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 C_{13}-fragment including the *ante*-iso moiety. The optically active C_{13}-fragment was synthesized asymmetrically by using the Wittig reaction from chiral synton 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 (C_{14}-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 C_{13}-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 $NaBH_4$. 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 CBr_4 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, C_{14}-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

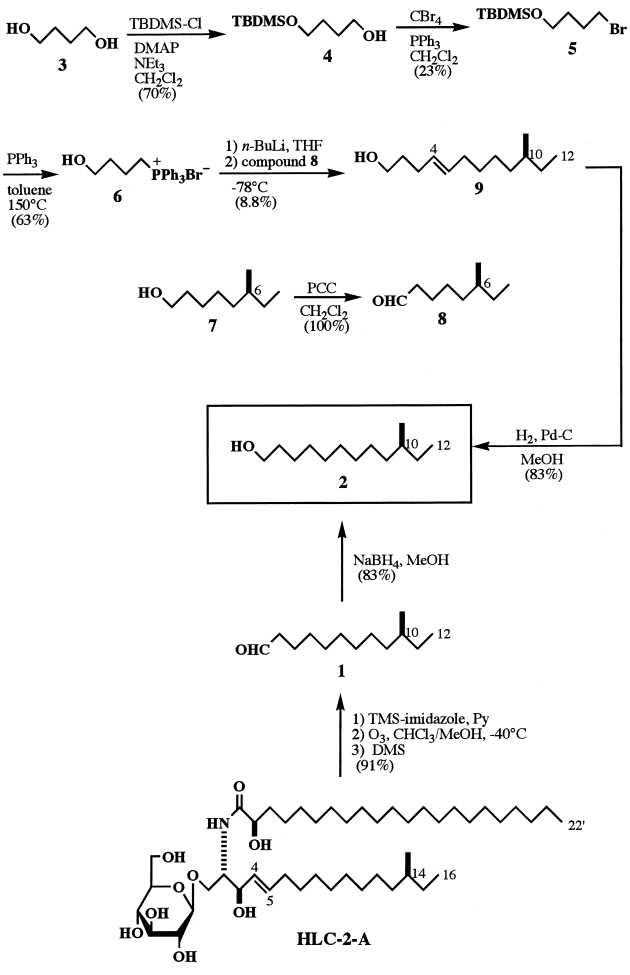

Chart 1

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25 °C. IR spectra were obtained on a Jasco FT/IR-410 infrared spectrophotometer. ^{1}H- and ^{13}C-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 $CHCl_3$–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 N_2 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. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, d, J=6.9, CH_3), 0.77 (3H, t, J=7.1, CH_3).

10-Methyl Dodecanol (Natural 2) Compound **1** (0.23 mg, 0.0012 mmol) was dissolved in MeOH (1 ml), and $NaBH_4$ (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. $[\alpha]_D$ +50.3° (c=0.018, 1-PrOH). ORD (c=0.0025 M, 1-PrOH) $[\phi]\times10^{-3}$ (nm): +3 (300), +3 (400), +4 (500), +4 (600). IR ($CHCl_3$) cm^{-1}: 3734 (OH). Positive-ion FAB-MS *m*/*z*: 223 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 3.62 (2H, t, J=6.6, 1-H_2), 0.84 (6H, m, 2×CH_3).

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 CH_2Cl_2 (150 ml) and the mixture was stirred for 15 h at room temperature under an N_2 atmosphere. The reaction mixture was washed successively with saturated aqueous NH_4Cl and $NaHCO_3$ solutions, dried over $MgSO_4$, 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. ^{1}H-NMR ($CDCl_3$) δ: 3.66 (4H, m, 2×OCH_2), 1.64 (4H, m, 2×CH_2), 0.90 (9H, s, *t*-Bu), 0.07 (6H, s, 2×CH_3).

(4-Bromo-butoxy)-*tert*-butyl-dimethyl-silane (5) To 150 ml of anhydrous CH_2Cl_2, compound **4** (7.0 g, 34 mmol), triphenylphosphine (10.7 g, 41 mmol), and CBr_4 (17.0 g, 51 mmol) were added, and the mixture was stirred for 5 min at room temperature under an N_2 atmosphere. The reaction mixture was washed successively with saturated aqueous $NaHCO_3$ and NaCl solutions, dried over $MgSO_4$, and the organic layer was concentrated. The crude reaction mixture was chromatographed on silica gel (solvent *n*-hexane–$CHCl_3$, 9 : 1 to 6 : 4) to yield **5** (2.1 g, 8.0 mmol, 23%) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 3.64 (2H, t, J=6.2, OCH_2), 3.45 (2H, t, J=6.8, CH_2Br), 1.94 (2H, m, CH_2), 1.66 (2H, m, CH_2), 0.89 (9H, s, *t*-Bu), 0.05 (6H, s, 2×CH_3).

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 N_2 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 ($CHCl_3$) cm^{-1}: 3365 (OH). ^{1}H-NMR ($CDCl_3$) δ: 7.74 (15H, m, 3×Ph), 3.45 (2H, m, OCH_2), 1.91 (4H, m, 2×CH_2), 1.73 (2H, m, CH_2).

(*S*)-6-Methyl Octanal (8) A solution of (*S*)-6-methyl octanol (**7**) (100 mg, 0.69 mmol) in anhydrous CH_2Cl_2 (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 CH_2Cl_2 (20 ml) and the mixture was stirred for 1.5 h at room temperature under an N_2 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. ^{1}H-NMR ($CDCl_3$) δ: 9.77 (1H, t, J=2.0, CHO), 2.43 (2H, dt, J=1.9, 10.4, 2-H_2), 1.63 (1H, m, 6-H), 1.59 (2H, m, CH_2), 1.31 (6H, m, 3×CH_2), 0.87 (6H, m, 2×CH_3).

(*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 N_2 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 NH_4Cl solution, and the organic layer was washed successively with saturated aqueous $NaHCO_3$ and NaCl solutions, dried over $MgSO_4$, 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. ^{1}H-NMR ($CDCl_3$) δ: 5.30, 5.05 (each 1H, m, 4-H, 5-H), 4.03, 3.62 (each 1H, m, 1-H_2), 2.27, 1.98, 1.93, 1.61 (each 2H, m, 4×CH_2), 1.20 (8H, m), 0.81 (6H, m, 2×CH_3).

(*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 H_2 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 H_2 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. $[\alpha]_D$ +53.3° (c=0.018, 1-PrOH), +21.0° (c=0.11, $CHCl_3$). ORD (c=0.0025 M, 1-PrOH) $[\phi]\times10^{-3}$ (nm): +4 (300), +4 (400), +3 (500), +4 (600). IR ($CHCl_3$) cm^{-1}: 3627 (OH). Positive-ion FAB-MS *m*/*z*: 223 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 3.62 (2H, t, J=6.6, 1-H2), 1.55 (3H, m), 1.29 (14H, m, 7×CH_2), 1.10 (2H, m, CH_2), 0.84 (3H, d, J=7.1, 10-CH_3), 0.82 (3H, t, J=3.2, CH_3). ^{13}C-NMR ($CDCl_3$) 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×CH_2), 19.2 (C_{10}-CH_3), 11.4 (C-12).

Acknowledgments We thank Mr. Y. Tanaka and Ms. Y. Soeda of the Faculty of Pharmaceutical Sciences, Kyushu University, for the NMR measurements. This work was supported in part by Grants-in-Aid for Scientific Research No. 13024260 (Priority Area A) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and No. 16510163 from the Japan Society for the Promotion of Science, which are gratefully acknowledged.

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