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Chemistry of Succinimido Esters. XXI^{*1}Determination of Sphingosine Bases as *N*-Arylacetyl
Derivatives by Normal Phase HPLC

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A sensitive determination of sphingolipid bases (1) was made using synthetic C_n -dihyrosphingosine and naturally occurring C_{18} -(1) aromatized with *N*-succinimidyl arylacetate (2a). The amino group in (1) easily reacted with (2a) to give the corresponding *N*-arylacetyl derivative (3a). The isomers of (1) were separated on a normal phase HPLC column in the form of (3a) which was detectable by the absorption in UV region. The isomer content of (1) as determined by the present method using (3a) having a different substituent showed agreement with that determined by GC method in the form of *N*-acetyl-*O*, *O*-bis(TMS) derivatives of (1) and the HPLC method in the form of *N*-arylcarbonyl derivative of (1), (3b).

1 Introduction

Analyses of sphingolipid bases (long chain bases) (1) are generally performed by GC using SE-30, OV-1, and OV-17 columns. The suitable compounds^{*2} are *N*-acetyl-*O*-TMS¹⁾ and *N*, *O*-TMS²⁾ derivatives. *N*-Acyl-*O*-TMS derivative of (1) is also applicable³⁾. Recently, we reported that the *erythro*/*threo* separation and the determination of *erythro* isomer content of synthetic dihyrosphingosine (DHSph) could be done by normal (in the form of *N*, *O*, *O*-triacyl and *N*-acetyl derivative⁴⁾ and reverse (in the form of free bases and their *N*-acetyl derivatives⁵⁾) phase HPLC. But, these methods had a disadvantage of low sensitivity even at low detection wavelengths (200 ~ 220 nm). This problem was overcome by aromatization of

DHSph with *N*-succinimidyl benzoate (2b)⁶⁾.

In the previous papers on the reaction of *N*-succinimidyl arylacetate (2a) with an amino compound in aqueous solution⁷⁾, we found out that (2a) was applicable to chemical modification of amino groups in proteins. In this paper, we extended this reaction to aromatization of (1) in organic solvent to give the corresponding *N*-arylacetyl derivative (3a) and carried out normal phase HPLC analysis of (3a) to confirm a

Ar = $\text{C}_6\text{H}_4\text{-X}$ or $\text{C}_6\text{H}_3\text{-X}_2$

R = $n\text{-C}_9\text{H}_{19}$ (C_{12} -DHSph),

$n\text{-C}_{13}\text{H}_{27}$ (C_{16} -DHSph),

$n\text{-C}_{13}\text{H}_{27}\text{-CH=CH-}$ (C_{18} -Sph),

DHSph : Dihyrosphingosine,

Phyto : Phytosphingosine

Su = *N*-Succinimidyl,

(2a), (3a) : $n=1$, (2b), (3b) : $n=0$

X = H, *o*-F, *m*-F, *p*-F, *o*-CH₃O, *m*-CH₃O, *p*-CH₃O,

o-Cl, *m*-Cl, *p*-Cl, *o*-Br, *m*-Br, *p*-Br, *o*-NO₂,

m-NO₂, *p*-NO₂, *o*-CH₃, *m*-CH₃, *p*-CH₃, *p*-CN, *p*-C₂H₅O,

2,4-diNO₂, 3,4-diNO₂, 2,4-diCl,

3,4-diCl, 2,6-diCl

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*2 Abbreviations

TMS : Trimethylsilyl, DSC :
N, *N'* - Disuccinimidyl carbonate,
DSO : *N*, *N'* - Disuccinimidyl ox-
alate, HMDS : Hexamethyl disila-
zane, TMCS : Trimethylchlorosi-
lane

sensitive determination of (1). Furthermore, the present method was compared with the previously reported HPLC method with the use of (2b) as the aromatizing reagent and GC method using *N*-acetyl-*O*-TMS derivative of (1).

2 Experiments

2.1 Materials

Phenylacetic acid and benzoic acid (purity >97%), DSC*² and DSO*² (SP for peptide synthesis), and 2-nitroethanol (purity: 98%) were purchased from Aldrich, Tokyo Kasei, Chemiscience, and E. Merck. Freshly distilled aldehydes (RCHO: R=*n*-C₉H₁₉, *n*-C₁₁H₂₃, and *n*-C₁₃H₂₇; purchased from Tokyo Kasei and Aldrich) were used for the synthesis of C₁₂-, C₁₄-, and C₁₆-DHSphs. The other bases C₁₈-(1), *erythro*-C₁₈-DHSph*³, C₁₈-DHSph (a mixture of *erythro*- and *threo*-C₁₈-DHSph), *D-erythro*-C₁₈-sphingosine (Sph), and *D-ribo*-C₁₈-phytosphingosine (Phyto), were purchased from Sigma.

Hexane and acetonitrile (SP for HPLC analysis) were purchased from Katayama Chemical and the other solvents were purified by the ordinary methods⁸. The TMS reagent (HMDS+TMCS/pyridine)*² was purchased from GL Science.

2.2 Products

2.2.1 *N*-Succinimidyl arylacetates (2a) and benzoates (2b)

The aromatizing (labeling) reagents having a different substituent in the aromatic ring, (2a) and (2b), were prepared by the coupling reaction of DSC with the corresponding acid in the presence of pyridine in acetonitrile according to the previously reported methods^{6),9)}.

2.2.2 Dihydrosphingosine (DHSph)

2-Amino-1,3-alkanediol (a mixture of *erythro*- and *threo*-C_{*n*}-DHSphs: *n*=12, 14 and 16)*³ was prepared by the catalytic hydrogenation of the corresponding 2-nitro-1,3-alkanediol according to the previously reported method¹⁰⁾. The obtained base was recrystallized from hexane.

* 3 According to "the nomenclature of lipids", recommended by IUPAC-IUB Commission on Biological Nomenclature [*Lipids*, 12, 455 (1977)], the configuration of naturally occurring *D-erythro*-C₁₈-dihydro-sphingosine is shown by (2*R*, 3*S*). In the present paper, *erythro* and *threo* therefore mean mixtures of (2*S*, 3*R*)+(2*R*, 3*S*) and (2*R*, 3*R*)+(2*S*, 3*S*), respectively.

threo- and *erythro*-C_{*n*}-DHSphs (*n*=12, 14 and 16) were prepared by the fractional crystallization of *N*-acetyl and *N*,*O*,*O*-triacyl derivatives of DHSph, followed by the alkaline hydrolysis¹¹⁾.

2.3 Preparation of (3) for normal phase HPLC analysis

A mixture of (2) (1~2 mg) and (1) (1~4 mM, 0.5 mL) dissolved in acetonitrile was heated on a hot plate at 50~60°C for 16~24h. An aliquot (0.2~1 μL) of the resulting solution was exposed to HPLC analysis.

A mixture (totally: 180 mg) of C₁₈-DHSph (*erythro*+*threo*), *D-erythro*-C₁₈-Sph, and *D-ribo*-C₁₈-Phyto (unknown ratio) was heated in acetonitrile (300 mL) at 60°C for 1h and filtered. The filtrate (0.5 mL) was added to (2) (1mg) and treated according to the above procedure. The resulting solution was used as a sample for the determination of isomer content of C₁₈-(1).

2.4 Analytical methods

HPLC analysis was carried by the same apparatus in the previous papers⁶⁾. Column: Inertsil SIL (particle size: 5 μm), 4.6 φ×250mm (GL Science); Mobile phase: 3~10 vol% ethanol/hexane where x vol% a/b means solvent b containing x vol% of solvent a in this paper; Flow rate: 1.0 mL/min; Detection wavelength: λ_{max} of *n*-C₁₂H₂₅NHCOCH₂Ar and *n*-C₁₂H₂₅-NHCOAr^{(6a)-(6c)} for (3a) and (3b), respectively. In the case that the amide compounds of dodecylamine did not have λ_{max}, 220 nm of the detection wavelength was employed.

UV analysis was done by the same apparatus in the previous paper^{6a)} at 25°C. Solvent: the same solvent for HPLC analysis; Light path length: 1 cm.

GC analysis was performed by the Hitachi 263-80 Gas Chromatograph (Detector: FID). Column: 1% Silicone gum SE-30/Gaschrom Q, 80~100 mesh (GL Science), 2 m; Column temperature: 170→240°C (5°C/min), 180→240°C (2°C/min), 190→250°C (2°C/min), and 200→270°C (2°C/min) for C₁₂-DHSphs, C₁₄-DHSphs, C₁₆-DHSphs, and C₁₈-(1), respectively; Flow rate of carrier (He): 50mL/min.

N-Acetyl-*O*-TMS derivative of (1) was prepared as follows: the solvent of substrate solution (0.5 mL) prior to the aromatization (2.3) was removed under a stream of nitrogen and the residue was dried in vacuo for 5h. The obtained

crystalline materials were treated with the previously reported method¹¹⁾.

The isomer contents of synthetic DHSph and the mixed C₁₈-(1) were calculated from the peak areas of the chromatograms in the HPLC and GC analyses.

3 Results and Discussion

3.1 Normal phase HPLC of C_n-DHSph in the form of (3a)

3.1.1 Retention volume

The amino group of DHSph (C₁₂~C₁₆) reacted with (2a) having a different substituent in the aromatic ring to give the corresponding *N*-arylacetyl compounds (3a), whose *erythro*/*threo* separation could be accomplished by normal phase using hexane containing ethanol. It was found that the elution order was C₁₆-(3a) > C₁₄-(3a) > C₁₂-(3a) [where c > d means that compound c is eluted faster than compound d in this paper]. A similar elution order was observed for (3b).

The net retention volumes (*V_N*) of *erythro*- and *threo*-(3)*⁴ were calculated by the following

equation :

$$V_N(3) = V(3) - V(\text{Solvent}) \quad (2)$$

where *V*(3) and *V*(Solvent) are the apparent retention volumes of (3) and solvent, respectively. As shown in Table-1, *V_N*(3a) was comparable with *V_N*(3b) in the case of X = *o*-Cl. (3a) was less retained than (3b) for X = *o*-NO₂ and *vice versa* for the other substituents.

3.1.2 separation factor (α)

Next, we calculated the separation factor (α) for normal phase HPLC of (3), defined by the following equation :

$$\alpha = \frac{V_N[\text{erythro}-(3)]}{V_N[\text{threo}-(3)]} \quad (3)$$

where *V_N*[*erythro*-(3)] and *V_N*[*threo*-(3)] are the net retention volumes of *erythro*- and *threo*-C_n-DHSphs in the form of (3), respectively. The results are summarized in Table-2, which shows good separation of *threo* and *erythro* isomers of DHSphs as (3). In the case of (3a) (X = 2,4-diNO₂) using 10 vol% ethanol/hexane, the *erythro*/*threo* separation was unsatisfactory, however, it could be accomplished by using 4 vol% ethanol/hexane as mobile phase.

Table-1 The *V_N*(3a)/*V_N*(3b) value of synthetic C_n-DHSph determined by normal phase HPLC^{a)}.

Substituent X in (3)	<i>V_N</i> (3a)/ <i>V_N</i> (3b)					
	<i>threo</i> -C ₁₂	<i>erythro</i> -C ₁₂	<i>threo</i> -C ₁₄	<i>erythro</i> -C ₁₄	<i>threo</i> -C ₁₆	<i>erythro</i> -C ₁₆
H	1.26	1.28	1.26	1.28	1.27	1.26
<i>o</i> -F	1.29	1.24	1.24	1.23	1.24	1.21
<i>m</i> -F	1.36	1.37	1.33	1.36	1.32	1.32
<i>p</i> -F	1.49	1.47	1.46	1.46	1.41	1.44
<i>o</i> -CH ₃ O	1.05	1.05	1.05	1.05	1.06	1.05
<i>m</i> -CH ₃ O	1.30	1.34	1.26	1.32	1.28	1.32
<i>p</i> -CH ₃ O	1.24	1.24	1.21	1.24	1.17	1.22
<i>o</i> -Cl	1.05	1.07	1.05	1.02	1.05	1.02
<i>p</i> -Cl	1.57	1.54	1.54	1.53	1.50	1.51
<i>o</i> -Br	1.02	0.99	1.02	1.00	1.02	1.00
<i>m</i> -Br	1.40	1.41	1.38	1.39	1.39	1.36
<i>p</i> -Br	1.60	1.60	1.57	1.56	1.54	1.54
<i>o</i> -NO ₂	0.91	0.86	0.92	0.87	0.92	0.87
<i>m</i> -NO ₂	1.57	1.50	1.57	1.49	1.56	1.48
<i>p</i> -NO ₂	2.12	2.01	2.08	1.97	2.03	1.94
2, 4-diCl	1.27	1.22	1.27	1.20	1.27	1.19
3, 4-diCl	1.66	1.63	1.61	1.60	1.58	1.58

a) Mobile phase : 10 vol% ethanol/hexane.

*4 *erythro*- and *threo*-(3) are *N*-arylacetyl derivatives of *erythro*- and *threo*-(1), respectively.

Table-2 Separation factor (α) of synthetic C_n -DHSph determined normal phase HPLC in form of (3)^a.

Substituent X in (3)	Separation factor (α)					
	C ₁₂ -DHSph		C ₁₄ -DHSph		C ₁₆ -DHSph	
	(3 a)	(3 b)	(3 a)	(3 b)	(3 a)	(3 b)
H	1.23	1.33	1.21	1.33	1.18	1.33
<i>o</i> -F	1.22	1.37	1.20	1.37	1.18	1.37
<i>m</i> -F	1.23	1.29	1.27	1.30	1.25	1.29
<i>p</i> -F	1.21	1.31	1.22	1.31	1.26	1.31
<i>o</i> -CH ₃ O	1.39	1.42	1.38	1.40	1.35	1.39
<i>m</i> -CH ₃ O	1.29	1.30	1.33	1.29	1.33	1.32
<i>p</i> -CH ₃ O	1.28	1.32	1.31	1.30	1.36	1.31
<i>o</i> -Cl	1.30	1.38	1.29	1.37	1.27	1.36
<i>m</i> -Cl	—	1.29	—	1.29	—	1.30
<i>p</i> -Cl	1.20	1.33	1.21	1.33	1.24	1.34
<i>o</i> -Br	1.31	1.37	1.29	1.36	1.28	1.33
<i>m</i> -Br	1.23	1.30	1.22	1.29	1.19	1.31
<i>p</i> -Br	1.20	1.33	1.20	1.33	1.22	1.38
<i>o</i> -NO ₂	1.17	1.25	1.17	1.25	1.17	1.25
<i>m</i> -NO ₂	1.09	1.19	1.09	1.20	1.09	1.20
<i>p</i> -NO ₂	1.08	1.21	1.08	1.21	1.07	1.21
<i>p</i> -C ₂ H ₅ O	1.29	—	1.30	—	1.28	—
<i>o</i> -CH ₃	—	1.33	—	1.33	—	1.33
<i>m</i> -CH ₃	—	1.34	—	1.35	—	1.35
<i>p</i> -CH ₃	—	1.35	—	1.36	—	1.36
<i>p</i> -CN	—	1.21	—	1.22	—	1.25
2, 4-diNO ₂	1.07 ^b	1.18	1.07 ^b	1.18	1.04 ^b	1.18
3, 4-diNO ₂	—	0.92 ^c	—	0.92 ^c	—	0.92 ^c
2, 4-diCl	1.24	1.38	1.21	1.40	1.19	1.41
3, 4-diCl	1.16	1.30	1.18	1.31	1.18	1.31
2, 6-diCl	1.33	—	1.33	—	1.33	—

a) Mobile phase : 10 vol% ethanol/hexane.
Detection wavelength : see Table-3.

b) Mobile phase : 4 vol% ethanol/hexane.

c) Mobile phase : 30 vol% ethanol/hexane.

Table-2 also shows that a variety of substituents (X) could be applicable to the analysis. The *threo* isomer of synthetic DHSph was eluted faster than its *erythro* one except (3 b) (X=3,4-diNO₂). It is reported that the elution order of *erythro*>*threo* is observed for *N*-acetyl DHSph and *vice versa* for *N, O, O*-triacyl derivative of DHSph in normal phase HPLC⁴. It is also reported that *threo*-(3 b) retained more strongly than *erythro*-(3 b) in reverse phase HPLC be-

cause the aromatic ring in (3 b) would interact with the reverse phase (C₁₈)^{6c}.

The α values were 1.08~1.39 (C₁₂-DHSph), 1.08~1.38 (C₁₄-DHSph), and 1.07~1.36 (C₁₆-DHSph), as (3 a) and 1.18~1.42 (C₁₂-DHSph), 1.18~1.40 (C₁₄-DHSph), and 1.18~1.39 (C₁₆-DHSph) as (3 b)*⁵. The α value of (3 a) was same as that of (3 b) in the case of X=methoxy (CH₃O), however, the former was smaller than the latter for the other substituents.

3.1.3 Determination of *threo* content of synthetic C_n-DHSph

Based on the above findings, the *threo* content of synthetic C_n-DHSph was determined in the form of (3 a) and compared with those determined by normal phase HPLC in the form of (3 b) and GC using *N*-acetyl-*O, O*-bis (TMS) derivative of DHSph. As shown in Table-3, an excellent coincidence was observed between the HPLC and GC methods.

3.2 Normal phase HPLC of C₁₈-(1)

3.2.1 Separation

We applied the present method using (2 a) as the aromatizing reagent to the determination of naturally occurring sphingolipid bases, C₁₈-(1). The analysis was carried out using commercially available bases (see 2.1 and 2.3).

The ratio, $V_N(3)/V_N(2)$ [where $V_N(2)$ and $V_N(3)$ are the net retention volumes of (2) and (1) as (3), respectively], was calculated to clarify the elution order of (1). As shown in Table-4, a good separation of (1) was obtained except for (3 a) (X=*m*- and *p*-NO₂) and C₁₈-(1) was eluted in the order of *threo*-C₁₈-DHSph > *erythro*-C₁₈-DHSph > *D-erythro*-C₁₈-Sph > *D-ribo*-C₁₈-Phyto (with an exception of *D-erythro*-C₁₈-Sph > *erythro*-C₁₈-DHSph for X=*o*-NO₂).

3.2.2 Determination of isomer content of C₁₈-(1)

Finally, we measured the isomer content of the mixed sample (2.3) to verify the sensitive determination of (1). The results are summarized in Table-5, which shows good agreement between the HPLC method in the form of (3) having a different substituent in the aromatic ring and the GC method using *N*-acetyl-*O*-TMS derivative of (1).

From the above observations, we concluded that (2 a) was applicable as the aromatization reagent for the determination of (1) by normal

*5 α^{-1} was 1.09 for (3 b) (X=3,4-diNO₂).

Table-3 The *threo* contents of synthetic C_n-DHSph determined by normal phase HPLC in the form of (3) and GC in the form of *N*-acetyl-*O*, *O*-bis(TMS) derivative of C_n-DHSph^{a)}.

Substituent X in (3)	<i>threo</i> content (%)						Detection	
	C ₁₂ -DHSph		C ₁₄ -DHSph		C ₁₆ -DHSph		wavelength (nm)	
	(3 a)	(3 b)	(3 a)	(3 b)	(3 a)	(3 b)	(3 a)	(3 b)
H	30.1	29.9	30.5	30.3	29.5	29.9	220	225
<i>o</i> -F	29.7	30.1	30.4	30.6	29.9	29.5	220	223
<i>m</i> -F	29.9	30.0	30.4	30.3	30.1	30.1	220	224
<i>p</i> -F	29.9	30.4	30.1	30.5	29.7	29.9	220	227
<i>o</i> -CH ₃ O	30.0	29.7	30.5	30.1	29.9	29.9	272	220
<i>m</i> -CH ₃ O	30.3	29.8	30.4	30.3	29.8	30.2	274	289
<i>p</i> -CH ₃ O	30.3	30.2	30.3	30.4	30.0	29.9	277	250
<i>o</i> -Cl	28.8	30.0	30.3	30.5	29.9	29.9	220	220
<i>m</i> -Cl	—	30.0	—	30.3	—	29.9	—	220
<i>p</i> -Cl	30.0	30.0	30.3	30.7	30.1	30.0	222	236
<i>o</i> -Br	30.2	30.1	30.3	30.4	29.8	30.1	225	225
<i>m</i> -Br	29.7	30.2	30.3	30.7	29.6	30.5	225	223
<i>p</i> -Br	30.1	30.2	30.3	30.3	29.9	29.9	222	220
<i>o</i> -NO ₂	29.9	29.8	30.4	30.0	30.0	29.8	255	245
<i>m</i> -NO ₂	30.1	29.8	30.1	30.3	29.3	29.4	260	246
<i>p</i> -NO ₂	29.6	30.3	30.2	30.4	30.2	29.8	269	263
<i>p</i> -C ₂ H ₅ O	29.8	—	30.4	—	30.0	—	228	—
<i>o</i> -CH ₃	—	29.9	—	29.9	—	29.8	—	225
<i>m</i> -CH ₃	—	30.2	—	30.5	—	29.7	—	230
<i>p</i> -CH ₃	—	30.1	—	30.1	—	29.8	—	234
<i>p</i> -CN	—	29.8	—	30.5	—	29.8	—	246
2,4-diNO ₂	29.9	29.8	30.6	30.3	29.8	29.8	238	230
3,4-diNO ₂	—	30.0	—	30.6	—	30.2	—	220
2,4-diCl	29.8	30.0	30.2	29.9	29.6	30.0	220	220
3,4-diCl	29.8	30.0	30.3	30.3	29.9	30.0	220	220
2,6-diCl	30.1	—	30.0	—	30.0	—	220	—

Method	Derivative	<i>threo</i> content (average) (%)					
		C ₁₂ -DHSph		C ₁₄ -DHSph		C ₁₆ -DHSph	
		(3 a)	(3 b)	(3 a)	(3 b)	(3 a)	(3 b)
HPLC	(3 a) or (3 b)	30.0±0.4	30.0±0.4	30.3±0.3	30.3±0.4	29.9±0.3	29.9±0.3
GC ^{a)}	N-Ac-O-TMS ^{b)}	29.9±0.4		30.3±0.5		29.7±0.6	

a) Average of 5~10 runs.

b) *N*-Acetyl-*O*, *O*-bis(TMS) derivative of C_n-DHSph.

Table-4 The ratio, $V_N(3)/V_N(2)$, of $C_{18}-(1)$ as (3) determined normal phase HPLC^{a)}.

Derivative (3)	$C_{18}-(1)$ X in (3)	$V_N(3)/V_N(2)$			
		<i>threo</i> -DHSph	<i>erythro</i> -DHSph	D- <i>erythro</i> -Sph	D- <i>ribo</i> -Phyto
(3 a)	<i>p</i> -CH ₃ O	0.64	0.86	0.92	1.36
	<i>p</i> -C ₂ H ₅ O	0.64 [1.34]	0.87 [1.99]	0.94 [2.18]	1.35 [3.48]
	<i>p</i> -Cl	0.70 [1.49]	0.89 [2.00]	0.95 [2.15]	1.48 [3.79]
	<i>p</i> -Br	0.71 [1.52]	0.89 [2.02]	0.95 [2.18]	1.48 [3.84]
	<i>o</i> -NO ₂	0.56	0.69	0.61	1.08
	<i>m</i> -NO ₂	0.66	0.74	0.76	1.23
	<i>p</i> -NO ₂	0.81	0.89	0.91	1.56
(3 b)	H	0.35 (0.32)	0.48 (0.44)	0.58 (0.51)	0.86 (0.73)
	<i>o</i> -F	0.28	0.41	0.47	0.61
	<i>m</i> -F	0.33 (0.31)	0.46 (0.41)	0.54 (0.49)	0.82 (0.68)
	<i>p</i> -F	0.33 (0.30)	0.45 (0.41)	0.55 (0.49)	0.84 (0.69)
	<i>m</i> -CH ₃	0.33	0.47	0.58	0.81
	<i>p</i> -CH ₃	0.34	0.47	0.58	0.82
	<i>p</i> -CH ₃ O	0.28 (0.34)	0.50 (0.44)	0.58 (0.52)	0.94 (0.79)
	<i>p</i> -Cl	0.31 (0.28)	0.44 (0.39)	0.54 (0.49)	0.78 (0.61)
	<i>p</i> -Br	0.30 (0.28)	0.43 (0.38)	0.54 (0.48)	0.76 (0.60)
	<i>o</i> -NO ₂	0.55	0.72	0.62	1.22
	<i>m</i> -NO ₂	0.38 (0.33)	0.48 (0.42)	0.54 (0.48)	0.90 (0.77)
	<i>p</i> -NO ₂	0.34 (0.32)	0.45 (0.38)	0.55 (0.48)	0.83 (0.70)

a) Mobile phase : 7 vol% ethanol/hexane. The numbers in the brackets and parentheses are the $V_N(3)/V_N(2)$ values determined using 3.0 and 8.5 vol% ethanol/hexane, respectively.

Table-5 The isomer contents of the mixed sample (2·3) determined by normal phase HPLC in the form of (3) and GC in the form of *N*-acetyl-*O*-TMS derivative of (1).

Derivative (3)	$C_{18}-(1)$ X in (3)	Content (%)			
		<i>threo</i> -DHSph	<i>erythro</i> -DHSph	D- <i>erythro</i> -Sph	D- <i>ribo</i> -Phyto
(3 a) ^{d)}	<i>p</i> -CH ₃ O ^{a)}	24.2	22.7	21.0	32.1
	<i>p</i> -C ₂ H ₅ O ^{b)}	23.7	21.4	20.8	34.1
	<i>p</i> -Cl ^{b)}	24.0	21.6	21.1	33.3
	<i>p</i> -Br ^{a)}	24.4	21.4	20.5	33.6
	<i>o</i> -NO ₂ ^{a)}	25.5	21.0	20.7	32.9
(3 b)	H ^{c)}	23.9	21.7	21.2	33.2
	<i>o</i> -F ^{a)}	23.4	22.5	21.1	33.1
	<i>m</i> -F ^{c)}	24.5	21.6	21.3	32.7
	<i>p</i> -F ^{c)}	24.7	21.8	20.9	32.6
	<i>m</i> -CH ₃ ^{a)}	24.3	21.9	20.3	33.5
	<i>p</i> -CH ₃ ^{a)}	24.6	21.7	20.9	33.1
	<i>p</i> -CH ₃ O ^{c)}	23.8	22.2	20.9	33.4
	<i>p</i> -Cl ^{c)}	25.2	21.7	20.5	32.4
	<i>p</i> -Br ^{c)}	25.1	21.3	20.9	32.7
	<i>o</i> -NO ₂ ^{c)}	25.3	21.8	20.0	32.9
	<i>m</i> -NO ₂ ^{c)}	25.4	21.9	20.9	31.8
	<i>p</i> -NO ₂ ^{c)}	24.0	21.7	21.6	32.9
Method	Derivative	Content (average) (%)			
HPLC	(3 a)	24.4±1.4	21.6±1.1	20.8±0.3	33.2±1.1
	(3 b)	24.5±1.1	21.8±0.7	20.8±0.8	32.8±1.0
GC ^{e)}	<i>N</i> -Ac- <i>O</i> -TMS	24.5±0.6	21.7±0.5	21.0±0.4	32.8±0.7

a) Mobile phase : 7 vol% ethanol/hexane.

b) Mobile phase : 3 vol% ethanol/hexane.

c) Mobile phase : 8.5 vol% ethanol/hexane.

d) The baseline separation was not obtained between *erythro*-DHSph and D-*erythro*-Sph for X = *m*-NO₂ and *p*-NO₂.

e) Average of five runs.

phase HPLC.

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スクシンイミドエステルの化学 (第 21 報)

N-アリールアセチル誘導体を用いたスフィンゴ塩基の順相 HPLC による定量

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スフィンゴ塩基 (1) の高感度定量法の確立を目的として、基質として合成したジヒドロスフィンゴシンと C_{18} - (1) を、芳香 (ラベル) 化剤として *N*-スクシンイミジルアリールアセテート (2a) を用いて、順相 HPLC 分析の検討を行った。(1) のアミノ基は (2a) と容易に反応して相当する *N*-アリールアセチル化合物 (3a) を生成した。(1) の異性体は (3a) の形にすると UV 領域に吸収を持つようになり、しかも、種々の置換基を持つ (3a) 形で順相 HPLC で分離ができることがわかった。また、順相 HPLC 分析から求めた (1) の異性体の組成が従来知られている (1) の *N*-アセチル-*O*-TMS 体の GC 分析及び (1) の *N*-アリールカルボニル体, (3b), の順相 HPLC の結果と良好な一致を示すことを認めた。