Synthesis of 1-Monoglycerides Having $C_{11\sim20}$ Branched Chain Fatty Acids and Their Hemolysis Effects

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Streptomyces sp. OCU-42815 produced several novel 1-monglycerides having $C_{17\sim20}$ branched chain fatty acids, which were first reported their antimicrobial activities against both bacteria and fungi[1,2]. However, similar monoglycerides having straight chain fatty acids did not show any antimicrobial activities at the concentrations up to 100 μg/ml. 1-Monoglycerides having $C_{14\sim16}$ branched chain fatty acids have other biological effects, such as platelet aggregation inhibition effect reported by ŌMURA *et al.*[3] On such research background, we have prepared a homologous series of 1-monoglycerides derived from $C_{11\sim20}$ *iso*- or *anteiso*-branched chain fatty acids to examine their biological effects.

The synthetic scheme adopted here is shown in Fig. 1. Racemic 1,2-isopropylidene-glycerol was esterified with the appropriate *iso*- or *anteiso*-branched chain fatty acids in carbon tetrachloride in the presence of 4-(dimethylamino)pyridine (DMAP) and *N*,*N*′-dicyclohexyl-carbodiimide (DCC) to afford 1,2-isopropylidene-3-acyl-glycerols (**I**)[4]. During hydrolysis of the protecting ketal group, acyl migration may occur to yield 2-acyl-glycerols especially at higher reaction temperature. The following deprotection therefore was performed by the procedure of KODALI[5], in which **I** on silica gel were exposed to hydrogen chloride (HCl) gas at −75°C for about 10 minutes. The reaction mixture was extracted three times with 50-ml portions of 30% (v/v) methanol in chloroform at 0°C. The combined organic extracts were washed with 50-ml of water before concentration

Fig. 1. Preparation of 1-monoglycerides with branched-chain fatty acids.

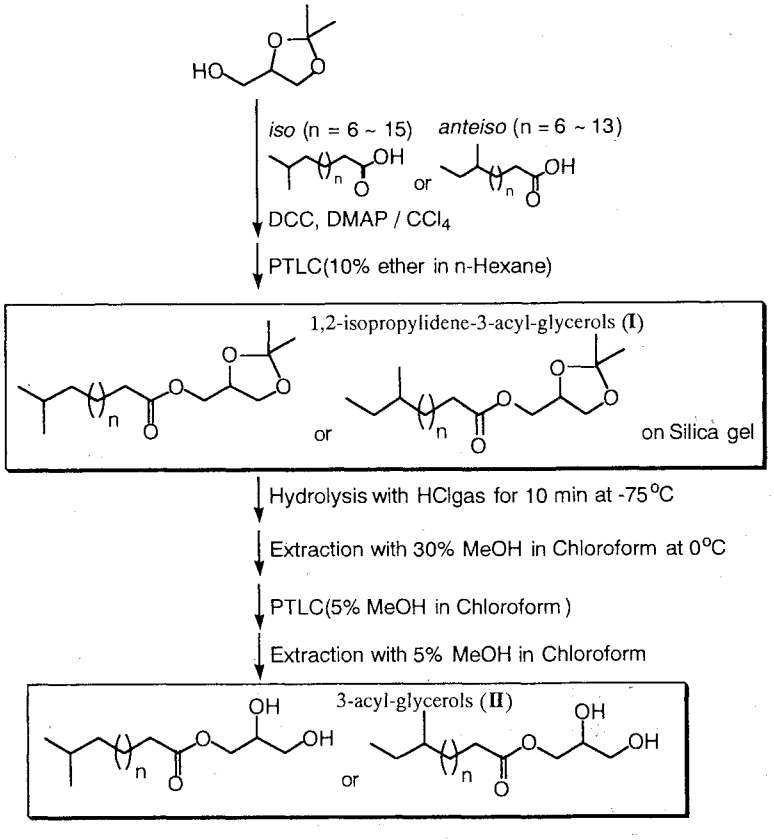

Table 1. Hemolysis effects of *iso*- and *anteiso*-branched chain $C_{11\sim20}$ fatty acids and their corresponding 1-monoglycerides.

Carbon number	O.D. at 410 nm[a]			
	Free fatty acids (100μg/ml)[b]		Corresponding 1-monoglycerides (100μg/ml)[b]	
	iso-	*anteiso*-	*iso*-	*anteiso*-
11	18.16	23.96	2.01	1.12
12	10.66	21.96	1.12	0.71
13	5.87	8.52	0.65	0.42
14	1.78	6.18	0.31	0.22
15	0.42	3.56	0.13	0.16
16	0.61	0.69	0.12	0.06
17	0.41	0.22	0.10	0.09
18	0.76	0.32	0.05	0.09
19	0.82	— [c]	0.05	—
20	0.36	—	0.09	—

[a] O.D. at 410 nm expressed here was the O.D. value subtracted from that of control (0.08).

[b] All samples were dissolved in methanol. The final concentration of methanol used in the experiment was 0.5%.

[c] Not tested.

in vacuo. The residue was purified by preparative thin-layer chromatography (PTLC) eluted with 5% (v/v) methanol in chloroform. Characterization of the synthetic 1-monoglycerides was performed by ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS). ^{1}H NMR spectrum of these monoglycerides were obtained on a JEOL JNM-LA 400, and summarized briefly as following: common signals for all compounds were observed at δ 2.35 (2H, t, J=7.6 Hz, $-CH_2C=O$), 3.59 (1H, dd, J=11.3, 5.8 Hz, $-CH_2OH$), 3.67 (1H, dd, J=11.3, 4.0 Hz, $-CH_2OH$), 3.91 (1H, tt, J=4.0, 5.6 Hz, $-CHOH$), 4.14 (1H, dd, J=11.6, 5.8 Hz, $-CH_2OCOR$), and 4.18 (1H, dd, J=11.6, 4.6 Hz, $-CH_2OCOR$). The signal at δ 0.85 (6H, d, J=6.7 Hz) was assigned to two methyl groups of *iso*-fatty acid, and the signals at δ 0.84 (3H, d, J=6.7 Hz) and δ 0.85 (3H, t, J=7.3 Hz) were assigned to two methyl groups of *anteiso*-fatty acid. ESI-MS data were obtained on a JEOL JMS-700T, and the mobile phase composition was methanol. The high resolution ESI mass spectra revealed sodiated molecular $[M+Na]^+$ ions, which were consistent with the structure of these monoglycerides (Data not shown).

As reported previously[2], AKD-2C, a model of these novel 1-monoglycerides having $C_{17\sim20}$ branched chain fatty acids isolated from *Streptomyces* sp. OCU-42815 exerts its antifungal actions through its effect on the fungal cell membrane. This time, we used animal cells instead of fungal cells as the target. Their hemolysis effects were evaluated as the biological effects of these synthetic 1-monoglycerides. Briefly, the erythrocytes were prepared from heparin-treated bovine blood by centrifuging the suspension for 15 minutes at 5,000 rpm in a refrigerated centrifuge. After incubation with each 1-monoglycerides (100 μg/ml) for 15 minutes at room temperature, the erythrocytes were centrifuged and hemolysis was monitored in a spectrophotometer by the absorbance of the supernatant at 410 nm indicating the hemoglobin released from erythrocytes described by TSONG.[6] As shown in Table 1, free fatty acids showed 10~20 times greater effects than their corresponding monoglycerides. Monoglycerides having $C_{11\sim14}$ both *iso*- and *anteiso*-fatty acids demonstrated obvious hemolysis effect. This effect decreased with the increasing of the carbon number of fatty acids. Interestingly, 1-monoglycerides having long carbon chain, $C_{17\sim20}$ reported with antimicrobial activity

previously[1,2], demonstrated weak hemolysis effects (O.D. around 0.1). It may be due to the difference of the membrane constitutes between animal cells and fungal cells. Further studies are now in progress.

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