報 文

Convenient Method for the Preparation of *N*-Acyl *O*-Trimethylsilyl Derivatives of Sphingosine Bases

Hirohumi HIRATA*, Alemka KISIC**, John BRABSON**,
and George J. SCHROEPFER, JR.**

* National Chemical Laboratory for Industry (Yatabe, Tsukuba, Ibaraki)

** Department of Chemistry and Biochemistry, Rice University
(Houston, Texas 77001, U.S.A.)

A convenient method for the preparation of *N*-acyl *O*-TMS derivatives of sphingolipid bases is described; *i.e.*, *O*-TMS derivatives of the bases, which are obtained by the HMDS+TMCS/pyridine treatment, are reacted with acid anhydride to give the desired *N*-acyl-*O*-TMS compounds.

The reaction of these bases with BSA/acetonitrile gave *N*, *O*-TMS derivatives which did not react with acid anhydrides, since the amino group was protected by the TMS group.

The above method was applied to several bases and the structures of the resulting products were determined by GLC and GLC-MS. *N*-Acyl *O*-TMS derivatives of synthetic *C_n*-dihydrosphingosines were prepared by the present method. The *erythro*/*threo* ratios of their derivatives were the same as those determined using BSA. The present method can be easily carried out in a very short period of time. MS analyses for both *O*-TMS and *N*-acyl *O*-TMS derivatives are possible without having to prepare the *N*-acyl derivatives of sphingosine bases.

1 Introduction

It has been reported that *erythro* and *threo* isomers of sphingolipid bases such as sphingosines and dihydrosphingosines could be separated on SE-30 and OV-1 columns. The suitable compounds are *N*-acetyl *O*-TMS^{1)~4)} and *N*, *O*-TMS¹⁾ derivatives. The structures of the TMS derivatives are determined by GLC-MS. The relative intensity (RI) of *N*-acetyl *O*-TMS derivatives (RI ca. 10%) are stronger than that of *N*, *O*-TMS derivatives (RI ca. 2%)^{1), 5)}. Thus, the former is better than the latter for GLC-MS.

The preparation of *N*-acetyl (or *N*-acyl) *O*-TMS derivatives of sphingolipid bases involves *N*-acetylation (or *N*-acylation) prior to *O*-trimethylsilylation. Although *N*-acetylation (or *N*-acylation) of the bases is already reported⁶⁾, a convenient preparation of *N*-acetyl *O*-TMS

derivatives of free bases has not been reported. In the present paper, the reactions of free bases such as DL-*erythro*-*C*₁₄-, D-*erythro*-*C*₁₈- and DL-*threo*-*C*₁₈-dihydrosphingosines, D-*erythro*-*C*₁₈-sphingosine and D-ribo-*C*₁₈-phytosphingosine with several acid anhydrides in the presence of TMS reagents in order to discover a convenient method for the preparation of *N*-acyl *O*-TMS derivatives of sphingosine bases.

2 Experimentals

2.1 Materials

Acetic anhydride (AR grade: Mallincknott) was purified by distillation (bp 139°C). Propionic, butyric, valeric, hexanoic, octanoic and decanoic anhydrides (GR grade) were purchased from Nakarai Chemicals (Osaka) and used without purification. Pyridine (AR grade: Mallincknott) was distilled under N₂ in the presence of potassium hydroxide and stored

Abbreviations

HMDS: Hexamethyldisilazane

TMCS: Trimethylchlorosilane

BSA: *N*, *O*-Bis (trimethylsilyl) acetamide

TMS: Trimethylsilyl

over potassium hydroxide. Other solvents were purified by the ordinary methods⁷⁾. The TMS reagents (HMDS+TMCS/pyridine and BSA/acetonitrile) were purchased from Gaschrom Kogyo (Tokyo) or prepared from the corresponding reagents and solvents. DL-erythro-C₁₄-Dihydrosphingosine was prepared previously⁸⁾ (mp 72~73°C, recrystallized from hexane). D-erythro-C₁₈-Sphingosine was isolated from bovine brain sphingolipid⁹⁾ or purchased from Serdary Research Laboratories, Inc. (SRL) and Sigma Chemical Company (Sigma). D-erythro-C₁₈-Dihydrosphingosine was purchased from Sigma and SRL. DL-threo-C₁₈-Dihydrosphingosine was purchased from Sigma.

N-Acetyl D-ribo-C₁₈-phytophingosine was isolated from yeast⁹⁾.

2.2 Analytical Methods

Melting points were recorded by a Thomas Hooper Capillary Melting Point Apparatus. GLC analyses were performed by a Hewlett Packard 402 High Efficiency Gas Chromatograph: Column 3% OV-17 on 80~100 mesh Gas-chrom Q, 6 ft, or 1% SE-30 on 80~100 mesh Gas-chrom Q, 2 m. GLC-MS analyses were carried out by a Finnigan GC/MS or a JEOL D-300 GC-MS: Column 3% OV-17 on 80~100 mesh Gas-chrom Q, 5 ft, or 1% SE-30 on 80~100 mesh Gas-chrom Q, 1 m; Ionization voltage 70 eV. TLC analyses were carried out by the similar methods in the previous papers^{1), 5)}.

2.3 Purification of N-acetyl D-ribo-C₁₈-phytosphingosine by medium pressure liquid chromatography (MPLC)

Partially purified N-acetyl D-ribo-C₁₈-phytosphingosine (1600 mg) dissolved in a mixture of chloroform and methanol (10 : 1 vol/vol, 18 ml) was loaded on a MPLC column (1000 mm×25 mm, I.D.) packed with Silica Gel (32~64 μm, Woelm). Then, the compounds were eluted with chloroform (Frs. # 1~10) and chloroform-methanol (19 : 1 vol/vol, Frs. # 11~80 and 10 : 1 vol/vol, Frs. # 81~290). Frs. # 102~120 were combined and evaporated to dryness. Yield 1348 mg (82.4%). Purity 99% (by GLC), mp 131~133°C. MS (*m/e*, measured by a Finnigan GC/MS at 70 eV): 500 (M-15, 15 : CH₃), 472 (M-103, C₂→C₁), 401 (M-174, C₃→C₂), 299 (M-276, C₄→C₃), 276 (C₄→C₃), 174 (C₃→C₂),

103 (C₂→C₁) and 73 (TMS : base peak ion) [as O, O, O-tri-TMS ether].

2.4 Hydrolysis of N-acetyl D-ribo-C₁₈-phytosphingosine (Preparation of D-ribo-C₁₈-phytosphingosine)

A mixture of N-acetyl D-ribo-C₁₈-phytosphingosine (500 mg), potassium hydroxide (2.5 g), water (5 ml) and methanol (45 ml) was refluxed for 8 h. After cooling to room temperature, water (50 ml) was added to the mixture. The compounds were extracted with five 100 ml portions of chloroform. The combined extracts were washed with three 150 ml portions of water and dried over anhydrous sodium sulfate. Removal of the solvents gave the desired free base, D-ribo-C₁₈-phytosphingosine, which was used without further purification in the present paper. Yield 90%. Purity 96% (by GLC). MS (*m/e*, measured by a Finnigan GC/MS at 70 eV): 590 (M-15), 502 (M-103, C₂→C₁), 412 (M-103-90), 401 (M-204, C₃→C₂), 299 (M-306, C₄→C₃), 204 (C₃→C₂) and 73 (TMS : base peak ion) [as N, O, O, O-tetra-TMS derivative]; 518 (M-15), 430 (M-103, C₂→C₁), 401 (M-132, C₃→C₂), 340 (M-103-90), 299 (M-234, C₄→C₃), 132 (C₃→C₂ : base peak ion) and 73 (TMS) [as O, O, O-tri-TMS derivative].

2.5 Established convenient method for the preparation of N-acyl O-TMS derivatives of sphingosine bases

A mixture of sphingolipid base (1~2 mg) and TMS reagent (HMDS+TMCS/pyridine, 200~400 μl) was left at room temperature for 2 min, heated at 60°C on a hot plate until the solution became clear (ca. 5 min), and then cooled to room temperature. To the resulting mixture was added acid anhydride (1~2 μl), and the solution was heated at 60°C for 5~10 min. An aliquot was injected to GLC and GLC-MS. The above reaction was carried out in a vial. In the cases of acetic, propionic and butyric anhydrides, the N-acylation was achieved at room temperature, however, heating was required for the reactions with heptanoic, caproic, octanoic and decanoic anhydrides.

2.6 C₁₆-Dihydrosphingosine (2-Amino-1,3-hexadecanediol)

A mixture of sodium salt of *aci*-2-nitro-1,3-hexadecanediol⁹⁾ (45 mmol) and glacial acetic

acid (100 ml) was stirred at room temperature until the salt was dissolved completely. Then, the catalyst (5% Pd-C, 4.0 g) was added. The resulting mixture was exposed to hydrogenation at room temperature at 2.8 kg/cm² of hydrogen pressure. After 48 h (the final hydrogen pressure: 2.0 kg/cm²), the catalyst, which was washed with three 20 ml portions of glacial acetic acid, was removed by filtration. The combined filtrates were concentrated under reduced pressure (ca. 55 g). To the residue previously cooled with a ice-bath was successively added water (200 ml), potassium hydroxide (55 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 60~63%. Purity 98% (by GLC).

C₁₂- and C₁₄-dihydrospingosines (2-amino-1, 3-dodecanediol and 2-amino-1, 3-tetradecanediol) were prepared by the same method described above or in the previous paper¹⁾. Recrystallization was carried out using hexane. Yield 55~65%. Purity 97~99% (by GLC).

2.7 N-Acyl derivatives of DL-erythro-C₁₄-dihydrospingosine

A mixture of DL-erythro-C₁₄-dihydrospingosine (20 mg) and HMDS+TMCS/pyridine (2 ml) was heated at 60°C for 2 min, and cooled to room temperature. Acid anhydride (30 μ l) was added. The reaction mixture was shaken gently for 10 min, heated at 60°C for 20 min, and cooled to room temperature. Water (20 ml) or a mixture of water and methanol (1 : 1 vol/vol, 20 ml) was added. After standing at room

temperature for 3~5 h, the compounds were extracted with three 50 ml portions of chloroform. The combined extracts were washed successively with two 50 ml portions of water, three 50 ml portions of 1 N hydrochloric acid, two 50 ml portions of water, three 50 ml portions of 1 M sodium carbonate, and three 50 ml portions of water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was crystallized from hexane.

3 Results

3.1 N-Acetylation of O-TMS derivatives of sphingolipid bases

The reactions of TMS derivatives of sphingolipid bases with acetic anhydride has been

Column: 1%SE-30

Temperature A~F: 170°C, G~I: 220°C

Substrates A~F: DL-erythro-Cis-Dihydrospingosine (DHSPH), G~I: D-ribo-Cis-Phytosphingosine (Phyto)

Products a: O,O-di-TMS, b: N,O,O-tri-TMS, c: N-Acetyl O,O-di-TMS, d: O,O,O-tri-TMS

e: N,O,O,O-tetra-TMS, f: N-Acetyl O,O,O-tri-TMS

Reactions A: DHSPH + (HMDS+TMCS pyridine) at room temperature (R.T.) for 2-40 min.

B: A + acetic anhydride at R.T. for 2 min or heated at 60°C for 2 min; D(R.T.) + acetic

anhydride at R.T. for 2 min or heated at 60°C for 2 min.

C: DHSPH + BSA acetonitrile, heated at 60°C for 5 min.

D: DHSPH + (HMDS+TMCS pyridine) at R.T. for 5 min and then heated at 60°C for 20 min.

E: N-Acetyl DHSPH (authentic) + (HMDS+TMCS pyridine) at R.T. for 2 min or heated at 60°C for 2 min.

F: C(R.T.) + acetic anhydride at R.T. for 20 min or heated at 60°C for 5 and 20 min.

G: Phyto + (HMDS+TMCS pyridine) at R.T. for 10 min and then heated at 60°C for 10 min.

H: G(R.T.) + acetic anhydride at R.T. for 2 min or heated at 60°C for 2 min.

I: N-Acetyl Phyto (authentic) + (HMDS+TMCS pyridine) at R.T. for 2 min or heated at 60°C for 2 min.

Fig.-1 Gas-liquid chromatograms of the products formed by the reactions of sphingolipid bases with acetic anhydride in TMS reagent/solvent.

studied using DL-*erythro*-C₁₄-, D-*erythro*-C₁₈- and DL-*threo*-C₁₈-dihydrosphingosines, D-*erythro*-C₁₈-sphingosine and D-ribo-C₁₈-phyto-sphingosines as substrates. The representative gas-liquid chromatograms of the products are illustrated in **Fig.-1**.

The bases reacted with HMDS+TMCS/pyridine to give O-TMS derivatives, which reacted partially with another molecule of TMS to give N, O-TMS-derivatives when the mixtures were heated. The N, O-TMS derivatives were prepared by the reaction with BSA/acetonitrile. In the case of HMDS+TMCS/pyridine, the resulting TMS derivatives reacted easily with acetic anhydride at room temperature to give N-acetyl O-TMS compounds whose structures were determined by GLC-MS analyses. The results are shown in **Fig.-2**. However, N-acetylation was not observed for the reactions of N, O-TMS ethers of the bases prepared using BSA/acetonitrile with acetic anhydride.

In the presence of excess acetic anhydride (anhydride/substrate=20 wt/wt), the N-acetyl O-TMS derivatives were decomposed when the mixture were heated (60°C, >2 h) or stored at

Table-1 Preparation of N-acyl derivatives of DL-*erythro*-C₁₄-dihydrosphingosine.

Acid anhydrides	N-Acyl	Yield (%)	mp (°C)
Acetic	CH ₃	83~93	121~123
Butyric	C ₃ H ₇	75~87	112~114
Hexanoic	C ₅ H ₁₁	81~88	95~ 97
Octanoic	C ₇ H ₁₅	85~90	96~ 98
Decanoic	C ₉ H ₁₉	73~83	87~ 89

Table-2 The *erythro*/*threo* ratios of N-acyl O-TMS derivatives of synthetic C_n-dihydrosphingosine.

N-Acyl	<i>erythro</i> (%) ^{a)}		
	C ₁₂ -DHSph ^{b)}	C ₁₄ -DHSph ^{b)}	C ₁₆ -DHSph ^{b)}
CH ₃	69.1	69.9	70.4
C ₂ H ₅	69.1	70.0	70.2
C ₃ H ₇	68.6	69.4	70.7
C ₄ H ₉	68.6	70.3	70.1
C ₅ H ₁₁	69.2	69.8	70.3
C ₇ H ₁₅	68.8	69.6	69.8
C ₉ H ₁₉	69.5	69.6	69.6
TMS ^{c)}	68.8	70.1	70.3

a) *erythro*+*threo*=100%.

b) DHSph : Dihydrosphingosine.

c) detected as N, O, O-tri-TMS derivatives.

TMS reagent : BSA/acetonitrile.

R₁-CH-CH-CH₂OTMS

TMSO NHTMS

R₁=C₈H₁₉, C₁₁H₂₃, C₁₃H₂₇

room temperature for a long time (>1 d). The N-acetyl O-TMS derivatives prepared by the present method were stable at least for several hours at the elevated temperature (>80°C) and for several days in a refrigerator. Virtually, no configurational change (e.g., *erythro* → *threo*) was observed.

3.2 N-Acylation of O-TMS derivatives of sphingolipid bases

The above N-acetylation was applied to N-acetylation of TMS derivatives of sphingolipid bases: the reactions of the TMS compounds with acid anhydrides have been studied using DL-*erythro*-C₁₄-dihydrosphingosine as a substrate, and propionic, butyric, pentanoic, caproic, octanoic and decanoic anhydrides as N-acyl reagents. The TMS derivative of the base prepared by using HMDS+TMCS/pyridine reacted with acid anhydrides to give the corres-

Fig.-2 Mass spectra of N-acetyl O-TMS derivatives of sphingolipid bases prepared by the present method.

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スフィンゴシン塩基の *N*-アシル
O-TMS誘導体の簡易調製法

平田博文*¹・A. KISIC*²・J. BRABSON*².
G.J. SCHROEPFER, JR.*²

*¹ 化学技術研究所 (茨城県筑波郡谷田部町東 1-1)

*² ライス大学生化学部 (アメリカ 合衆国 テキサス
州ヒューストン市)

スフィンゴシン塩基の分析は一般に *N*-アセチル *O*-TMS 誘導体として GLC や GLC-MS によって行われるが、その化合物の簡便な調製法は今までに報告されていない。

本報ではスフィンゴシン塩基の *N*-アシル *O*-TMS 誘導体の簡易調製法について検討した。スフィンゴシン塩基と HMDS+TMCS/ピリジンとの反応から生ずる *O*-TMS 誘導体と酸無水物とを反応させると目的の *N*-アシル化が起こり、*N*-アシル *O*-TMS 化合物が得られた。BSA/アセトニトリルとの反応から生ずる *N*, *O*-TMS 体は、*N*-TMS 基がアミノ基の保護基として作用するため、酸無水物とは反応しなかった。上記の *N*-アシル *O*-TMS 化反応について数種のスフィンゴ塩基について検討したところ、すべての場合について起こることが認められた。生成物の構造は GLC, GLC-MS から決定した。合成した C_n -ジヒドロスフィンゴシン ($n=12, 14, 16$) を *N*-アシル *O*-TMS 体とし、その *erythro/threo* 比を求めたところ、従来法で求めた結果 (BSA を用い、*N*, *O*-TMS 体とし GLC で定量) と一致した。本報の特色は、スフィンゴシン塩基の *N*-アシル *O*-TMS 体が簡便に調製でき、かつ、短時間の反応で行うことができる点にある。しかも、GLC-MS の分析の際、スフィンゴシン塩基の *N*-アシル体を別途合成することなく、*O*-TMS 体と *N*-アシル *O*-TMS 体両方の測定ができる利点がある。