ORIGINAL

Synthesis of DL-*threo*- and DL-*erythro*-2-*d*- C₁₄-Dihydrosphingosines

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The synthesis of DL-*threo*- and DL-*erythro*-2-*d*-C₁₄-dihydrosphingosines [*threo*-(1) and *erythro*-(1)] was carried out. The catalytic reduction of sodium salt of *aci*-2-nitro-1, 3-tetradecanediol in acetic acid-*O-d* gave 2-amino-2-*d*-1, 3-tetradecanediol (1). *Threo*-(1) and *erythro*-(1) were separated by the fractional crystallization of the *N, O, O*-triacyl and *N*-acetyl derivatives of (1) [(2) and (3)]. The structures and deuterium content (ca. 60%) of *threo*-(1) and *erythro*-(1) were determined by mass spectrometry.

1 Introduction

Deuterium compounds are very powerful for assignment of their structures in NMR and MS studies. It was reported that the synthesis of 3-*d*-C₁₈-sphingosine (C₃-*d*) involved the keto intermediate which could be reduced by NaBD₄¹⁾ and that, on the other hand, 3-oxo carboxylates were the intermediates for the syntheses of long chain bases such as C₁₈-sphingosines and C₁₈-dihydrosphingosines^{2), 3)}. This suggests a possibility for the preparation of C₁-*d*₂ and C₃-*d* compounds, whereas C₂-*d* compound has not been reported. In the present paper, we selected DL-*threo*- and DL-*erythro*-2-*d*-C₁₄-dihydrosphingosines as substrates to discover a possibility of synthetic methods for C₂-*d* compounds with long chain bases.

2 Experimentals

2.1 Materials and analytical methods

Deuterium oxide (D% : 99.8%) was purchased from Merck, Canada. Sodium salt of *aci*-2-nitro-1, 3-tetradecanediol was prepared by the previously reported method⁴⁾. GLC and TLC analyses were carried out by the similar way in the previous papers^{5)~7)}.

2.2 Acetic acid-*O-d*

A mixture of acetic anhydride (4 mol : bp 139~140°C) and deuterium oxide (4 mol) was stirred for 4 h at room temperature, refluxed for 1 h, and then distilled. bp 117~118°C.

2.3 2-Amino-2-*d*-1, 3-tetradecanediol (1)

Sodium salt of *aci*-2-nitro-1, 3-tetradecanediol (35 mmol) was dissolved in acetic acid-*O-d* (90 ml) at room temperature. Then, 2.2 g of 5%Pd-C was added to the solution which was exposed to hydrogenolysis at room temperature. After 48 h, the catalyst was filtered off, which was washed five 10 ml portions of acetic acid-*O-d*. The combined filtrates were concentrated under reduced pressure to afford crude material in ca. 50 g. To the residue (cooling with ice-bath) were added successively water (200 ml), potassium hydroxide (50 g) and methanol (200 ml). The compounds were extracted with three 200 ml portions of chloroform. The combined extracts were washed with three 200 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from hexane (150 ml). Yield 65

%, *erythro* content 72~73% (determined by GLC as *N*, *O*, *O*-tris-TMS and *N*-acetyl *O*, *O*-bis-TMS derivatives)^(6),7).

2.4 *N*, *O*, *O*-Triacetyl 2-amino-2-*d*-1, 3-tetradecanediol (2)

A mixture of 2-amino-2-*d*-1, 3-tetradecanediol (1) (20 mmol), pyridine (50 ml) and acetic anhydride (25 ml) was allowed to stand at room temperature for 20 h. Then, water (100 ml) was added to the solution and the mixture was cooled with ice-bath for 5 h, precipitating colorless crystals which were filtered, dried *in vacuo* (over P₂O₅) and recrystallized from hexane (150 ml). Yield 66% [*erythro*-rich (2)]. The mother liquor (hexane solution) was evaporated to dryness to afford 16% of *threo*-rich (2). The filtrate of the reaction mixture was extracted with three 100 ml portions of chloroform. The combined extracts were washed with three 200 ml portions of 5% hydrochloric acid and three 100 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness. Yield 14% [*threo*-rich (2)]. The combined *threo*-rich triacetate was exposed to the following mild alkaline hydrolysis (2.6).

2.5 *N*-Acetyl DL-*erythro*-2-*d*-C₁₄-dihydrosphingosine [*erythro*-(3)] [Mild alkaline hydrolysis of *erythro*-rich (2)]

A mixture of *erythro*-rich triacetate (2) (14 mmol), 10% sodium hydroxide solution (30 ml) and methanol (250 ml) was left at room temperature. After 18.5 h, water (250 ml) was added to the solution. The compounds were extracted with three 250 ml portions of chloroform. The combined extracts were washed with water (250 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The residue was suspended in hexane (500 ml), filtered, washed with three 100 ml portions of hexane and dried *in vacuo* (over P₂O₅) to afford 94% of the crude product. The resulting crystals were recrystallized from a mixture of hexane and ethanol (10 : 1 vol/vol, 600 ml). Yield 64%, *erythro* content 99.7% (determined by GLC as *O*, *O*-bis-TMS derivative)⁽⁸⁾, mp 117~119°C.

2.6 *N*-Acetyl DL-*threo*-2-*d*-C₁₄-dihydrosphingosine [*threo*-(3)] [Mild alkaline hydrolysis of *threo*-rich (2)]

A mixture of *threo*-rich triacetate (2) (6.5

mmol), 10% sodium hydroxide solution (20 ml) and methanol (180 ml) was allowed to stand at room temperature for 17 h. Then, water (200 ml) was added to the solution. The compounds were extracted with three 200 ml portions of chloroform. The combined extracts were washed with water (200 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The residue was crystallized from hexane (100 ml) (Yield 86%, *threo* 77%). Further recrystallization from hexane-diethyl ether-ethyl acetate (6 : 5 : 1 vol/vol/vol, 960 ml) gave the desired *N*-acetyl DL-*threo*-2-*d*-C₁₄-dihydrosphingosine [*threo*-(3)]. Yield 43%, *threo* content 98% (determined by GLC as *O*, *O*-bis-TMS derivative)⁽⁸⁾, mp 93~94°C.

2.7 DL-*erythro*-2-*d*-C₁₄-Dihydrosphingosine [*erythro*-(1)]

A mixture of *erythro*-(3) (9 mmol), potassium hydroxide (13 g), water (26 ml) and methanol (234 ml) was refluxed for 7 h. Then, water (500 ml) was added to the mixture. The compounds were extracted with three 250 ml portions of chloroform. The combined extracts were washed with three 250 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness (Yield 95%). The residue was recrystallized from hexane (300 ml). Yield 94%, mp 71~72°C.

2.8 DL-*threo*-2-*d*-C₁₄-dihydrosphingosine [*threo*-(1)]

A mixture of *threo*-(3) (2 mmol), potassium hydroxide (3 g), water (6 ml) and methanol (54 ml) was refluxed for 7 h. The mixture was treated by the same manner in 2.7. Recrystallization of the product from hexane (150 ml) gave the desired free base. Yield 90%, mp 89~90°C.

3 Results and Discussion

3.1 Insertion of deuterium to C₂ of 2-amino-1,3-tetradecanediol

Catalytic reduction (5%Pd-C) of sodium salt of *aci*-2-nitro-1,3-tetradecanediol in acetic acid-*O*-*d* gave 2-amino-2-*d*-1,3-tetradecanediol. This suggests that sodium salt of *aci*-2-nitro-1,3-tetradecanediol reacts with acetic acid-*O*-*d* to give 2-nitro-2-*d*-1,3-tetradecanediol followed by catalytic reduction of its nitro group to

amino moiety. This is very interesting because the preparation of C_2 - d has not been reported, although C_3 - d derivatives were already reported⁹.

3.2 The *erythro*/*threo* separation of 2- d - C_{14} -dihydrosphingosines

The separation of DL-*erythro*- and DL-*threo*-2- d - C_{14} -dihydrosphingosines [*erythro*-(1) and *threo*-(1)] was performed by the (re)crystallization of *N*, *O*, *O*-triacyl and *N*-acetyl derivatives of (1) [(2) and (3)]. Crystallization of *N*, *O*, *O*-triacyl derivative of (1) in a cold aqueous solution gave *erythro*-rich (2) in crystal and *threo*-rich compound in the aqueous solution. Recrystallization of the former from hexane gave more *erythro*-rich (2). On the

other hand, the *erythro*-rich *N*-acetyl compound was recrystallized from hexane-ethanol (10 : 1 vol/vol) to give pure *erythro*-(3) while the *threo*-rich (3) was afforded pure *threo*-(3) by the same manner with hexane-diethyl ether-ethyl acetate (6 : 5 : 1 vol/vol/vol). Alkaline hydrolysis of *N*-acetyl compounds (3) gave pure DL-*erythro*- and DL-*threo*-2- d - C_{14} -dihydrosphingosines [*erythro*-(1) and *threo*-(1)].

3.3 Mass spectra of the synthetic deuterium compounds

Mass spectra of TMS derivatives of above

Fig.-1 Mass spectra of *O*, *O*-bis-TMS derivatives of *erythro*-(1) and *threo*-(1).

Fig.-2 Mass spectra of *N, O, O*-tris-TMS derivatives of *erythro*-(1) and *threo*-(1).

Fig.-3 Mass spectra of *O, O*-bis-TMS derivatives of *erythro*-(3) and *threo*-(3).

Compounds A: *erythro*-(3), B: *erythro*-(3-h).

Fragment ions () = $m/e(C_2-d) - m/e(C_2-h)$

- a: CDH_2OH (1), b: $CH_3CONHCD=CH_2$ (1),
 c: $CH_3CONHCDCH_2OH$ [$C_3 \rightarrow C_2$] (1),
 d: $HOCHCD(NHAc)CH_2OH$ (1), e: $C_{11}H_{23}CH(OH)CDNH_2$ (1),
 f: $g-H_2O$ (1), g: $M-31(CH_2OH)$ [$C_2 \rightarrow C_1$] (1),
 h: $M+1$ (1).

Fig.-4 Mass spectra (DI-MS) of *erythro*-(3) and *N*-acetyl *DL-erythro*- C_{14} -dihydroshingosine [*erythro*-(3-h)].

free bases [*erythro*-(1) and *threo*-(1)] and *N*-acetyl derivatives (3) prepared above are shown in **Figs.-1~3**. DI-MS of *erythro*-(3) is also shown in **Fig.-4**. These shows that deuterium is inserted at C_2 and that deuterium content is *ca.* 60% although it may be controlled by the ratio of [sodium salt of *aci*-2-nitro-1,3-tetradecanediol]/[acetic acid-*O*-*d*].

The results in the present paper can be applied to the ^{13}C -NMR assignment of C_2 carbon atom of dihydroshingosines. This will be in due course.

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DL-*threo*- 及び DL-*erythro*-2-*d*- C_{14} -ジヒドロスフィンゴシンの合成

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3 位にジュウテリウムをもつ長鎖塩基の合成については既に報告があるが、2 位にジュウテリウムをもつ化合物の報告はない。そこで本報では、2 位にジュウテリウムをもつ DL-*threo*- 及び DL-*erythro*-2-*d*- C_{14} -ジヒドロスフィンゴシン [*threo*-(1) 及び *erythro*-(1)] の合成法について研究を行った。*aci*-2-ニトロ-1,3-テトラデカンジオールを酢酸-*O*-*d* 中で接触還元すると 2-アミノ-2-*d*-1,3-テトラデカンジオール (1) が得られた。(1) の *N*, *O*, *O*-トリアセチル体 (2) 及び *N*-アセチル体 (3) を分別結晶すると, *threo*/*erythro* の分離ができ、これを更に加水分解して目的の *threo*-(1) 及び *erythro*-(1) を得た。*threo*-(1) 及び *erythro*-(1) の構造、ジュウテリウム含量の決定は質量分析から行った。