

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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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 synthon 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 laboratory.^{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 determined. In this paper, we report 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

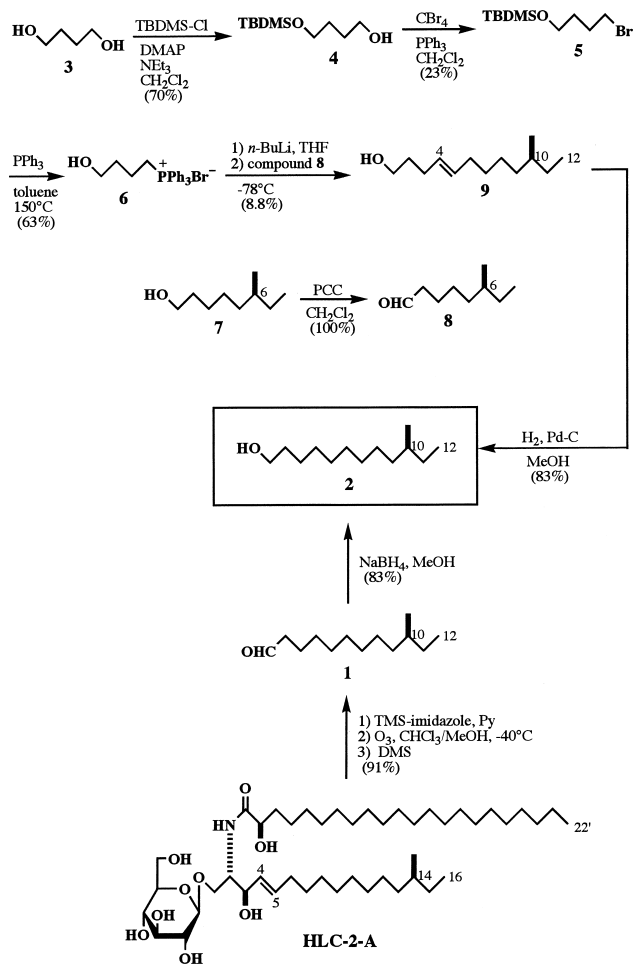


Chart 1

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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 Dodecanol (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 CHCl₃-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, 8 : 2) to give **1** (0.23 mg, 0.0012 mmol, 91%) as colorless oil. ¹H-NMR (CDCl₃) δ: 0.81 (3H, d, *J*=6.9, CH₃), 0.77 (3H, t, *J*=7.1, CH₃).

10-Methyl Dodecanol (Natural 2) 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 6 h, it was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (solvent *n*-hexane-AcOEt, 8 : 2) to yield natural **2** (0.20 mg, 0.001 mmol, 83%) as colorless oil. [α]_D²⁰ +50.3° (*c*=0.018, 1-PrOH). ORD (*c*=0.0025 M, 1-PrOH) [φ] × 10⁻³ (nm): +3 (300), +3 (400), +4 (500), +4 (600). IR (CHCl₃) cm⁻¹: 3734 (OH). Positive-ion FAB-MS *m/z*: 223 [M+Na]⁺. ¹H-NMR (CDCl₃) δ: 3.62 (2H, t, *J*=6.6, 1-H₂), 0.84 (6H, m, 2 × CH₃).

4-(*tert*-Butyl-dimethyl-silanyloxy)-butan-1-ol (4) 1,4-Butanediol (**3**) (8.8 g, 98 mmol), TBDMS-Cl (16.3 g, 108 mmol), triethylamine (16.4 ml, 118 mmol), 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 NH₄Cl and NaHCO₃ solutions, dried over MgSO₄, and the organic layer was evaporated. The residue was purified by silica gel column chromatography (solvent *n*-hexane-AcOEt, 7 : 3) to afford **4** (14.1 g, 69 mmol, 70%) as colorless oil. ¹H-NMR (CDCl₃) δ: 3.66 (4H, m, 2 × OCH₂), 1.64 (4H, m, 2 × CH₂), 0.90 (9H, s, *t*-Bu), 0.07 (6H, s, 2 × CH₃).

(4-Bromo-butoxy)-*tert*-butyl-dimethyl-silane (5) To 150 ml of anhydrous CH₂Cl₂, compound **4** (7.0 g, 34 mmol), triphenylphosphine (10.7 g, 41 mmol), and CBr₄ (17.0 g, 51 mmol) were added, and the mixture was stirred for 5 min at room temperature under an N₂ atmosphere. The reaction mixture was washed successively with saturated aqueous NaHCO₃ and NaCl solutions, dried over MgSO₄, and the organic layer was concentrated. The crude reaction mixture was chromatographed on silica gel (solvent *n*-hexane-CHCl₃, 9 : 1 to 6 : 4) to yield **5** (2.1 g, 8.0 mmol, 23%) as colorless oil. ¹H-NMR (CDCl₃) δ: 3.64 (2H, t, *J*=6.2, OCH₂), 3.45 (2H, t, *J*=6.8, CH₂Br), 1.94 (2H, m, CH₂), 1.66 (2H, m, CH₂), 0.89 (9H, s, *t*-Bu), 0.05 (6H, s, 2 × CH₃).

Triphenylphosphonium Salt (6) Bromide (**5**) (320 mg, 1.2 mmol) and triphenylphosphine (310 mg, 1.2 mmol) were added to toluene (5 ml), and the mixture was refluxed for 19.5 h at 150 °C under an N₂ stream. The reaction mixture was filtered and the collected crude product was washed with *n*-hexane at 70 °C to give **6** (314 mg, 0.76 mmol, 63%) as white powder. IR (CHCl₃) cm⁻¹: 3365 (OH). ¹H-NMR (CDCl₃) δ: 7.74 (15H, m, 3 × Ph), 3.45 (2H, m, OCH₂), 1.91 (4H, m, 2 × CH₂), 1.73 (2H, m, CH₂).

(*S*)-6-Methyl Octanol (8) A solution of (*S*)-6-methyl octanol (**7**) (100 mg, 0.69 mmol) in anhydrous CH₂Cl₂ (1 ml) was added to a suspension of anhydrous AcONa (56.5 mg, 0.69 mmol), Celite (300 mg), and PCC (297 mg, 1.38 mmol) in anhydrous CH₂Cl₂ (20 ml) and the mixture was stirred for 1.5 h at room temperature under an N₂ atmosphere. The reaction mixture was filtered with Celite, the filtrate was concentrated, and the residue was purified by silica gel column chromatography (solvent *n*-hexane-AcOEt, 9 : 1) to afford **8** (98 mg, 0.69 mmol, 100%) as colorless oil. ¹H-NMR (CDCl₃) δ: 9.77 (1H, t, *J*=2.0, CHO), 2.43 (2H, dt, *J*=1.9, 10.4, 2-H₂), 1.63 (1H, m, 6-H), 1.59 (2H, m, CH₂), 1.31 (6H, m, 3 × CH₃), 0.87

(6H, m, 2 × CH₃).

(*S*)-10-Methyl Dodec-4-en-1-ol (9) A solution of *n*-BuLi (141 mg, 2.2 mmol) in *n*-hexane (1.4 ml) was added to a solution of compound **6** (534 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: 5.30, 5.05 (each 1H, m, 4-H, 5-H), 4.03, 3.62 (each 1H, m, 1-H₂), 2.27, 1.98, 1.93, 1.61 (each 2H, m, 4 × CH₂), 1.20 (8H, m), 0.81 (6H, m, 2 × CH₃).

(*S*)-10-Methyl Dodecanol (Synthetic 2) 5% Pd-C (20 mg) was added to MeOH (10 ml) and the mixture was stirred for 30 min under an H₂ atmosphere. Compound **9** (7.0 mg, 0.035 mmol) in MeOH (5 ml) was added and the mixture was stirred a further 15 h under an H₂ atmosphere. The reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by silica gel column chromatography (solvent *n*-hexane-AcOEt, 8 : 2) to afford synthetic **2** (5.8 mg, 0.029 mmol, 83%) as colorless oil. [α]_D²⁰ +53.3° (*c*=0.018, 1-PrOH), +21.0° (*c*=0.11, CHCl₃). ORD (*c*=0.0025 M, 1-PrOH) [φ] × 10⁻³ (nm): +4 (300), +4 (400), +3 (500), +4 (600). IR (CHCl₃) cm⁻¹: 3627 (OH). Positive-ion FAB-MS *m/z*: 223 [M+Na]⁺. ¹H-NMR (CDCl₃) δ: 3.62 (2H, t, *J*=6.6, 1-H₂), 1.55 (3H, m), 1.29 (14H, m, 7 × CH₂), 1.10 (2H, m, CH₂), 0.84 (3H, d, *J*=7.1, 10-CH₃), 0.82 (3H, t, *J*=3.2, CH₃). ¹³C-NMR (CDCl₃) δ: 63.1 (C-1), 34.4 (C-10), 36.6, 32.8, 30.1, 29.6, 29.5, 29.4, 27.1, 25.7 (9 × CH₂), 19.2 (C₁₀-CH₃), 11.4 (C-12).

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- R* isomer could not be synthesized since (*R*)-**7** was not available.