Determination of the Absolute Configurations of the Anteiso Acid Moieties of Glycoglycerolipid S365A Isolated from Corynebacterium aquaticum

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Received March 4, 2002; Accepted March 19, 2002

The absolute configurations of the two acid moieties, 12-methyltetradecanoate and 14-methylhexadecanoate, of glycoglycerolipid S365A isolated from Corynebacterium aquaticum were determined by an HPLC analysis after their conversion with the chiral fluorescent labeling reagents, (1S,2S)- and (1R,2R)-2-(2,3-anthracenedicarboximido)cyclohexanol. Both anteiso acids had the S configuration.

Key words: branched-chain fatty acid; chiral conversion; high-performance liquid chromatography; absolute configuration

The chiral discrimination of both naturally occurring and synthetic materials has received considerable attention because the enantiomers usually have different biological properties.1-3) Therefore, determination of the absolute stereochemistry is very important for studies on chiral compounds.

Although methyl branched fatty acids and alcohols have been found in many natural products,4-6) it had been very difficult or practically impossible to discriminate their enantiomers from the very small amounts of materials. Therefore, many natural products have been left without determining of their absolute configurations. We have recently reported (1S,2S)- and (1R,2R)-2-(2,3-anthracenedicarboximido)cyclohexanols [(1S,2S)- and (1R,2R)-1, Fig. 1] as strong fluorescent chiral conversion reagents.7) These reagents made it possible to separate the diastereomeric derivatives of anteiso fatty acids having a methyl group from the C2 to C12 position by reversed-phase HPLC and to detect them at the 10^-15 mol level by fluorescence detection. These reagents were successfully applied to determine the absolute configuration of a novel methyl branched-chain fatty acid derived from a ceramide isolated from the epiphytic dinoflagellate, Coolia mononis.8) However, there are many methyl branched fatty acids which have a chiral center at the carbon more remote than the C12 position from the carbonyl group.9-12) S365A (Fig. 1) has been reported as a glycoglycerolipid produced by Corynebacterium aquaticum having 12-methyltetradecanoic acid (2) and 14-methylhexadecanoic acid (3) as components.13) Since the absolute configurations of the acids have not previously been determined, we report here the absolute configuration of these acid moieties by using HPLC after conversion with (1S,2S)- and (1R,2R)-1.

Experimental

General remarks. (1R,2R)- and (1S,2S)-1 were prepared by the method previously reported.7) The solvents used for the HPLC analysis were of HPLC grade purchased from Kanto Chemical Co. (Tokyo, Japan). The other reagents and solvents were purchased from Wako Pure Chemical Co. (Osaka, Japan) and Tokyo Kasei Organic Chemicals (Tokyo, Japan). Authentic optically pure (S)-2 was prepared by the method previously reported.7) Glycoglycerolipid S365A, which had been isolated from Corynebacterium aquaticum, was presented by Prof. A. Yoshimoto of Hiroshima University (Japan).

All the synthesized products were purified by silica
gel column chromatography. High-resolution mass spectra were measured in the EI mode or FAB mode by using 4-nitrobenzaldehyde as a matrix with a JMS HX-105 instrument (Jeol, Tokyo, Japan). 1H-NMR spectra were measured in CDCl3 by a Varian Unity INOVA 500 instrument, and IR spectra were measured by Impact 410 FT-IR apparatus (Nicolet, U.S.A.). Optical rotation was measured by a DIP-370 polarimeter (Japan Spectroscopic Co., Japan).

Synthesis of optically pure (S)-3. Sodium hydride (6.0 g, 0.25 mol) was added to 1,12-undecanediol (50 g, 0.25 mol) in 1000 ml of DMF at 0°C, and the mixture was stirred for 1 h at 0°C and then for 14 h at r.t., before 42.3 g (0.25 mol) of benzylbromide was added, and the mixture was stirred for 1 h to give 12-benzyloxy-1-bromoundecane (29.2 g, 67 mmol) in THF to give the corresponding Grignard reagent. (3)-2-Methylbutyl tosylate (1.6 g, 6.2 mmol) was stirred for 1 h to give 12-benzyloxy-12-bromoundecane (22.4 g, 88.7 mmol). This bromide (4.7 g, 19 mmol) was reacted with magnesium (1.6 g, 67 mmol) in THF to give the corresponding Grignard reagent. (S)-2-Methylbutyl tosylate (1.6 g, 6.2 mmol) and CuBr2 (28.3 g, 85 mmol) were dissolved in 1000 ml of CH2Cl2, and the mixture was stirred for 30 min at 0°C. Triphenylphosphine (28.0 g, 0.11 mol) was then added, and the mixture was stirred for 1 h to give 12-benzyloxy-1-bromoundecane (22.4 g, 88.7%). This bromide (4.7 g, 19 mmol) was reacted with magnesium (1.6 g, 67 mmol) in THF to give the corresponding Grignard reagent. (S)-2-Methylbutyl tosylate (1.6 g, 6.2 mmol) and CuBr2 (28.3 g, 85 mmol) were dissolved in 1000 ml of CH2Cl2, and the mixture was stirred for 30 min at 0°C. Triphenylphosphine (28.0 g, 0.11 mol) was then added, and the mixture was stirred for 1 h to give 12-benzyloxy-1-bromoundecane (22.4 g, 88.7%). This bromide (4.7 g, 19 mmol) was reacted with magnesium (1.6 g, 67 mmol) in THF to give the corresponding Grignard reagent.

HPLC instruments. The HPLC pump used was a Jasco PU-980 unit (Japan Spectroscopic Co., Tokyo, Japan) equipped with a Rheodyne 7125 sample injector with a 20-µl sample loop. The fluorescence detector was a Jasco FP-920 unit with a 5-µl flow cell, and the integrator was a Chromatocorder 12 (System Instrument, Tokyo, Japan). Cryocool CC100-II was used to control the column temperature.

Sample preparation procedure from S365A. Glycoglycerolipid S365A (7 mg) was hydrolyzed for 4 h in 40 ml of ethanol/10%aq. NaOH (3:1). After evaporating the ethanol under reduced pressure, the resulting residue was added to 10%aq. H3PO4 (50 ml) and extracted with 60 ml of ether. After drying with MgSO4, the solvent was evaporated under reduced pressure, and the residue was dissolved in 0.5 ml of toluene. An aliquot (50 µl) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 5 mg) were added to the mixture of (1S,2S)- or (1R,2R)-I (2 mg) and 4-dimethylaminopyridine (DMAP, 2 mg) in toluene/acetoni- trile (1:2, 300 µl). The mixture was kept overnight at 40°C. An aliquot (30 µl) was loaded onto a silica gel TLC plate (10 cm length, silica gel 60 F254, Art-5744) and developed with n-hexane/ethyl acetate (4:1, v/v). The fluorescent band where the authentic 1-derivatives of 2 and 3 had been developed was collected and packed in a Pasteur pipette, before eluting with ethyl acetate/methanol (3:1, v/v, 1 ml). An aliquot (10 µl) was injected into a reversed-phase HPLC column (Develosil C-30-UG-5, 5 µm, 4.6 mm I.D.×150 mm, Nomura Chemical Co., Japan) and eluted with methanol at 1.0 ml min−1 and 30°C. Each peak at 9.8 and 14.0 min, where the authentic 1-derivatives of 2 and 3 had respectively been eluted, was collected. An aliquot (1 µl) was used for the chiral analysis.

HPLC separation of each diastereomeric 1-derivative of 2 and 3. The 1-derivatives of the anteiso acids were separated in an ODS column (Develosil ODS-3, 3µm, 4.6 mm I.D.×150 mm, Nomura Chemical Co., Japan). The compounds were detected by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm). To separate the 2-derivatives, a mixture of acetoni- trile/THF/n-hexane (100:100:5, v/v/v) was used as a mobile phase with a flow rate of 0.15 ml min−1, the column being kept at −50°C. To separate the 3-derivatives, a mixture of acetoni- trile/THF/n-hexane (150:200:15, v/v/v) was used as a mobile phase with a flow rate of 0.15 ml min−1, the column being kept at −45°C.

Results and Discussion

Preparation of the authentic anteiso acids

Authentic optically pure (S)-2 was prepared by the method previously reported,19 while authentic (S)-3 was prepared from commercially available optically pure (S)-2-methylbutyl tosylate. The tosylate and 12-benzyloxyundecanyl magnesium bromide were coupled to give (S)-1-benzyloxy-14-methylhexadecane. After debenzylation, the alcohol was oxidized with KMnO4 to give (S)-3.

Although both (S)-2 and (S)-3 gave significant [α]D values, they were too small to determine their absolute configuration with the small amount of each sample by measuring the [α]D values of the acids derived from natural products. Fluorescence conversion, however, allowed us to follow the target com-
Determination of the absolute conformation of the methyl-branched fatty acids derived from S365A

S365A has been reported as a glycerolipid with 12-methyltetradecanoate at the 6' position and 14-methylheptadecanoate at the 3 position as the main acyl components. Both anteiso acids were obtained by the hydrolysis of S365A under alkaline conditions, and the mixture was labeled with (1S,2S)- and (1R,2R)-1 in the presence of EDC and DMAP in a 1:2 mixture of acetonitrile and toluene. After removing the excess reagents by silica gel TLC, the acids were separated and purified by HPLC with a C-30 reversed-phase column at 30°C. Under these conditions, each 1-derivative of authentic (S)-2, (S)-3, lauric, myristic, palmitic and stearic acids gave a single peak, and these could be completely separated from each other, while the 2 and 3-derivatives of rac-1 could not be separated into the diastereomeric isomers. The fatty acid mixture obtained from S365A consisted of 2 (55.0%), 3 (36.2%), palmitic acid (3.3%) and some minor components (less than 3% each), and no other anteiso acid was detected in the mixture. Both (1S,2S)- and (1R,2R)-1 were respectively used to prepare the converted sample.

As shown in Fig. 2, the (1R,2R)-1 derivative of 2 from S365A had the same retention time as that of authentic (S)-12-methyltetradecanoate that had been labeled with (1R,2R)-1, and no peak was apparent at the retention time at which the (S)-2 derivative labeled with (1S,2S)-1 had been eluted. On the other hand, the (1S,2S)-1 derivative of 2 derived from S365A had the same retention time as that of the authentic (S)-2 derivative labeled with (1S,2S)-1. These results unequivocally show that the absolute configuration of 12-methyltetradecanoate from S365A was of (S)-type. In the same manner, the 14-methylhexadecanoic acid from S365A was determined to be of (S)-configuration (Fig. 3).

We have recently made it possible to separate the enantiomers of anteiso fatty acids having a methyl branch up to the 26 position, and this will be reported elsewhere. (1S,2S)- and (1R,2R)-1 should be powerful tools to determine the absolute configuration of carboxylic acids having a chiral methyl branch in naturally occurring products because of its high sensitivity and resolution.
Acknowledgments

We thank Prof. Akihiro Yoshimoto of Hiroshima University (Japan), for kindly presenting glyco-glycerolipid S365A. This work was supported in part by grant aid for scientific research from the Ministry of Education, Science, and Culture of Japan and by research project funding from the Institute for the Development of Kansei and Human Welfare at Tohoku Fukushi University.

References


