Note

Synthesis and Immunoadjuvant Activity of *N*-[2-*O*-(1,5-Anhydro-2,3-dideoxy-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine and Its Derivatives[†]

Akira HASEGAWA, Eiji SEKI, Koji KIGAWA,
Makoto KISO and Ichiro AZUMA*

Department of Agricultural Chemistry,
Gifu University,
Gifu 501-11, Japan

*Institute of Immunological Science,
Hokkaido University,
Sapporo 060, Japan

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In the continuing efforts to elucidate the relationships between the biological activities of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP) and the structure of the carbohydrate moiety, and to obtain glycopeptide adjuvants that exhibit strong activity and low toxicity, it has been demonstrated that replacement¹⁻⁴⁾ of the hydroxyl group at C-1 of the sugar skeleton by a thiol or an acylthio group, and a hydrogen atom caused strong immunoadjuvant activities, comparable to that of MDP, but that these compounds showed very weak pyrogenic activity in rabbits. In addition, it was observed that the introduction⁵⁻⁸⁾ of a lipophilic character at C-2 or C-6 in MDP caused potent antitumor or anti-infection activity, as well as strong immunoadjuvant activities. In view of these facts, we now describe the synthesis of 1-deoxy-*N*-octa-

decanoylmuramoyl-L-alanyl-D-isoglutamine and its derivatives.

Hydrogenolytic removal of the *S*-acetyl group in 1-*S*-acetyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-2-octadecanoylamino-1-thio-β-D-glucopyranose⁹⁾ (1) with hydrogen in the presence of Raney Ni (W-2) catalyst, in ethanol, gave 1,5-anhydro-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-2-octadecanoylamino-D-glucitol (2) in a 62% yield. Saponification of 2 with 0.2 M aqueous potassium hydroxide in 1,4-dioxane gave the free acid, which was used for the next reaction without purification. Coupling of the acid with L-alanyl-D-isoglutamine methyl ester trifluoroacetate was conducted with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) as the activating agents to afford *N*-[2-*O*-(1,5-anhydro-2,3-dideoxy-4,6-*O*-isopropylidene-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (3) in a 96% yield. *O*-Deisopropylidenation of 3 by mild acid hydrolysis gave compound 4, which was converted, on saponification with 0.2 M aqueous potassium hydroxide, into *N*-[2-*O*-(1,5-anhydro-2,3-dideoxy-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine (5) in an excellent yield. On the other hand, when treated with octadecanoyl chloride in pyridine-dichloromethane at -40°C, compound 4 yielded *N*-[2-*O*-(1,5-anhydro-2,3-dideoxy-6-*O*-octadecanoyl-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (6).

The immunoadjuvant activity to *N*-acetyl-L-tyrosine-3-azobenzene-4'-arsonate in guinea-pigs has been examined.¹⁰⁾ Surprisingly, all of the compounds (4~6) had completely lost the activity, although the 2-*N*-(fatty acyl)-MDP analogs exhibited strong immunoadjuvant activities.⁵⁾ The results indicate that the position for the introduction of lipophilicity into the sugar moiety of 1-deoxy-MDP is limited for the manifestation of activity, rather than MDP.

EXPERIMENTAL

Melting points (mp) were determined with a Yanagimoto micro melting-point apparatus and are uncorrected.

[†] Studies on Immunoadjuvant Active Compounds. Part XXX. For part XXIX, see ref. 1.

Specific rotations were determined with a Union PM-201 polarimeter at 25°C, and IR spectra were recorded with a Jasco A-100 spectrophotometer. NMR spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer. Evaporations were conducted *in vacuo*. Preparative chromatography was performed on silica gel (Waco Co., 200 mesh) with the solvent systems specified.

1,5-Anhydro-2-deoxy-4,6-O-isopropylidene-3-O-[D-1-(methoxycarbonyl)ethyl]-2-octadecanoylamino-D-glucitol (2). 1-*S*-Acetyl-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[D-1-(methoxycarbonyl)ethyl]-2-octadecanoylamino-1-thio- β -D-glucopyranose⁹ (1, 300 mg) was dissolved in ethanol (3 ml); Raney Ni (W-2) catalyst (300 mg) was added, and hydrogen was bubbled through while the mixture was stirred for 5 hr at room temperature; at that time, the reaction was complete. After removing the catalyst by filtration, the filtrate was evaporated. The residue was chromatographed on a column of silica gel (10 g) with chloroform, and 200:1 chloroform-methanol. The latter eluate gave 2 (165 mg, 62%) as crystals; mp 48°C, $[\alpha]_D +23.5^\circ$ ($c=0.45$, chloroform); IR $\nu_{\max}^{\text{Nujol}} \text{cm}^{-1}$: 3300 (NH), 1740 and 1260 (ester), 1640 and 1550 (amide), and 850 (Me_2C); NMR (CDCl_3): δ 0.88 (near t, 3H, $J_{\text{Me},\text{CH}_2}$ 5.5 Hz, MeCH_2), 1.20~1.35 (m, 30H, 15 CH_2), 1.40 (d, 3H, $J_{\text{Me},\text{CH}}$ 7.4 Hz, MeCH), 1.40, 1.50 (2s, 6H, Me_2C), 3.78 (s, 3H, MeO).

Anal. Found: C, 67.22; H, 10.31; N, 2.48. Calcd. for $\text{C}_{31}\text{H}_{57}\text{NO}_7$: C, 66.99; H, 10.34; N, 2.52%.

N-[2-O-(1,5-Anhydro-2,3-dideoxy-4,6-O-isopropylidene-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (3). To a solution of 2 (100 mg) in 1,4-dioxane (6 ml) and methanol (3 ml) was added 0.2 M potassium hydroxide (4.5 ml); the mixture was stirred for 5 min at room temperature, treated with Amberlite IRC-50 (H^+) resin before the resin was filtered off, and finally washed with methanol. The filtrate and washings were combined, and then evaporated. The residue was dissolved in dry 1,4-dioxane (2 ml), and DCC (88 mg) and HOSu (37 mg) were added to the mixture. The mixture was stirred for 15 min, at which time the acid was converted into the activated ester. L-Alanyl-D-isoglutamine methyl ester trifluoroacetate (100 mg) and triethylamine (0.2 ml) were added to the mixture, which was stirred for 30 min at room temperature; the precipitates were filtered off, and washed with 1,4-dioxane. The filtrate and washings were combined, and evaporated to a syrup which was extracted with chloroform. The extract was washed with water, dried (sodium sulfate), and evaporated. The residue was purified by chromatography on a column of silica gel 10 g with 30:1 chloroform-methanol, to give 3 (130 mg, 96%) as a syrup; $[\alpha]_D +6.5^\circ$ ($c=0.6$, chloroform); IR $\nu_{\max}^{\text{film}} \text{cm}^{-1}$: 3300 (NH), 2940 and 2850 (Me, methylene), 1740 and 1250 (ester), 1660 and 1540 (amide), and 850 (Me_2C); NMR (CDCl_3): δ 0.88 (near t, 3H, $J_{\text{Me},\text{CH}_2}$ 5.4 Hz, MeCH_2), 1.20~1.33 (m, 30H, 15 CH_2), 1.32~1.40 (m,

12H, 2MeCH, Me_2C), 3.68 (s, 3H, MeO), 5.98, 6.90, 7.05, and 7.45 (5H, 3NH, NH_2).

Anal. Found: C, 62.11; H, 9.25; N, 7.36. Calcd. for $\text{C}_{39}\text{H}_{70}\text{N}_4\text{O}_{10}$: C, 62.04; H, 9.35; N, 7.42%.

N-[2-O-(1,5-Anhydro-2,3-dideoxy-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (4). A solution of 3 (100 mg) in 80% aqueous acetic acid (3 ml) was heated for 2 hr at 45°C, the course of the reaction being monitored by TLC. The mixture was lyophilized to give 4 in a quantitative yield, which showed a single spot in TLC; mp 176~178°C, $[\alpha]_D +3.7^\circ$ ($c=0.2$, chloroform); IR $\nu_{\max}^{\text{Nujol}} \text{cm}^{-1}$: 3400~3280 (OH, NH) 2940 and 2850 (Me, methylene), 1730 and 1250 (ester), and 1660, 1640, 1550, and 1540 (amide).

Anal. Found: C, 60.38; H, 9.46; N, 7.84. Calcd. for $\text{C}_{36}\text{H}_{66}\text{N}_4\text{O}_{10}$: C, 60.48; H, 9.31; N, 7.84%.

N-[2-O-(1,5-Anhydro-2,3-dideoxy-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine (5). To a solution of 4 (32 mg) in methanol (4 ml) was added 0.2 M aqueous potassium hydroxide (2.5 ml). The solution was stirred for 10 min at room temperature, treated with Amberlite IR-120 (H^+) resin, and then evaporated to afford 5. Crystallization from ether gave a pure sample (29 mg, 93%); mp 148~149°C, $[\alpha]_D -7.4^\circ$ ($c=0.5$, chloroform); IR $\nu_{\max}^{\text{KBr}} \text{cm}^{-1}$: 3400~3280 (OH, NH), 2930 and 2850 (Me, methylene), 1720 (C=O), and 1660 and 1550 (amide).

Anal. Found: C, 60.21; H, 9.33; N, 7.86. Calcd. for $\text{C}_{35}\text{H}_{64}\text{N}_4\text{O}_{10}$: C, 59.98; H, 9.20; N, 7.99%.

N-[2-O-(1,5-Anhydro-2,3-dideoxy-6-O-octadecanoyl-2-octadecanoylamino-D-glucitol-3-yl)-D-lactoyl]-L-alanyl-D-isoglutamine methyl ester (6). To a solution of 4 (71 mg) in dry dichloromethane (2 ml) and dry pyridine (0.5 ml) was added, with stirring, a solution of octadecanoyl chloride (55 mg) in dry dichloromethane (3.5 ml) at -40°C. The mixture was stirred for 10 hr at -20°C, methanol (1 ml) was added to the mixture, and it was finally evaporated. The residue was chromatographed on a column of silica gel (5 g) with (a) 150:1, (b) 100:1, (c) 80:1, and (d) 50:1 chloroform-methanol. Eluant (d) gave 6 (41 mg, 43%); mp 178~180°C, $[\alpha]_D -3.5^\circ$ ($c=0.2$, chloroform); IR $\nu_{\max}^{\text{KBr}} \text{cm}^{-1}$: 3350~3300 (OH, NH), 2940 and 2850 (Me, methylene), 1740 and 1240 (ester), and 1660 and 1540 (amide); NMR (CDCl_3): δ 0.88 (near t, 6H, $J_{\text{Me},\text{CH}_2}$ 5.2 Hz, 2 MeCH_2), 1.11~1.44 (m, 66H, 30 CH_2 , 2MeCH), and 3.69 (s, 3H, MeO).

Anal. Found: C, 66.53; H, 10.36; N, 5.70. Calcd. for $\text{C}_{54}\text{H}_{98}\text{N}_4\text{O}_{11}$: C, 66.22; H, 10.09; N, 5.72%.

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