July 1994 Communications to the Editor *Chem. Pharm. Bull.* **42**(7) 1531—1533 (1994)

THREE NOVEL POLYACETYLENE TRIGLYCERIDES, LYCOGARIDES A-C, FROM THE MYXOMYCETES *LYCOGALA EPIDENDRUM*

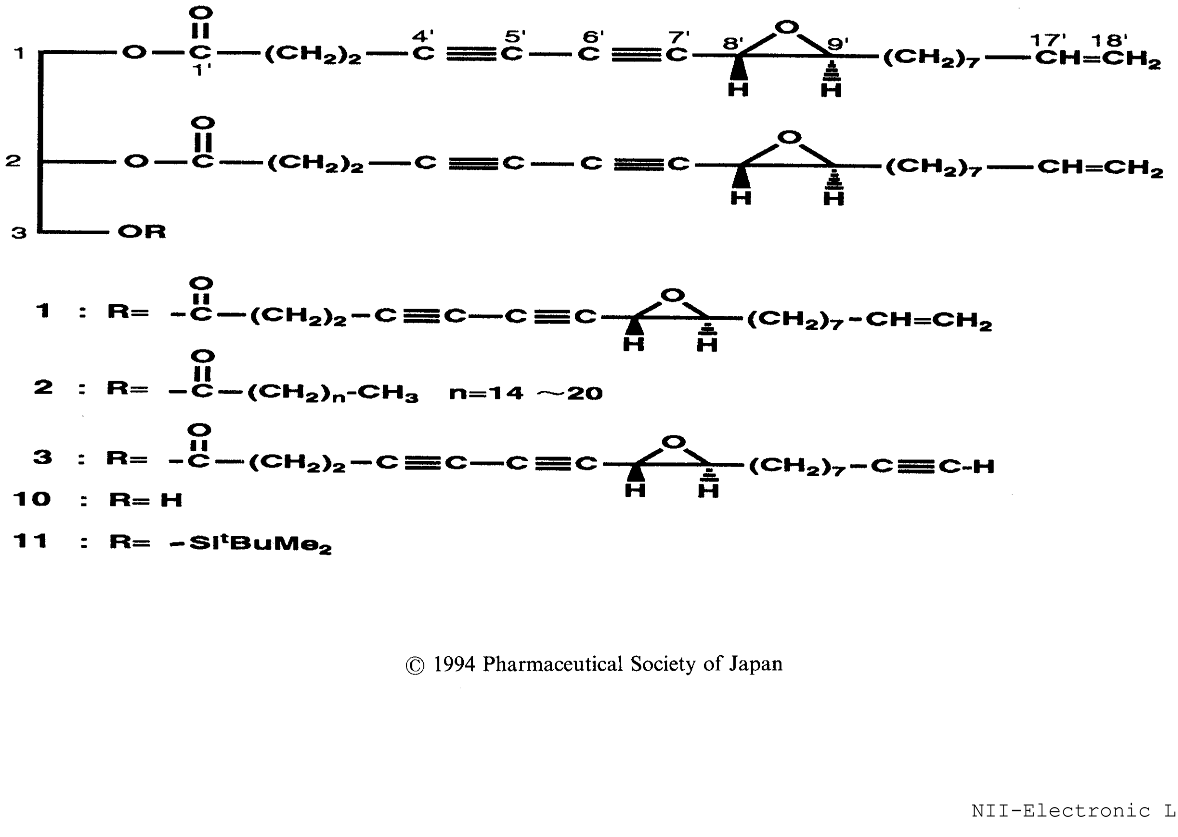
Toshihiro HASHIMOTO,[a] Kyoko AKAZAWA,[a] Motoo TORI,[a] Yukiko KAN,[a]
Takenori KUSUMI,[b] Haruko TAKAHASHI,[b] and Yoshinori ASAKAWA*,[a]
Faculty of Pharmaceutical Sciences, Tokushima Bunri University,[a] Yamashiro-cho, Tokushima 770, Japan
Faculty of Pharmaceutical Sciences, Tokushima University,[b] Sho-machi, Tokushima 770, Japan

Three novel polyacetylene triglycerides, named lycogarides A-C (**1**-**3**), have been isolated from the Myxomycetes *Lycogala epidendrum.* Their relative structures have been established by a combination of two-dimension NMR spectroscopy and chemical degradation. The absolute structures of compounds **1** and **2** were deduced from the modified Mosher's and dibenzoate exiton chirality methods of their derivatives.

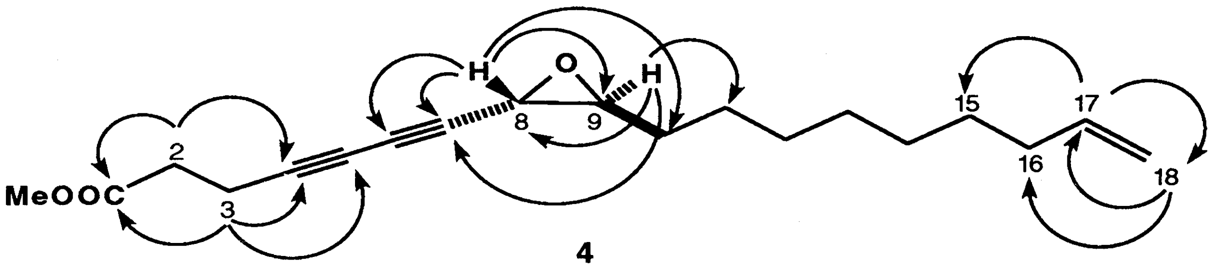
KEYWORDS Myxomycetes; *Lycogala epidendrum*; polyacetylene triglyceride; lycogarides A-C; two dimension NMR spectroscopy; modified Mosher's method

In our previous paper, we reported the isolation and structure elucidation of three novel dimethyl pyrroledicarboxylates, named lycogarubins A-C, having anti-HSV-I virus activity from fruit bodies of slime moulds (Myxomycetes), *Lycogala epidendrum.*1) In this paper, we report the structures of three novel polyacetylene triglycerides, lycogarides A-C (**1**-**3**), from *L. epidendrum.*

The AcOEt extract (57.9 g) of fresh fruit bodies (1.65 kg) of *L. epidendrum* collected in Tokushima in 1990 was subjected repeatedly to column chromatography on Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) and on silica gel ($CHCl_3$-AcOEt gradient) to afford lycogarides A (**1**, 13.6 g),2) B (**2**, 2.8 g),3) and C (**3**, 1.4 g).4)

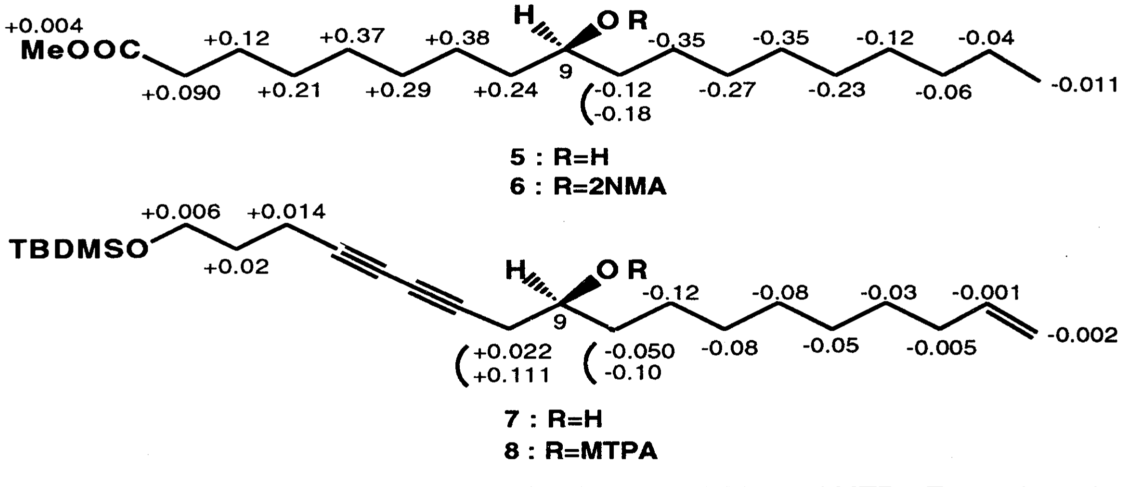


The IR and UV spectra of lycogaride A (**1**, $C_{57}H_{74}O_9$) indicated the presence of a conjugated diacetylenic (2250 cm^{-1}; λ_{max} 233, 245, 259 nm) and an ester (1740, 1240 cm^{-1}) group. The ^{1}H NMR spectra of **1** showed the presence of a C-1 and C-3 symmetrical glyceride [δ_H 4.19 (dd, J=5.9, 12.0 Hz), 4.36 (dd, J=4.2, 12.0 Hz) , 5.30 (dd, J= 4.2, 5.9 Hz)], a vinyl group [δ_H 4.93 (dd, J=1.5, 11.2 Hz), 4.98 (dd, J=1.5, 17.1 Hz) and 5.81 (m)] and two methine groups bearing an epoxide [δ_H 3.08 (dt, J=2.0, 5.6 Hz), 3.12 (d, J=2.0 Hz)]. Methanolysis (0.5N-MeONa, MeOH-benzene, 50°C, 20 min.) of **1** afforded the sole methyl ester **4** [$C_{19}H_{26}O_3$ (HRMS: m/z 302.1863) ; δ_H 3.63 (s)]. The planar structure and the relative configuration of **1** were derived from a careful analysis of the 2D NMR spectra including the HMBC (Fig. 1) and NOESY spectra of **1** and **4**. Hydrogenation (H_2/10 % Pd-C) of **4** afforded (+)-methyl 9(*S*)-hydroxyoctadecanoate (**5**) [mp 52.5-53.5°C; $[\alpha]_D^{23}$ +0.16° (c 7.7, MeOH)] [lit.[5]; mp 53.0-53.5°C; $[\alpha]_D^{20}$ +0.17° (c 3.3, MeOH)]. The modified Mosher's method[6] applied to a 2NMA (2-naphthylmethoxyacetic acid) ester **6** of **5**, and an MTPA ester **8** of **7** derived from **1** in two steps (1. $LiAlH_4$ / Et_2O; 2. (*t*-Bu)Me_2SiCl /DMAP / NEt_3) led to the conclusion of the (*S*)-configuration of 9-hydroxy groups of **5** and **7** as shown in Fig. 2. Thus, the structure of lycogaride A including the absolute configuration was established as shown in formula **1.**



4

Fig. 1. The Long-Range Correlation Detected by HMBC Spectrum of **4** in $CDCl_3$



5 : R=H
6 : R=2NMA

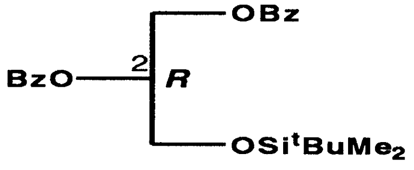
7 : R=H
8 : R=MTPA

Fig. 2. The Δδ Values [δ(-)-δ(+)] Obtained for the 2NMA and MTPA Esters (**6** and **8**) in 600MHz ^{1}H NMR Spectra in $CDCl_3$

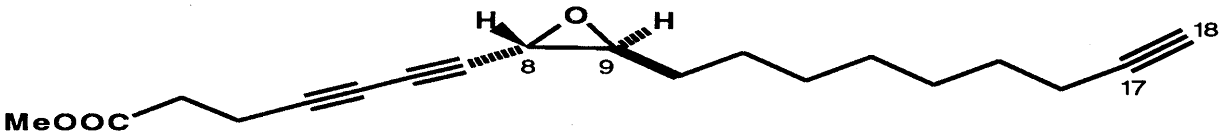
The spectral data (^{1}H and ^{13}C NMR, IR and UV) of lycogaride B (**2**, $C_{57}H_{72}O_9$) were similar to those of lycogaride A (**1**). Methanoylsis of **1** afforded 2 eq of **4** and 1 eq of a mixture **9** of saturated fatty acid methyl esters, which consisted of n=14 (14%), n=15 (4%), n=16 (39%), n=17 (3%), n=18 (23%), n=19 (9%), and n=20 (8%) judging from GC-MS analysis. Selective enzymatic hydrolysis [lipase (porcine pancreas), 1M Tris-HCl buffer (pH 8.0), 40°C] of **2** gave an alcohol **10**, which was treated with *t*-butyldimethylsilyl chloride and triethylamine in CH_2Cl_2 to afford **11**. Compound **11** was treated with sodium methoxide in methanol-benzene, followed by benzoylation with benzoic anhydride in pyridine to afford a dibenzoate **12**. The absolute configuration at C-2 of glycerol of lycogaride B (**2**) was determined as (*S*)-configuration by the dibenzoate exiton chirality method of (*R*)-**12** [CD (MeOH) λ_{max}nm ($\Delta\varepsilon$): 236 (+2.60), 220 (−1.58)][lit7); CD (MeOH) λ_{max}nm ($\Delta\varepsilon$): 236 (+3.09), 220 (−2.00)].

The spectral data (^1H and ^{13}C NMR, IR and UV) of lycogaride C (**3**) resembled those of **1** except for the presence of an ethynyl [(3300 cm^{-1}, δ_H1.94(t, J=2.7Hz)] group. Methanolysis of **3** afforded 2 eq of **4** and 1 eq of **13**. Since compound **3** was not symmetric, the planar structure and relative configuration were determined as depicted in formula **3**. As selective enzymatic hydrolysis of **3** with lipase did not proceed, the determination of absolute configuration of **3** is in progress.

$CH_3-(CH_2)_n-COOMe$
n=14~20
9



12



13

Lycogarides A-C (**1**-**3**) completely inhibited germination of the root and second coleoptile of rice in husk at 100 ppm. The other bioassay is in progress.

REFERENCES AND NOTES

1) T. Hashimoto, A. Yasuda, K. Akazawa, S. Takaoka, M. Tori, Y. Asakawa, *Tetrahedron Lett.*, **35**, 2559 (1994).

2) mp 51.5-54.0°C; $[\alpha]_D^{23}$-15.7°(c 1.5, $CHCl_3$); FAB-MS(+): 903 (M^++1); IR (KBr) ν cm^{-1}: 2250 (C≡C), 1740 (CO), 1640 ($CH=CH_2$); UV (dioxane) λ_{max}nm (logε): 233 (3.64), 245 (3.63), 259 (3.43); *Anal.* Calcd for $C_{57}H_{74}O_9$: C, 75.79; H, 8.25. Found: C, 75.52; H, 8.21.

3) mp 48.0-51.0°C; $[\alpha]_D^{23}$-4.0°(c 0.5, $CHCl_3$); IR (KBr) ν cm^{-1}: 2250 (C≡C), 1740 (CO), 1640 ($-CH=CH_2$); UV (dioxane) λ_{max}nm (logε): 234 (2.65), 246 (2.70), 259 (2.52).

4) mp 53.5-55.5°C; $[\alpha]_D^{23}$-12.5°(c 0.9, $CHCl_3$); FAB-MS(+): 901 (M^++1); IR (KBr) ν cm^{-1}: 3300 (C≡CH), 2220 (C≡C), 1740 (CO), UV (dioxane) λ_{max}nm (logε): 253 (3.69), 268 (4.05), 284 (3.97): *Anal.* Calcd for $C_{57}H_{72}O_9$: C, 75.96; H, 8.05. Found: C, 75.66; H, 8.12.

5) G. J. Schroepfer, Jr., K. Bloch, *J. Biol. Chem.*, **240**, 54 (1965).

6) T. Kusumi, H. Takahashi, Xu Ping, T. Fukushima, Y. Asakawa, T. Hashimoto, Y. Kan, *Tetrahedron Lett.*, in press (1994).

7) H. Uzawa, Y. Nishida, H. Ohrui, H. Meguro, *Biochem. Biophys. Res. Commun.*, **168**, 506 (1990).

(Received May 9, 1994; accepted June 15, 1994)