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Preliminary communication

Synthesis and immunoadjuvant activities of muramoyl-L-alanyl-D-isoglutamine and some carbohydrate analogs*

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Since the discovery that *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (**4**) is the minimal structure required for the immunoadjuvant activity of bacterial, cell-wall peptidoglycans (Ellouz *et al.*[1], 1974; Merser *et al.*[2] and Kotani *et al.*[3], 1975), a great number of analogs thereof have been synthesized by the same groups[4,5], and their immunological and antitumor activities investigated[6,7].

Interest in elucidating the mode of action, especially the role of the carbohydrate moiety in muramoyl dipeptides, has stimulated our recent studies on the synthesis of a variety of carbohydrate analogs[8]. In the course of our investigations, it has been found that not only is the sugar moiety essential for adjuvant activity[6a,8c], but also that the position and configuration of the linkage between the lactoyl-dipeptide and the sugar moiety are critically important[8d,9]. On the other hand, as described in an accompanying communication[10], the 6-hydroxyl group can be replaced by an amino or acylamino group. In this communication, the chemical modification of the C-2 substituent in the sugar moiety and the resulting variation of immunoadjuvant activity are presented.

Benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside[11] (**1a**) was prepared by treatment of the 5,6-diol with 2,2-dibenzyloxypropane in *N,N*-dimethylformamide containing a trace of *p*-toluenesulfonic acid. We found that, when 2,2-dimethoxypropane was used, 2-deoxy-D-*arabino*-hexose produced the methyl 2-deoxy-5,6-*O*-isopropylidene-D-*arabino*-hexofuranosides (**3a**), $[\alpha]_D$ +33.2° (*c* 2.6, $CHCl_3$), in 40% yield after column chromatography. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**2a**) was synthesized by a conventional method, using acetone–sulfuric acid[12].

The furanoid derivatives **1a**, **2a**, and **3a** were condensed with L-2-chloropropanoic

*Studies on Immunoadjuvant Active Compounds, Part IV. For Part III, see ref. 8*c*.
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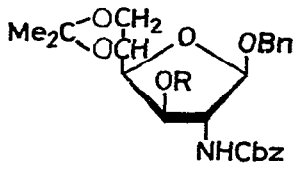
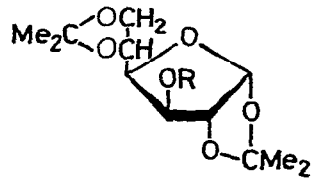
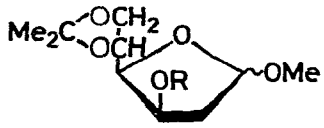


1a-c



2a-c



3a-c

Bn = $PhCH_2$; Cbz = $PhCH_2OCO$ 1-3

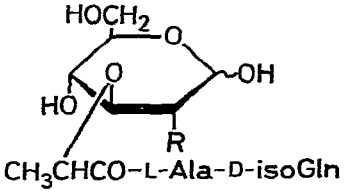
a, R = H

b, R = $-CH(CH_3)CO_2H$

c, R = $-CH(CH_3)CO$-L-Ala-D-isoGln-OBn

acid in the presence of sodium hydride, to give the corresponding 3-*O*-(D-1-carboxyethyl) derivatives: **1b**, $[\alpha]_D$ −28° (*c* 0.5, $CHCl_3$); **2b**, $[\alpha]_D$ +2° (*c* 1, $CHCl_3$); and **3b**, $[\alpha]_D$ +63.7° (*c* 1.25, $CHCl_3$), respectively. Couplings of **1b**–**3b** with L-alanyl-D-isoglutamine benzyl ester[4a] were conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as activating agents, to afford the corresponding lactoyldipeptide derivatives: **1c**, m.p. 148.5°, $[\alpha]_D$ −39° (*c* 0.7, $CHCl_3$); **2c**, $[\alpha]_D$ −9.6° (*c* 1.46, $CHCl_3$); and **3c**, $[\alpha]_D$ +34° (*c* 0.96, $CHCl_3$), in high yields.

Hydrolytic removal of the isopropylidene group of **1c** with 60% acetic acid, and subsequent hydrogenation with 10% palladium–carbon catalyst, followed by treatment with 0.1M hydrochloric acid, gave muramoyl-L-alanyl-D-isoglutamine hydrochloride (**5**), *m.p.* 159–163° (dec.), $[\alpha]_D$ +55° (*c* 0.58, H_2O; equil.). Compounds **2c** and **3c** were first treated with 80–90% trifluoroacetic acid, to convert them into the pyranoid structures, and then hydrogenated, to yield the desired 2-hydroxy (**6**) {$[\alpha]_D$ +60.7° (*c* 0.5, MeOH; equil.)} and 2-deoxy (**7**) {$[\alpha]_D$ +27.6° (*c* 0.284, MeOH; equil.)} analogs, as amorphous materials after freeze-drying.



4 R=NHAc
5 R=NH_2·HCl
6 R=OH
7 R=H

Immunoadjuvant activities (on the induction of delayed-type hypersensitivity to *N*-acetyl-L-tyrosine-3-azobenzene-4′-arsonate) of 4–7 were examined in guinea pigs[13]. Compound 5 showed potent activity that was even stronger than that of *N*-acetylmuramoyl-dipeptide 4. The D-glucose analog (6) had strong activity, comparable to that of 4, whereas the 2-deoxy-D-*arabino*-hexose analog (7) did not show any adjuvant activity. These results suggest that, for activity, the substituent on C-2 is not restricted to the acetamido group, but that the presence of a substituent such as an amino or a hydroxyl group at C-2 is essential for adjuvant activity.

New compounds obtained gave elemental analyses and i.r. and n.m.r. data in agreement with the structures assigned.

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