Short Communication

Synthesis of Positional and Configurational Isomers of *N*-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP), and Their Immunoadjuvant Activities[†]

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Received April 14, 1980

In the course of studies^{1~6)} on the relationship between the structure of the carbohydrate moiety and activity of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), which is the minimal structure^{7,8)} required for the immunoadjuvant activity of bacterial cell-wall peptidoglycans, we have demonstrated that the carbohydrate moiety is essential for the adjuvant activity,⁴⁾ but not restricted to 2-acetamido-2-deoxy-D-glucose. It can be replaced by 2-amino-2-deoxy-D-glucose,⁵⁾ 2-acetamido-6-amino- and -6-(acylamino)-2,6-dideoxy-D-glucose,^{1,6)} 2-acetamido-2-deoxy-D-mannose,³⁾ etc. without decreasing the activity. However, the activity is abolished almost completely by replacement of the substituent at the C-2 or C-6 position by a hydrogen atom.^{5,6)} We now report the syntheses of 2-acetamido-2-deoxy-4- and -6-*O*-(D-2-propionyl-L-alanyl-D-isoglutamine)-D-glucopyranoses (positional isomers of MDP, as to the lactyl-dipeptide linkage), and D-allo-, D-gulo- and D-galacto-types of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (configurational isomers of MDP bearing lactyl-

dipeptide at the C-3 position). The resulting variation of immunoadjuvant activity is also described.

Benzylation of **1**²⁾ which was prepared by a unique acetonation of benzyl 2-acetamido-2-deoxy- α -D-glucopyranoside^{2,9)} with 2,2-dimethoxypropane, *N,N*-dimethylformamide and *p*-toluenesulfonic acid, gave crystalline **2** [mp 135~137°C, $[\alpha]_D + 149^\circ$ ($c=1$, CHCl₃); lit.¹⁰⁾ mp 127~128°C, $[\alpha]_D + 112^\circ$ ($c=1.2$, CHCl₃)] in a high yield. Hydrolytic removal of the 4,6-*O*-isopropylidene group of **2** with 60% acetic acid, and subsequent selective benzylation by the procedure of Jacquinet and Sinaÿ,¹¹⁾ gave benzyl 2-acetamido-3,6- and -3,4-di-*O*-benzyl-2-deoxy- α -D-glucopyranosides (**5**) [mp 207~208°C, $[\alpha]_D + 127^\circ$ ($c=0.5$, CHCl₃); lit.¹¹⁾ mp 204~205°C, $[\alpha]_D + 121^\circ$ ($c=1$, CHCl₃)] and (**6**) [mp 146~147°C, $[\alpha]_D + 110^\circ$ ($c=0.5$, CHCl₃); lit.¹¹⁾ mp 145~145.5°C, $[\alpha]_D + 114^\circ$ ($c=1$, CHCl₃)]. Compounds **5** and **6** were condensed with L-2-chloropropionic acid in the presence of sodium hydride, to give the corresponding 4-*O*- and 6-*O*-(D-1-carboxyethyl) derivatives **7** [mp 139~140°C, $[\alpha]_D + 127^\circ$ ($c=1$, CHCl₃)] and **8** [mp 165~166°C, $[\alpha]_D + 75^\circ$ ($c=0.3$, CHCl₃)] in high yields, respectively. Coupling of **7** and **8** with L-alanyl-D-isoglutamine benzyl ester was conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as activating agents, to afford the corresponding lactyl-dipeptide derivatives **9** [$[\alpha]_D + 67^\circ$ ($c=1$, 1:1 MeOH-CHCl₃)] and **10** [$[\alpha]_D + 64^\circ$ ($c=0.9$, 1:1 MeOH-CHCl₃)] in almost quantitative yields. Hydrogenolytic removal of the protecting groups of **9** or **10** in solutions of 10:1 (v/v) MeOH-AcOH and then 10:10:1 (v/v/v) MeOH-H₂O-AcOH containing 10% Pd/C catalyst, yielded the desired "positional" isomers of MDP; **11** [$[\alpha]_D + 55^\circ$ ($c=0.3$, MeOH, equil.)] and **12** [$[\alpha]_D + 24^\circ$ ($c=1$, MeOH, equil.)] as amorphous materials.

Benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene- α -D-allopyranoside (**13**) [mp 163°C, $[\alpha]_D + 128^\circ$ ($c=1$, CHCl₃)] was prepared from **3** according to the procedure of Reckendorf.¹²⁾ On the other hand, benzyl 2-

[†] Studies on Immunoadjuvant Active Compounds. Part VIII. For Parts VI and VII, see ref. 1.

acetamido-2-deoxy- α -D-gulo and -D-galactopyranosides were synthesized by the procedures of Parquet and Sinay¹³⁾ and Gross *et al.*,¹⁴⁾ respectively, *via* benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-(methylsulfonyl)- α -D-glucopyranoside which was prepared from **4** [mp 136~137°C, $[\alpha]_D +103^\circ$ ($c=1$, MeOH)]. The kinetically controlled isopropylidenation of benzyl 2-acetamido-2-deoxy- α -D-gulo and -D-galactopyranosides with 2,2-dimethoxypropane, *N,N*-dimethylformamide and *p*-toluenesulfonic acid, gave **16** [mp 165~166°C, $[\alpha]_D +139^\circ$ ($c=1$, MeOH)] and **19** [syrup, $[\alpha]_D +141^\circ$ ($c=1.8$, CHCl₃)], respectively. Compounds **13**, **16** and **19** were condensed with L-2-chloropropionic acid, and the resulting **14** [mp 161~162°C, $[\alpha]_D +56^\circ$ ($c=1$, CHCl₃)] and **17** [mp 167~169°C, $[\alpha]_D +90^\circ$ ($c=1$, MeOH)] were coupled with L-alanyl-D-isoglutamine benzyl ester by the procedures just described above, to afford the corresponding dipeptide derivatives **15** [$[\alpha]_D +25^\circ$ ($c=1$, CHCl₃)] and **18** [$[\alpha]_D +10^\circ$ ($c=1$, CHCl₃)] as amorphous materials in near quantitative yields. Since the 4,6-*O*-iso-

propylidene group of **20** was very labile, the synthesis of the D-galacto analog was carried out *via* **20'** to give **21** [mp 213~214°C, $[\alpha]_D +58^\circ$ ($c=0.5$, MeOH)] in a 95% yield. De-*O*-isopropylidenation of **15** and **18**, and subsequent hydrogenation on 10% Pd/C catalyst, gave the desired "configurational" isomers of MDP; D-allo (**22**) [$[\alpha]_D -26.4^\circ$ ($c=0.5$, MeOH, equil.)] and D-gulo (**23**) [$[\alpha]_D -45^\circ$ ($c=0.5$, MeOH, equil.)] analogs. Similarly, the D-galacto analog **24** [$[\alpha]_D +40.7^\circ$ ($c=0.4$, MeOH, equil.)] was obtained from **21**.

The immunoadjuvant activities of the synthetic muramyl dipeptide analogs (**11**, **12** and **22**~**24**) on the induction of delayed-type hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4'-arsonate in guinea pigs¹⁵⁾ were examined. Only **24** showed potent activity comparable to that of MDP, whereas the others exhibited weak or no adjuvant activity. The results show that the position of the lactyl-dipeptide linkage must be at C-3 and that the configuration seems to be very critical for manifestation of the immunoadjuvant activity. However, the configuration of the hydroxyl

group at the C-4 position is not essential for the activity. The details of the biological activity will be published elsewhere. All new compounds were characterized by IR and NMR spectra, and had elemental compositions that coincided satisfactorily with theoretical ones.

Acknowledgment. This work was supported, in part, by a cancer research grant (no. 401537) from the Japanese Ministry of Education.

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