our knowledge, hanishenol B (**3**) is the first example of a C-branched enol ether of glycerol. Assuming correct taxonomic identifications of *A. carteri* in the literature,[11] variability of secondary metabolism for this sponge according to geographic location is implied; degraded oroidin-family alkaloids to be reported

elsewhere for our sponge reinforce this point. This was the reason to name these enol ethers from the place of collection. Linked to this, the notion that marine bacteria are capable of de novo synthesis of branched fatty acids [12] gives some hint that at least part of the fatty acid chain of **3** may have bacterial origin.

Experimental Section

General. All evaporations were carried out at reduced pressure. Yields are given on reacted reagents. Flash-chromatography (FC): Merck Si-60 (15- 25 μm). TLC: Merck Kieselgel 60 PF_{254} plates. Reversed-phase HPLC: 25x1 cm columns with Merck LiChrospher RP18 (7 μm), UV monitoring at λ 220 nm, solvent flux 5 ml min^{-1}, if not otherwise specified. UV: Perkin-Elmer Lambda-3 spectrophotometer, λ_{max} in nm, ϵ in $mol^{-1}\cdot l\cdot cm^{-1}$. CD: Jasco J-710 spectropolarimeter, λ_{max} in nm, $\Delta\epsilon$ in $mol^{-1}\cdot l\cdot cm^{-1}$. Polarimetric data: Jasco-DIP-181 polarimeter. NMR: Varian XL-300 spectrometer, ^1H at 300 MHz, ^{13}C at 75.4 MHz, δ values in ppm rel. to internal $SiMe_4$ (= 0 ppm), *J* values in Hz; multiplicities from DEPT; [13] assignments from $^1H,^1H$ COSY,[14] $^{13}C,^1H$ COSY,[15] and differential NOE (5s preirradiation). EI-MS and HR-EI-MS spectra: Kratos-MS80 mass spectrometer equipped with a home-built computerized acquisition data system.

Collection and Isolation. The sponge, *Acanthella carteri* Dendy, 1889 (Demospongiae, Axinellida, Axinellidae), was collected by scuba diving along the northern coast of the Hanish islands, Yemen, in South Red Sea, at a depth of 35 m in November 93 and was immediately immersed in EtOH. The sponge was identified by Dr. J. Vacelet, Station Marine d'Endoume, Marseille, France, where specimens are deposited under label AP931105-YA1/695M. Repeated extraction of the sponge with fresh EtOH and evaporation of the combined extracts gave 13.8 g of residue which was subjected to FC with hexane/ EtOAc gradient elution, collecting fractions 1-11 of 50 ml each, then EtOAc-MeOH gradient elution, collecting fractions 12-20, and finally MeOH, collecting fractions 21-27. Fractions 17-19 were combined and subjected to reversed-phase HPLC first with MeOH/ H_2O 9:1 and then with MeOH, solvent flux 6 ml min^{-1}, collecting fractions at retention time 19.6 and 18.2 min which were further subjected to HPLC with hexane/ i-PrOH 92:8 giving spectroscopically pure hanishenol A (**1**), (t_R 11.0 min; 8.5 mg) and hanishenol B (**3**), (t_R 15.2 min; 2.3 mg).

Hanishenol A (**1**). Colourless oil. $[\alpha]_D^{25}$ = + 4.0 , $[\alpha]_{365}^{25}$ = + 18.0, (*c* 0.12, MeOH). EI-MS: *m* z (%) 424 (M^+, 4), 332 ($M^+ - C_3H_8O_3$, 6), 131 (11), 111 (17), 103 (100), 97 (34), 83 (43), 75 (45), 71 (20), 57 (99). HR-EI-MS: *m* z 424.391±0.005; $[C_{27}H_{52}O_3]^+$ calc. 424.392.

Hanishenol B (**3**). Colourless oil. $[\alpha]_D^{25}$ = + 5.0, $[\alpha]_{365}^{25}$ = +18.8, (*c* 0.10, MeOH). EI-MS: *m* z (%) 438 (M^+, 20), 346 ($M^+ - C_3H_8O_3$, 3), 145 (43), 103 (54), 97 (28), 83 (32), 75 (48), 71 (100), 57 (69). HR-EI-MS: *m* z 438.407±0.005; $[C_{28}H_{54}O_3]^+$ calc. 438.407.

Ozonolysis of **1**. Ozone-enriched oxygen was allowed bubbling through a solution of hanishenol A (**1**) (5.6 mg in 4 ml of dry CH_2Cl_2 and 0.1 ml of MeOH) at - 78 °C until persistent blue colour followed by N_2 bubbling and methyl sulfide (0.1 ml) addition. The mixture was left overnight at r.t., added of water (2 ml) and extracted with CH_2Cl_2 (3x5 ml). The combined organic phases were evaporated, the residue was subjected to FC on silica gel with hexane/ EtOAc 8:2 and the first eluate was evaporated to give NMR-pure 1-octadecanal, as revealed by comparison (RP18 HPLC, MeOH/ H_2O 95:5, solvent flux 1 ml min^{-1}, Waters 410 differential refractometer, t_R 11.0 min) with an authentic sample obtained by PCC oxidation of commercial 1-octadecanol; δ_H ($CDCl_3$): 9.74 (t; 1.9), 2.40 (td; 7.3, 1.9), 1.62 (quint; 7.2), 1.24 (br.s), 0.87 (t; 7.2); δ_C ($CDCl_3$): 202.86 (d), 43.89 (t), 29.68-29.00 (series of t), 22.48 (t), 14.09 (q). EI-MS: 268 (M^+, 0.4), 250 (1), 239 (1), 224 (1), 123 (12), 111 (18), 109 (21), 97 (33), 95 (40), 83 (40), 81 (40), 71 (42), 69 (57).

Determination of the absolute configuration. A solution of hanishenol A (**1**) (1.6 mg) in dry pyridine (0.5 ml),

containing 4-(dimethylamino)pyridine (0.5 mg) was treated with an excess of benzoyl chloride . After 4 h at r.t. the mixture was added of a saturated aqueous $NaHCO_3$ solution (1 ml) and CH_2Cl_2 (3 ml), then filtered through a phase separator filter (Whatman) and evaporated. The residue was subjected to reversed-phase HPLC with MeOH, UV monitoring at λ 230 nm, to give pure di-*O*-benzoate derivative **2** (t_R 5.8 min, 2.1 mg, 88%). UV (MeOH): 230 (20500). CD (MeOH): $\Delta\epsilon(\lambda)$ = -3.7 (237), +2.1 (222). δ_H ($CDCl_3$) : 5.98 (dt; 6.2, 1.5, H-1), 4.40 (dt; 6.2, 7.4, H-2), 2.02 (m; H_2-3, H_2-5, H_2-8), 1.30 (m; H_2-4), 5.34 (m; H-6, H-7), 1.24 (br. s; H_2-9 -H_2-23), 0.87 (t; 7.0, H_3-24), 3.99 (m; H_2-1'), 5.59 (pseudo quint.; 5.1, H-2'), 4.69 (dd; 11.8, 4.0) and 4.59 (dd; 11.8, 6.3), H_2-3', 7.75 (d; 8.7, Ph), 7.42 (d; 8.7, Ph). EI-MS: 550(0.5), 298(1), 239(4), 151(5), 105(7), 77(7), 73(25), 57(40).

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