Ceratodictyols, 1-Glyceryl Ethers from the Red Alga−Sponge Association *Ceratodictyon spongiosum/Haliclona cymaeformis*

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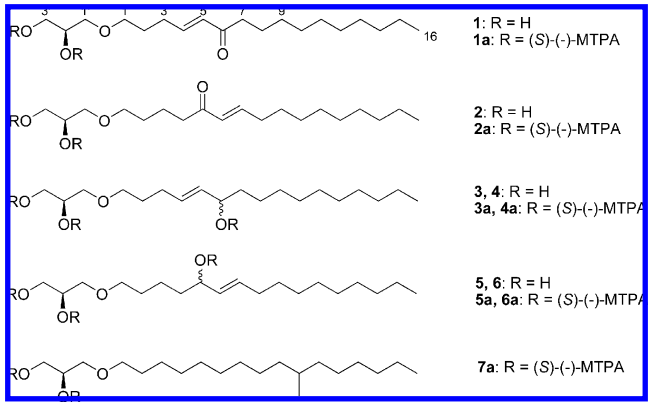
Six 1-glyceryl ethers (**1**−**6**) were isolated from the red alga−sponge assemblage *Ceratodictyon spongiosum/Haliclona cymaeformis*. Structural assignments were conducted by interpretation of spectroscopic data and the modified Mosher's method. Four allylic alcohols were obtained as a pair of epimeric mixtures (**3/4** and **5/6**). These glyceryl ethers exhibited weak cytotoxic activity against HeLa human cervical cancer cells.

The association between the sponge *Haliclona cymaeformis* and the red alga *Ceratodictyon spongiosum* is frequently found in shallow waters of tropical Indo-Pacific reefs where nutrients are limited.[1] In spite of its wide distribution and abundance, the chemistry of the association is under-explored.[2] In the course of our screening for cytotoxic compounds against HeLa human cervical cancer cells, the association collected off Amami-oshima exhibited activity. From the extract of the sponge/alga assemblage, we obtained **1**, **2**, mixtures of **3** and **4**, and mixtures of **5** and **6** as the active constituents.



The MeOH extract of the sponge/alga association (360 g) was partitioned between H_2O and $CHCl_3$, and the residue from the $CHCl_3$ layer was further partitioned between 90% MeOH and *n*-hexane. The material from the 90% MeOH layer was fractionated by ODS column chromatography, reversed-phase HPLC, silica gel column chromatography, and ODS HPLC to afford ceratodictyol A (**1**), ceratodictyol B (**2**), and a mixture of ceratodictyols C−F (**3**−**6**). The mixture was further separated into two peaks (mixtures of **3** and **4** and of **5** and **6**) only by HPLC using π-NAP stationary phase, naphthylethyl-bonded silica gel.

Ceratodictyol A (**1**) had the molecular formula $C_{19}H_{36}O_4$, which was determined by HRESIMS. Analysis of the ^1H and ^{13}C NMR data (Table 1) indicated the presence of one terminal methyl, numerous methylenes, three of which were oxygenated, one oxymethine, one disubstituted olefin, and one ketone. The COSY spectrum revealed three partial structures: the first unit (C1′ to C3′ portion) was assigned as the 1-substituted glyceryl group on the basis of the ^1H and ^{13}C chemical shift values; the second unit comprised a 1-oxygenated 4-pentenyl group with a polarized olefin (C1 to C5); the last unit (C7 to C16 portion) was deduced to be a decyl group, because one terminal methyl and nine methylenes were left. With the ^1H NMR signals of the C1 to C3 portion well resolved, the location of the C4−C5 olefinic bond was unambiguously assigned by analysis of the COSY data. The connection of these units was established by analysis of the HMBC data. The linkage of C1′ and C1 through an ether bond was shown by the HMBC correlations $H_2$1/C1′ and $H_2$1′/C1. The ketone was inserted between C5 and C7 on the basis of HMBC cross-peaks, H5/C6, H4/C6, $H_2$7/C6, and $H_2$8/C6. The geometry of the C4−C5 double bond was assigned as *E* on the basis of the coupling constant of 15.8 Hz between H-4 and H-5. The configuration of the glyceryl portion of **1** was determined by the modified Mosher's method.[3] Compound **1** was converted to the bis-(*S*)-(−)-MTPA ester (**1a**), whose ^1H NMR data of the glyceryl portion matched well with those of the bis-(*S*)-(−)-MTPA ester of 2′*S*-glyceryl ether in the literature (**7a**).[4] Therefore, a 2′*S* configuration was assigned for **1** (Table 2).

The molecular formula of ceratodictyol B (**2**) was identical with that of **1**. ^1H and ^{13}C NMR data suggested that **2** had the same sets of functional groups as those of **1**. Three partial structures (C1′ to C3′, C1 to C4, and C6 to C16) were identified by analysis of the COSY and HSQC data together with the mass spectrometric data. The presence of four contiguous methylene carbons (C1−C4) was assigned by COSY data. HMBC correlations, (H3, H4, H6, and H7)/C5, H1/C1′, and H1′/C1, revealed that **2** was isomeric with **1**, differing in the position and orientation of the enone moiety. The geometry of the C6−C7 double bond was assigned as *E* on the basis of the $^1H-^1H$ coupling constant of 15.8 Hz. The 2′*S*-configuration was determined by comparing the ^1H NMR spectrum of the bis-(*S*)-MTPA ester (**2a**) with those of **7a** (Table 2).

Ceratodictyols C and D (**3** and **4**) were obtained as an epimeric mixture with the molecular formula $C_{19}H_{38}O_4$. We initially considered the mixture to be homogeneous because it exhibited only one set of NMR signals. Analysis of the NMR data suggested that the ketone in **1** was replaced by a secondary alcohol. The olefin was no longer polarized and one of the olefinic protons was coupled with an oxymethine at δ 3.96. The *E* geometry of the C4−C5 double bond was assigned on the basis of the $^1H-^1H$ coupling constant of 15.0 Hz. We attempted to elucidate the configurations of the two chiral centers by the modified Mosher's method. We were surprised to find that the ^1H NMR spectrum of the tris-(*S*)-(−)-MTPA ester (**3a** and **4a**) exhibited two sets of signals in a 1:1 ratio (Supporting Information, Table S1). The duplication of signals occurred around C6, suggesting that the starting triol was an epimeric mixture differing in configuration at C6. However, from the ^1H NMR data of the mixture of **3a** and **4a**, we were able to assign the 2′*S* configuration (Supporting Information, Table S2).

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Table 1. ^{1}H and ^{13}C NMR Data of **1**−**6** in CD_3OD

	1		**2**		**3** + **4**		**5** + **6**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	71.7	3.50, m	72.3	3.48, m	72.0	3.48, m	72.7	3.48, m
2	29.4	1.76, quint (7.1)	30.6	1.59, quint (7.1)	30.5	1.66, quint (7.1)	30.5	1.59, quint (6.9)
3	30.4	2.34, q (7.1)	22.3	1.66, quint (7.1)	29.9	2.12, q (7.1)	23.4	1.39, m
4a	149.4	6.95, dt (15.8, 7.1)	40.4	2.63, t (7.1)	131.9	5.62, dt (15.0, 7.1)	38.4	1.45, m
4b								1.53, m
5	131.6	6.14, d (15.8)	203.6		135.1	5.44, dd (15.0, 7.1)	73.8	3.96, q (7.1)
6	203.8		131.4	6.12, d (15.8)	73.9	3.96, q (7.1)	134.5	5.41, dd (15.8, 7.1)
7a	40.8	2.59, t (7.1)	150.0	6.93, dt (15.8, 7.1)	38.6	1.43, m	132.7	5.61, dt (15.8, 7.1)
7b						1.51, m		
8	25.7	1.58, quint (7.1)	33.7	2.25, q (7.1)	26.8	1.30, m	33.4	2.04, q (7.1)
9	30.5	1.30, m	29.4	1.49, quint (7.1)	30.5	1.30, m	30.4	1.39, m
10	30.5	1.30, m	30.5	1.33, m	30.5	1.30, m	30.5	1.31, m
11	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m
12	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m
13	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m	30.5	1.30, m
14	33.2	1.30, m	33.2	1.30, m	33.2	1.30, m	33.2	1.30, m
15	23.9	1.30, m	23.9	1.30, m	23.9	1.30, m	23.9	1.30, m
16	14.6	0.90, t (6.9)	14.6	0.90, t (6.9)	14.6	0.90, t (6.9)	14.6	0.90, t (6.9)
1′a	73.4	3.42, dd (10.0, 5.8)	73.4	3.42, dd (10.0, 5.8)	73.4	3.42, dd (10.0, 5.8)	73.4	3.42, dd (9.6, 6.2)
1′b		3.48, m		3.47, m		3.48, m		3.48, m
2′	72.4	3.75, m	72.4	3.74, m	72.4	3.75, m	72.4	3.75, m
3′a	64.7	3.51, m	64.7	3.51, m	64.7	3.51, m	64.7	3.51, m
3′b		3.57, dd (11.0, 4.8)		3.57, dd (11.3, 5.2)		3.58, dd (11.4, 5.2)		3.58, dd (11.3, 5.2)

Table 2. ^{1}H NMR Data of the Glyceryl Portion **1a**, **2a**, and **7a** in $CDCl_3$

	1a	**2a**	**7a**[a]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	3.39, m	3.40, m	3.39, m
1′a	3.56, dd (10.3, 5.1)	3.56, dd (10.3, 5.2)	3.56, dd (10.2, 4.9)
1′b	3.61, dd (10.3, 6.4)	3.61, dd (10.3, 6.2)	3.61, dd (10.2, 6.9)
2′	5.49, m	5.48, m	5.49, m
3′a	4.37, dd (12.4, 5.0)	4.37, dd (12.4, 5.2)	4.37, dd (12.1, 4.4)
3′b	4.62, dd (12.4, 3.7)	4.63, dd (12.4, 3.4)	4.63, br dd

[a] Data from ref 4.

The structures of ceratodictyols E and F (**5** and **6**) were analyzed in the same manner. The mixture of **5** and **6** appeared homogeneous by NMR, and its planar structure was assigned as described for **3** and **4**. The ^{1}H NMR spectrum of the tris-MTPA ester (**5a** and **6a**) disclosed that the material was a 1:1 mixture of 5*S* and 5*R* isomers with the 2′*S* configuration (Supporting Information, Tables S1 and S2).

Ceratodictyols A (**1**) and B (**2**) and mixtures of ceratodictyols C and D (**3** and **4**) and ceratodictyols E and F (**5** and **6**) exhibited cytotoxic activity against HeLa human cervical cancer cells with an IC_{50} value of 67 μM for each.

Glyceryl ethers are widely distributed in nature, usually as minor lipid components.[5] They have attracted the attention of medicinal chemists due to their antineoplastic and other pharmacological activities.[6] Monoalkyl glyceryl ethers with notable alkyl substituents have been isolated from marine sponges.[7] To the best of our knowledge, ceratodictyols are the first 1-alkyl-glyceryl ethers with oxygenation in the alkyl chain.[8,9]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a Shimadzu BioSpec-1600 spectrophotometer. NMR spectra were recorded on a JEOL delta 600 NMR spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks at δ_H 3.31 and δ_C 49.15 for CD_3OD and at δ_H 7.16 for C_6D_6.

Animal Material. The red alga−sponge association *Ceratodictyon spongiosum*/*Haliclona cymaeformis* was collected at Kurosaki (20°07′ N; 129°20′ E), Amami-Oshima, Kagoshima Prefecture. The specimens were frozen on site and kept at −20 °C until used. A voucher specimen was deposited at the Zoological Museum, University of Amsterdam (ZMAPOR 20129).

Extraction and Isolation. The specimen (360 g wet weight) was extracted with MeOH (1 L × 3), and the extracts were combined and concentrated *in vacuo*. The MeOH extract was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ residue was partitioned between 90% MeOH and *n*-hexane. The 90% MeOH layer was concentrated and separated by ODS flash column chromatography by a stepwise elution with mixtures of MeOH and H_2O to give five fractions (A−E). Fraction C (90% MeOH eluate; 209 mg) was further separated by reversed-phase HPLC (Inertsil ODS-3, 10 × 50 mm; 70% MeOH to MeOH, linear gradient). One of the fractions was subjected to silica gel column chromatography using a stepwise gradient system using $CHCl_3$, MeOH, and H_2O. The $CHCl_3$−MeOH (98:2) fraction (26.9 mg) was further separated by reversed-phase HPLC (COSMOSIL 5C_{18}-AR-II, 20 × 250 mm; isocratic elution with 70% MeOH) to give ceratodictyol A (**1**, 0.7 mg) and ceratodictyol B (**2**, 0.6 mg). Another fraction in the ODS HPLC was subjected to two rounds of reversed-phase HPLC with COSMOSIL πNAP (10 × 250 mm; isocratic elution with 65% MeOH) to give a mixture of ceratodictyols C and D (**3** and **4**, 0.8 mg) and a mixture of ceratodictyols E and F (**5** and **6**, 0.6 mg).

Ceratodictyol A (1): colorless oil; $[\alpha]^{22}_D$ −33 (*c* 0.01, MeOH); UV (MeOH) 224 nm (ϵ 17 000); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 351.2496 (calcd. for $C_{19}H_{36}O_4$, 351.25113).

Ceratodictyol B (2): colorless oil; $[\alpha]^{23}_D$ −27 (*c* 0.02, MeOH); UV (MeOH) 224 nm (ϵ 15000); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 351.2523 (calcd. for $C_{19}H_{36}O_4$, 351.25113).

Mixture of Ceratodictyols C and D (3 and 4): $[\alpha]^{24}_D$ −27 (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 353.2680 (calcd. for $C_{19}H_{38}O_4$, 353.26678).

Mixture of Ceratodictyols E and F (5 and 6): $[\alpha]^{24}_D$ −26 (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRESIMS *m*/*z* 353.2680 (calcd for $C_{19}H_{38}O_4$, 353.26678).

Assay for the Cytotoxity against HeLa Human Cervical Cancer Cells. Cytotoxic activity was determined as described.[4]

Preparation of MTPA Ester. MTPA esters were prepared and purified as described.[4]

1a, 2a, 3a, 4a, 5a, and 6a. ^{1}H NMR ($CDCl_3$ and C_6D_6) data, see Supporting Information, Tables S1 and S2.

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Supporting Information Available: NMR spectra for compounds **1**−**6** and ^{1}H NMR data for the MTPA esters. This material is available free of charge via the Internet at http://pubs.acs.org.

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