Alkyl Glycerol Monoethers in the Marine Sponge *Desmapsamma anchorata*[1]

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1-*O*-Hexadecylglycerol (chimyl alcohol), 1-*O*-heptadecylglycerol and 1-*O*-octadecylglycerol (batyl alcohol) have been identified as the major native constituents of a mixture of free alkyl glycerol ethers isolated from the contained water and the methanolic extract of the sponge *Desmapsamma anchorata*. Minor components were the free C_{14}, C_{15}, C_{19}, C_{20} and C_{21} alkyl glycerol monoethers. The alkyl glycerol monoethers were analyzed and identified by gas chromatography/mass spectrometry of their isopropylidene derivatives. This is the first report on the occurrence of free C_{15}, C_{19}, C_{20} and C_{21} alkyl glycerol monoethers in a sponge.
***Lipids* 29, 731–734 (1994).**

The saturated monoalkyl ethers of glycerol, 1-*O*-hexadecyl-*sn*-glycerol(**1c**; chimyl alcohol) (see Fig. 1); and 1-*O*-octadecyl-*sn*-glycerol(**1e**; batyl alcohol) commonly occur esterified to fatty acids in a wide variety of marine organisms (1–5). They have also been found in some marine sponges in the unesterified form (6–11). Thus, free chimyl alcohol **1c** was recently found in *Stylopus australis* (Poecilosclerida:Hymedesmiidae) (6), *Tethya aurantia* (Hadromerida:Tethyidae) (7) and *Tedania ignis* (Poecilosclerida:Myxillidae) (8), while batyl alcohol **1e** was found in *Ulosa ruetzleri* (Axinellida:Axinellidae) (9) and *T. ignis* (8). The first report on the occurrence of a free alkyl glycerol monoether in a sponge was that on 1-*O*-tridecyl-*sn*-glycerol (**1i**) from a sponge of the family Plocamiidae (10). At about the same time, the branched-chain alkyl glycerol monoesters **5a** and **5b** were isolated from a Taiwanese marine sponge of genus *Aaptos* (Hadromerida:Suberitidae) (11) and **1j** and **1k** from *T. aurantia* (7).

Desmapsamma anchorata (Poecilosclerida:Esperiopsidae) first described by Carter in 1882 (12) and more recently by Green *et al.* (13), is a *Desmapsamma* ramose sponge erected on an encrusting base. Alive, it is pale pink externally and dark orange internally, and it is smooth and "spongy." *Desmapsamma anchorata* inhabits its hexacoralia plates and *Acropora* cemeteries on the leeward side of coral reefs in the Gulf of Mexico and Caribbean Sea. It has previously been shown that the Caribbean *D. anchorata* contains interesting fatty acids with unusual unsaturation and methyl branching as well as the common C_{14} to C_{26} fatty acids and common sponge sterols; but no free alkyl glycerol ethers were found (14). We report here on the isolation of free saturated monoalkyl ethers of glycerol from *D. anchorata* harvested in the Gulf of Mexico.

[1]Contribution No. 1241 from the Instituto de Química, UNAM. This work was presented in part at the First Lanbio Symposium, Trends in Natural Products Research: Prospects for Pharmacological and Agrochemical Applications, Asunción, Paraguay, August 6–10, 1993.

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Abbreviations: GC/MS, gas chromatography/mass spectrometry; MS, mass spectrometry; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography; TMS, tetramethylsilane.

EXPERIMENTAL PROCEDURES

Sponge collection. Approximately 30 specimens of *D. anchorata* were collected at depths of 2 to 15 m and at a water surface temperature of 28°C near the La Anegada de Afuera reef (Veracruz, Ver., Mexico) in June 1989. A voucher specimen is being kept at the Laboratorio de Farmacología Marina, Instituto de Ciencias del Mar y Limnología (UNAM). *Desmapsamma anchorata* is a shallow water species which shows no association with symbiotic algae but considerable affinity for *Holopsamma helwigi* at the same location. *Desmapsamma anchorata* is a very abundant species near the reefs of Veracruz, Mexico.

Isolation. The sponge (6.0 kg) was squeezed, and the water from the sponge was filtered through a Büchner funnel using a celite layer as filter aid to remove insoluble materials. The filtrate was extracted with ethyl acetate (4 × 500 mL). The insoluble material was washed from the celite layer first with petroleum ether (4 × 2 L) and then with methanol (4 × 2 L). The ethyl acetate extract and the petroleum ether and methanol washings were taken to dryness *in vacuo* to yield 1.42, 0.43 and 0.94 g of material, respectively. The residues were then combined as they were found to be similar as judged by thin-layer chromatography (TLC) using the developing solvent dichloromethane/acetone (9:1, vol/vol).

To the remaining sponge material (545.5 g dry wt), methanol (5 × 2.5 L) was added, and the mixture was filtered through celite. The aqueous methanol phase was brought to dryness and then extracted with dichloromethane and ethyl acetate (4 × 2 L each). The organic extracts were concentrated *in vacuo* to yield a reddish-brown oil (35 g). The residue from the aqueous phase of the sponge was chromatographed on a column (4.5 × 35.0 cm) of 60 g of silica gel, while the residue from the remaining sponge material was separated on a column (6.0 × 35.0 cm) containing 170 g of silica gel, and the columns were eluted with petroleum ether, petroleum ether/ethyl acetate mixtures, and methanol. Elution with petroleum ether yielded 135 mg of a mixture of fatty acid methyl esters which were likely formed during methanol extraction. Gas chromatography/mass spectrometry (GC/MS) analysis of the mixture indicated the presence of C_{14} to C_{21} fatty acids which had previously been identified in this species (14). Petroleum ether/ethyl acetate (9:1, vol/vol) yielded 4.2 g of a mixture of sterols (m.p. 134–140°C). Acetylation of the

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sterol mixture (60 mg) gave the corresponding acetates. GC/MS of the acetates confirmed the presence of the sterols previously identified in this species (14), i.e., desmosterol (3.9%), cholesta-5,22-dienol (9.2%), cholesterol (44.6%), 24-methylcholesta-5,22-dienol (18.3%), 24-methylcholesterol (6.2%), stigmasterol (4.4%) and sitosterol (11.1%). Elution with petroleum ether/ethyl acetate (4:1 and 3:1, vol/vol) yielded a mixture of the monoalkyl ethers of glycerol (10 mg from aqueous phase, and 300 mg from sponge) as an amorphous solid (**1a–h**). The compounds found in the aqueous and sponge residues were similar.

Optical rotations were measured on $CHCl_3$ solutions using a Jasco DIP-360 Digital polarimeter (Japan Spectroscopic Co., Tokyo, Japan). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra on $CDCl_3$ solutions, with tetramethylsilane (TMS) as internal standard, were recorded at 300 MHz or 200 MHz, either on a Varian VXR-300S instrument or a Varian Gemini-200 instrument (Varian, Palo Alto, CA). Mass spectra (MS) were recorded using a Hewlett-Packard MS5985B mass spectrometer (Palo Alto, CA). GC/MS was done using a PAS-1701 (ECD tested 1701 silicone) column (25 × 0.32 mm × 0.25 μm) in a Hewlett-Packard 5890 Series II gas chromatograph linked to a JEOL JMS-AX505HA mass spectrometer with a data system (Tokyo, Japan). Samples were analyzed using temperature programming: first isothermal at 100°C for 1 min, then increased at 10°C/min to 280°C, and finally kept at 280°C for 10 min. TLC was done on silica gel plates impregnated with fluorescent dye (Merck Silica Gel 60 F_{254}; Merck, Darmstadt, Germany).

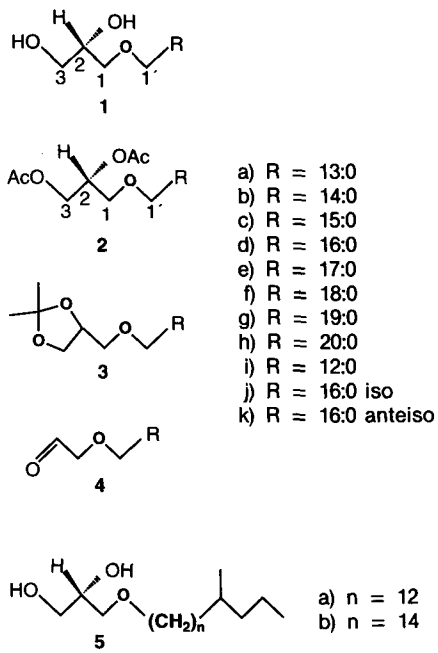

FIG. 1. Structures of alkyl glycerol monoethers (1a–h) from *Desmapsamma anchorata* and of some structural analogs (2a–h, 3a–h) as well as of some related compounds from other sponges (1i–k, 5a–b).

*Monoalkyl ethers of glycerol (Fig. 1, **1a–h**).* ^1H NMR ($CDCl_3$, 300 MHz) δ 0.88 *t*, *J* = 6.7 Hz, CH_3; 1.26, *s*, $(CH_2)_n$, aliphatic chain; 1.57, *quint*, *J* = 6.9 Hz, H-2′; 3.46, AB *m*; *apparent td*, *J* = 6.6 Hz, *J* = 1.2 Hz, H-1′; 3.48, *dd*, *J* = 9.6 Hz, *J* = 6.0 Hz, H-1a; 3.53, *dd*, *J* = 9.6 Hz, *J* = 4.2 Hz, H-1b; 3.63, *dd*, *J* = 11.7 Hz, *J* = 5.4 Hz, H-3a; 3.71, *dd*, *J* = 11.7 Hz, *J* = 3.9 Hz, H-3b; and 3.86, *pseudo quint*, *J* = 5.4 Hz, H-2. ^{13}C NMR δ ($CDCl_3$, 75 MHz) δ 14.13, 22.70, 26.08, 29.36, 29.47, 29.58, 29.59, 29.62, 29.67, 29.70, 31.92, 64.23, 70.48, 71.83, 72.43. The chemical shifts for H-1 and H-3 are reverse compared to those described in Ref. 15. Assignments for H-2 and H-3 were confirmed by the downfield shifts of the signals in the ^1H NMR spectrum of the acetylation product.

Acetylation of the monoalkyl ethers of glycerol. An aliquot of monoalkyl ethers (10 mg) was treated with Ac_2O (0.2 mL) and pyridine (0.1 mL) at room temperature overnight. The excess reactants were removed on a rotary evaporator under reduced pressure. The residue was chromatographed on a column of silica gel to yield the diacetates (Fig. 1, **2a–h**) (5 mg) as a yellowish oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 0.89, *t*, CH_3; 1.26, *s*, CH_2, aliphatic chain; 1.56, *quint*, H-2′; 2.07, *s*, AcO; 2.10, *s*, AcO; 3.44, AB *m*, H-1′; 3.56, *d*, *J* = 5.3 Hz, H-1; 4.16, *dd*, *J*= 11.5 Hz, *J* = 5.8 Hz, H-3b; 4.34, *dd*, *J* = 11.5 Hz, *J* = 4.0 Hz, H-3a; and 5.19, *m*, H-2. ^{13}C NMR δ ($CDCl_3$, 75 MHz) 14.15, 20.81, 21.08, 22.71, 26.03, 29.37, 29.46, 29.53, 29.62, 29.64, 29.72, 31.94, 62.99, 68.82, 70.31, 71.76.

Isopropylidene derivatives of monoalkyl ethers of glycerol. To a solution of monoalkyl ethers of glycerol (20 mg) in dry acetone (12 mL), dry $CuSO_4$ was added in excess and refluxed with stirring for 2 h. The reaction was monitored by TLC. The mixture was filtered, and the filtrate was directly subjected to silica gel column chromatography. Elution with petroleum ether/CH_2Cl_2 (4:1, vol/vol) yielded the isopropylidene derivatives (Fig. 1, **3a–h**). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.88, *t*, CH_3; 1.26, *s*, CH_2, aliphatic chain; 1.37, *s*, 3H, 1.43, *s*, 3H, gem dimethyl; 1.56, *quint*, H-2′; 3.41 and 3.52, *dd*, *J* = 9.8 Hz, *J* = 5.6 Hz, H-1b and H-1a; 3.46, AB *m*, H-1′a and H-1′b; 3.73 and 4.07, *dd*, *J* = 8.2 Hz, *J* = 6.4 Hz, H-3b and H-3a; and 4.28, *pseudo quint*, *J* = 6.2 Hz, H-2. ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.07, 22.65, 25.38, 26.01, 26.73, 29.33, 29.43, 29.56, 29.65, 29.99, 31.89, 66.93, 71.81, 71.89, 74.75, 109.37.

Periodate oxidation of the monoalkyl ethers of glycerol. A solution of monoalkyl ethers of glycerol (50 mg) in diethyl ether was treated with 38% periodic acid (10 mL) at room temperature overnight, and the reaction mixture was purified by column chromatography to yield the respective glycolaldehyde ethers (Fig. 1, **4a–h**) (10 mg). ^1H NMR ($CDCl_3$, 200 MHz) δ 0.9, *t*, CH_3, *J* = 7.0 Hz; 1.25, *s*, CH_2, aliphatic chain; 1.57, *m*, H-2′; 3.53 *t*, *J* = 6.5 Hz, H-1′; 4.05, *d*, *J* = 1.0 Hz, H-1; and 9.74, *t*, *J* = 1.0 Hz, H-2.

RESULTS AND DISCUSSION

The monoalkyl ethers of glycerol were isolated as a waxy solid (m.p. 57–58°C; $[\alpha]_D$ + 2.0° at c = 0.2 in $CHCl_3$) and

were identified by ^1H NMR and GC/MS of the isopropylidene derivatives. The ^1H NMR spectrum exhibited signals for an aliphatic long chain and signals associated with the presence of the glycerol monoether moiety. Three methylenes and one methine were observed attached to oxygen atoms: a two proton AB multiplet (an apparent triplet of doublets, J = 6.6 Hz, J = 1.2 Hz H-1′) at 3.46 ppm, two AB doublets of doublets centered at 3.48 ppm (J = 9.6 Hz, J = 6.0 Hz, H-1a) and 3.53 ppm (J = 9.6 Hz, J = 4.2 Hz, H-1b), two AB doublets of doublets centered at 3.63 ppm (J = 11.7 Hz, J = 5.4 Hz, H-3a) and 3.71 ppm (J = 11.7 Hz, J = 3.9 Hz, H-3b) and a pseudo quintuplet at 3.86 ppm ($J \approx 5.4$ Hz) for the methine proton (H-2). The ^{13}C NMR spectrum was in agreement with this structure and showed three methylene carbons at 72.43 (C-1), 71.83 (C-1′) and 64.23 (C-3), and a methine at 70.48 (C-2). These assignments are based on multipulse APT and 2D ^1H-^{13}C correlation experiments.

Proton assignments were confirmed by converting the diols to the corresponding diacetates. The ^1H NMR showed two sharp singlets at 2.07 and 2.10 ppm corresponding to the acetyl protons, as well as a downfield shift of the two H-3 doublets of doublets (4.16, J = 11.5 Hz, J = 5.8 Hz, H-3a; 4.34, J = 11.5 Hz, J = 4.0 Hz, H-3b) and the methine proton (H-2, 5.19 ppm).

Oxidative cleavage of the diols with periodic acid gave the corresponding glycoaldehyde alkyl ethers confirming the presence of a 1,2-diol group. The ^1H NMR spectrum exhibited the aldehydic proton at 9.74 ppm as a triplet (J = 1.0 Hz) coupled with the methylene doublet at 4.05 ppm (J = 1.0 Hz).

The above assignments are in good agreement with those published for chimyl and batyl alcohol (**1c, 1e**) as well as other monoalkyl ethers of glycerol **1j, 1k** (7,15,16).

Although useful anomalous ion peaks at one mass unit above the molecular weight [M + 1] have been reported to occur in the spectra of various ethers, alcohols, glycols, amines and nitriles (17), the presence of such a peak has not been reported previously for alkylglycerol ethers (6–11,15). We noted that the electron impact mass spectrum measured at 70 eV on the mixture of monoalkyl ethers of glycerol isolated from *D. anchorata* showed a useful [M + 1] peak at *m/z* 317 (0.9%) and other significant peaks at *m/z* 285 $[M - CH_3O]^+$ (0.6%), 255 $[M - C_2H_5O_2]^+$ (0.9%) and 225 $[M - C_3H_7O_3]^+$ (3.1%) characteristic of chimyl alcohol **1c** as the major component. The mass spectrum also showed additional weak [M + 1] ion peaks at *m/z* 331 (0.2%) and 345 (0.1%) indicative of the presence of C_{17} and C_{18} analogs. Similar results were obtained at 12 eV and in the chemical ionization mode (data not shown).

While mass spectrometry using the direct insertion mode, of the intact alkyl glycerol monoethers indicated the presence of glycerol ethers with 16:0, 17:0 and 18:0 hydrocarbon chains as major constituents, GC/MS of the isopropylidene derivatives identified glycerol ethers with hydrocarbon chain lengths from C_{14} to C_{21}. GC/MS of the isopropylidene derivatives did not give molecular ion peaks, but it showed the $[M - 15]^+$ ion and the characteristic base peak at *m/z* 101 (18). The major saturated 1-*O*-alkyl glycerol ethers in decreasing order of abundance were: C_{16} (56.48%), C_{17} (21.98%), and C_{18} (17.24%), followed by C_{14} (1.65%), C_{15} (1.44%), C_{19} (0.77%), C_{20} (<0.5%) and C_{21} (<0.5%). GC/MS furthermore indicated the presence of two C_{16}, four C_{17}, three C_{18} and two C_{19} saturated alkyl isomers, suggesting the presence of iso, anteiso, and/or other branched structures. It was not possible to identify the position of the methyl branching in the hydrocarbon side chain. It should be noted that GC/MS of the methyl esters of the fatty acids isolated from this sponge also indicated the presence of carbon chain lengths from C_{14} to C_{21} and the presence of saturated branched isomers (14).

Sponges such as *D. anchorata* have been shown to be a good source of phospholipids with fatty acids having unusual unsaturation and methyl branching which is typically not found in terrestrial systems (14). Chimyl alcohol (**1c**), batyl alcohol (**1e**) and their unsaturated analogs are widely distributed in nature and have been found in elasmobranch fish, human milk and bone marrow, atherosclerotic human aorta, pig spleen, in the fat of neonatal calves, in colostrum and milk of cows, goats, pigs and sheep (9), and more recently in reptile skin gland secretions (18). Unesterified alkyl glycerol monoethers have been isolated from marine sponges, although screening of several marine sponges of the class Demospongiae has shown that this type of ether lipid is not widely distributed among sponges (7). Studies on the antimicrobial activity of alkyl glycerol ethers have shown that these compounds inhibit bacterial growth (19). Their role in sponges is still uncertain, although it has been proposed that alkyl glycerol monoethers may play some part in the defense mechanism in the life of the sponge (7).

ACKNOWLEDGMENTS

Specimens were collected with the help of L.M. Mitchell and J.A. Mercado. We also thank R. Patiño for optical rotation measurements, R Gaviño for 200 MHz NMR spectra, I. Chávez for 300 MHz NMR measurements; and L. Velasco and F.J. Pérez for mass spectra and GC/MS. The authors thank the International Foundation for Science (IFS) Stockholm, Sweden, for financial support (F. Cruz), Research Grant Agreement No. F/1684-1.

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[Received February 4, 1994, and in final revised form July 7, 1994; Revision accepted August 26, 1994]