Short Communication

Biological Activities of Fundamental, Carbohydrate Skeleton of Lipid A Containing Amide-linked 3-Hydroxytetradecanoic Acid

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The cell-wall lipopolysaccharide (LPS) of Gram-negative bacteria contains a unique, hydrophobic component called lipid A. Chemical investigations[1,2] have shown that the general, fundamental structure of lipid A from *Salmonella* and *Escherichia coli* is a β-(1,6)-linked disaccharide of 2-deoxy-2-(D-3-hydroxytetradecanoylamino)-D-glucopyranose esterified with fatty acyl and phosphoryl groups. The amide-linked D-3-hydroxytetradecanoic acid[3] is a characteristic marker of lipid A. In addition to the peculiarity of structure, it has been suggested that the lipid A component plays an important role in a variety of biological actions (endotoxicity, immunoadjuvancy and antitumor activity *etc.*) of LPS.[4] In view of this fact, the synthetic work[5～7] of lipid A and related compounds should be important in elucidating the relationship between chemical structure and activity, as well as in possible providing a new source of biologically-active carbohydrates. In this communication, we wish to report some interesting results regarding the chemical structure and biological activity of lipid A.

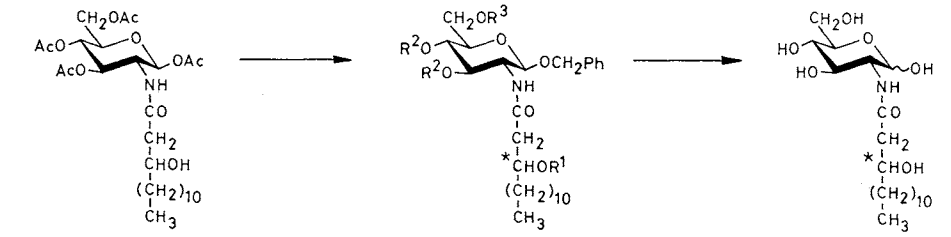
2-Deoxy-2-(D- and L-3-hydroxytetradecanoylamino)-D-glucose [**6**(D) and **6**(L)],*1 *i.e.* diastereoisomers of the fundamental, monosaccharide skeleton of lipid A have been synthesized, stepwise, from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(DL-3-hydroxytetradecanoylamino)-β-D-glucopyranose (**1**)*2 as shown in Fig. 1. We found that the one-step glycosylation[9] of benzyl alcohol (5 mol equiv.) with compound **1** (1 mol equiv.) in the presence of anhydrous ferric chloride (1.5 mol equiv.) in dichloromethane gave the corresponding β-glycoside **2** in a quantitative yeild. When the treatment of **1** with ferric chloride was conducted without benzyl alcohol, a new oxazoline **7** was obtained in an excellent yield [a syrup, NMR $\delta^{CDCl_3}_{Me_4Si}$ at 90 MHz: 0.88 (3H, t, CH_3), 1.1～1.8 (20H, m, CH_2), 2.08, 2.11 (9H, 2s, OAc), 2.35～2.55 (2H, m, α-CH_2 of the *N*-acyl moiety), 4.90 (1H, dq, $J_{4,5}=9.0$, $J_{2,4}=1.5$ Hz, H-4), 5.25 (1H, ～t, $J_{2,3}\simeq J_{3,4}=2.0$～2.2 Hz, H-3), 5.96 (1H, d, $J_{1,2}=7.4$ Hz, H-1)]. Conversion of compound **2** into **6**(D) or **6**(L) was achieved by the procedure reported previously.[6b]

The disaccharide derivative **8** and 2-deoxy-2-(hexadecanoylamino)-D-glucose (GlcNPam)[5] were readily prepared by deprotection of benzyl 6-*O*-[2-(DL-3-acetoxytetradecanoylamino)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-2-(DL-3-acetoxytetradecanoylamino)-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside[6c] and benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(hexadecanoylamino)-D-glucose,[6a] respectively.

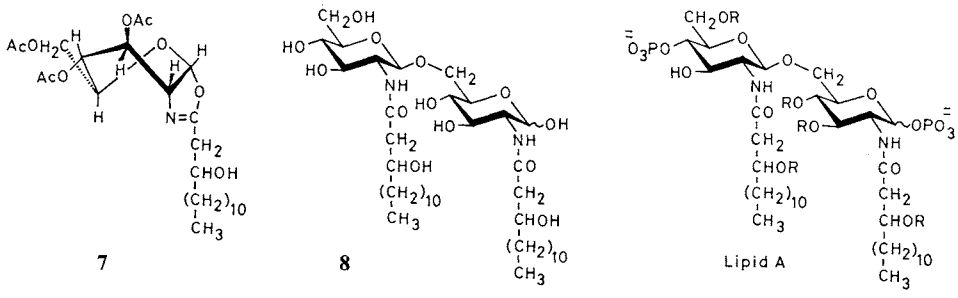
Endotoxic activity of the synthetic, lipid A analogs [**6**(D), **6**(L), **8** and GlcNPam] was examined by limulus test. As shown in Table I, compounds **6**(D) and **8** had potent gelation activity comparable to that of LPS in spite of lacking both *O*-esterified fatty acyl and phosphoryl groups. On the other hand, GlcNPam was 10^{-3}～10^{-5} times less active in this assay than the others. Interestingly, the gelation sensitivity appears to correlate with the suppression activity of tumor growth in mice

*1 (D) or (L) indicates the configuration around the asymmetric carbon (C_3) of th 3-hydroxytetradecanoyl group.

*2 Compound **1** was easily prepared by treatment of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose[8] with *N*-(DL-3-hydroxytetradecanoyloxy)-succinimide[6b] in anhydrous tetrahydrofuran (THF).



1 **2** $R^1=H$, $R^{2,3}=Ac$ **6**(D)
3 $R^1=MEM$, $R^{2,3}=Ac$ **6**(L)
4 $R^1=MEM$, $R^{2,3}=H$
5(D), **5**(L) $R^1=MEM$, $R^2=H$, $R^3=Tr$
[MEM = $MeO(CH_2)_2OCH_2$, Tr = Ph_3C]



7 **8**

(R = fatty acyl residue or H)

TABLE I. GELATION SENSITIVITY BY LIMULUS TEST

Compound No.	Concentration (μg/ml)			
	10^{-1}	10^{-3}	10^{-4}	10^{-5}
6(D)	+	+	+	+
6(L)	+	+	±	−
8	+	+	+	+
GlcNPam[a]	±	−	−	−
LPS (*E. coli*, 055 : B5)	+	+	+	+
H_2O (Endotoxin-free)	−			

[a] GlcNPam, 2-deoxy-2-(hexadecanoylamino)-D-glucose.

TABLE II. SUPPRESSION OF TUMOR (METH-A FIBROSARCOMA) GROWTH IN BALB/*c* MICE WITH SYNTHETIC GLYCOLIPIDS[a]

Compound No.	Dose (μg)	No. of tumor-free mice/No. of mice survived[b]
Exp. 1		
6(D)	100	6/10
6(L)	100	1/10
GlcNPam	100	4/10
Control[c]	—	0/10
Exp. 2		
8	100	8/10
Control[c]	—	2/10

[a] A mixture of tumor cells (2×10^5 in Exp. 1, and 1×10^5 in Exp. 2) and glycolipids suspended in phosphate buffer solution was inoculated intradermally in ten BALB/*c* female mice, and tumor growth at the inoculated site was measured.
[b] At 4 weeks after inoculation.
[c] Only tumor cells were inoculated.

(Table II). Among synthetic glycolipids, **6**(D) and **8** showed significant activities and in the mice treated with the latter, the growth of reinoculated (rechallenged) tumor cells was suppressed completely. These experimental results strongly suggest that the amide-linked 3-hydroxytetradecanoic acid seems to be critically important for the manifestation of the activity of lipid A. Especially, it is noteworthy that the D-3-hydroxytetradecanoic acid derivative showed higher activities in both biological tests than the corresponding L-derivative. Further, synthetic and biological investigations of lipid A and related compounds[10,11] are now progressing.

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