Constituents of Holothuroidea, 16.1) Determination of Absolute Configuration of the Branched Methyl Group in Ante-iso Type Side Chain Moiety on Long Chain Base of Glucocerebroside from the Sea Cucumber Holothuria leucospilota

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Received April 21, 2005; accepted June 27, 2005

The absolute configuration of the branched methyl group in ante-iso type side chain moiety on the long chain base of glucocerebroside, HLC-2-A, which was isolated from the sea cucumber Holothuria leucospilota was determined. Oxidation of the glucocerebroside with ozone afforded C13-fragment including the ante-iso moiety. The optically active C13-fragment was synthesized asymmetrically by using the Wittig reaction from chiral syn
ton for comparison with the natural fragment.

Key words glycosphingolipid; glucocerebroside; absolute configuration; sea cucumber; Holothuria leucospilota

In our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structural elucidation of the GSLs from sea cucumber species have been performed in our laborat
ory.2–12) In the preceding paper, we reported the isolation of a sphingosine-type glucocerebroside (HLC-2-A in Chart 1) with ante
iso type side chain of the long-chain base (LCB) moiety from the whole bodies of the sea cucumber Holothuria leucospilota (Nisekuronamako in Japanese).1) However, the absolute configuration of the branched methyl group (C14-Me) in the ante
iso moiety has not yet been deter
mined. In this paper, we report the determination of the absolute configuration of the branched methyl group of HLC-2-A.

Since study on the cerebroside itself was regarded as difficult, we focused on a fragment which included the ante
iso moiety from the parent cerebroside. Trimethylsilylation followed by ozone oxidation of HLC-2-A gave C13-fragment (1) which was released from the cerebroside by the fission of C4–C5 bond. Compound 1 was converted to alcohol (natural 2) by reduction with NaBH4. The absolute configuration of natural 2, 10-methyl dodecanol, was elucidated by comparison with synthetic optically active 2 as follows (Chart 1).

One of the primary alcohols of 1,4-butanediol (3) was protected by TBDMS ether to give 4. The remaining hydroxy group of 4 was converted to bromide with CBr4 under standard conditions, producing 5. The triphenylphosphonium salt (6) was synthesized from 5 with elimination of the TBDMS group using the usual process. The Wittig reaction with 6 and (S)-6-methyl octanal (8), which was synthesized from commercially-available (S)-6-methyl octanol (7) by PCC oxidation, yielded (S)-10-methyl dodecenol (9). Finally, hydrogenation of 9 with Pd–C gave (S)-10-methyl dodecanol (synthetic 2).13)

Comparison of the optical rotations of natural 2 (+50.3°) and synthetic 2 (+53.3°) suggests the former is also (S)-10-methyl dodecanol. Furthermore, their ORD spectra are identical. Therefore, the branched methyl group, C14-Me, in the ante
iso moiety of HLC-2-A must be S configuration as shown in Chart 1.

The present study is, to the best of our knowledge, the first regarding determination of the absolute configuration of branched methyl group in the ante
iso type of side chain of sphingolipids and thus worthy of noting.

Experimental

Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 25 °C. ORD spectra were taken with a Jasco J-720W spectropolarimeter at

Chart 1

October 2005

Notes


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25 °C. IR spectra were obtained on a Jasco FT/IR-410 infrared spectrophotometer. 1H- and 13C-NMR spectra were recorded on a JEOL GX-270 spectrometer (270, 67.8 MHz) or a Varian Unity-500 spectrometer (500, 125 MHz). Positive-ion FAB-MS spectra were acquired with a JEOL JMS-SX102 mass spectrometer (xenon atom beam; matrix, m-nitrobenzyl alcohol). (S)-6-Methyl octanol (7) was purchased from Tokyo Kasei Kogyo Co., Ltd.

Preparation of 10-Methyl Dodecanal (1) from HLC-2-A. HLC-2-A (1.00 mg, 0.0013 mmol) was heated with TMS-imidazole (50 µl)-pyridine (50 µl) for 4 h at 70 °C, and the reaction mixture was concentrated in vacuo. The residue (TMS ether) was dissolved to CH₂Cl₂-MeOH (1 : 1 (1 ml)) and the mixture was treated with ozone for 30 min at −40 °C. Superfluous ozone was driven out with an N₂ stream, DMS (1 mg) was added, and the mixture was stirred for 2 h at room temperature and concentrated. The residue was chromatographed on silica gel (solvent n-hexane–AcOEt, 9 : 1) to afford 1 (0.010 mg, 0.0006 mmol, 48%) as colorless oil. 1H-NMR (CDCl₃) δ: 0.82 (6H, m, 2 × CH₃), 1.06 (8H, m), 1.91 (2H, m, CH₂), 2.51 (2H, t, J = 6.8, 1-H), 3.58 (2H, m, CH₂). Positive-ion FAB-MS m/z: 223 [M + Na]+.

(10-Bromo-butoxy)-tert-butyl-dimethyl-silanyloxy)-butan-1-ol (4) (300 mg, 1.2 mmol) in MeOH (1 ml) and NaBH₄ (2 mg) was added. After stirring the mixture at room temperature for 2 h, it was concentrated in vacuo. The crude product was purified by column chromatography (solvent n-hexane–AcOEt, 8 : 2) to yield 4 (141 mg, 0.77 mmol, 27%) as colorless oil. 1H-NMR (CDCl₃) δ: 0.68 (6H, m, 2 × CH₃), 0.90 (9H, s, t-Bu), 1.07 (3H, t, J = 7.1, CH₃).

2-Methyl-butanal (2) (8.8 g, 98 mmol), triethylamine (16.4 ml, 0.12 mol), and DMAP (1.2 g, 9.8 mmol) were dissolved to anhydrous CH₂Cl₂ (150 ml) and the mixture was stirred for 15 h at room temperature under an N₂ atmosphere. The reaction mixture was washed successively with saturated aqueous NaHCO₃ and NaCl solutions, dried over MgSO₄, and concentrated. The crude reaction mixture was chromatographed on silica gel (solvent n-hexane–AcOEt, 9 : 1 to 8 : 2) to yield 2 (7.0 mg, 0.035 mmol, 8.8%) as colorless oil. 1H-NMR (CDCl₃) δ: 3.62 (2H, t, J = 6.6, 1-H), 1.55 (3H, m, 1-PrOH), 1.01 (9H, s, t-Bu), 0.73 (3H, t, J = 7.1, CH₃). ORD (0.018, 1-PrOH), [α]D = +16.6° (c = 0.018, 1-PrOH). 1H-NMR (CDCl₃) δ: 3.62 (2H, t, J = 6.6, 1-H), 0.84 (6H, m, 2 × CH₂).

10-Methyl Dodecanal (Natural Compound) 1 (0.23 mg, 0.0012 mmol) was dissolved in MeOH (1 ml) and NaBH₄ (2 mg) was added. After stirring the mixture at room temperature for 5 min at room temperature under an N₂ atmosphere. The reaction mixture was refluxed for 19.5 h at 150 °C under an N₂ stream. The reaction mixture was partitioned between CHCl₃ and water. The organic layer was washed successively with saturated aqueous NaHCO₃ and NaCl solutions, dried over MgSO₄, and concentrated. The crude reaction mixture was chromatographed on silica gel (solvent n-hexane–AcOEt, 9 : 1 to 8 : 2) to yield 9 (7.0 mg, 0.035 mmol, 8.8%) as colorless oil. 1H-NMR (CDCl₃) δ: 3.62 (2H, t, J = 6.6, 1-H), 1.55 (3H, m, 1-PrOH), 1.01 (9H, s, t-Bu), 0.73 (3H, t, J = 7.1, CH₃).

(5)-10-Methyl Dodec-4-en-1-ol (9) A solution of n-BuLi (141 mg, 2.22 mmol) in n-hexane (1.4 ml) was added to a solution of compound 6 (354 mg, 1.3 mmol) in anhydrous THF (15 ml) at −78 °C under an N₂ stream. After being stirred for 30 min at the same temperature, compound 8 (79 mg, 0.4 mmol) in THF (1.0 ml) was added and the stirring was continued for another 2.5 h at −78 °C. The reaction mixture was partitioned between AcOEt and saturated aqueous NH₄Cl solution, and the organic layer was washed successively with saturated aqueous NaHCO₃ and NaCl solutions, dried over MgSO₄, and concentrated. The crude reaction mixture was chromatographed on silica gel (solvent n-hexane–AcOEt, 9 : 1 to 8 : 2) to yield 9 (7.0 mg, 0.035 mmol, 8.8%) as colorless oil. 1H-NMR (CDCl₃) δ: 3.62 (2H, t, J = 6.6, 1-H), 1.55 (3H, m, 1-PrOH), 1.01 (9H, s, t-Bu), 0.73 (3H, t, J = 7.1, CH₃).

References and Notes