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SYNTHESIS OF (2S,3R,4E,8E)-N-(2'R)-2'-HYDROXYHEXADECANOYL-9-METHYL-4,8-SPHINGADIENINE, THE CERAMIDE PORTION OF THE FRUITING-INDUCING CEREBROSIDE IN A BASIDIOMYCETE SCHIZOPHYLLUM COMMUNE

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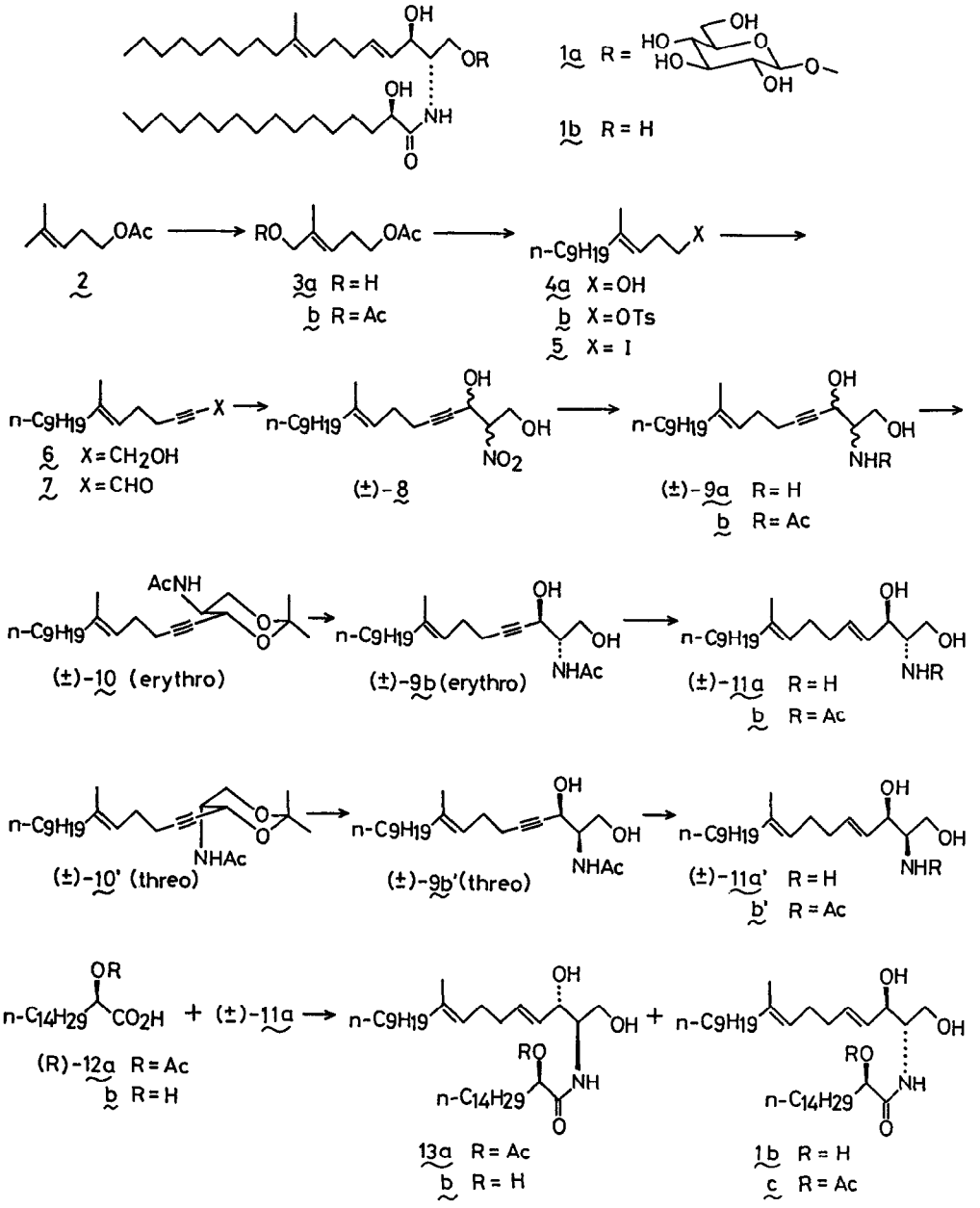
Summary : A total synthesis of the natural enantiomer of the title compound was accomplished, which confirmed the structure proposed for the fruiting-inducing cerebroside of Schizophyllum commune.

Fruiting body formation in Basidiomycetes is indeed a spectacular phenomenon especially to those who love to taste mushrooms. Its mechanism, however, is still a mystery in spite of the tremendous efforts to clarify it. Very recently Kawai and Ikeda found that the fruiting body formation of Schizophyllum commune (Japanese name : Suéhiro také) can be stimulated by some cerebrosides in its mycelia.[2)] They then identified one of the active substances as (2S,3R,4E,8E)-N-(2'R)-2'-hydroxyhexadecanoyl-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine 1a,[3)] which had previously been isolated from a sea anemone (Metridium senile) by Karlsson et al.[4)] Such a minute amount of 1a as 0.1 μg induced the fruiting body formation of S.commune, and the corresponding ceramide 1b lacking the sugar portion was also active.[3)]

This remarkable bioactivity of 1a prompted us to synthesize it so as to confirm the proposed structure.[5)] In this communication will be described a synthesis of the ceramide 1b with correct stereochemistry. Our synthetic 1b was highly active in inducing the fruiting body formation of S.commune.

Construction of the sphingadienine portion of 1b started from the known homoprenyl acetate 2.[6)] Oxidation of 2 with SeO_2 was followed by $NaBH_4$ reduction to give 3a, whose acetylation (Ac_2O/C_5H_5N) yielded 3b (38.0 % from 2). This was treated with n-$C_8H_{17}MgBr$/THF in the presence of Li_2CuCl_4 to give 4a (94.2 %). The corresponding tosylate 4b was submitted to the Finkelstein reaction (NaI/acetone) to afford 5 (96.2 %). Alkylation of $HC{\equiv}CCH_2OTHP$ with 5 was effected with n-BuLi in THF/HMPA (-15°, 1.5 hr; 0°, 1.5 hr), yielding 6 (44.3 %) after the removal of the THP group with p-TsOH/MeOH (room temp, 18 hr). The alcohol 6 gave an aldehyde 7 (92.4 %) upon treatment with MnO_2 in pet ether.

Condensation of 7 with 2-nitroethanol in the presence of K_2CO_3 in MeOH[7)]



afforded a nitro diol 8 as a diastereomeric mixture. Because of the instability of 8, its chromatographic separation into the erythro- and threo-isomers was unsuccessful. Conversion of 8 into the corresponding acetonide[8] was not successful either due to the retro-aldol reaction. The diastereomeric nitro diol 8 was therefore directly reduced with Zn/EtOH-conc HCl. The resulting 9a was acetylated (Ac_2O/MeOH) to give 9b, whose EtOAc soln deposited a crystalline mass, mp 95.5~96.5° (24.6% from 7). This was later shown to be (±)-threo-9b' (vide infra). The mother liquor was treated with $Me_2C(OMe)_2$ and

PPTS in acetone to give a mixture of (±)-10 and 10'. This was separated by medium pressure LC (SiO_2) to give (±)-erythro-10 (21.5% from 7) and (±)-threo-10' (4.7% yield from 7). The assignment of the relative configuration between C-2 and C-3 of 10 was made possible by ^{1}H-NMR spectroscopy according to our previous observation.[8,9] The one with an equatorial -NHAc [δ ($CDCl_3$) 4.58(1H, d,J=7 Hz, -CHC≡CCH$_2$-)] was the erythro-isomer (±)-10, while the other with an axial-NHAc[δ($CDCl_3$) 4.80 (1H,d,J=2 Hz, -CHC≡CCH$_2$-)] was the threo-isomer (±)-10'. The aforementioned crystalline (±)-9b' yielded (±)-10' upon acetonide formation, manifesting its threo-stereochemistry. Removal of the acetonide protective group of (±)-10 was effected by treatment with p-TsOH/i-PrOH (40°, 40 min) to give (±)-erythro-9b. Prior to the reduction of the triple bond, (±)-erythro-9b was treated with KOH/aqMeOH (reflux, 6 hr) to remove the N-acetyl group. The resulting (±)-erythro-9a was reduced with LAH/THF to give (±)-erythro-11a (42.6% from 9b). Similarly (±)-threo-9b' yielded (±)-threo-11a' (52.7% from 9b'). These two were separately N-acetylated (Ac_2O/MeOH) to give (±)-(4E,8E)-2,3-erythro-2-acetamino-9-methyl-4,8-octadecadiene-1,2-diol 11b and its threo- isomer 11b'. The ^{1}H- and ^{13}C-NMR spectra of these two products were compared with those of the N-acetylnonadecasphingadienine derived from the natural cerebroside 1a of S.commune. A part of ^{13}C-NMR data as shown in Table 1 indicated that the sample derived from the natural product possesses erythro- stereochemistry. The 400 MHz ^{1}H-NMR spectrum of (±)-erythro-11b was identical with that of the sample of the natural origin.[10]

The remaining task was to prepare (R)-2-acetoxyhexadecanoic acid 12a and to N-acylate (±)-11a with (R)-12a. By employing (R)-12a as the acylating agent, we expected the separation of the resulting diastereomeric mixture in a later stage. The desired acid (R)-12a was prepared by the acetylation (Ac_2O/C_2H_5N) of the corresponding hydroxy acid (R)-12b, mp 92∿93°; $[\alpha]_D^{20}$ -2.9° (c= 1.03, $CHCl_3$)[lit.[12] mp 93.3∿93.5°; $[\alpha]_D$ -3.2° ($CHCl_3$)].[11∿14] This, in turn, was obtained by deaminating (R)-2-aminohexadecanoic acid, which was prepared by the enzymatic resolution (amino acylase)[15] of the known racemate.[16] Acylation of (±)-11a with (R)-12a in the presence of EtN=C=N(CH$_2$)$_3$NMe$_2$·HCl[14] gave a diastereomeric mixture of 13a and 1c (45.6% from 11a). This was dissolved in $CHCl_3$ and treated with NaOH/MeOH (room temp, 1 hr) to give a mixture

Table 1. ^{13}C-NMR data of the natural and synthetic 2-acetamino-9-methyl-4,8-octadecadiene-1,2-diol

Carbon (No.)	Natural ($CDCl_3$, 100 MHz)	Synthetic ($CDCl_3$, 22.6 MHz) (±)-erythro-11b	(±)-threo-11b'
-CH$_2$O-(C-1)	62.19	62.1	63.7
>CHN< (C-2)	54.36	54.5	54.9
>CHO- (C-3)	74.48	74.2	72.5

of 13b and 1b. Chromatographic separation (Merck Lobar column, Lichroprep® Si 60 (40∿63 μm); Elution with $CHCl_3$-MeOH=150:1) of the mixture gave 13b (22.3 % from 11a), $[\alpha]_D^{21}$+10.6° (c=0.54, $CHCl_3$), and 1b, mp 62∿64°; $[\alpha]_D^{21}$+6.4° (c=0.76, $CHCl_3$). An authentic sample of 1b, prepared from 1a by the method of Hammarström[17] showed mp 59∿61°; $[\alpha]_D^{21}$+7.3±0.4° (c=0.25, $CHCl_3$). The identity of the natural and synthetic 1b was confirmed by the comparison of IR, ^{1}H-NMR (400 MHz), ^{13}C-NMR (25 MHz) and HPTLC using three different solvent systems.[18] The final proof of the identity of our synthetic 1b with the ceramide 1b of natural origin was its very strong fruiting-inducing activity on S.commune. Indeed the specific activity of our 1b (15,000 units/mg) was higher than that (10,000 units/mg) of the natural cerebroside 1a itself. The diastereomer 13b was less active (2,000 units/mg).[19]

In conclusion, the structure 1a proposed for the fruiting-inducing cerebroside of Schizophyllum commune was confirmed by synthesizing 1b.

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10) ^{1}H-NMR data (400 MHz, $CDCl_3$) of (±)-erythro-11b:δ 0.88 (3H,t,J=7.0Hz),1.24 (12H,br.s), 1.36 (2H,m), 1.58 (3H,s), 1.94 (2H,t,J=8.0 Hz), 2.03 (3H,s), 2.08 (4H,br.s), 3.50 (1H,br.s), 3.58 (1H,br.s), 3.68 (1H,deformed d, J=11.0 Hz), 3.88 (1H,dt,J_1=7.6 Hz, J_2=3.8 Hz), 3.92 (1H,dd,J_1=11.0 Hz, J_2=3.8 Hz), 4.30(1H,br.s), 5.09 (1H,t,J=6.0 Hz), 5.53 (1H,dd,J_1=15.6 Hz, J_2=6.4 Hz), 5.79 (1H,ddd,J_1=15.6 Hz, J_2=6.4 Hz, J_3=5.5 Hz), 6.55 (1H,d,J=7.0 Hz).
11) (R)-2-Hydroxyhexadecanoic acid 12b was known as a constituent of wool wax.[12,13] Its (S)-enantiomer was synthesized by Horn et al.,[13] while Hammarström reported the synthesis of (R)-12b (without experimental details).[14]
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18) Physical data of synthetic 1b: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (6H,t,J=7.0 Hz), 1.20∿1.45 (40H,m),1.58 (3H,s), 1.95 (2H,t,J=7.5 Hz),2.08 (4H,br,s), 3.20 (1H,br.s), 3.55 (1H,br.s), 3.70 (1H,br,s),3.74 (1H,deformed d, J=11.0 Hz), 3.87 (1H,dd,J_1=11.0 Hz, J_2=4.0 Hz), 3.91 (1H,dt,J_1=8.0 Hz, J_2=4.0 Hz), 4.11 (1H,dd,J_1=7.5 Hz, J_2=3.5 Hz), 4.27 (1H,br,s), 5.09 (1H,t,J=6.0 Hz), 5.52 (1H,dd,J_1=15.5 Hz, J_2=6.5 Hz), 5.79 (1H,dt,J_1=15.5 Hz, J_2=6.0 Hz), 7.21 (1H,d,J=8.0 Hz);^{13}C-NMR(25 MHz, $CDCl_3$) δ 14.1, 16.0, 22.7, 25.2, 27.6, 28.1, 29.4, 29.6, 29.7, 31.9, 32.6, 34.7, 39.7, 54.5, 61.9, 72.5, 74.1, 123.1, 128.6, 134.0, 136.2, 175.5; HPTLC (Merck Kieselgel 60F_{254}) R_f 0.53 ($CHCl_3$-MeOH=9:1), R_f 0.60 (n-hexane-acetone=1:1), R_f0.16 (C_6H_6-EtOAc=1:4).
19) We thank Mr.G.Kawai of Noda Institute for Scientific Research, Noda,Chiba, for his kind gift of 1a and for the bioassay of our synthetic materials.

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