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Synthesis of Ceramides Bearing DL-*erythro*- and DL-*threo*- C_n -Dihydrosphingosines, and the Determination of the *erythro* and *threo* Isomers by Mass Spectrometry

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DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines ($n=12, 14$, and 16) were separated from the corresponding 2-amino-1, 3-alkanediols by repeated recrystallization of the *N,O,O*-triacyl and *N*-acetyl derivatives. Ceramides having DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines were prepared by reactions of the bases with *N*-acyloxysuccinimides in tetrahydrofuran. GLC-MS analysis of *O, O*-di-TMS derivatives of the ceramides was carried out to find differences between *threo* and *erythro* isomers in mass spectra. One characteristic difference was observed in the relative intensity (RI) at $m/e=M-103$; i.e., the relative intensities of *threo* compounds (RI 40~80%) were stronger than those of *erythro* isomers (RI 6~14%). The ratios, RI ($M-103$)/RI($M-15$), were 3.7~6.8 and 0.8~1.3 for *threo* and *erythro* compounds, respectively. These observations demonstrate the possibility of determining *erythro* and *threo* isomers by mass spectrometry.

1 Introduction

Ceramides (*N*-Acyl derivatives of long chain bases) have played important roles as intermediates in the biosynthesis of sphingolipids such as sphingomyelin and cerebroside, and are the most simple compounds in them¹⁾.

Several methods have been reported for the synthesis of ceramides. Acylation of long chain bases by an acyl chloride leads to formation of the *N,O,O*-triacyl compounds, which provides the desired ceramides after mild alkaline hydrolysis^{2),3)}. Selective *N*-acylation of long chain base is performed by using *N*-hydroxysuccinimide esters of fatty acids (*N*-acyloxysuccinimides)⁴⁾, or free fatty acids in the presence of the coupling reagents such as 1-ethyl-3-(*N,N*-dimethylamino) propylcarbodiimide⁵⁾ and triphenylphosphine +2, 2'-dipyridyl disulfide⁶⁾. However, the previous papers described above are limited to the synthesis of ceramides bearing C_{18} -dihydrosphingosine or C_{18} -sphingosine, because the chemical synthesis is focussed on the naturally major occurrences.

The structures of ceramides are determined

by GC-MS as *O*-TMS ethers. Although the fragment ions of the *O*-TMS ethers are assigned⁷⁾, no information is available about comparison between *threo* and *erythro* isomers in mass spectra.

In the present paper, the separation of DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines ($n=12, 14$, and 16) from the corresponding 2-amino-1, 3-alkanediols (mixtures of DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines) was performed, ceramides bearing DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines were prepared, and GC-MS analysis of *O,O*-di-TMS derivatives of the ceramides was carried out in order to discover a possibility of the determination of *erythro* and *threo* isomers by mass spectrometry.

2 Results and Discussion

2.1 The *erythro*/*threo* separation of C_n -dihydrosphingosines from 2-amino-1, 3-alkanediols

The separation of DL-*threo*- and DL-*erythro*- C_n -dihydrosphingosines (*threo*-4 and *erythro*-4) from the corresponding 2-amino-1, 3-alkane-

diols (1) was carried out by repeating recrystallization of the *N,O,O*-triacyl and *N*-acetyl derivatives (2 and 3) (Fig.-1). Recrystallization of *N,O,O*-triacyl derivatives (2) from hexane gave the *erythro* rich crystals and *threo* rich compounds were obtained from the mother liquors. The *erythro* rich *N*-acetyl compounds were recrystallized from a mixture of hexane and ethanol (10 : 1 vol/vol) to give pure *erythro*-3. The same solvent did not give pure *threo* isomers. The *N*-acetyl derivatives of the *threo* compounds were obtained by the recrystallization from a mixture of diethyl ether-hexane (3 : 1 vol/vol) or hexane-diethyl ether-ethyl acetate (6 : 5 : 1 vol/vol/vol). Alkaline hydrolysis of *N*-acetyl derivatives (3) purified by the recrystallization gave pure *DL-erythro*- and *DL-threo*- C_n -dihydrosphingosines (*erythro*-4 and *threo*-4). Physical properties of pure free bases (4), and the *N,O,O*-triacyl and *N*-acetyl derivatives (2 and 3) are summarized in Table -1.

2.2 Ceramide bearing *DL-threo*- and *DL-erythro*- C_n -dihydrosphingosines (*threo*-6 and *erythro*-6).

Ceramides bearing *DL-threo*- and *DL-erythro*- C_n -dihydrosphingosines (*threo*-6 and *erythro*-6) were prepared in order to clarify the physical properties. The *N*-acyl reagents, *N*-acyloxysuccinimides (5), were prepared by the

hydrolysis of *N*-acetyl derivatives (3) purified by the recrystallization gave pure *DL-erythro*- and *DL-threo*- C_n -dihydrosphingosines (*erythro*-4 and *threo*-4). Physical properties of pure free bases (4), and the *N,O,O*-triacyl and *N*-acetyl derivatives (2 and 3) are summarized in Table -1.

Table-1 Physical properties of *DL-threo*- and *DL-erythro*- C_n -dihydrosphingosines (*threo*-4 and *erythro*-4), and the *N, O, O*-triacyl and *N*-acetyl derivatives (2 and 3).

Compounds R	Derivatives	<i>DL-erythro</i>		<i>DL-threo</i>		MS (70 eV) <i>m/e</i>
		mp(°C)	Solvent	mp(°C)	Solvent	
	Free base	65~ 67	Hexane	85~ 86	Hexane	346(M-15), 258(M-103), 132(base peak ion) ^{a)}
C_9H_{19}	<i>N</i> -Acetyl	118~120	Hexane-EtOH (10 : 1 vol/vol)	99~101	Et ₂ O-Hexane (3 : 1 vol/vol)	418(M-15), 330(M-103), 204(base peak ion) ^{b)}
	<i>N, O, O</i> - Triacyl	72~ 72	Hexane	—	—	388(M-15), 300(M-103), 73(base peak ion) ^{a)}
	Free base	71~ 72	Hexane	91~ 93	Hexane	374(M-15), 286(M-103), 132(base peak ion) ^{a)}
$C_{11}H_{23}$	<i>N</i> -Acetyl	119~121	Hexane-EtOH (10 : 1 vol/vol)	101~103	Et ₂ O-Hexane- EtOAc (6 : 5 : 1 vol/ vol/vol)	446(M-15), 328(M-103), 204(base peak ion) ^{b)}
	<i>N, O, O</i> - Triacyl	80~ 82	Hexane	—	—	416(M-15), 328(M-103), 73(base peak ion) ^{a)}
	Free base	78~ 80	Hexane	94~ 95	Hexane	402(M-15), 314(M-103), 132(base peak ion) ^{a)}
$C_{13}H_{27}$	<i>N</i> -Acetyl	119~120	Hexane-EtOH (10 : 1 vol/vol)	98~ 99	Et ₂ O-Hexane- EtOAc (6 : 5 : 1 vol/ vol/vol)	474(M-15), 386(M-103), 204(base peak ion) ^{b)}
	<i>N, O, O</i> - Triacyl	88~ 89	Hexane	—	—	444(M-15), 357(M-103), 73(base peak ion) ^{a)}

^{a)} *O,O*-di-TMS derivatives.

^{b)} *N,O,O*-tri-TMS derivatives.

Table-2 Physical properties of *N*-acyloxysuccinimides (**5**).

R'	Yield (%)	mp(°C)	Solvent for recrystallization	R _f ^{a)}	Solv./Sub. ^{b)}	GLC-MS ^{g)} , ^{h)} m/e
C ₇ H ₁₅	66	62~63 ^{c)}	Hexane	0.41	200/0.3	98(Su), 127(RCO : base peak ion), 241(M)
C ₉ H ₁₉	27	71~72	Ethanol	0.42	250/0.3	98(Su), 155(RCO : base peak ion), 269(M)
C ₁₁ H ₂₃	57	78~79 ^{d)}	Ethanol	0.44	250/0.3	98(Su : base peak ion), 183(RCO), 297(M)
C ₁₃ H ₂₇	63	85~86	Ethanol	0.46	250/0.15	98(Su : base peak ion), 211(RCO), 325(M)
C ₁₅ H ₃₁	70	90~91 ^{e)}	Ethanol	0.49	300/0.15	98(Su : base peak ion), 239(RCO), 353(M)
C ₁₇ H ₃₅	72	94~95 ^{f)}	Ethanol	0.52	400/0.15	98(Su : base peak ion), 267(RCO), 381(M)

a) Solvent system : CHCl₃ + EtOH + AcOH (99 : 1 : 1 vol/vol/vol).

b) [Sub] = [HOSu] + [RCOOH] + [DCC], [HOSu] = [RCOOH] = [DCC] = 0.1 or 0.05 mol, [Solv]/[DCC] = 100/0.1 (ml/mol).

c) mp 63°C¹²⁾, d) mp 75°C¹²⁾, e) mp 90°C¹²⁾, f) mp 93 °C¹²⁾, g) Su : *N*-Succinimidyl.

h) RIs(M) were less than 1%.

Fig.-1 Separation of DL-*threo*- and DL-*erythro*-C_n-dihydrosphingosines (*threo*-**4** and *erythro*-**4**) from 2-amino-1, 3-alkanediols.

coupling reaction of *N*-hydroxysuccinimide with the corresponding fatty acids in the presence of *N*, *N'*-dicyclohexylcarbodiimide (DCC) in dioxane or tetrahydrofuran (THF). The physical properties of **5** are summarized in **Table-2**. *N*-Acylation of dihydrosphingosines (**4**) with **5**⁴⁾ was carried out in THF at room temperature. The results are listed in **Table-3**. Obviously, no *O*-acyl derivatives were detected by TLC, GLC and GC-MS analyses.

2.3 GC-MS of *O*, *O*-di-TMS derivatives of ceramides bearing DL-*threo*- and DL-*erythro*-C_n-dihydrosphingosines (**6**).

GLC-MS analyses of *O*, *O*-di-TMS derivatives of **6** were carried out in order to discover differences between *threo* and *erythro* isomers in mass spectra. A comparison between *N*-hexadecanoyl DL-*threo*- and DL-*erythro*-C₁₄-dihydrosphingosines (**6**, R=C₁₁H₂₃, R'=C₁₅H₃₁) is shown in **Fig.-2**. This shows a characteristic difference in the relative intensities at M-103. The *threo* compound shows a stronger intensity (RI ca. 66%) relative to the *erythro* isomer (RI ca. 11%). Other ceramides showed a similar tendency as shown in **Table-4**. The ratios, RI(M-103)/RI(M-15), were 3.7~6.8 and 0.8~1.3 for *threo* and *erythro* compounds (**Table-4**). *N*-Acetyl *O*,

Table-3 Yields and mps of ceramides bearing DL-*threo*- and DL-*erythro*-C_n-dihydrosphingosines (*threo*-6 and *erythro*-6).

Compounds		<i>erythro</i> -6			<i>threo</i> -6		
R	R'	Yield (%)	mp (°C)	Solvent for recrystallization	Yield (%)	mp (°C)	Solvent for recrystallization
C ₉ H ₁₉	C ₇ H ₁₅	73	98~99	—	78	73~74	—
	C ₉ H ₁₉	86	99~100	MeOH-H ₂ O(7:4)	93	79~80	—
	C ₁₁ H ₂₃	85	100~101	MeOH-H ₂ O(8:3)	92	82~84	—
	C ₁₃ H ₂₇	87	101~102	MeOH-H ₂ O(16:5)	92	83~85	—
	C ₁₅ H ₃₁	90	101~102	MeOH-H ₂ O(17:4)	95	78~80	—
	C ₁₇ H ₃₅	88	100~101	MeOH-H ₂ O(8:2)	94	79~80	MeOH-H ₂ O(5:1)
C ₁₁ H ₂₃	C ₇ H ₁₅	77	101~103	MeOH-H ₂ O(8:4)	82	69~70	—
	C ₉ H ₁₉	85	97~99	MeOH-H ₂ O(7:3)	92	80~81	—
	C ₁₁ H ₂₃	87	96~99	MeOH-H ₂ O(8:2)	76	86~87	MeOH-H ₂ O(8:2)
	C ₁₃ H ₂₇	88	99~101	MeOH-H ₂ O(9:2)	83	87~89	—
	C ₁₅ H ₃₁	87	96~98	MeOH-H ₂ O(9:1)	71	88~90	—
	C ₁₇ H ₃₅	90	94~96	MeOH-H ₂ O(9:1)	68	88~89	MeOH-H ₂ O(9:1)
C ₁₃ H ₂₇	C ₇ H ₁₅	75	95~98	Hexane-EtOH(250:1)	85	70~72	Hexane
	C ₉ H ₁₉	85	92~94	Hexane-EtOH(250:2)	88	78~80	Hexane
	C ₁₁ H ₂₃	86	92~94	Hexane-EtOH(100:1)	92	84~86	Hexane
	C ₁₃ H ₂₇	87	97~98	Hexane-EtOH(100:1)	85	87~89	Hexane-EtOH(100:1)
	C ₁₅ H ₃₁	90	97~99	Hexane-EtOH(100:1)	90	93~95	Hexane-EtOH(100:1)
	C ₁₇ H ₃₅	93	98~99	Hexane-EtOH(100:1)	91	92~93	Hexane-EtOH(100:1)

O-di-TMS derivatives of D-*erythro*- and DL-*erythro*-C₁₈-dihydrosphingosines gave almost same RI (M-103) and RI (M-103)/RI(M-15), differing from the DL-*threo* derivative as shown in Table-4. These observations suggest a possibility for the determination of the *erythro* and *threo* isomers of dihydrosphingosines by mass spectrometry.

Fig.-2 Mass spectra of O, O-di-TMS derivatives of N-hexadecanoyl DL-*threo*- and DL-*erythro*-C₁₄-dihydrosphingosines.

3 Experimentals

3.1 Materials

Solvents were purified by the ordinary methods⁸⁾. The TMS reagent (HMDS+TMCS/pyridine) was purchased from Gasukuro Kogyo. N-Hydroxysuccinimide (GR grade) was purchased from Wako Pure Chemicals. N, N'-Dicyclohexylcarbodiimide (DCC: SP grade), fatty acids (GR grade) and acid anhydrides (GR grade) were purchased from Nakarai Chemicals. D-*erythro*-, DL-*erythro*- and DL-*threo*-C₁₈-dihydrosphingosines were purchased from Sigma Chemicals. Eluents for HPLC analysis were filtered with a 0.45 μm membrane filter (Tokyo Roshi Co. Ltd.)⁹⁾.

3.2 Analytical methods

GLC analyses were performed by a Hewlett Packard 402 High Efficiency Gas Chromatograph or a Hitachi 263-80 Gas Chromatograph: Column 1%SE-30 on 80~100 mesh Gas-chrom Q, 1 m or 2 m. GLC-MS analyses were carried out by a JEOL D-300 GC-MS: Column 1%SE-30 on 80~100 mesh Gas-chrom Q, 1 m;

Table-4 The relative intensities at M-103 and M-15, and the ratios, RI(M-103)/RI(M-15), for *O*, *O*-di-TMS derivatives of ceramides bearing DL-*threo*- and DL-*erythro*-C_n-dihydrosphingosines (*threo*-**6** and *erythro*-**6**).

Compounds		<i>erythro</i> - 6			<i>threo</i> - 6		
R	R'	RI(M-103)	RI(M-15)	RI(M-103)/ RI(M-15)	RI(M-103)	RI(M-15)	RI(M-103)/ RI(M-15)
C ₉ H ₁₉	CH ₃	8.8	8.7	1.04	40.7	9.8	4.2
	C ₇ H ₁₅	11.5	11.7	0.98	57.7	10.6	5.4
	C ₉ H ₁₉	11.6	10.8	1.07	39.6	6.1	6.5
	C ₁₁ H ₂₃	12.4	12.4	1.00	49.0	8.7	5.6
	C ₁₃ H ₂₇	10.6	10.6	1.00	62.3	10.9	5.7
	C ₁₅ H ₃₁	11.7	11.6	1.01	50.9	8.1	6.3
	C ₁₇ H ₃₅	9.3	7.9	1.18	53.6	8.9	6.0
C ₁₁ H ₂₃	CH ₃	8.7	9.0	0.97	43.3	11.0	3.9
	C ₃ H ₇	12.0	11.0	1.09	44.2	8.5	5.2
	C ₅ H ₁₁	13.3	10.4	1.28	51.1	7.5	6.8
	C ₇ H ₁₅	11.0	11.3	0.97	67.7	11.8	5.7
	C ₉ H ₁₉	11.1	9.9	1.12	78.6	14.1	5.6
	C ₁₁ H ₂₃	12.0	11.5	1.04	40.6	7.3	5.6
	C ₁₃ H ₂₇	12.7	12.4	1.02	63.3	10.7	5.9
	C ₁₅ H ₃₁	11.2	10.8	1.04	65.6	10.9	6.0
	C ₁₇ H ₃₅	13.1	12.5	1.05	46.0	7.8	5.9
C ₁₃ H ₂₇	CH ₃	6.9	8.2	0.84	31.6	8.7	3.7
	C ₇ H ₁₅	12.5	12.5	1.00	66.7	11.3	5.9
	C ₉ H ₁₉	12.6	12.7	0.99	62.2	10.8	5.8
	C ₁₁ H ₂₃	11.1	10.7	1.04	53.4	8.9	6.0
	C ₁₃ H ₂₇	11.5	11.3	1.02	50.7	9.3	5.5
	C ₁₅ H ₃₁	11.8	11.7	1.01	56.6	9.7	5.8
	C ₁₇ H ₃₅	10.4	9.5	1.09	62.7	10.9	5.8
C ₁₅ H ₃₁	CH ₃	10.3	8.7	1.18	40.8	11.0	3.7
	CH ₃ ^{a)}	6.7	7.2	0.93	—	—	—

^{a)} *N*-Acetyl D-*erythro*-C₁₈-dihydrosphingosine

Ionization voltage 70 eV. TLC analyses were carried out by the similar methods in the previous papers^{4), 10)~12)}. HPLC analyses were performed by a Triotar III High Pressure Liquid Chromatograph (Japan Spectroscopic Co. Ltd.: JASCO) equipped with a variable wavelength UVDEC-100-III UV detector (JASCO) using a reverse phase column, Finepak SIL C₁₈, 4.6 mm×250 mm (JASCO); Eluent CH₃CN+H₂O or CH₃OH+H₂O containing 0.1% phosphoric acid.

3.3 Products

3.3.1 2-Amino-1,3-alkanediols

2-Amino-1,3-dodecanediol (**1a**), 2-amino-1,3-tetradecanediol (**1b**), and 2-amino-1,3-

hexadecanediol (**1c**) were prepared by the similar method in the previous paper¹¹⁾. The crude products were recrystallized from hexane. Yield 55~75%. Purities were 98~99% and the *erythro* contents were 68~72% (estimated by GLC as *N*-acetyl *O*,*O*-di-TMS derivatives)¹¹⁾.

3.3.2 *N*-Acyloxysuccinimides

N-Acyl reagent, *N*-acyloxysuccinimide (**5**), were prepared by a modification of the method by Lapidot *et al.*¹²⁾ as follows: To a stirring solution of *N*-hydroxysuccinimide (0.05~0.1 mmol), fatty acid (0.05~0.1 mmol), and dioxane (100~350 ml) was added a solution of *N,N'*-dicyclohexylcarbodiimide (0.05~0.1 mmol) in dioxane (50~100 ml) during 30~50

min, precipitating the colorless crystals of *N*, *N'*-dicyclohexylurea. The stirring was continued for further 12~16 h at room temperature. The reaction mixture was filtered to remove the urea product, which was washed with three 20 ml portions of dioxane. The combined filtrates were evaporated to dryness. The residue was dissolved in a boiling hexane (100~200 ml). The hot solution was filtered to remove insoluble impurities and the filtrate was left at room temperature. The crystalline products were filtered and recrystallized from hexane or ethanol. The solvent/substrate ratios are summarized in **Table-2**.

The above reaction was also carried out in THF and almost same treatment as in dioxane gave pure (**5**). Purities of (**5**) were estimated by HPLC and GLC analyses. TLC analysis gave a single spot.

3.3.3 DL-*erythro*-C_n-Dihydrosphingosines (*erythro*-4)

A mixture of 2-amino-1, 3-hexadecanediol (**1c**, 80 mmol) and acetic anhydride (120 ml) in pyridine (240 ml) was left at room temperature for 16~20 h. The reaction mixture was poured into a cold water (500 ml). The mixture was stirred for 7 h at room temperature. Then, the compounds were extracted with four 150 ml portions of chloroform. The combined extracts were successively washed with two 100 ml portions of water, three 200 ml portions of 10% hydrochloric acid, two 200 ml portions of water, two 200 ml portions of 1 M sodium carbonate and three 200 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from two 400 ml portions of hexane. Crystalline product (*erythro*-2 **c**): Yield 60~63% (as *N*, *O*, *O*-triacetate). The mother liquors of the recrystallization were combined and evaporated to dryness to give pasty products (*threo*-2 **c**). Yield 20~25% (as *N*, *O*, *O*-triacetate). Triacetate (*threo*-2 **c**) was used for the preparation of DL-*threo*-C₁₆-dihydrosphingosine (*threo*-4 **c**).

N, *O*, *O*-Triacetyl derivatives (*erythro*-2 **a**, *erythro*-2 **b**, *threo*-2 **a** and *threo*-2 **b**) were prepared by the similar method described above. Yield: *erythro*-2 **a** 52~55%, *threo*-2 **a** 30~34%, *erythro*-2 **b** 56~67%, and *threo*-2 **b** 26~28% (as *N*, *O*, *O*-triacetyl derivatives).

A mixture of *erythro*-2 **c** (12.5 mmol), 10% sodium hydroxide (25 ml) and methanol (475 ml) was allowed to stand at room temperature for 16~20 h. The solution was concentrated under reduced pressure (to ca. 200 g). Water (250 ml) was added to the concentrated solution. Then, the compounds were extracted with three 250 ml portions of chloroform. The combined extracts were washed with two 250 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was suspended with a 250 ml of hexane, filtered and recrystallized from two 110 ml/g crystals portions of a mixture of hexane and ethanol (10 : 1 vol/vol) to give pure *N*-acetyl DL-*erythro*-C₁₆-dihydrosphingosine (*erythro*-3 **c**). Yield 60~70%. The *erythro* content was 99.9% (estimated by GLC as *O*, *O*-di-TMS derivative).

N-Acetyl DL-*erythro*-C₁₂-dihydrosphingosine (*erythro*-3 **a**) and *N*-acetyl DL-*erythro*-C₁₄-dihydrosphingosine (*erythro*-3 **b**) were prepared by the similar method described above. Crude *erythro*-3 **a** and *erythro*-3 **b** were recrystallized from two 70 ml and 100 ml/g crystals portions of hexane-ethanol (10 : 1 vol/vol), respectively. Yields: *erythro*-3 **a** 58~74% and *erythro*-3 **b** 61~68%. The *erythro* contents were more than 99.8% (estimated by GLC as *O*, *O*-di-TMS derivatives).

A mixture of *erythro*-3 **c** (6.5 mmol), potassium hydroxide (10 g), water (20 ml), and methanol (180 ml) was refluxed for 7~8 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. The residue was recrystallized from 150 ml/g crystals of hexane to give pure DL-*erythro*-C₁₆-dihydrosphingosine (*erythro*-4 **c**). Yield 95~97%.

DL-*erythro*-C₁₂- and C₁₄-Dihydrosphingosines (*erythro*-4 **a** and *erythro*-4 **b**) were prepared similarly: *erythro*-4 **a** 94~96% and *erythro*-4 **b** 95~97%.

3.3.4 DL-*threo*-C_n-Dihydrosphingosines (*threo*-4)

Triacetates (*threo*-2) were hydrolyzed to *N*-acetyl derivatives (*threo*-3) as follows: A mix-

ture of *threo-2c* (19 mmol), 10% sodium hydroxide (45 ml) and methanol (470 ml) was allowed to stand at room temperature for 15~19 h. Water (500 ml) was added to the solution. The compounds were extracted with three 500 ml portions of chloroform. The combined extracts were washed with two 500 ml portions of water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was crystallized from 100 ml of hexane and recrystallized from two 600 ml/g crystals portions of a mixture of hexane, diethyl ether, and ethyl acetate (6:5:1 vol/vol/vol) to give pure *N*-acetyl DL-*threo*-C₁₆-dihydrosphingosine (*threo-3c*). Yield 45~55%. The *threo* content was 99.3~99.8%.

Triacetates (*threo-2a* and *threo-2b*) were hydrolyzed by the similar method described above. Crude *N*-acetyl DL-*threo*-C₁₂- and C₁₄-dihydrosphingosines (*threo-3a* and *threo-3b*) crystallized from hexane were recrystallized from two 400 ml/g crystals and 600 ml/g crystals portions of diethyl ether-hexane (3:1 vol/vol) and hexane-diethyl ether-ethyl acetate (6:5:1 vol/vol), respectively. Yields: *threo-3a* 33~42% and *threo-3b* 47~68%. The *threo* contents were more than 99.5% (estimated by GLC as *O*, *O*-di-TMS derivatives).

DL-*threo*-C_n-Dihydrosphingosines (*threo-4a*, *threo-4b*, and *threo-4c*) were obtained from the alkaline hydrolysis of the corresponding *N*-acetyl derivatives (*threo-3*) by the similar method described in 3.3.3. Yields: *threo-4a* 95~98%, *threo-4b* 96~99% and *threo-4c* 98~99%.

3.3.5 Ceramides bearing DL-*erythro*- and DL-*threo*-C_n-dihydrosphingosines (*erythro-6* and *threo-6*)

To a stirring solution of dihydrosphingosine (**4**) (1 mmol) in THF (15 ml) was added dropwise a solution of *N*-acyloxysuccinimide (**5**) (1 mmol) dissolved in THF (15 ml). The reaction mixture was stirred for further 10~15 h. Then, the mixture was poured into water (300 ml) and left at room temperature for 2~5 h to precipitate completely the desired *N*-acyl compounds. The resulting crystals were collected by filtration, washed with four 25 ml portions of water, dried in vacuo and recrystallized from an appropriate solvent as shown in Table-3.

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DL-*erythro*- 及び DL-*threo*-C_n-ジヒドロ スフィンゴシンを骨格に有するセラミ ドの合成と質量分析法によるそれらの *erythro* 及び *threo* の決定法

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DL-*erythro*- 及び DL-*threo*-C_n-ジヒドロスフィンゴシン ($n=12, 14, 16$) を相当する 2-アミノ-1,3-アルカンジオールの *N*, *O*, *O*-トリアセチル及び *N*-アセチル誘導体を再結晶することにより分離できた。DL-*threo*- 及び DL-*erythro*-C_n-ジヒドロスフィンゴシンを骨格に持つセラミドは、これらの塩基と *N*-アシロキシスクシンイミドをテトラヒドロフラン中で反応させて得た。*threo* 体と *erythro* 体の MS スペクトルの差異を調べるため、このセラミドの *O*, *O*-di-TMS 誘導体の GC-MS の分析を行った。最も顕著な違いは $m/e=M-103$ の相対強度 (RI) に見られた、すなわち、*threo* 体では RI=40~80%, *erythro* 体では RI=6~14% と、前者の方が後者に比べて数倍高い。また、RI(M-103)/RI(M-15) は、*threo* 体では 3.7~6.8 であり、*erythro* 体では 0.8~1.3 であった。以上の結果から、質量分析によってジヒドロスフィンゴシンの *erythro*, *threo* が決定できることがわかった。