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# Phosphocholine-Bonded Galactosylceramides Having a Tri-Unsaturated Long-Chain Base from the Clam Worm, *Marphysa sanguinea*

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**Seven glycosphingolipids were obtained in the pure state from the clam worm, *Marphysa sanguinea*, by preparative HPLC with a reversed-phase column in a recycling mode. Their structures were elucidated based on detailed analyses of $^{1}H$- and $^{13}C$-NMR spectra of the intact compounds. The position and the geometry of double bonds in the long-chain base (LCB) were determined by the two-dimensional NMR (COSY, NOESY, and HMBC) analyses. All compounds are phosphocholine-bonded monogalactosylceramides and two of them have tri-unsaturated long-chain bases.**

**Key words: glycosphingolipid, *Marphysa sanguinea*, phosphocholine-galactosylceramide, recycling HPLC, tri-unsaturated long-chain base.**

Recent studies on the lipid composition of phylum Annelids, such as *Pheretima hilgendorfi* (*1*), *Pheretima asiatica* (*2, 3*), *Marphysa sanguinea* (*4*), and *Neanthes diversicolor* (*5*) have revealed that they contain a novel type of glycosphingolipids. In contrast to glycosylceramides so far reported, these have a phosphocholine group at the C-6 position of the galactose moiety. Due to the difficulty of isolation of homogeneous compounds from complex mixtures of analogs and/or homologs, their structures have been determined conventionally by using such mixtures. Our recent work showed that the recycling HPLC technique with a reversed-phase column using various chloroform/methanol mixtures as a mobile phase was effective for isolating intact glycosphingolipids. We successfully isolated three, two, and five novel galactosylceramides in the pure state from *Pheretima asiatica* (*3*), *Marphysa sanguinea* (*4*), and *Neanthes diversicolor* (*5*), respectively, and characterized their full structures on the basis of chemical and spectral data.

As a part of our continuing research aimed at the discovery of biologically active compounds in annelids, particularly glycosphingolipids, we have made a more detailed survey of a glycosylceramide fraction obtained from the clam worm, *Marphysa sanguinea*. Four new monogalactosylceramides in addition to three known ones were obtained in the pure state by application of the recycling HPLC technique. High resolution NMR (600 MHz) spectroscopic analysis revealed that all compounds are phosphocholine-linked monogalactosylceramides and two of them possess a tri-unsaturated LCB residue. The location and geometry of the double bonds were determined from the NOESY and HMBC spectra (*6, 7*). This paper deals with the isolation and structure of these compounds.

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Abbreviations: EI-MS, electron impact ionization mass spectrometry; FAB-MS, fast atom bombardment mass spectrometry; LCB, long-chain base; Rt, retention time; HMBC, two-dimensional heteronuclear multiple bond connectivity spectroscopy; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; COSY, two-dimensional shift correlation spectroscopy.

## MATERIALS AND METHODS

*Extraction and Fractionation of the Crude Glycosphingolipid Fraction*—Sold as commercial bait for fishing, *Marphysa sanguinea* (3 kg) were purchased from Meitokuya (Aichi Prefecture, March, 1992). The live materials were soaked in 5 liters of chloroform/methanol (1 : 1), then in 5 liters of methanol, each for one day at room temperature. The extracts were combined and precipitates were removed by filtration. The filtrate was evaporated *in vacuo* to dryness to give an extractive (198 g), which was shaken with 900 ml of chloroform/methanol/water (2 : 1 : 1). The lower phase gave, on evaporation, a fraction (49 g), which was placed on a silica gel column (bed volume 500 ml) and eluted successively with the following: chloroform/methanol (8 : 2→7 : 3)→chloroform/methanol/water (7 : 3 : 0.5→6 : 4 : 1→5 : 5 : 1). The eluates were monitored by HPTLC (mobile phase: chloroform/methanol/water, 6 : 4 : 1) and those showing a positive tailing band on spraying with Dittmer-Lester's reagent (*8*) were combined and evaporated to give a crude substance (6.5 g). This was subjected to column chromatography on a reversed-phase gel (bed volume 380 ml, Cosmosil $75C_{18}$-OPN, Nacalai Tesque) using methanol→chloroform/methanol (1 : 1) as the eluent to afford a crude glycosphingolipid fraction (2.8 g), which showed a reddish violet tailing band ($R_f$ *ca.* 0.2) on HPTLC (silica gel 60, E. Merck, Art 5556, mobile phase: chloroform/methanol/water, 5 : 5 : 1) on spraying with 5% $H_2SO_4$ in methanol, followed by heating.

*Isolation of Galactosylceramides*—The crude glycosphingolipid fraction was subjected to preparative HPLC on a reversed-phase column (size, 2 cm i.d.×25 cm; 10 μm, Inertsil prep-ODS, GL Sciences) using chloroform/meth-

anol (0.9 : 20) as an eluent. Recycling HPLC was conducted with a JASCO 880-PU pump equipped with a JASCO recycling valve by use of chloroform/methanol (1 : 20) as a mobile phase.

*NMR Spectroscopy*—The NMR spectra were recorded on a GE NMR OMEGA 600 instrument at 600 MHz ($^1$H) and 150 MHz ($^{13}$C) and a JEOL JMN GSX 400 instrument at 400 MHz ($^1$H) and 100 MHz ($^{13}$C), using a solution of *ca.* 10 mg in 0.4 ml methanol-$d_4$ with tetramethylsilane as the internal standard, at a probe temperature of 35°C. The NOESY spectrum was obtained using a mixing time of 500 ms. The HMBC spectrum was recorded at 600 MHz with 32 scans ($^{2,3}J_{CH}$ = 7 Hz).

*Mass Spectrometer*—The mass spectra were taken on a JEOL JMS DX-300 spectrometer equipped with a JMA 3500 data system. (FAB-MS: accelerating voltage, 3 kV; matrix, glycerin; collision gas, Xe; EI-MS: ionization voltage, 30 eV; accelerating voltage, 3 kV.)

*Determination of Fatty Acid*—Each compound was treated with 7.5% methanolic HCl at 90°C for 1 h. The fatty acid methyl ester produced was extracted with *n*-hexane, then analyzed by GLC [fused silica capillary column bonded MPS-50 (Quadrex), 0.25 mm × 50 m; column temp. 230°C (hold, 12 min) −240°C at 1°C/min] and EI-MS.

## RESULTS

The glycosphingolipid fraction (2.8 g) was obtained from live clam worm, *Marphysa sanguinea* (3 kg) as described in "MATERIALS AND METHODS." Figure 1 shows a chromatogram of reversed-phase HPLC of the glycosphingolipid fraction. It gave three major peaks, I-III, accompanied by some minor ones. Four portions corresponding to peaks I-IV were collected and their purity was examined by the positive ion FAB-MS and the $^{13}$C-NMR spectra. Fractions I and II each exhibited only one $[M+H]^+$ ion peak in their FAB-MS spectra, and no carbon signals ascribable to analogous compounds appeared in their $^{13}$C-NMR spectra. These observations indicated that fractions I (**1**, 10.9 mg, *m/z*: 861 $[M+H]^+$, $[\alpha]_D$ +3.9° in MeOH) and II (**2**, 165 mg, *m/z*: 863 $[M+H]^+$) were homogeneous. On the other hand, fractions III and IV exhibited several pseudo-molecular ion peaks, and they were regarded as being mixtures of homologs and/or analogs. For instance, the positive ion FAB-MS spectrum of fraction IV showed pseudo-molecular ion peaks at *m/z* 903, 891, and 879, and its $^{13}$C-NMR spectrum gave four major signals along with some minor ones due to contaminants (Fig. 2). Fraction IV was further subjected to HPLC in a recycling mode with chloroform/methanol (1 : 20) as a mobile phase, and finally compounds **5** (20 mg, *m/z*: 903 $[M+H]^+$, $[\alpha]_D$ +14.0° in MeOH) (11 cycles), **6** (22 mg, *m/z*: 891 $[M+H]^+$, $[\alpha]_D$ + 8.7° in MeOH), and **7** (20 mg, *m/z*: 879 $[M+H]^+$) (23 cycles) were obtained in the pure state. Fraction III was separated in the same manner to yield homogeneous compounds **3** (12 mg, *m/z*: 877 $[M+H]^+$, $[\alpha]_D$ +5.9° in MeOH) and **4** (85 mg, *m/z*: 865 $[M+H]^+$) (21 cycles).

Three of these compounds, **2**, **4**, and **7**, were identified

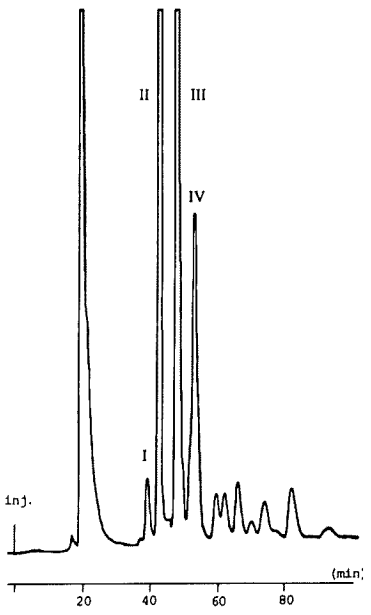


Fig. 1. **HPLC chromatogram of glycosphingolipid fraction.** Column, Inertsil Prep-ODS (20 × 250 mm); solvent, $CHCl_3$/MeOH (1 : 10); flow rate, 3 ml/min; detector, differential refractive index detector.

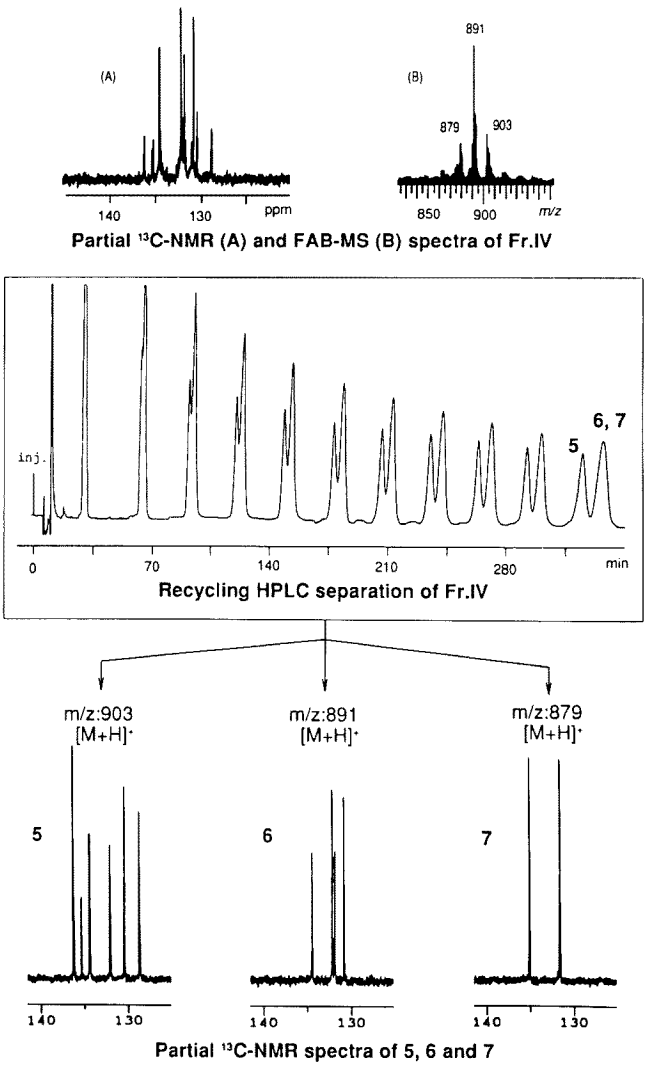


Fig. 2. **Recycling HPLC separation profile.**

respectively as *N*-hexadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*, 8*E*)-sphingadiene, *N*-hexadecanoyl-, and *N*-heptadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-sphingenine obtained previously (*5*) by comparison with authentic samples (Fig. 3).

The $^1$H-NMR spectrum of **5** showed five olefinic proton signals ($\delta$ 5.3–6.1) together with a signal ascribable to a methyl group ($\delta$ 1.70, 3H, s), but other characteristic signals, such as those due to fatty acid, galactose, and phosphocholine units, gave almost the same chemical shifts and coupling constants as those of **2**, **4**, and **7** (Fig. 4). The $^{13}$C-NMR spectrum of **5** exhibited six signals due to olefinic carbons and the signals assignable to C-5 and C-6 of the galactose unit and to C-1″ and C-2″ of the phosphocholine group appeared as doublets owing to coupling with $^{31}$P (*4*, *5*). Hence, it was proved that **5** is an analog of the monogalactosylceramide carrying a phosphocholine group so far obtained, and that it has three unsaturated C-C bonds and a branched methyl group. Methanolysis of **5** liberated a fatty acid methyl ester, which was identified as methyl octadecanoate ($R_t$, 9.23 min) by GLC analysis. In view of its molecular weight (M.W. 902) and components (galactose 6-*O*-phosphocholine and octadecanoyl units), the LCB of **5** was considered to be a tri-unsaturated carbon chain ($C_{19:3}$).

To clarify the position and the geometry of the double bonds in the LCB unit of **5**, its $^1$H-NMR spectrum was analyzed by the application of $^1$H-$^1$H COSY. The $^1$H-$^1$H COSY spectrum of **5** showed correlation peaks between H-3 ($\delta$ 4.08, t, $J$=7.0 Hz) and H-4 ($\delta$ 5.51, dd, $J$=7.0, 15.0 Hz), H-4 and H-5 ($\delta$ 5.72, ddd, $J$=7.0, 7.0, 15.0 Hz), H-5 and $H_2$-6 ($\delta$ 2.11, m), $H_2$-6 and $H_2$-7 ($\delta$ 2.22, m), and $H_2$-7 and H-8 ($\delta$ 5.35, dd, $J$=7.0, 7.0 Hz), successively. In addition, the H-8 signal was also correlated with methyl protons at $\delta$ 1.70, (3H, s). Hence, it is reasonable to suppose that the branched methyl group is located at C-9. The olefinic proton at $\delta$ 6.03 (d, $J$=15.0 Hz) was correlated with that at $\delta$ 5.56 (ddd, $J$=7.0, 7.0, 15.0 Hz), and the latter showed a correlation with neighboring methylene protons at $\delta$ 2.07 (m). From these findings and from their splitting patterns, the signals at $\delta$ 6.03, 5.56, and 2.07 could be assigned to H-10, H-11, and $H_2$-12, respectively.

All carbon signals arising from **5** were analyzed by the $^1$H-$^{13}$C COSY spectrum, and then the HMBC spectrum was

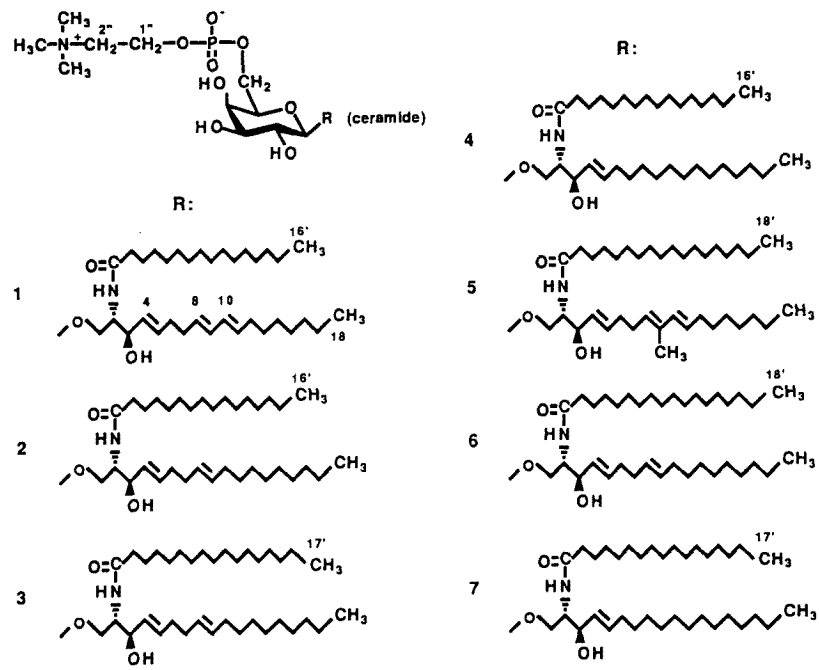


Fig. 3. **Structures of 1–7.**

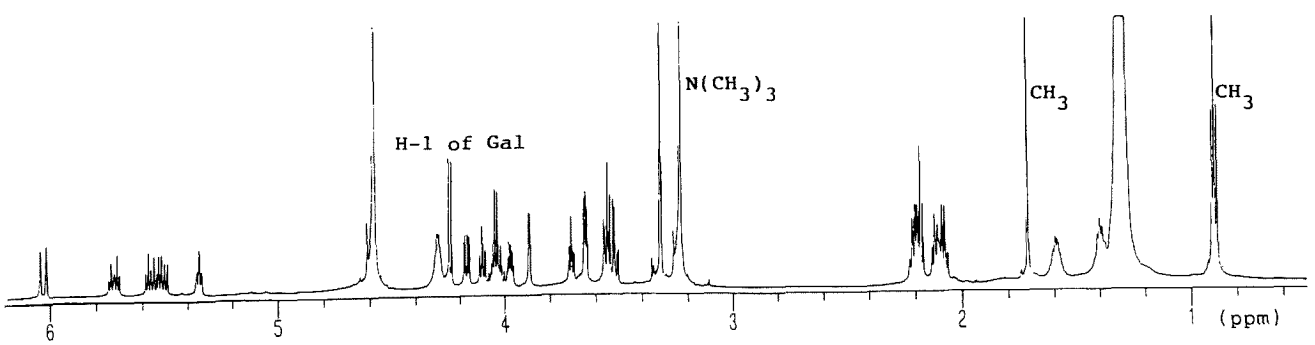


Fig. 4. **$^1$H-NMR spectrum of 5 (600 MHz, $CD_3OD$).**

taken. Figure 5 clearly shows long-range correlation between the methyl protons and three carbons (C-8, 9, and 10) along with other significant correlation peaks, and these were evidenced that the branched methyl group is located at C-9, and that the positions of the three double bonds are C-4, C-8, and C-10. Tables I and II show the chemical shifts of almost all carbon and proton signals.

With regard to their geometry, the three double bonds were all considered to have *trans* forms in view of their coupling constants (Table I). This was confirmed by the NOESY spectrum. As was expected, clear correlation peaks were found between H-8 and H-10, and $CH_3$-9 and H-11 besides other diagnostic correlation peaks (Fig. 6). From the above results, the structure of **5** is characterized as *N*-hexadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-$\beta$-D-galactopyranosyl-(4*E*,8*E*,10*E*)-9-methylsphingatriene (Fig. 3).

The $^1$H-NMR spectrum of **1** differed from that of **5** in the disappearance of the signal due to a branched methyl group, and the replacement of the five olefinic protons in **5** by six proton signals at $\delta$ 5.4-6.0. A fatty acid methyl ester liberated by methanolysis gave, on GLC, a single peak ($R_t$, 6.34 min) identical with that of methyl hexadecanoate. Together with the FAB-MS (M.W. 860) and the $^1$H-NMR spectral data, this finding indicated that the LCB of **1** was another tri-unsaturated ($C_{18:3}$) straight-carbon chain.

Precise assignments of proton signals arising from **1** were achieved from the $^1$H-$^1$H and $^1$H-$^{13}$C COSY spectra. The H-4, H-5, $H_2$-6, and $H_2$-7 and the corresponding carbon signals could be readily assigned by the correlation peaks starting from the characteristic signal due to H-3 in the same manner as described for **5**. Furthermore, the $H_2$-7 signals showed a correlation with one of the remaining four olefinic proton signals, and the other three olefinic protons showed successive correlation. These observations revealed, similar to **5**, the presence of a conjugated double bond in the LCB part. Discrimination between H-8 and H-11, and between H-9 and H-10 was difficult because of their overlapping. However, the corresponding carbon signals were observed separately, and we then measured the HMBC spectrum.

In the HMBC spectrum, $H_2$-7 ($\delta$ 2.13) gave a long-range correlation with a carbon signal at $\delta$ 132.06 (the corresponding proton signal at $\delta$ 5.56), which was also correlated with proton signals at $\delta$ 5.99 and 5.98 together with those at $\delta$ 2.13. Likewise, good correlation peaks between $H_2$-12 and two carbon signals at $\delta$ 131.75 and 133.53 were observed (Fig. 7). The carbon signal at $\delta$ 131.75 also gave long-range correlation peaks with the proton at $\delta$ 5.56, and, therefore, the carbon signals at $\delta$ 131.75, 132.06, 132.29, and 133.53 could be assigned to C-10, C-8, C-9, and C-11, respectively. Thus all olefinic proton as well as carbon signals were assigned without ambiguity (Tables I and II). The NOESY spectrum of **1** exhibited unequivocal cross

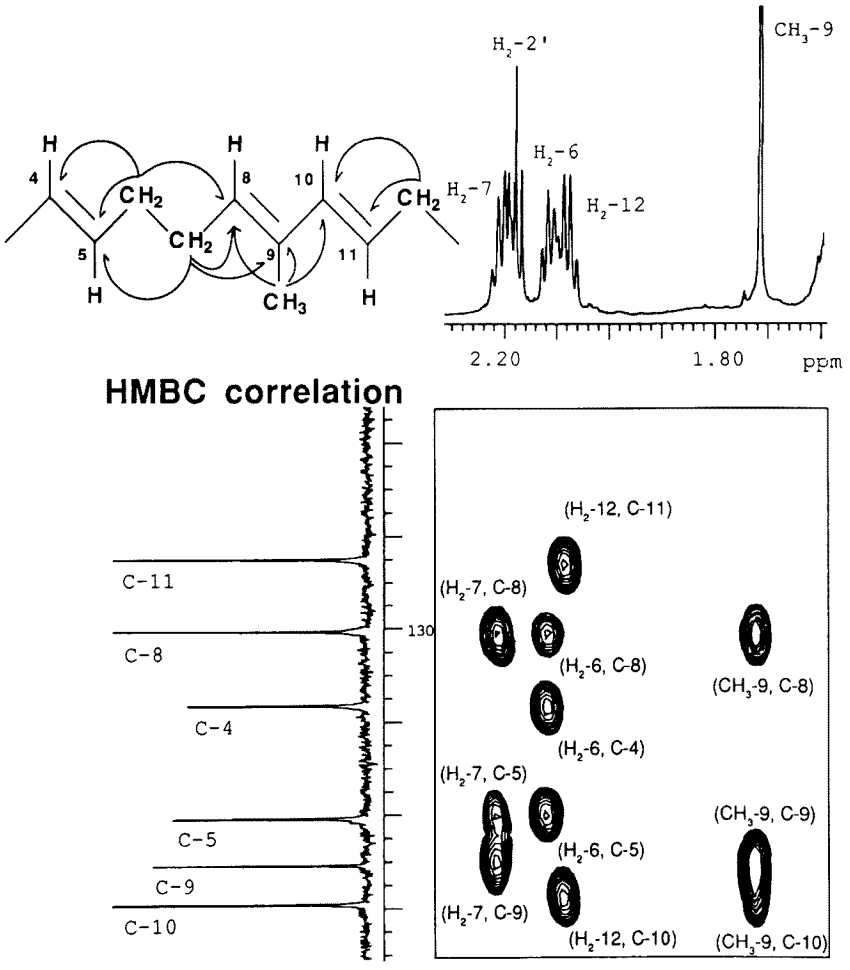


Fig. 5. **Partial HMBC spectrum of 5.**

TABLE I. **$^1$H-NMR chemical shifts of 1, 3, 5, and 6 (400 MHz).** Spectra were taken with *ca.* 10 mg in 0.4 ml of solution ($CD_3OD$); $\delta$ in ppm from tetramethylsilane and coupling constants ($J$) in Hz are given in parentheses. All assignments were based on $^1$H-$^1$H shift correlated two-dimensional spectra. s, singlet; d, doublet; dd, double-doublet; ddd, double-double-doublet; t, triplet; td, triple-doublet; m, multiplet; Cho, choline residue.

| Proton No. | 1 | 3 | 5 | 6 |
|---|---|---|---|---|
| 1 | 3.52 (1H, dd, *J*=6.6, 10.0) | 3.52 (1H, dd, *J*=6.6, 10.0) | 3.52 (1H, dd, *J*=6.6, 10.0) | 3.52 (1H, dd, *J*=6.6, 10.0) |
| | 4.17 (1H, dd, *J*=4.2, 10.0) | 4.18 (1H, dd, *J*=4.0, 10.0) | 4.18 (1H, dd, *J*=4.0, 10.0) | 4.18 (1H, dd, *J*=4.0, 10.0) |
| 2 | 3.96 (1H, ddd, *J*=4.2, 6.6, 7.4) | 3.96 (1H, ddd, *J*=4.0, 6.6, 7.0) | 3.91 (1H, ddd, *J*=4.0, 6.6, 7.0) | 3.91 (1H, ddd, *J*=4.0, 6.6, 7.0) |
| 3 | 4.08 (1H, t, *J*=7.4) | 4.08 (1H, t, *J*=7.0) | 4.08 (1H, t, *J*=7.0) | 4.07 (1H, t, *J*=7.0) |
| 4 | 5.49 (1H, dd, *J*=7.4, 15.0) | 5.47 (1H, dd, *J*=7.0, 15.0) | 5.51 (1H, dd, *J*=7.0, 15.0) | 5.47(1H, dd, *J*=7.0, 15.0) |
| 5 | 5.70 (1H,ddd, *J*=6.1, 6.1, 15.0) | 5.71 (1H,ddd, *J*=7.0, 8.0, 15.0) | 5.72 (1H,ddd, *J*=7.0, 7.0, 15.0) | 5.72 (1H,ddd, *J*=7.0, 8.0, 15.0) |
| 6 | 2.12 (2H, m) | 2.08 (2H, m) | 2.11 (2H, m) | 2.07 (2H, m) |
| 7 | 2.13 (2H, m) | 2.08 (2H, m) | 2.22 (2H, m) | 2.07 (2H, m) |
| 8 | 5.56 (1H, m) | 5.43 (1H, m) | 5.35 (1H, dd, *J*=7.0, 7.0) | 5.43 (1H, m) |
| 9 | 5.99 (1H, m) | 5.43 (1H, m) | | 5.43 (1H, m) |
| 10 | 5.98 (1H, m) | 1.98 (2H, m) | 6.03 (1H, d, *J*=15.0) | 1.99 (2H, m) |
| 11 | 5.55 (1H, m) | | 5.56 (1H,ddd, *J*=7.0, 7.0, 15.0) | |
| 12 | 2.05 (2H, td, *J*=7.0, 7.3) | | 2.07 (2H, m) | |
| 13 | 1.37 (2H, m) | | 1.37 (2H, m) | |
| 18 | 0.90 (3H, t, *J*=7.0) | 0.90 (3H, t, *J*=7.0) | 0.90 (3H, t, *J*=7.0) | 0.90 (3H, t, *J*=7.0) |
| 9-$CH_3$ | | | 1.70 (3H, s) | |
| 2' | 2.17 (2H, t, *J*=7.3) | 2.17 (2H, t, *J*=7.0) | 2.18 (2H, t, *J*=7.0) | 2.18 (2H, t, *J*=7.0) |
| 3' | 1.58 (2H, m) | 1.58 (2H, m) | 1.58 (2H, m) | 1.58 (2H, m) |
| 16' | 0.90 (3H, t, J=7.0) | | | |
| 17' | | 0.90 (3H, t, *J*=7.0) | | |
| 18' | | | 0.90 (3H, t, *J*=7.0) | 0.90 (3H, t, *J*=7.0) |
| Gal -1 | 4.24 (1H, d, *J*=7.1) | 4.24 (1H, d, *J*=7.0) | 4.24 (1H, d, *J*=7.1) | 4.24 (1H, d, *J*=7.0) |
| 2 | 3.54 (1H, dd, *J*=7.1, 9.5) | 3.54 (1H, dd, *J*=7.0, 9.5) | 3.54 (1H, dd, *J*=7.1, 9.5) | 3.54 (1H, dd, *J*=7.0, 9.5) |
| 3 | 3.50 (1H, dd, *J*=2.8, 9.5) | 3.50 (1H, dd, *J*=3.0, 9.5) | 3.51 (1H, dd, *J*=2.2, 9.5) | 3.51 (1H, dd, *J*=2.0, 9.5) |
| 4 | 3.86 (1H, dd, *J*=0.7, 2.8) | 3.86 (1H, dd, *J*=0.7, 3.0) | 3.86 (1H, dd, *J*=0.7, 2.2) | 3.87 (1H, dd, *J*=0.7, 2.0) |
| 5 | 3.72 (1H, td, *J*=0.7, 6.1) | 3.71 (1H, td, *J*=0.7, 6.0) | 3.71 (1H, td, *J*=0.7, 6.1) | 3.71 (1H, td, *J*=0.7, 6.0) |
| 6 | 4.03 (2H, t, *J*=6.1) | 4.02 (2H, t, *J*=6.0) | 4.03 (2H, t, *J*=6.1) | 4.03 (2H, t, *J*=6.0) |
| Cho -1" | 4.30 (2H, m) | 4.30 (2H, m) | 4.31 (2H, m) | 4.31 (2H, m) |
| 2" | 3.65 (2H, broad t, *J*=4.6) | 3.65 (2H, broad t, *J*=4.5) | 3.65 (2H, broad t, *J*=4.9) | 3.66 (2H, broad t, *J*=4.7) |
| $CH_3$ | 3.23 (9H, s) | 3.23 (9H, s) | 3.23 (9H, s) | 3.23 (9H, s) |

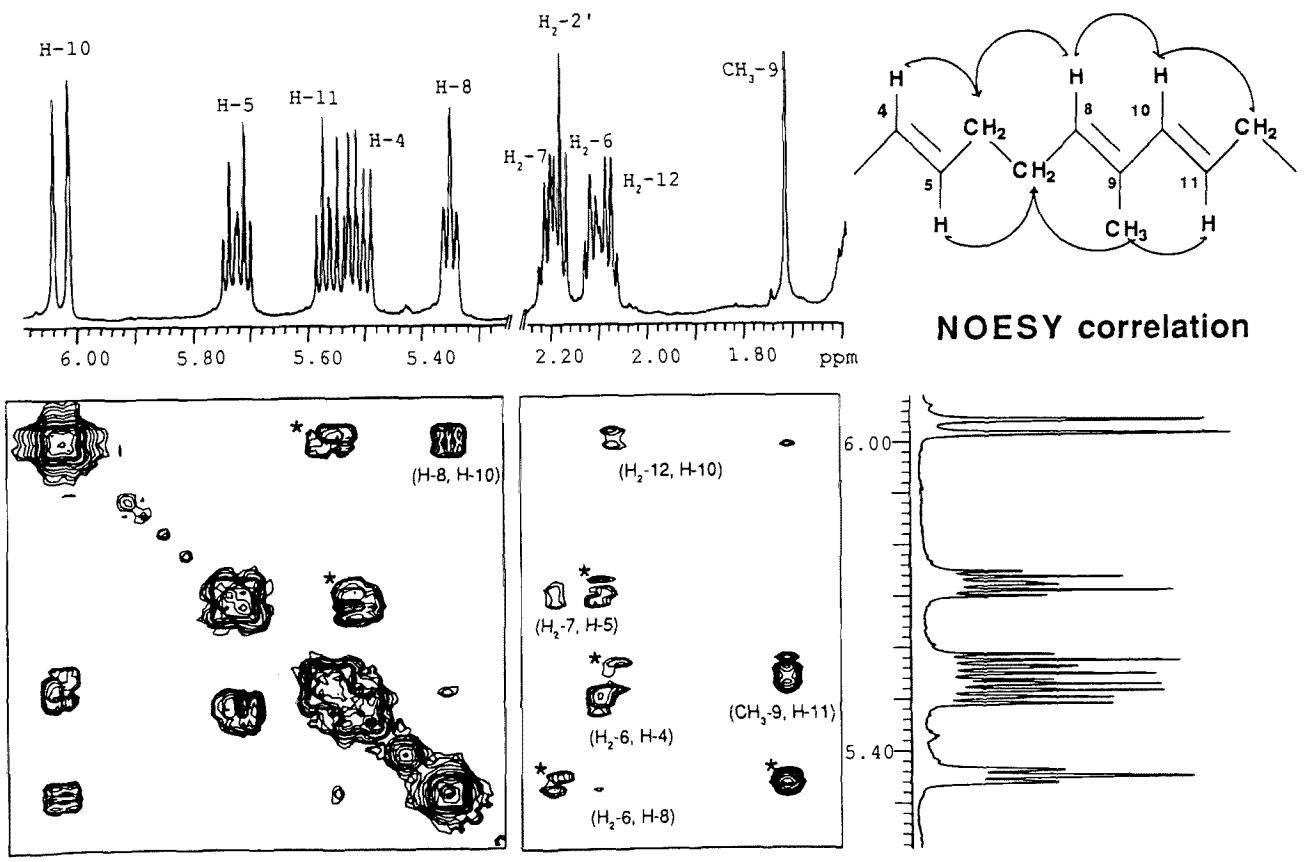


Fig. 6. **Partial NOESY spectrum of 5.** The correlation peaks marked with asterisks are observed in the $^1$H-$^1$H COSY spectrum.

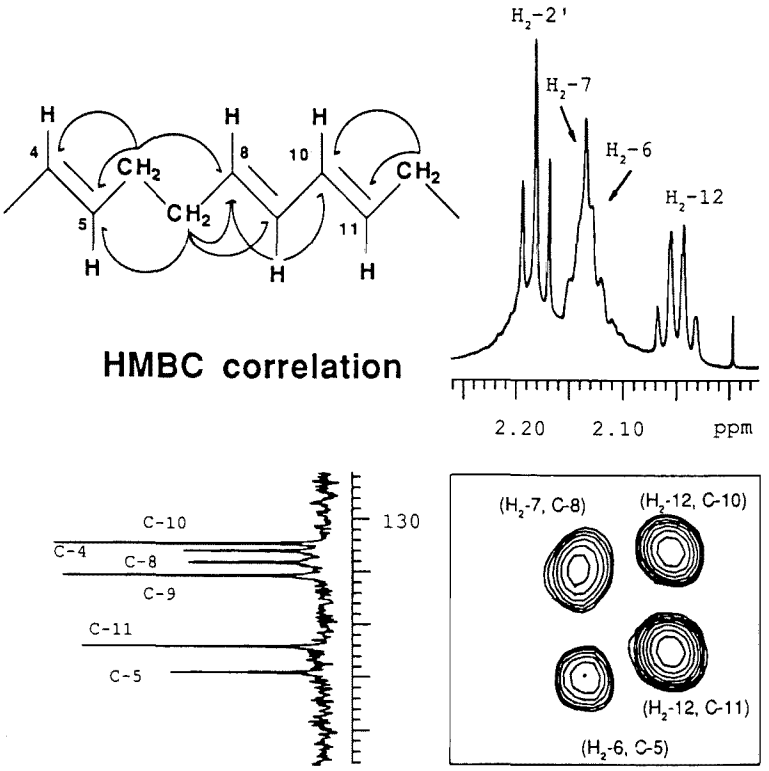


Fig. 7. **Partial HMBC spectrum of 1.**

TABLE II. **$^{13}$C-NMR chemical shifts of 1, 3, 5, and 6 (100 MHz).** Spectra were taken with *ca.* 10 mg in 0.4 ml of solution ($CD_3OD$); $\delta$ in ppm from TMS. Assignments of carbon signals were based on $^1$H-$^{13}$C heteronuclear shift correlated two-dimensional spectra. The signals marked with * appeared as doublets, $J$=5-8 Hz, owing to coupling with $^{31}$p.

| Carbon No. | 1 | 3 | 5 | 6 |
|---|---|---|---|---|
| 1 | 70.17 | 70.39 | 70.19 | 70.16 |
| 2 | 54.75 | 54.78 | 54.86 | 54.75 |
| 3 | 72.86 | 72.86 | 72.84 | 72.89 |
| 4 | 131.94 | 131.77 | 131.94 | 131.72 |
| 5 | 134.14 | 134.41 | 134.26 | 134.30 |
| 6 | 33.41 | 33.77 | 33.49 | 33.71 |
| 7 | 33.35 | 33.73 | 28.89 | 33.64 |
| 8 | 132.06 | 132.03 | 130.28 | 132.00 |
| 9 | 132.29 | 130.66 | 135.22 | 130.65 |
| 10 | 131.75 | 33.50 | 136.13 | 33.05 |
| 11 | 133.53 | | 128.57 | |
| 12 | 33.67 | | 33.99 | |
| 17 | 23.73 | 23.79 | 23.73 | 23.71 |
| 18 | 14.45 | 14.50 | 14.47 | 14.41 |
| 9-$CH_3$ | | | 12.84 | |
| 1′ | 175.96 | 175.91 | 175.91 | 175.90 |
| 2′ | 37.43 | 37.42 | 37.45 | 37.40 |
| 3′ | 27.19 | 27.23 | 27.19 | 27.16 |
| 16′ | 14.45 | | | |
| 17′ | | 14.50 | | |
| 18′ | | | 14.47 | 14.41 |
| Gal-1 | 105.49 | 105.48 | 105.49 | 105.48 |
| 2 | 72.72 | 72.61 | 72.72 | 72.71 |
| 3 | 74.67 | 74.61 | 74.65 | 74.65 |
| 4 | 69.95 | 69.96 | 69.93 | 69.93 |
| 5 | 75.31* | 75.32* | 75.29* | 75.28* |
| 6 | 65.72* | 65.80* | 65.70* | 65.70* |
| Cho-1″ | 60.50* | 60.52* | 60.50* | 60.46* |
| 2″ | 67.62* | 67.53* | 67.61* | 67.61* |
| $CH_3$ | 54.84 | 54.81 | 54.80 | 54.82 |

peaks between H-8 and H-10, and between H-9 and H-11, and the double bonds are proved to be all *trans* forms (Fig. 8). Consequently, the structure of compound **1** is defined as *N*-hexadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)-phosphinate-$\beta$-D-galactopyranosyl-(4*E*,8*E*,10*E*)-sphingatriene (Fig. 3).

The structures of compounds **3** and **6** were elucidated in the same manner as described for **1** and **5**, and their chemical and spectral data revealed that both compounds have the same (4*E*,8*E*)-sphingadiene moiety as **2**, and that they differ only in the fatty acid residue. That is, **3** and **6** are characterized as *N*-heptadecanoyl- and *N*-octadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-$\beta$-D-galactopyranosyl-(4*E*,8*E*)-sphingadiene, respectively.

## DISCUSSION

Tri-unsaturated LCBs were found first in a tentaculate, *Lingula unguis* (*9*) by Mishima *et al.* They showed the presence of 2-amino-4,8,10-octadecatriene-1,3-diol in hydrolysis products of glucosylceramide mixture, but the geometry of the double bonds was not discussed. Recently, Irie and co-workers isolated two tri-unsaturated long-chain aldehydes along with others released from the glucosylceramides fraction obtained from the spermatozoa of the starfish, *Asterias amurensis* (*10*), by the method of Gaver and Sweeley (*11*), and characterized their full structures by means of $^1$H-NMR. Two of the tri-unsaturated LCBs were identified as (4*E*,8*E*,10*E*)-2-amino-4,8,10-octadecatriene and (4*E*,8*E*,10*E*)-2-amino-9-methyl-4,8,10-octadecatriene-1,3-diol, which correspond to the LCB moieties of compounds **1** and **5** obtained in this study. With regard to parent glycosphingolipids carrying a tri-unsaturated LCB, Higuchi *et al.* obtained a homologous mixture of glucosyl-

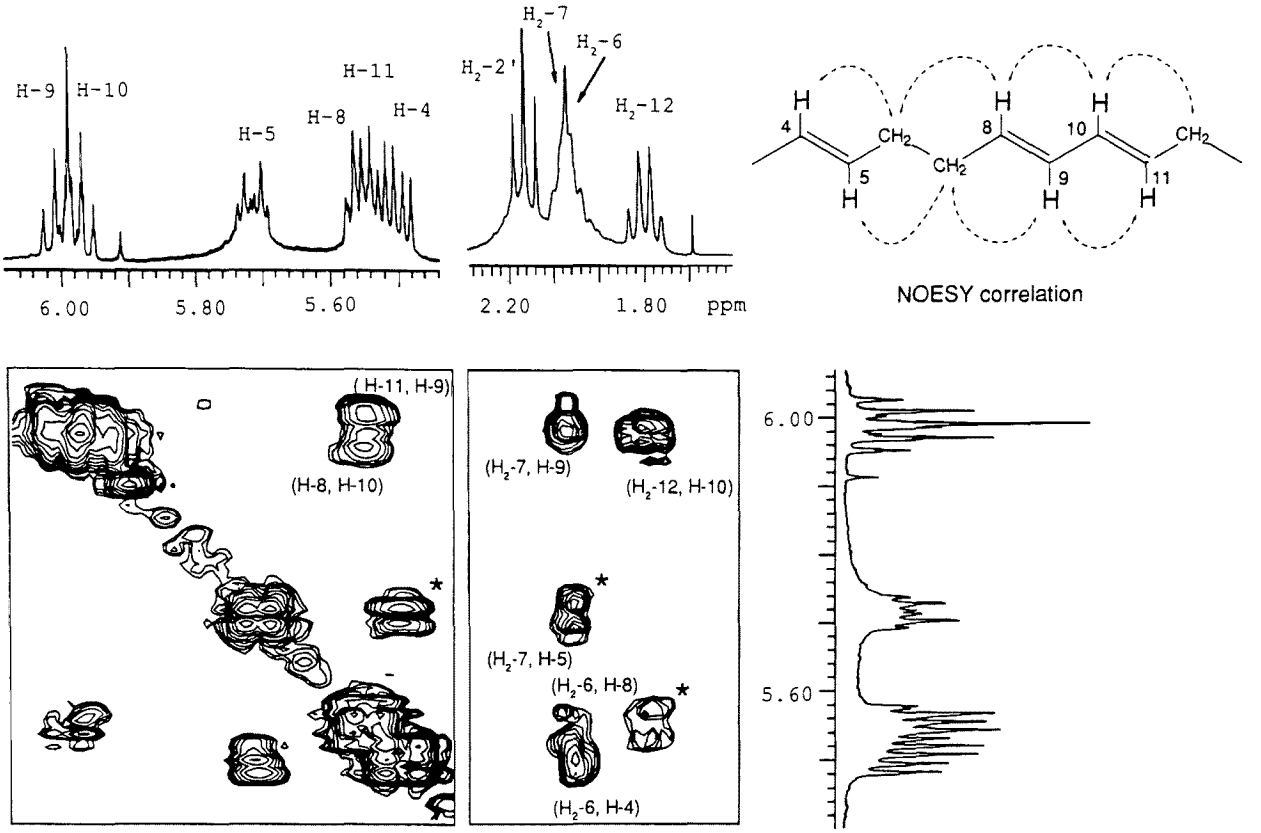


Fig. 8. **Partial NOESY spectrum of 1.** The correlation peaks marked with asterisks are observed in the $^1H$-$^1H$ COSY spectrum.

ceramides possessing the same LCB unit as that of compound **5** from another starfish, *Stellaster equestris* (*12*). Furthermore, Jin *et al.* have recently reported the isolation of five monoglucosylceramides, which have the same LCB unit as that of **5**, from a sea star, *Ophidiaster ophidiamus* (*13*).

Seven compounds isolated in this investigation are phosphocholine-bonded galactosylceramides, so-called zwitterionic-type glycosphingolipids. In contrast to the usual glycosylceramides obtained so far, these compounds could not be separated by preparative HPTLC and conventional HPLC, because of their high polarity and the tailing of bands (*5*).

To overcome this difficulty, we applied repeated recycling of preparative HPLC and succeeded in isolation of the seven phosphocholine-linked monogalactosylceramides. Their structures were characterized mainly on the bases of COSY, NOESY, and HMBC spectral data. Compound **1** is the first example of a glycosphingolipid having a triunsaturated LCB, (4*E*,8*E*,10*E*)-sphingatriene unit. Jin *et al.* reported (*13*) that five monoglucosylceramides possessing a (4*E*,8*E*,10*E*)-9-methylsphingatriene group showed strong cytotoxicity against L1210 leukemia. In addition, Kawai and his colleagues have reported that the presence of 9-methyl and 8-ene functional groups is essential for a LCB in glycosylceramide to induce fruiting body formation in the fungus *Schizophyllum commune* (*14-17*).

The fact that the compounds now isolated are zwitterionic glycosphingolipids, in conjunction with the findings stated above, lead us to expect that they have unique biological functions and roles. The biological activities of these compounds will be examined.

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