March 1989 Communications to the Editor *Chem. Pharm. Bull.* 37(3) 849—851 (1989) 849

# HETEROSIGMA-GLYCOLIPIDS I AND II, TWO NEW GALACTOLIPIDS CONTAINING OCTADECATETRAENOYL AND EICOSAPENTAENOYL RESIDUES, FROM A RAPHIDOPHYTE DINOFLAGELLATE *HETEROSIGMA* SP.

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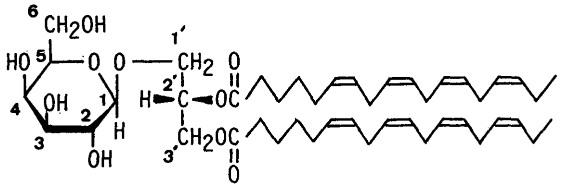
A new galactolipid containing an eicosapentaenoyl (EPA) residue, named heterosigma-glycolipid II (2), was isolated from a cultured raphidophycean dinoflagellate *Heterosigma* sp., together with another new galactolipid heterosigma-glycolipid I (1). Based on enzymatic hydrolysis using lipase and physicochemical evidence, the structures of heterosigma-glycolipids I (1) and II (2) have been determined as (2'S)-2',3'-di-O-(6,9,12,15-octadecatetraenoyl)-glyceryl β-D-galactopyranoside and (2'S)-2'-O-(6,9,12,15-octadecatetraenoyl)-3'-O-(5,8,11,14,17-eicosapentaenoyl)-glyceryl β-D-galactopyranoside, respectively.

KEYWORDS heterosigma-glycolipid I; heterosigma-glycolipid II; dinoflagellate raphidophycean; *Heterosigma* sp.; galactolipid; eicosapentaenoic acid; enzymatic hydrolysis; lipase

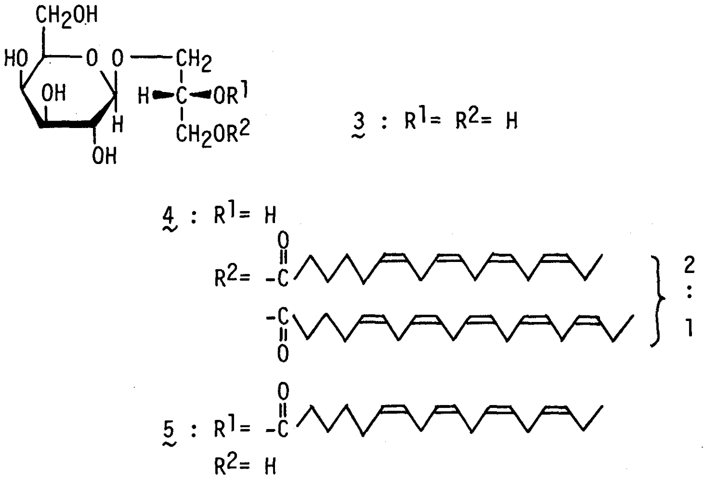
The low incidence of acute myocardial infarction in Eskimos in Greenland[1] has been believed to be due to the high content of 5,8,11,14,17-eicosapentaenoic acid (EPA) in their diet of fish. In recent years, EPA has been shown to exhibit various biological activities.[2] In search of new biologically active marine natural products,[3] we have been engaged in studies of phytoplankton metabolites, and have found a new EPA-containing galactolipid named heterosigma-glycolipid II (2) together with another new galactolipid, heterosigma-glycolipid I (1), from a cultured raphidophycean dinoflagellate *Heterosigma* sp. The raphidophycean dinoflagellates of *Heterosigma* sp.[4,5] are widely distributed in coastal waters, where they may often cause "red-tide" phenomena and extensive mortalities for cultured fish and other marine organisms.[6] This paper communicates the evidence consistent with the structures of heterosigma-glycolipids I (1) and II (2).

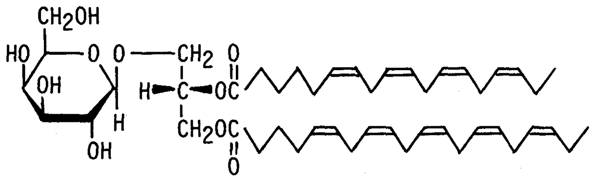
A raphidophycean dinoflagellate, *Heterosigma* sp., which was isolated from the coastal water of Suma-ura, Osaka Bay, was grown in the mixed media of ASP-2[7] and ES[8] in 20 l glass bottles at 21°C. The cultivation was carried out in a 16:8 h light to dark cycle using homolux lamps FL40S-PG (National) for 40 days. The combined 60 l culture was partitioned with EtOAc and the EtOAc-soluble portion was evaporated under reduced pressure to give 1.2 g of the extractive. Silica gel column chromatography ($CHCl_3$-MeOH=20:1) of the extractive furnished a glycolipid fraction (120 mg) as the major constituent, which showed a single spot on thin-layer chromatogram (TLC). The glycolipid fraction was further separated by HPLC (ZORBAX ODS, MeOH-$H_2O$) to afford two major glycolipids: heterosigma-glycolipid I (1) (15 mg) and heterosigma-glycolipid II (2) (46 mg).

Heterosigma-glycolipid I (1), $[\alpha]_D^{25}$ -3.6° ($CHCl_3$), IR ($CHCl_3$): 3425, 1725 $cm^{-1}$, gives a molecular ion peak at m/z 793 ($M+Na^+$) in FAB-MS. The $^1H$-NMR (500 MHz) and the $^{13}C$-NMR[9] of 1 shows signals which are characteristic of a glycolipid (Table I). Treatment of heterosigma-glycolipid I (1) with 15 % NaOMe in MeOH afforded a glyceryl galactoside and methyl 6,9,12,15-octadecatetraenoate, which was identified by the analysis of its Mass, $^1H$-NMR,[10] and $^{13}C$-NMR spectra. The glyceryl galactoside, $[\alpha]_D^{23}$ -8° ($H_2O$) was shown to be identical in all respects with (2'R)-1-O-glyceryl β-D-galactopyranoside (3), which was previously obtained by NaOMe treatment of an anti-inflammatory galactolipid M-5[11] isolated from the Okinawan marine sponge *Phyllospongia foliascens*. The comparisons in detail of $^1H$-NMR and $^{13}C$-NMR data[9,12] for heterosigma-glycolipid I (1) and 3 have shown that the fatty acid residues in 1 attach to C-2' and C-3' in the glycerol moiety (Table I). Thus, the carbon signal due to C-1' of the glycerol moiety in 1 is obtained with higher ppm (*ca* 3 ppm)[13] compared to that in 3, while signals ascribable to C-2' and C-3' of 1 and 3 occur



heterosigma-glycolipid I (1)





heterosigma-glycolipid II (2)

Table I. $^{1}$H-NMR Data for 1, 2, 3, 4, and 5[a]

| $^{1}$H | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 4.74(d,J=7.9) | 4.83(d,J=7.6) | 4.88(d,J=7.6) | 4.82(d,J=7.6) | 4.79(d,J=7.6) |
| 2 | 4.32[b] | 4.44[b] | 4.48(dd,J=7.6,9.5) | 4.42[b] | 4.39(dd,J=7.6,9.2) |
| 3 | 4.06(dd,J=9.5,3.4) | 4.15(dd,J=9.6,2.9) | 4.13(dd,J=9.5,3.4) | 4.11(dd,J=9.5,3.3) | 4.09[b] |
| 4 | 4.46(d,J=3.4) | 4.57(d,J=2.9) | 4.52(d,J=3.4) | 4.50(d,J=3.3) | 4.47(d,J=3.1) |
| 5 | 3.98(dd,J=5.8,5.5) | 4.08[b] | 4.04(dd,J=6.4,5.5) | 4.03[b] | 3.99(dd,J=6.1,6.1) |
| 6 | 4.32(2H,m) | 4.44(2H,m) | 4.40(2H,m) | 4.36(2H,m) | 4.34(2H,m) |
| 1' | 4.29(dd,J=10.7,5.2) | 4.37(dd,J=10.8,5.3) | 4.44(dd,J=10.1,6.2) | 4.37[b] | 4.34[b] |
|  | 4.01(dd,J=10.7,5.0) | 4.08[b] | 4.22(dd,J=10.1,4.3) | 4.04(dd,J=10.4,5.2) | 4.12(dd,J=11.6,4.7) |
| 2' | 5.58(m) | 5.67(m) | 4.40(m) | 4.42(m) | 5.54(m) |
| 3' | 4.62(dd,J=11.9,3.1) | 4.70(dd,J=11.9,3.4) | 4.10(d,J=4.6) | 4.51(d,J=5.5) | 4.42(dd,J=10.8,5.2) |
|  | 4.45(dd,J=11.9,5.2) | 4.54(dd,J=11.9,6.6) | 4.09(d,J=5.5) | 4.50(d,J=3.1) | 4.17(dd,J=10.8,5.0) |

a) All compounds were measured in $d_5$-pyridine (treated with 1 drop of $D_2O$) at 500 MHz and the assignments were based on decoupling experiments. b) These signals overlapped within the same vertical column.

at similar chemical shifts. Consequently, the chemical structure of heterosigma-glycolipid I (1) has been expressed as (2'S)-2',3'-di-O-(6,9,12,15-octadecatetraenoyl)-glyceryl β-D-galactopyranoside (1).

Heterosigma-glycolipid II (2), $[\alpha]_D^{25}$ -4° ($CHCl_3$), shows a molecular ion peak at m/z 819 ($M+Na^+$) in FAB-MS. The $^{1}$H-NMR and $^{13}$C-NMR[14)] spectra of 2 closely resemble those spectra of heterosigma-glycolipid I (1) except for signals due to the fatty acid moieties. Treatment of heterosigma-glycolipid II (2) with NaOMe as carried out for heterosigma-glycolipid I (1) furnished the same glyceryl β-D-galactoside (3) and a mixture of fatty acid methyl esters. The fatty acid composition in heterosigma-glycolipid II (2) was determined by GLC analysis of the above methyl esters to be a 1:1 mixture of methyl 6,9,12,15-octadecatetraenoate and methyl 5,8,11,14,17-eicosapentaenoate, the latter being identified with an authentic sample (Sigma) by GLC, HPLC, and $^{1}$H-NMR.[15)] Furthermore, the $^{1}$H-NMR and $^{13}$C-NMR analysis of heterosigma-glycolipid II (2) in comparison with heterosigma-glycolipid I (1) indicated that the fatty acid residues in 2 were attached to the 2'-OH and 3'-OH of the glycerol moiety.

In order to determine the sequence of these fatty acid residues in heterosigma-glycolipid II (2), we first attempted partial removal of the fatty acid residues in 2. Reduction of heterosigma-glycolipid II (2) with $LiAlH(Ot\text{-}Bu)_3$ in benzene at 40°C for 1.5 h, provided a 3'-O-acylated galactolipid 4. The $^{1}$H-NMR spectrum of 4 showed the signal of 2'-H at δ 4.42 (m) which was at higher fields than the signal of 2'-H of 2, and treatment of 4 with NaOMe furnished a 2:1 mixture of methyl 6,9,12,15-octadecatetraenoate and methyl 5,8,11,14,17-eicosapentaenoate. So it appeared that $LiAlH(Ot\text{-}Bu)_3$ reduction of heterosigma-glycolipid II (**2**) proceeded less regioselectively, since acyl migration of the fatty acid residue from 2'-OH to 3'-OH of the glycerol moiety in **2** might occur when the 3'-O-acyl residue in **2** was removed. We next subjected heterosigma-glycolipid II (**2**) to enzymatic hydrolysis. On enzymatic hydrolysis using Lipase type XIII (from Pseudomonas sp., Lot 67F-09641, Sigma) in dioxane-$H_2O$ (1:1) at 37°C for 4 h, **2** furnished quantitatively a 2'-O-acylated galactolipid **5** and 5,8,11,14,17-eicosapentaenoic acid which was identified by $CH_2N_2$ treatment as the methyl ester. As shown in Table I, the $^1$H-NMR spectrum of **5** showed the signals due to 3'-$H_2$ at higher fields than those in **2**, and treatment of **5** with NaOMe liberated methyl 6,9,12,15-octadecatetraenoate. So it was concluded that residues of 6,9,12,15-octadecatetraenoyl and 5,8,11,14,17-eicosapentaenoyl were attached to 2'-OH and 3'-OH respectively of the glycerol moiety in **2**. Consequently, the chemical structure of heterosigma-glycolipid II (**2**) has been determined as (2'S)-2'-O-(6,9,12,15-octadecatetraenoyl)-3'-O-(5,8,11,14,17-eicosapentaenoyl)-glyceryl β-D-galactopyranoside (**2**).

It is noted here that the enzymatic hydrolysis of **2** using lipase in dioxane-water has quantitatively proceeded and the regioselective partial hydrolysis of **2** has occurred at the C-3' position of the glycerol moiety without acyl migration. In addition, it appears from the view point of food-chains in marine organisms that the lipid (e.g. heterosigma-glycolipid II (**2**)) initially produced by marine unicellular algae may be one of the origins of EPA contained abundantly in certain kinds of fish.

ACKNOWLEDGEMENTS The authors are grateful to Dr. Y. Hara, Institute of Biological Sciences, University of Tsukuba, for identification of plankton and to Prof. T. Momose, Faculty of Pharmaceutical Sciences, Kinki University, for measuring the FAB-MS. They also thank the Ministry of Education, Science, and Culture of Japan for financial support.

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9) The $^{13}$C-NMR data for **1** (125 MHz, $CD_3OD$, $\delta_C$): 105.3 (C-1), 72.4 (C-2), 74.9 (C-3), 70.2 (C-4), 76.8 (C-5), 62.4 (C-6), 68.7 (C-1'), 71.8 (C-2'), 64.0 (C-3'), 174.9, 174.8 (-O-CO-).
10) The $^1$H-NMR data for methyl 6,9,12,15-octadecatetraenoate (500 MHz, $CDCl_3$, δ): 2.32 (2H, t, J=7.5 Hz, 2-H), 1.65 (2H, tt, J=7.5, 8.8 Hz, 3-H), 1.45 (2H, tt, J=8.8, 8.8 Hz, 4-H), 2.09 (4H, m, 5-H and 17-H), 5.40 (8H, m, olefinic H), 2.84 (6H, m, 8,11,14-H), 1.00 (3H, t, J=7.5 Hz, 18-H).
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12) The $^{13}$C-NMR data for **3** (22.5 MHz, $CD_3OD$, $\delta_C$): 105.1 (C-1), 72.5 (C-2), 74.8 (C-3), 70.3 (C-4), 76.7 (C-5), 62.5 (C-6), 72.0 (C-1'), 72.1 (C-2'), 64.0 (C-3').
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14) The $^{13}$C-NMR data for **2** (125 MHz, $CD_3OD$, $\delta_C$): 105.3 (C-1), 72.4 (C-2), 74.9 (C-3), 70.2 (C-4), 76.8 (C-5), 62.4 (C-6), 68.7 (C-1'), 71.8 (C-2'), 64.1 (C-3'), 174.8, 174.6 (-O-CO-).
15) The $^1$H-NMR data for methyl 5,8,11,14,17-eicosapentaenoate (500 MHz, $CDCl_3$, δ): 2.33 (2H, t, J=7.7 Hz, 2-H), 1.72 (2H, tt, J=7.7, 8.6 Hz, 3-H), 2.11 (4H, m, 4-H and 19-H), 5.40 (10H, m, olefinic H), 2.85 (8H, m, 7,10,13,16-H), 1.00 (3H, t, J=7.5 Hz, 20-H).

(Received January 20, 1989)