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Studies on Amino-hexoses. XV. Synthesis of Deoxy-*N*-acetyl-muramic Acid

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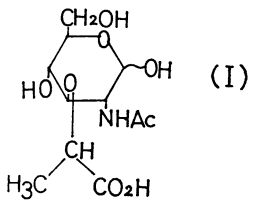
Benzyl 2-acetamido-6-*O*-acetyl-4-chloro-2,4-dideoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-D-galactopyranoside and benzyl 2-acetamido-4,6-dichloro-2,4,6-trideoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-D-galactopyranoside were prepared by the reaction of sulfuryl chloride on *N*-acetyl-muramic acid derivatives. 2-Acetamido-2,4-dideoxy-3-*O*-(D-1-carboxyethyl)-D-xylohexo-pyranose (*N*-acetyl-4-deoxy-muramic acid) and 2-acetamido-2,4,6-trideoxy-3-*O*-(D-1-carboxyethyl)-D-xylohexo-pyranose (*N*-acetyl-4,6-dideoxy-muramic acid) were prepared by the reduction of these chlorodeoxy derivatives with tri-*n*-butyltin hydride.

N-Acetyl-muramic acid (Formula 1), now known to be a constituent of the cell walls of both gram-positive and gram-negative bacteria, was isolated at first as a nucleotide derivative that plays the role of precursor in cell wall biosynthesis.[1] Lindberg and Agback prepared some analogues of muramic acid with variations of the lactic side chain for the purpose of inspecting antibacterial activity.[2] Diana synthesized 6-deoxy analogues of muramic acid for the same purpose.[3] As is well-known, *N*-acetyl-muramic acid is glycosidically bound at C–4 with *N*-acetyl-D-glucomine in the bacterial cell wall mucopeptides, and the



(I)

Formula 1

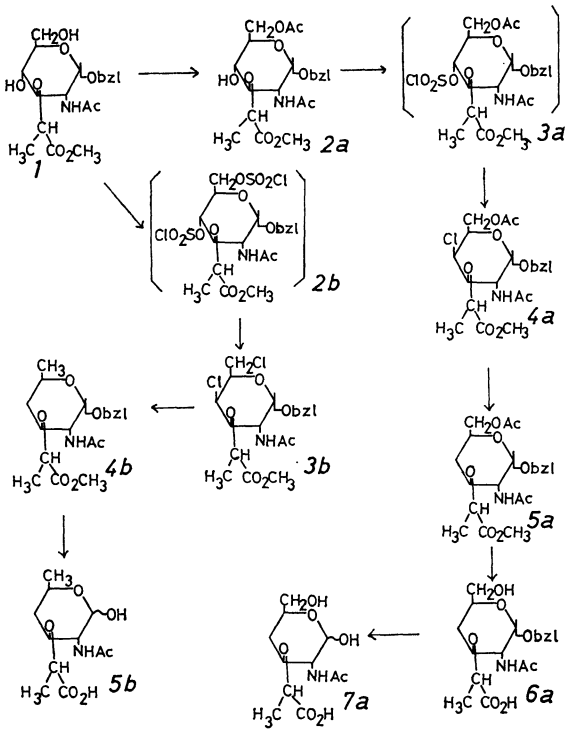
hydroxyl group at C–4 of muramic acid seems to be indispensable for cell wall biosynthesis. We attempted to prepare 4-deoxy- and 4,6-dideoxy-*N*-acetyl-muramic acid on the basis of the stereospecific synthesis of muramic acid reported by Matsushima and Park.[4] Introduction of chlorodeoxy group at C–4 or C–6 of

1) J. T. Park, *J. Biol. Chem.*, **194**, 885 (1952).
2) B. Lindberg and H. Agbach, *Acta Chem. Scand.*, **18**, 185 (1964).
3) G. D. Diana, *J. Org. Chem.*, **35**, 1910 (1970).
4) Y. Matsushima and J. T. Park, *ibid.*, **27**, 3581 (1962).

N-acetyl-muramic acid was performed by the reaction with sulfuryl chloride in pyridine.[8] The chlorodeoxy sugars were successfully reduced by tri-*n*-butyltin hydride and α-α'-azobisisobutyronitrile as an initiator of the radical reaction.

Results and Discussion

The route of the synthesis is shown in the Scheme 1. Intermediate **1** was prepared according to the method of Flowers and Jeanloz.[5] Partial acetylation giving 6-*O*-acetyl compound of **1** was successful, its structure being confirmed by NMR spectrometry. Two methyl signals appeared in the region δ 2.0—2.1, one being assigned as *N*-acetyl (δ 2.03) and the other as 6-*O*-acetyl (δ 2.0). No signal for 4-*O*-acetyl was observed. Complete selectivity of acetylation between C–6 and C–4 may be due to the influence of a lactyl side chain at C–3. We found no such selectivity between primary and secondary hydroxyl groups under the same reaction conditions.[6,7] Treatment of **1** and **2a** with sulfuryl chloride resulted in chlorodeoxy compounds **4a** and **3b**. The chloro groups were introduced most probably through the intermediates **3a** and **2b**. The configuration of the chloro group at C–4 of both **4a** and **3b** was inferred to be galacto-type on the basis of the data presented by Jennings and Jones.[8] Reduction of **4a** with tri-*n*-butyltin hydride proceeded without producing any by-product under mild conditions, but reduction of **3b** required refluxing overnight to complete the reaction. As in the case of reduction of chlorodeoxy neutral sugars, no reaction occurred without co-existence of a small amount of α,α'-azobis-isobutyronitrile.[7] Compounds **5a** and **4b** were deacetylated with sodium hydroxide and debenzylated by catalytic reduction with palladium on charcoal. The structures of the synthetic substances were confirmed by NMR spectrometry. The NMR assignment of compound **5a** is as follows (Fig. 1). Irradiation at δ 1.40 caused the quartet of CH_3CH-$COOCH_3$ at δ 4.10 to collapse to a singlet. Conversely, irradiation at δ 4.10 caused the doublet at δ 1.40 to collapse to a singlet. Irradiation of H–2 at δ 3.60 caused the doublet of H–1 at δ 5.38 to collapse to a singlet. Integration suggested that H–4e existed in the region δ 1.8–2.3 overlapping the signal of the *N*-acetyl group. Similarly H–4a existed in the region δ 1.2—1.8 overlapping the signal of CH_3-$CHCOOCH_3$. Furthermore, integration suggested that H–3 and H–5 existed in the region δ 3.7—4.1 overlapping CH_3-$CHCOOCH_3$. The spectrum of compound **4b** is shown in Figs. 2 and 3. Irradiation at δ 1.37 caused the quartet of $CH_3CHCOOCH_3$ at δ 4.10 to collapse to a singlet. Irradiation of H–5 at δ 3.98 caused the doublet of CH_3–5 at δ 1.16 to collapse to a singlet. Irradiation of H–2 at δ 3.65 also caused the doublet of H–1 at δ 5.30 to collapse to a singlet. Integration suggested that H–3 existed in the region δ 3.7—4.1, which overlapped the signal of $CH_3CHCOOCH_3$.



Scheme 1.

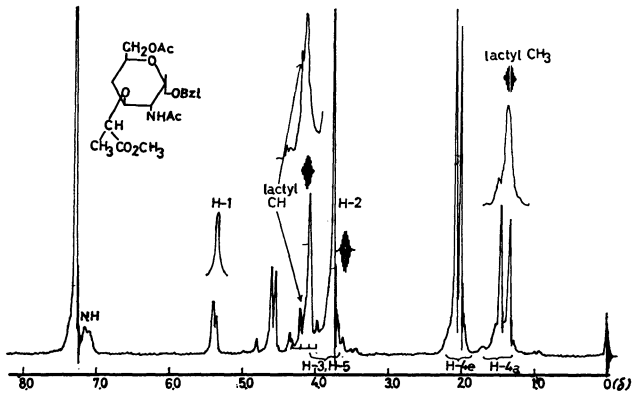


Fig. 1. The NMR spectrum of **5a** in $CDCl_3$.

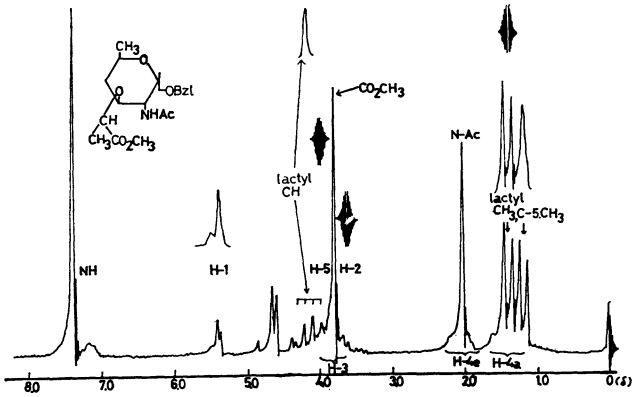


Fig. 2. The NMR spectrum of **4b** in $CDCl_3$.

5) H. M. Flowers and R. W. Jeanloz, *J. Org. Chem.*, **28**, 2983 (1963).

6) H. Arita and Y. Matsushima, *J. Biochem.*, **70**, 795 (1971).

7) H. Arita, N. Ueda, and Y. Matsushima, This Bulletin, **45**, 567 (1972).

8) H. J. Jennings and J. K. N. Jones, *Can. J. Chem.*, **40**, 1408 (1962).

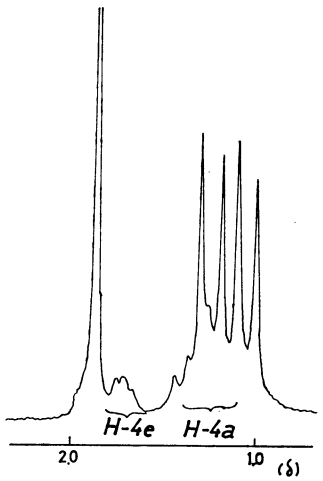


Fig. 3. The NMR spectrum of **4b** in $CDCl_3$+Benzene-d_6.

Integration also suggested that H–4e, H–4a existed in the region δ 1.8—2.3 and δ1.1—1.7 overlapping the signals of C–5 CH_3 and $\underline{CH_3}CHCOOCH_3$, respectively. The assignment of H–4e was confirmed by the addition of benzene-d_6 to the solution of **4b** in $CDCl_3$ (Fig. 3). The signal of H–4e appeared in a higher field.

Experimental

General Methods. All the melting points were uncorrected. Nuclear magnetic resonance spectra were recorded using the specimens dissolved in chloroform-*d* with tetramethylsilane as an internal standard. A Varian T-60 spectrometer was employed. Thin layer chromatagraphy (tlc) was performed with silica gel G (Merck). The spots were detected by spraying with 5% sulfuric acid in methanol and heating at 150°C. Tri-*n*-butyltin hydride was prepared by thermal decomposition of tri-*n*-butyltin formate according to the method of Okawara and Ohara.[9]

*Benzyl 2-Acetamido-2-deoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-*D*-glucopyranoside (**1**).* Compound (**1**) was prepared from benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[D-1-methyl carboxylate ethyl]-2-deoxy-α-D-glucopyranoside by treatment in a 80% aqueous acetic acid at 60°C for 2 hr.[5] $[\alpha]_D^{46}$+127° (*c* 1.08, in chloroform)

*Benzyl 2-Acetamido-6-*O*-acetyl-2-deoxy-3-[D-1-(methyl carboxylate)ethyl]-α-D-glucopyranoside(**2a**).* Compound **1** (13 g) was dissolved in a mixture of anhydrous chloroform (50 m*l*) and pyridine (50 m*l*), and the solution was cooled in a dry ice-acetone bath. Acetic anhydride (3.75 m*l*) was added dropwise to the solution, and the reaction solution was kept at −20°C for 40 hr. A few drops of water was added to destroy residual acetic anhydride and the solution was dried up *in vacuo*. The residual syrup was extracted with chloroform and the extract was washed with water several times. The chloroform layer was dried up *in vacuo* to give a slightly colored syrup (13.5 g), which showed a single spot on tlc with the solvent system *n*-BuOH–EtOH–H_2O (3 : 1 : 1). $[\alpha]_D^{20}$+91.2° (*c* 1.25, in chloroform) NMR (in $CDCl_3$); δ 1.4 (3H d; *J* 7 Hz $\underline{CH_3}CHCOOCH_3$) 2.10 (3H s, OAc) 2.04 (3H s, NAc) 2.34 (1H s, OH) 3.74 (3H s, $-CO_2CH_3$) 5.33 (1H d, *J* 3 Hz H-1) 7.6 (1H d, *J* 5 Hz NH)

*Benzyl 2-Acetamido-6-*O*-acetyl-4-chloro-2,4-dideoxy-3-*O*-[D-1-(methylcarboxylate) ethyl]-α-D-galactopyranoside(**4a**).* Compound **2a** (13 g) was dissolved in anhydrous pyridine (30 m*l*) and the solution was cooled in an ice-water bath. Sulfuryl chloride (2.9 m*l*) was added dropwise, and the solution was kept in a refrigerator overnight and then kept at room temperature for 3 hr. The reddish reaction solution was extracted with chloroform and the extract was washed with water several times. Evaporation *in vacuo* gave a syrup which was co-distilled with toluene until residual pyridine was completely removed. Rapid crystallization occurred, and recrystallization with 2-propanol gave colorless needles (8.4 g) which melted at 139—140°C and had $[\alpha]_D^{20}$+182° (*c* 1.0 in chloroform) Found: C, 55.10; H, 6.20; N, 3.12; Cl, 7.59%. Calcd for $C_{21}H_{28}O_8NCl$: C, 55.07; H, 6.16; N, 3.06; Cl, 7.74%. NMR (in $CDCl_3$) δ 1.42 (3H d, *J* 7 Hz $\underline{CH_3}CHCOOCH_3$) 2.0 (3H s, OAc) 2.03 (3H, s, NAc) 3.72 (3H s, $COOCH_3$) 4.0 (1H unresolved. H-2) 4.03 (1H q, *J* 7 Hz $\underline{CH_3}CHCOOCH_3$) 5.43 (1H d, *J* 3 Hz H-1) 7.1 (1H d, *J* 5 Hz NH)

*Benzyl 2-Acetamido-6-*O*-acetyl-2,4-dideoxy-3-*O*-[D-1-(methyl carboxylate) ethyl]-α-D-xylohexopyranoside(**5a**).* Six grams of **4a** was dissolved in anhydrous toluene under nitrogen atmosphere. Tri-*n*-butyltin hydride (5 m*l*) and α,α′-azobisisobutyronitrile (10 mg) were added to the solution. The mixture was heated at 80°C under stirring for 2 hr. Evaporation *in vacuo* gave a syrup, which was chromatographed on silica gel column with ethyl acetate–toluene (1 : 1) as an eluant. A colorless syrup (4.5 g) was obtained, which failed to crystallize. The syrup was re-chromatographed on a silica gel column with the same solvent. $[\alpha]_D^{20}$+154° (*c* 1.57 in chloroform) Found: C, 58.71; H, 6.72; N, 3.29%. Calcd for $C_{21}H_{29}O_8N$: C, 59.56; H, 6.90; N, 3.31%. NMR (in $CDCl_3$) δ 1.4 (3H d, *J* 7 Hz $\underline{CH_3}CHCOOCH_3$) *ca.* 1.5 (1H H-4a) *ca.* 2.2 (1H H-4e) 2.01 (3H s, OAc) 2.04 (3H s, NAc) *ca.* 3.6 (1H unresolved H-2) 3.74 (3H s, CO_2CH_3) 4.10 (1H q, *J* 7 Hz $\underline{HC}(CH_3)COOCH_3$) 5.38 (1H d, *J* 3 Hz H-1) 7.1 (1H d, 5 Hz NH)

*2-Acetamido-2,4-dideoxy-3-*O*-(D-1-carboxyethyl)-D-xylohexopyranose (**7a**).* Two grams of **5a** was dissolved in a mixture of methanol (25 m*l*) and 2N aqueous sodium hydroxide (2 m*l*), and the solution was kept at room temperature for 40 hr. It showed no color reaction of ester,[10] and was neutralized with glacial acetic acid. Reduction with hydrogen over 10% palladium on charcoal was carried out at room temperature. The reduction was complete within 24 hr. Palladium charcoal were filtered off, and the filtrate was passed through a column of Dowex-50X8 (H^+) to remove sodium ion and then dried up *in vacuo* to a colorless syrup (1.2 g), which failed to crystallize. The syrup was further purified on a Bio-gel P-2 column (3.0×200 cm) with 0.02 M acetic acid as an eluant. $[\alpha]_D^{20}$+71° (*c* 1.22 in water) Found: C, 47.32; H, 6.85; N, 4.78%. Calcd for $C_{11}H_{19}O_7N$: C, 47.65; H, 6.91; N, 5.05%. NMR (in D_2O) δ 1.4 (3H d, *J* 7 Hz $\underline{CH_3}COOCH_3$) *ca.* 1.68 (1H H-4a) 2.05 (3H s, NAc) *ca.* 2.3 (1H H-4e) 5.34 (1H d, *J* 3 Hz H-1).

*Benzyl 2-Acetamido-4,6-dichloro-2,4,6-trideoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-D-galactopyranoside(**3b**).* Four grams of **1** was dissolved in anhydrous pyridine (40 m*l*) and the solution was cooled in an ice-water bath. Sulfuryl chloride (2.0 m*l*) was then added to the solution, which was kept in a refrigerator overnight and then kept at room temperature for 3 hr. The reaction solution was extracted with chloroform and the extract was washed several times with water.

9) R. Okawara and M. Ohara, *J. Organometal. Chem.*, **3**, 484 (1965).

10) N. Abdel-Akher and F. Smith, *J. Amer. Chem. Soc.*, **73**, 5869 (1951).

The chloroform extract was evaporated *in vacuo* to a syrup, which was extracted with toluene–ligroin (3 : 1) several times on a boiling water bath. The extracts were collected and dried up *in vacuo*. Crystals appearing in methanol were recrystallized with the same solvent to give colorless needles (2.5 g) melting at 131—135°C and had $[\alpha]_D^{20}+103°$ (*c* 1.0, in chloroform). Found, C, 52.52, H, 5.69; N, 3.26; Cl, 15.94%. Calcd for $C_{19}H_{25}O_6NCl_2$: C, 52.54; H, 5.80; N, 3.23; Cl, 16.33%.

*Benzyl 2-Acetamido-2,4,6-trideoxy-3-O-[D-1-(methyl carboxylate)ethyl]-α-D-xylohexopyranoside(**4b**).* Two grams of **3b** was dissolved in anhydrous toluene (50 m*l*) under nitrogen atmosphere and tri-*n*-butyltin hydride (3 m*l*) and α,α′-azobisisobutyronitrile (*ca.* 10 mg) were added. The solution was refluxed for 24 hr. The slightly yellow solution was passed through silica gel column with a solvent mixture of ethylacetate and toluene (1 : 1) as an eluant. The syrup obtained was pure as judged by tlc (ethylacetate–toluene 1 : 1), its Beilstein test being negative. $[\alpha]_D^{20}+177°$ (*c* 1.0, in chloroform) Found: C, 63.01; H, 7.38; N, 3.81%. Calcd for $C_{19}H_{27}O_6N$: C, 62.45; H, 7.45; N, 3.83%.

NMR (in $CDCl_3$) δ 1.16 (3H d, *J* 7 Hz CH_3-5) 1.37 (3H d, *J* 7 Hz $CH_3CHCOOCH_3$) *ca.* 1.5 (1H H-4a) 2.0 (3H s, NAc) *ca.* 2.1 (1H H-4e) *ca.* 3.65 (1H H-2) 3.73 (3H s, CO_2CH_3) 4.1 (1H q, *J* 7 Hz $CH_3CHCOOCH_3$) 3.98 (1H q, *J* 7 Hz H-5) 5.31 (1H d, *J* 3 Hz H-1) 7.15 (1H d, *J* 5 Hz NH).

*2-Acetamido-2,4,6-trideoxy-3-O-(D-1-carboxyethyl)-D-glucopyranose (**5b**).* Compound **4b** (800 mg) was dissolved in 10 m*l* of methanol, and 2 N aqueous sodium hydroxide (1 m*l*) was added. The solution was kept at room temperature for 40 hr and then neutralized with glacial acetic acid. Evaporation *in vacuo* gave a syrup which was dissolved in 2-propanol (50 m*l*) and reduced with hydrogen over palladium charcoal at room temperature. The reaction was complete after 24 hr. The charcoal was filtered off and the filtrate dried up *in vacuo*. The residual syrup was dissolved in 3 m*l* of water and the solution was passed through a column of Dowex-50X8 (H^+) to remove sodium ion. Although the product showed a single spot on tlc (BuOH–EtOH–water 3 : 1 : 1), it failed to crystallize. $[\alpha]_D^{20}+63°$ (*c* 1.7, in water) Found: C, 49.75; H, 7.32; N, 5.24%. Calcd for $C_{11}H_{19}O_6N$: C, 50.56; H, 7.33; N, 5.36%.