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Glycerol enol ethers of the brachiopod *Gryphus vitreus* from the Tuscan archipelago

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Abstract. Long-chain free glycerol enol ethers, as well as monounsaturated and saturated β-methoxy-substituted glycerol ethers, have been isolated from *Gryphus vitreus* (Born, 1778) (Brachiopoda Articulata, Terebratulidae) collected in Tuscan archipelago waters, Thyrrenian Sea. This is the first description of secondary metabolites from brachiopods.
Key words. *Gryphus vitreus*; Brachiopoda; glycerol ethers; glycerol enol ethers.

In the active search for new secondary metabolites from marine organisms, brachiopods have been completely neglected. This is understandable because of their generally scarce occurrence[1] and difficulty of collection, and very low specific biomass of these invertebrates, which hamper natural product studies. On the other hand active interest in the ecology of brachiopods has emerged from a recent work[2] and a review[3] showing that articulate brachiopods, even when offered freed of the shell, repel potential predators such as sea stars, fish, crabs and snails. Mussels were always preferred. This suggested[2] the presence in brachiopods of distasteful compounds as an adaptive response which might have saved these mostly sessile invertebrates from complete extinction in their decline since the Paleozoic, when they outnumbered the molluscs. Because of these ecological[2] and phylogenetic[4] implications, brachiopods represent a potentially interesting group of invertebrates for natural product studies.

Materials and methods

Materials and instrumentation. High-pressure liquid chromatography (HPLC): Merck LiChrosorb Si60 (7 μm, 25 × 0.4 cm; solvent flux 1 ml/min; UV monitoring). Flash chromatography (FC): Merck Kieselgel Si60, 15–25 μm. Reversed-phase flash chromatography (RPFC): Merck LiChrosorb RP-18, 25–40 μm. Thin-layer chromatography (TLC): Merck Kieselgel 60 PF_{254}. Optical rotation data: JASCO-DP-181 polarimeter, $[\alpha]_D$ values in 10^{-1} deg cm^2 g^{-1}. Fourier transform (FT) ^{1}H- and ^{13}C-NMR spectra at 299.94 and 75.43 MHz, respectively on a Varian XL-300 spectrometer; δ(ppm) rel. to internal Me_4Si (= 0 ppm) and J in Hz; multiplicities from DEPT[5,6]; assignments confirmed by ^{1}H-^{13}C COSY[7]. Mass spectrometry (MS): Kratos MS80 mass spectrometer equipped with a home-built data system and a Vacumetrics DIP Gun for FAB spectra. All evaporations were carried out at reduced pressure at room temperature (RT).

Collections and work-up. Unidentified brachiopods were collected by beam trawl during the Marion Dufresne-30 cruise in 1982, west of Cochons island, depth 240 m, and Possession island, depth 200 m (South Indian Ocean). *Gryphus vitreus* (Born, 1778) (Brachiopoda, Articulata, Terebratulida, Terebratulacea, Terebratulidae) was collected by dredging either off Corsica, Ligurian Sea, on the oceanographic vessel Korotneff in 1985, or by beam trawl in the Tuscan archipelago on several occasions from 1990 to 1992, during survey cruises. A large collection (640M) of *G. vitreus* (80% of which proved to be alive) was thus made on 14 May 1992/survey 12, north of Capraia island, in an area very unusually rich in this brachiopod, from N 43° 11.30′, E 9° 55.30′ to N 43° 6.50′, E 9° 50.00′, at a mean depth of 179 m. The brachiopods filling a 120-l tank were crushed and the slurry was filtered through a plankton net, retaining the solid material, which was then immersed in a total of 12 l ethanol and stored at −20 °C in the dark for 12 months. This slurry was then filtered and the solvent evaporated to give 4.5 g of residue that was subjected to RP-FC, gradient MeOH/H_2O from 0/100 to 100/0%. Central fractions were evaporated to give 3.5 g of a residue that was subjected to FC, gradient elution AcOEt/petroleum ether from 0/100 to 100/0%, collecting 15 fractions. Central fractions were evaporated to give a residue (0.38 g) that was subjected to preparative TLC with petroleum ether-EtOAc 1:1 to give, from the R_F 0.27 band, 60 mg of a mixture that was further subjected to TLC with petroleum ether/EtOAc 2:3. Three bands were collected at R_F 0.20 (8 mg of a 2:1 mixture of **3** and **4**), 0.41 (32 mg of a mixture of several polyunsaturated, non-conjugated glycerol monoesters), and 0.56 (6 mg of a 1:5 mixture of **1** and **2** as an amorphous solid). HPLC with hexane-isopropyl alcohol 96:4, λ 220 nm, (t_R 25 and 26 min for **1** and **2**, respectively) gave mixtures enriched enough in **1** or **2** to be suitable for NMR and MS assignments. Catalytic hydrogenation of the above 2:1

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mixture of **3** and **4** was carried out with H_2, 1 atm, in EtOH-AcOEt 1:1 in the presence of PtO_2 at RT for 4 h. The mixture was filtered and the solvent evaporated, obtaining practically pure **4**.

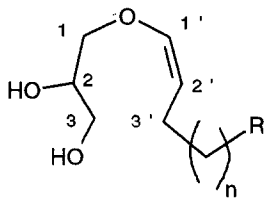
Results

Mixture of compounds 1 and 2. The optically active R_F 0.56 fraction ($[\alpha]_D^{20}$ −2.7, c = 0.15 g/100 ml, EtOH) showed ^{1}H-NMR signals in $CDCl_3$ attributable to **1** and **2** (see below) besides a triplet at δ_H 0.88 for a glycerol enol ether bearing a long, linear alkyl chain. This ether could not be freed from **1** and **2**, so that superimposition of NMR signals prevented discriminating between a C-17 or a C-18 hydrocarbon chain; should the former apply, this compound would correspond to the enol ether portion of the phospholipid isolated from the sponge *Tethia aurantia*[8]. The small amount of **1** and **2** and purification difficulties prevented a thorough stereochemical study. However these compounds, only differing structurally from one another in regions far from chiral centres, are expected to show the same (negative) sign of rotation[9]. Comparison with (−)(*S*)-synthetic analogues differing from **1** and **2** only in the hydrocarbon chain[10,11], suggests an *S* configuration for the latter.

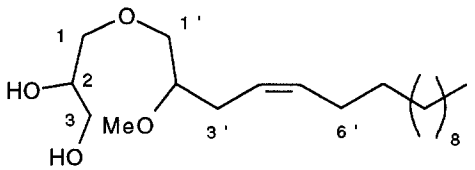
Compound 1. Mixtures enriched in **1** showed three sets of ^{1}H-NMR signals for protons at oxygen-bearing carbons in $CDCl_3$, suggesting a free glycerol monoether. Thus, a multiplet at δ_H 3.92 ppm, attributable to H-C(2), is coupled to both the δ_H 3.80 and 3.79 signals for 2H-C(1) and the δ_C 3.75 and 3.65 signals for 2H-C(3). The two double triplets at δ_H 5.94 and 4.41 are consistent with a *cis*-disubstituted C=C bond: on the C(2′) side (δ_C 108.34) by an alkyl chain and on the C(1′) side (δ_C 144.46) by O-C(1) of the glycerol moiety. A multiplet at δ_H 1.51, coupled to two equivalent methyl groups, establishes an isopropyl hydrocarbon chain. These data, and HREIMS on the *m/z* 342 peak, suggest the structure 1-(16-methylheptadec-1-enyloxy)-2,3-propanediol for **1**. The ^{13}C-NMR spectrum of **1** in $CDCl_3$ consists of the following signals: 144.46 [d, C(1′)], 108.34 [d, C(2′)], 23.24 [t, C(3′)], several signals close to 29 [t, C(4′)-C(15′)], 27.95 [d, C(16′)], 22.65 [q, C(17′) and C(18′)], 73.18 [t, C(1)], 70.58 [d, C(2)], 69.59 [t, C(3)]; ^{1}H-NMR: 5.94 [td, $J_{1',2'} = 6.0$, $J_{1',3'} = 1.5$, H-C(1′)], 4.41 [td, $J_{2',1'} = J_{2',3'}$ 6.0, H-C(2′)], 2.05 [qd, $J_{3',2'} = J_{3',4'} = 6.0$, $J_{3',1'} = 1.5$, 2H-C(3′)], 1.25 [br.s, 2H-C(4′) to 2H-C(15′)], 1.51 [m, H-C(16′)], 0.86 [d, $J = 6.5$, 3H-C(17′) and 3H-C(18′)], 3.80, 3.79 [CD, 2H-C(1)], 3.92 [X of ABCDX, m, H-C(2)], 3.75, 3.65 [AB of ABCDX, $J_{AB} = 11.0$, $J_{AX} = 3.5$, $J_{BX} = 5.5$, 2H-C(3)]. The EI mass spectrum showed signals at *m/z* (%) 342 (0.8), 328 (0.3), 311 (0.6), 267 (1.4), 251 (4.7), 57 (100). High resolution EIMS: found M^+, 342.31266 ± 0.005; $C_{21}H_{42}O_3$ requires 342.31339.

Compound 2. Both the ^{1}H- and ^{13}C-NMR spectra of **2** in $CDCl_3$ proved practically superimposable on those of **1** except for the signal δ_H 0.83 [d, $J = 6.5$, 3H-C(16′) and 3H-C(17′)]. EIMS showed signals at *m/z* (%) 328 (0.9), 297 (0.6), 253 (1.5), 237 (4.6), 57 (100) and the composition could be ascertained by HREI: found M^+, 328.29789 ± 0.005; $C_{20}H_{40}O_3$ requires 328.29774. From this structure 1-(15-methylhexadec-1-enyloxy)-2,3- propanediol is assigned to compound **2**.

Compound 3. Although the ^{1}H-NMR signals for a 2:1 mixture of compounds **3** and **4** in $CDCl_3$ were too crowded to allow analysis, and 2D maps proved insufficiently resolved, the change to C_5D_5N as solvent led to spectra detailed enough for the assignment of all hydrogens for both compounds. Thus, COSY maps pointed to a glycerol ether of a methoxylated long hydrocarbon chain, indicating the partial structure $\sim OCH_2CH(OMe)CH_2CH{=}CHCH_2(CH_2)_n\sim$. FAB-MS spectra gave the molecular ions at *m/z* 367 $(M+23)^+$ and *m/z* 345 $(M+1)^+$, establishing, in combination with the NMR data below, compound **3** as 1-(2-methoxyhexadec-4-enyl-1-oxy)-2,3-propanediol. The full ^{1}H-NMR spectrum of **3** in C_5D_5N consists of δ 4.00, 3.93 [AB of ABCDX, $J_{AB} = 10.0$, $J_{AX} = 5.0$, $J_{BX} = 6.0$, 2H-C(1)]; 4.43 [X of ABCDX m, H-C(2)]; 4.19, 4.15 [CD of ABCDX, $J_{CD} = 11.0$, $J_{DX} = 5.0$, $J_{B'X} = 6.0$, 2H-C(3)]; 3.73, 3.70 [AB of ABX, $J_{AB} = 10.0$, $J_{A'X} = 4.3$, $J_{B'X} = 5.8$, 2H-C(1′)]; 3.54 [qd, X of ABX, further coupled, $J_{XA} = 4.3$, $J_{XB} = 5.8$, $J_{2',3'} = 5.8$, H-C(2′)]; 3.44 [s, MeOC(2′)]; 2.48 [br.t, $J_{3',2'} = 5.8$, $J_{3',4'} = 7.0$, $J_{3',5'}$ small, 2H-C(3′)]; 5.60 [dtt,



1 n=12, R=-CHMe$_2$
2 n=11, R=-CHMe$_2$



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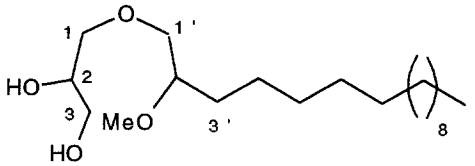

4

$J_{4',3'} = 7.0$, $J_{4',5'} = 11.0$, $J_{4',6'}$ small, H-C(4')]; 5.50 [dtt, $J_{5',4'} = 11.0$, $J_{5',6'} = 7.0$, $J_{5',3'}$ small, H-C(5')]; 2.11 [br.q, $J_{6',5'} = 7.0$, $J_{6',7'} = 6.5$, 2H-C(6')]; 1.2 [br.s, from 2H-C(7') to 2H-C(15')]; 0.87 [t, $J_{16',15'} = 6.5$, 3H-C(16')]. 13C-NMR spectrum in C_5D_5N: 73.59 [t, C(1')], 80.59 [d, C(2')], 57.50 [q, MeO-C(2')], 30.00 [t, C(3')], 126.00 [d, C(4')], 132.33 [d, C(5')], 27.81 [t, C(6')], several signals close to 30 [t, C(7')-C(15')], 14.44 [q, C(16')], 74.45 [t, C(1)], 72.16 [d, C(2)], 64.88 [t, C(3)].

Compound 4. The ^{1}H-NMR of **4** in C_5D_5N was very similar to that of compound **3** except for lack of signals for unsaturation. The following resonances could be recorded: 4.01, 3.94 [AB of ABCDX, $J_{AB} = 10.0$, $J_{AX} = 5.0$, $J_{BX} = 6.0$, 2H-C(1)]; 4.44 [X of ABCDX, m, H-C(2)]; 4.20, 4.16 [CD of ABCDX, $J_{CD} = 11.0$, $J_{A'X} = 5.0$, $J_{DX} = 6.0$, 2H-C(3)]; 3.71, 3.66 [AB of ABX, $J_{AB} = 10.0$, $J_{A'X} = 4.3$, $J_{B'X} = 5.8$, 2H-C(1')]; 3.46 [qd, X of ABX, further coupled, $J_{XA} = 4.3$, $J_{XB} = 5.8$, $J_{2',3'} = 5.8$, H-C(2')]; 3.44 [s, MeOC(2')]; 1.61 [m, 2H-C(3')]; 1.2 [br.s, from 2H-C(4') to 2H-C(15')]; 0.87 [t, $J_{16',15'} = 6.5$, 3H-C(16')]. ^{13}C-NMR spectrum in C_5D_5N: 74.14 [t, C(1')], 80.59 [d, C(2')], 57.58 [q, MeO-C(2')], 32.26 [t, C(3')], several signals close to 30 [t, C(4')-C(15')], 14.44 [q, C(16')], 74.45 [t, C(1)], 72.16 [d, C(2)], 64.88 [t, C(3)]; FAB mass spectra gave peaks at m/z 369 $(M + 23)^+$ and m/z 347 $(M + 1)^+$. Comparison of ^{1}H-NMR data in $CDCl_3$ for compound **4** with those for synthetic diastereomeric (2'*S*,2*S*)- and (2'*R*,2*S*)-1-*O*-(2-methoxyhexadecyl) glycerols[12] allowed us to assign the configuration (2'*S*,2*R*) or (2'*R*,2*S*) to **4**. We favour the latter on the basis that compound **4** in THF solution showed a positive sign of rotation, albeit of small specific magnitude, $[\alpha]_{577} = +8.75$; $[\alpha]_{546} = +6.25$ (*c* 0.08 g/100 ml); that these are affected by large uncertainty is due to scarcity of material and thus small measured optical rotations.

Discussion

It has been shown above that *G. vitreus* contains, besides long-chain olefinic and saturated glyceryl ethers and polyunsaturated glycerol esters, free glycerol enol ethers possessing a long, branched, C-chain (**1–2**). The latter belong to a rare class of compounds, particularly **2** because of its odd C long-chain. More commonly encountered are saturated glycerol ethers of even or odd C-chains, generally occurring as diesters. In unesterified form they are typical of marine organisms, mostly sponges[8,13,14] and anthozoans, such as batyl alcohols in the gorgonian *Plexaura flexuosa*[15] and both batyl and chimyl alcohol in the zoanthid *Palythoa liscia*[16].

Polyunsaturated analogues have been isolated from an unidentified sponge of Brittany waters[17]. Long-chain enol ethers of glycerol have been found in marine sponges, either in the phospholipid fraction[8], or as free dienyne[18,19] or enediyne derivatives[20,21].

Besides the enol ethers, *G. vitreus* also contains methoxy-alkyl glycerol ethers (**3** and **4**), which belong to a class of compounds massively present in shark and ratfish liver oil[22], where they are accompanied by saturated, monoene, or polyene analogues with chain lengths C_{14}-C_{22}. In trace amounts they also occur in herring, mackerel, cod liver oil, crayfish, shrimp, and mussels[23,24]. It is interesting that 1-*O*-(2-methoxyhexadecyl) glycerols inhibit tumour growth and metastasis formation when added to the diet of mice[25]. Biological activity has been noticed for long-chain saturated *S* glycerol ethers, especially antimicrobial[8] and ichthyotoxic effects. In contrast, a synthetic *R* analogue proved devoid of ichthyotoxicity[13]. Of special relevance is the feeding-deterrence activity displayed by chimyl alcohol (=1-*O*-hexadecyl glycerol) against the Antarctic sea star *Odontaster validus*, which may account for protection of the nudibranch *Tritoniella belli* Eliot[26]. In this light, it is appealing to consider the glycerol ethers isolated here as adaptive metabolites evolved for defense of *G. vitreus*. However, studies of predation on *G. vitreus* and in vitro tests with its glycerol ethers would be difficult and very expensive to carry out because of the great labour required to collect the brachiopod and the low yield of natural compounds; it might be more practical to solve the problem of securing these compounds through total synthesis[27].

Brachipods are suspension feeders – via the lophophore and the mantle lobes – probably thriving on plankton and colloidal and dissolved organic matter. This is a largely obscure area, however, so we cannot judge whether the metabolites isolated from *G. vitreus* may have a dietary source. However, we are not aware of any similar product from typical parasites or symbionts of brachiopods such as trematodes, copepods, and ciliated protists, the latter being under active study in our laboratories[28–30].

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1 Emig, C. C., and García-Carrascosa, M. A., Sci. Mar. *55* (1991) 385.
2 Thayer, C. W., Science *228* (1985) 1527.
3 Pietra, F., A Secret World. Natural Products of Marine Life, p. 156. Birkhäuser Verlag, Basel 1990.
4 Pietra, F., Chem. Soc. Rev. *24* (1995) 65.
5 Doddrell, D. H., Pegg, D. T., and Bendall H. R., J. magn. Reson. *48* (1982) 323.
6 Pegg, D. T., Doddrell, D. M., and Bendall, M. R., J. chem. Phys. *77* (1982) 2745.
7 Bax, A., J. magn. Reson. *53* (1983) 517.
8 Smith, G. M., and Djerassi, C., Lipids *22* (1987) 236.
9 Legrand, M., and Rougier, M. J., in: Stereochemistry, Vol. 2, p. 179, Ed. H. B. Kagan. G. Thieme Verlag, Stuttgart 1977.
10 Vtorov, I. B., Serebrennikova, G. A., and Evstigneeva, R. P., Tetrahedron Lett. (1971) 4605.

11 Gigg, J., and Gigg, R., J. chem. Soc. (C) (1968) 16.
12 Stallberg, G. A. M., Acta chem. scand. *44* (1990) 368.
13 Myers, B. L., and Crews, P., J. org. Chem. *48* (1983) 3583.
14 Do, M. N., and Erickson, K. L., Tetrahedron Lett. *24* (1983) 5699.
15 Kind, C. A., Bergmann, W., J. org. Chem. *7* (1942) 424.
16 Pettit, G. R., and Fujii, Y., J. nat. Prod. *45* (1982) 640.
17 Mancini, I., Guella, G., and Pietra, F., Helv. chim. Acta *74* (1991) 941.
18 Guella, G., Mancini, I., and Pietra, F., Helv. chim. Acta *70*, (1987) 1050.
19 Guella, G., Mancini, I., and Pietra, F., Helv. chim. Acta *70* (1987) 1400.
20 Perry, N. B., Becker, G. E., Blunt, J. W., Lake, R. J., and Munro, M. H. G., J. nat. Prod. *53* (1990) 732.
21 Iguchi, K., Kitade, M., Kashiwagi, T., and Yamada, Y., J. org. Chem. *58* (1993) 5690.
22 Hayashi, K., and Takagi, T., Nippon Suisan Gakkaishi *48* (1982) 1347.
23 Hallgren, B., Niklasson, A., Stallberg, G., and Thorin, H., Acta chem. scand. Ser. B *28* (1974) 1035.
24 Hallgren, B., Niklasson, A., Stallberg, G., and Thorin, H., Acta chem. scand. Ser. B *28* (1974) 1029.
25 Boeryd, B., and Hallgren, B., Acta pathol. microbiol. scand. Sect. A *88A* (1980) 11.
26 McClintock, J. B., Slattery, B. J., Heine, J. N., Bryan, P. J., Yoshida, W., Davies-Coleman, M. T., and Faulkner, D. J., J. chem. Ecol. *20* (1994) 3361.
27 Pfaendler, H. R., and Muller, F. X., Synthesis (1992) 350.
28 Dini, F., Guella, G., Giubbilini, P., Mancini, I., and Pietra F., Naturwissenschaften *80* (1993) 84.
29 Guella, G., Dini, F., Tomei, A., and Pietra, F., J. chem. Soc., Perk. Trans. *1* (1994) 161.
30 Guella, G., Dini, F., Erra, F., and Pietra F., J. Chem. Soc., chem. Comm. (1994) 2585.