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SYNTHESIS OF 2-AMINO-3-*O*-(D-1-CARBOXYETHYL)-2-DEOXY-D-GALACTOSE (D-*galacto* ANALOG OF MURAMIC ACID) AND OF ITS (L-1-CARBOXYETHYL) ANALOG*

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ABSTRACT

The synthesis of syrupy 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-galactose ("galactomuramic acid") and of crystalline 2-amino-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-galactose ("galactoisomuramic acid"), starting from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside, is described.

INTRODUCTION

A new disaccharide has been isolated recently from the lysozyme digest of *Micrococcus lysodeikticus* cell-wall. It was shown to be composed of a 2-amino-2-deoxy-D-glucose residue and of a new amino sugar similar to, but not identical with, 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid). Degradation of this sugar indicated a structure having the D-*manno* configuration[1,2]. In order to study the separation by paper, thin-layer, and gas–liquid chromatography of compounds having compositions similar to that of muramic acid, the D-*galacto* analogs of both muramic acid and isomuramic acid [2-amino-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-glucose] have been synthesized.

DISCUSSION

The synthesis, similar to that recently described for the preparation of muramic acid[3], was started from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[4] (1), with the exception that pure L-2-chloropropionic acid and D-2-

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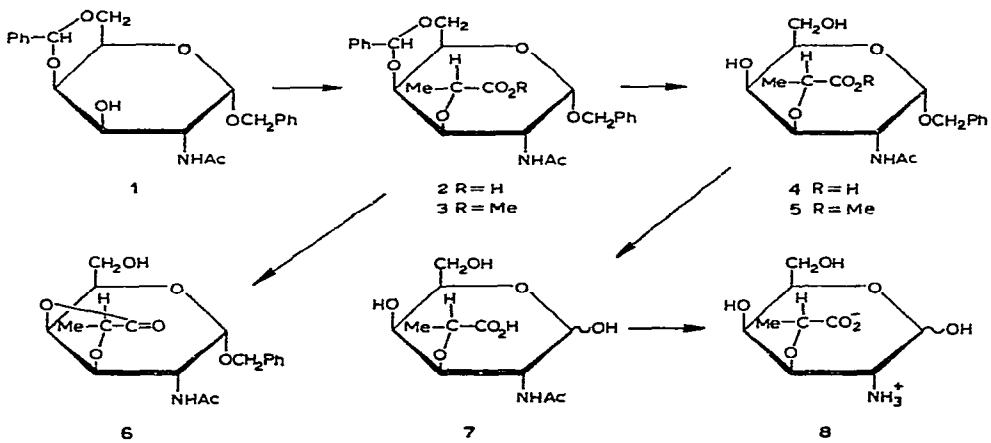
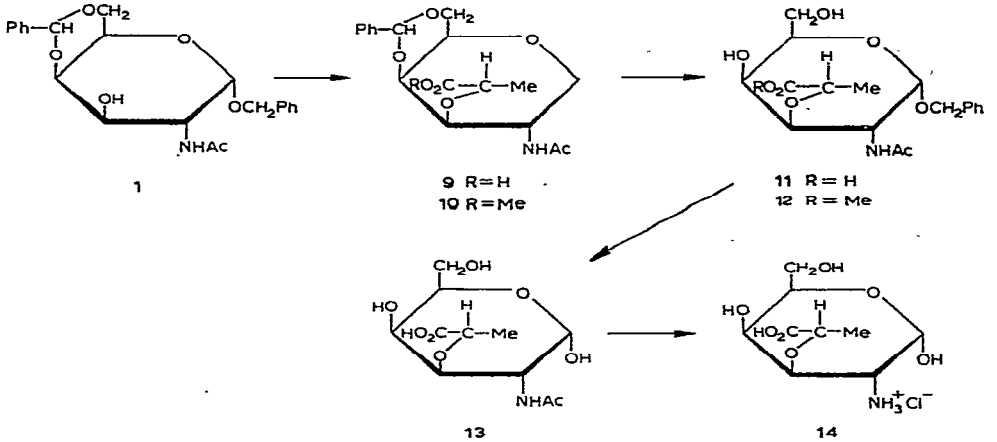
chloropropionic acid, instead of DL-2-chloropropionic acid, were used. Both acids were prepared by a modification[5] of the method of Fischer and Raske[6]. After condensation of **1** with L-2-chloropropionic acid, benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**2**) was isolated in 80% yield. Esterification of **2** with diazomethane gave the crystalline ester **3**, whereas removal of the benzylidene group of **2** with acetic acid transformed **2** into crystalline benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**4**). An attempt to obtain the methyl ester **5** from **4** by treatment with diazomethane was, surprisingly, unsuccessful. An attempt to obtain the methyl ester **5** from **3** by treatment with dilute acetic acid was also disappointing, since the methyl ester group was partially hydrolyzed to give **4**, with the concomitant formation of a small proportion of internal ester **6**.



Hydrogenolysis of the protective benzyl glycoside group of **4** gave amorphous 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-galactose ("*N*-acetylgalactomuramic acid") (**7**), which was further hydrolyzed to afford amorphous 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-galactose ("galactomuramic acid") (**8**).

A similar sequence of reactions starting with D-2-chloropropionic acid gave the 3-*O*-(L-1-carboxyethyl) derivatives **9** to **14**. It is of interest that, in this case, treatment of benzyl 2-acetamido-3-*O*-(L-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**11**) with diazomethane gave the methyl ester **12** in excellent yield, in contrast to the results obtained with the 3-*O*-(D-1-carboxyethyl) derivative **4**. This difference in reactivity of the carboxylic group toward diazomethane might be explained by a change of the pK due to hydrogen bonding[7]. The configurational factors are, however, not clear at the present time, since the 3-*O*-(D-1-carboxyethyl) derivative having the D-*gluco* configuration is reactive[8], but those having D-*galacto* and D-*manno*[9] configurations are not, whereas the 3-*O*-(L-1-carboxyethyl) derivative having the D-*galacto* configuration is reactive, but not that having the D-*gluco* configuration[10]. Hydrogenolysis of the benzyl group of **11** gave crystalline 2-acetamido-3-*O*-(L-1-carboxyethyl)-2-deoxy-α-D-

galactose ("*N*-acetylisogalactomuramic acid") (**13**), which was further hydrolyzed into 2-amino-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-galactose ("isogalactomuramic acid"), isolated as the crystalline hydrochloride **14**.



In the attribution of the D configuration to the lactyl residue linked to O-3 of the 2-amino-2-deoxy-D-glucose moiety in muramic acid, it was assumed[11] that a D-lactyl side-chain would give a more dextrorotatory contribution to the molecule than does an L-lactyl side-chain. This assumption is not always correct, as shown by a comparison of the optical rotation of derivatives **2** and **9** in pyridine solution: the 3-*O*-(L-carboxyethyl) derivative **9** is more dextrorotatory ($[\alpha]_D$ +223°) than the 3-*O*-(D-carboxyethyl) derivative **2** ($[\alpha]_D$ +174°). This result shows the necessity for more unequivocal evidence regarding the configuration of this part of the muramic acid molecule.

EXPERIMENTAL

Melting points were determined on a hot stage equipped with a microscope, and correspond to "corrected melting points". Optical rotations were determined with a Perkin–Elmer No. 141 polarimeter. The chloroform used was A.R. grade and contained approximately 0.75% of ethanol. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model 237. N.m.r. spectra were recorded with a Varian T-60 n.m.r. Spectrometer. The homogeneity of the compounds synthesized was determined by chromatography on plates covered with a thin layer of a 3:1 mixture of silica gel G (Merck) and silica gel GF (Merck). Column chromatography was performed on "Silica Gel Davison", from the Davison Co., Baltimore, Maryland 21201 (grade 950, 60–200 mesh), which was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was conducted by heating to 170–200° (manufacturer's instructions). The sequence of eluants was hexane, benzene (or 1,2-dichloroethane), ether, ethyl acetate, acetone, and methanol, individually or in binary mixtures. The ratio of weight of substance

to weight of adsorbent was 1:50 to 1:100. The ratio of weight of substance (in g) to volume of fraction of eluant (in ml) was 1:100. The ratio of diameter to length of the column was 1:20. Evaporations were conducted *in vacuo*, with the bath temperature below 45°. Volumes of volatile solvent smaller than 20 ml were evaporated under a stream of dry nitrogen. The microanalyses were performed by Dr. M. Manser, Zurich, Switzerland.

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-(D-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**2**). — To a stirred solution of dry benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[3] (**1**) (0.44 g) in dry *p*-dioxane (30 ml), freshly distilled in the presence of sodium, was added sodium hydride in oil suspension (0.25 g) (50% of sodium hydride by weight, Alfa Inorganics, Beverly, Massachusetts). The mixture was kept for 1 h at 95°, and then the temperature was decreased to 65°, and L-2-chloropropionic acid (0.62 g) dissolved in a small volume of dry *p*-dioxane was added. L-2-Chloropropionic acid was prepared by a modification[5] of the method of Fischer and Raske[6] for D-2-bromopropionic acid. After 1 h, a further addition of the 50% suspension of sodium hydride (1 g) was made, and the stirring was continued overnight at 65°. To the cooled reaction mixture, water (15 ml) was carefully added, to decompose the excess of sodium hydride. The lower layer, alkaline and dark-colored, was discarded, and the upper, organic layer was decanted, filtered, partially concentrated (about 30 ml was evaporated off), and diluted with 10 ml of water. This aqueous solution was extracted once with chloroform, to remove the mineral oil and any trace of unreacted starting material; it was then acidified at 0° with 2.5M hydrochloric acid until pH 3 was reached. The resulting precipitate was immediately extracted with chloroform. The chloroform extracts were combined, washed with water, and evaporated, to give a crystalline residue (0.42 g, 80%) of a product that was practically pure, as shown by t.l.c. in 2:1 acetone–methanol; after recrystallization from ethyl acetate, it had m.p. 219–220°, $[\alpha]_D^{20}$ +174° (*c* 0.32, pyridine), $[\alpha]_D^{20}$ +188° (*c* 0.40, methanol).

Anal. Calc. for $C_{25}H_{29}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.73; H, 6.26; N, 3.03.

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-[D-1-(methoxycarbonyl)ethyl]-2-deoxy-α-D-galactopyranoside (**3**). — The crude, crystalline product **2** (0.42 g) was dissolved in methanol (25 ml), and esterified by addition of a slight excess of diazomethane in ether. The course of the reaction was followed by t.l.c. in 2:1 acetone–methanol. Evaporation of the solution, and crystallization of the residue from ethyl acetate, gave 254 mg (60%), m.p. 198–200°, $[\alpha]_D^{20}$ +180° (*c* 1.75, chloroform). Slow crystallization from methanol gave long needles, m.p. 201.5–203.5°.

Anal. Calc. for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44; N, 2.88; O, 26.36. Found: C, 64.24; H, 6.50; N, 3.03; O, 26.62.

Benzyl 2-acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**4**). —A solution of compound **2** (242 mg) in 60% acetic acid (3.2 ml) was heated for 1 h on a boiling water-bath. After being cooled to room temperature, the solution was evaporated to a glassy residue, and the last traces of acetic acid and benzaldehyde were

removed by adding and evaporating several portions of water. Finally, the water was removed by addition of toluene, followed by evaporation, to give a residue (190 mg) which showed partial formation of an internal ester on examination by t.l.c. in 4:5:1 butyl alcohol–acetone–water.

The internal ester was hydrolyzed by treatment of the residue, dissolved in methanol (5.5 ml), with 2M sodium hydroxide (0.5 ml). The mixture was kept for 3 h at room temperature, and then diluted with water (6 ml). Amberlite IRC-50 (H^+) ion-exchange resin was added until a pH of approximately 6 was reached, the resin was filtered off and washed with 50% aqueous methanol, and the filtrate was evaporated to a colorless solid (138 mg, 71%) which crystallized from methanol–ethyl acetate as plates, m.p. 176-178°, $[\alpha]_D^{20}$ +149° (*c* 1.42, methanol).

Anal. Calc. for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65; O, 33.38. Found: C, 56.36; H, 6.39; N, 3.76; O, 33.31.

Attempts at preparation of benzyl 2-acetamido-3-O-[D-1-(methoxycarbonyl)-ethyl]-2-deoxy-α-D-galactopyranoside (5) A. *From* 4. — Compound 4 (288 mg) was dissolved in the minimal volume of methanol, and an excess of a freshly prepared solution of diazomethane in ether was added. The yellow color persisted from the beginning of the addition. The solution was kept at room temperature, and, after the yellow color had become very faint, an excess of diazomethane solution in ether was added. After evaporation under a stream of nitrogen, the residue (277 mg) was shown by t.l.c. in 4:5:1 butyl alcohol–acetone–water to consist mostly of unreacted starting material 4. It was dissolved in ethyl acetate containing the minimal volume of methanol, and chromatographed on silica gel (20 g). Acetone–methanol (2:1) eluted 208 mg (72%) of 4.

B. *From* 3. — A solution of compound 3 (100 mg) in 60% acetic acid (2 ml) was heated for 45 min on a boiling water-bath. After being cooled to room temperature, the solution was evaporated to a syrup, and the last traces of acetic acid and benzaldehyde were removed by addition of water followed by distillation. T.l.c. of the residue (19:1 chloroform–methanol) indicated that partial hydrolysis of the methyl ester had occurred. The residue was chromatographed on a silica-gel column (8 g). Ethyl acetate eluted 14 mg (18%) of benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-α-D-galactopyranoside 4-(internal ester) (6), which was recrystallized from acetone to give needles, m.p. 229–230°, $[\alpha]_D^{20}$ +117° (*c* 0.09, chloroform).

Anal. Calc. for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 58.94; H, 6.28; N, 3.87.

Earlier fractions eluted with 1:1 ethyl acetate–acetone contained 5 (26 mg) as a syrup which, by t.l.c. (19:1 chloroform–methanol), had a mobility identical with that of 5. Attempts to crystallize this syrup were unsuccessful. N.m.r. data (Me_2SO-d_6, tetramethylsilane as the external standard): τ 8.88, 8.77 (3-proton doublet, $J_{2',3'}$ 7 Hz (lactyl side-chain); τ 8.28 (3-proton singlet, Ac); τ 6.97 (3-proton singlet, Me ester); and τ 2.8–3.0 (5 protons, Ph).

Later fractions, eluted with 1:1 ethyl acetate–acetone and then with methanol, contained 4 (40 mg, 50%).

Hydrolysis of **3** for 15 min increased the proportion of **5** to 64%, after chromatographic separation on a silica gel column as previously described. The amount of recovered internal ester **6** decreased to 8%, and of acid **4** to 28%. Hydrolysis of **3** for 7 min gave, after preparative t.l.c. in 47:3 chloroform–methanol, 17 mg (60%) of **5** and traces of starting material **3** and acid **4**.

2-Acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-D-galactose (*N-acetylgalactomuramic acid*) (**7**). — A solution of compound **4** (174 mg) in 90% methanol (or ethanol) (25 ml) was hydrogenolyzed for 2 days at room temperature and normal pressure in the presence of 10% palladium-on-charcoal (100 mg). After filtration of the suspension, the filtrate was evaporated, and the residue (126 mg, 94%) was shown, by t.l.c. (4:5:1 butyl alcohol–acetone–water), to be homogeneous. It was dissolved in methanol and precipitated by addition of ethyl acetate, to give a colorless, amorphous product, m.p. about 150° (dec.), $[\alpha]_D^{20}$ +66° (*c* 0.67, 70% ethanol).

Anal. Calc. for $C_{11}H_{19}NO_8$: C, 45.04; H, 6.53; N, 4.78; O, 43.64. Found: C, 45.21; H, 6.83; N, 4.54; O, 43.29.

This material was shown to be homogeneous by descending paper chromatography on Whatman No. 1 paper. $R_{N\text{-}acetylmuramic\ acid}$ 0.67 in 6:4:3 butyl alcohol–pyridine–water, 0.80 in butyl alcohol–acetic acid–water (25:6:25, upper phase), and 0.88 in 5:2:2 butyl alcohol–acetic acid–water. The spots detected with the silver nitrate reagent[12] were faint, but they could be detected very conveniently by the procedure of Sharon and Seifter[13].

2-Amino-3-O-(D-1-carboxyethyl)-2-deoxy-D-galactose (**8**). — Compound **7** (122 mg) was hydrolyzed with 2.5M hydrochloric acid (3 ml) for 4 h on a boiling water-bath. The solution was decolorized with activated charcoal (Merck), and then evaporated to dryness. A small volume of water was several times added and evaporated off under vacuum, the residue (91 mg) was dissolved in a small volume of water, and the solution was shaken at 0° with an excess of Dowex-1 x8 (OH^-) ion-exchange resin. The resin was filtered off, washed with water, and then stirred with water. Hydrochloric acid (M) was added to this suspension until pH 5.7 (checked with a pH meter) was reached. T.l.c. on a cellulose MN-400 plate with 5:1:2 butyl alcohol–acetic acid–water showed an R_F 0.21 (R_F of muramic acid[14], 0.28). Descending paper-chromatography on Whatman No. 1 paper showed $R_{muramic\ acid}$ 0.73 in 5:1:2 butyl alcohol–acetic acid–water, and 0.58 in 4:5:1 butyl alcohol–ethanol–water. The spots were detected with ninhydrin.

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-(L-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**9**). — A solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**1**, 1.06 g) was treated as described for the preparation of **2**. The D-2-chloropropionic acid was synthesized in the same way as for the L-analog. After acidification of the aqueous solution containing the sodium salt of the condensation product, the resulting precipitate (755 mg) was immediately filtered off, and extensively washed with water. The filtrate was extracted many times with chloroform, and the chloroform extracts were combined, washed with water, dried, and evaporated to give additional crystalline material (127 mg); total yield, 882 mg (70%). The

materials were combined, and recrystallized from methanol–ethyl acetate to give needles, m.p. 250–251.5°, $[\alpha]_D^{20}$ +166° (*c* 0.22, methanol), +223° (*c* 0.30, pyridine).

Anal. Calc. for $C_{25}H_{20}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 64.02; H, 6.36; N, 2.85.

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-[L-1-(methoxycarbonyl)ethyl]-2-deoxy-α-D-galactopyranoside (**10**). — Compound **9** (25 mg) was dissolved in a small volume of methanol, and esterified by addition of a slight excess of diazomethane in ether. Evaporation of the solution, and crystallization of the residue from ethyl acetate, gave 18 mg (72%), m.p. 232–236°, $[\alpha]_D^{20}$ +150° (*c* 0.32, methanol).

Anal. Calc. for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44; N, 2.88; O, 26.36. Found: C, 64.24; H, 6.55; N, 2.89; O, 26.82.

Benzyl 2-acetamido-3-O-(L-1-carboxyethyl)-2-deoxy-α-D-galactopyranoside (**11**). — A solution of compound **9** (274 mg) in 60% acetic acid (5 ml) was heated for 3 h on a boiling water-bath. After being cooled to room temperature, the solution was evaporated to a glassy residue, and the last traces of acetic acid and benzaldehyde were removed by several additions of water, followed by evaporation. Removal of the water by addition of toluene, followed by evaporation, gave a colorless residue (210 mg). T.l.c. in 4:5:1 butyl alcohol–acetone–water showed the presence of a small amount of internal ester as a contaminant. Crystallization from methanol–ethyl acetate gave needles (150 mg, 71%), having a double m.p.: 199–200° and 230–232°, $[\alpha]_D^{20}$ +143° (*c* 0.31, methanol).

Anal. Calc. for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65. Found: C, 56.35; H, 6.60; N, 3.65.

Benzyl 2-acetamido-3-O-[L-1-(methoxycarbonyl)ethyl]-2-deoxy-α-D-galacto-pyranoside (**12**). — Crude compound **11** (70 mg) was dissolved in the minimal volume of methanol, and an excess of ethereal diazomethane solution was added. The solution was evaporated under a stream of nitrogen at room temperature. The colorless residue (62 mg) was crystallized from acetone–ether–pentane, to give needles having a complex m.p., sintering at 202–206°, filamentous needles appearing at 209–210°, and final m.p. at 234–237°, $[\alpha]_D^{20}$ +104° (*c* 0.27, chloroform).

Anal. Calc. for $C_{19}H_{27}NO_8$: C, 57.42; H, 6.85; N, 3.52; O, 32.21. Found: C, 57.34; H, 6.92; N, 3.48; O, 32.14.

2-Acetamido-3-O-(L-1-carboxyethyl)-2-deoxy-α-D-galactose (*N-acetylisogalacto-muramic acid*) (**13**). — Hydrogenolysis of compound **12** (66 mg), as described for the preparation of **7**, gave in quantitative yield a product that was crystallized from methanol–ethyl acetate, m.p. 110–120° (slight dec.), $[\alpha]_D^{20}$ +27 (5 min)→+9° (equil., 20 h, *c* 0.59, 70% ethanol).

Anal. Calc. for $C_{11}H_{19}NO_8$: C, 45.05; H, 6.53; N, 4.78. Found: C, 45.17; H, 6.63; N, 4.71.

The material was shown to be homogeneous by descending paper-chromatography on Whatman No. 1 paper: $R_{\text{N-acetylmuramic acid}}$ 0.64 in 6:4:3 butyl alcohol–

pyridine–water, 0.75 in butyl alcohol–acetic acid–water (25:6:25, upper phase), and 0.87 in 5:2:2 butyl alcohol–acetic acid–water.

2-Amino-3-O-(L-1-carboxyethyl)-2-deoxy-α-D-galactose hydrochloride (**14**). — Compound **13** (10 mg) was hydrolyzed with 2.5M hydrochloric acid (1 ml) for 4 h on a boiling water-bath. The solution was evaporated to dryness, the residue was dissolved in a small volume of water, and the solution was evaporated to dryness; this procedure was repeated several times. The crystalline residue was recrystallized from methanol–acetone–ether to give needles (7 mg, 71%) that did not melt below 300°, $[\alpha]_D^{20}+107.5$ (5 min) → +89° (equil., 20 h, *c* 0.34, 70% ethanol); ν_{max}^{KBr} 3485, 3350, 3065, 2940, 2380, 1925, 1725, 1610, 1525, 1405, 1360, 1230, 1170, 1035, and 780 cm^{-1}.

Anal. Calc. for $C_9H_{18}ClNO_7$: C, 37.65; H, 6.31; N, 4.88. Found: C, 37.43; H, 6.40; N, 4.80.

The material was shown to be homogeneous by descending paper chromatography on Whatman No. 1 paper; $R_{muramic\ acid}$ 0.45 in 6:4:3 butyl alcohol–pyridine–water and 0.68 in 5:1:2 butyl alcohol–acetic acid–water; the spots were detected with the ninhydrin reagent.

REFERENCES

1 R. W. JEANLOZ, *Pure Appl. Chem.*, 14 (1967) 57.
2 O. HOSHINO AND R. W. JEANLOZ, *Abstracts Papers Intern. Congr. Biochem. 7th, Tokyo*, D-54 (1967).
3 R. W. JEANLOZ, E. WALKER, AND P. SINAŸ, *Carbohyd. Res.*, 6 (1968) 184.
4 P. H. GROSS, F. DU BOIS, AND R. W. JEANLOZ, *Carbohyd. Res.*, 4 (1967) 244.
5 P. H. GROSS AND R. W. JEANLOZ, unpublished data.
6 E. FISCHER AND K. RASKE, *Ber.*, 39 (1906) 3981.
7 R. LAMBERT AND F. ZILLIKEN, *Ber.*, 93 (1960) 2915.
8 H. M. FLOWERS AND R. W. JEANLOZ, *J. Org. Chem.*, 28 (1963) 2983.
9 P. SINAŸ, R. W. JEANLOZ, AND P. H. GROSS, *Abstracts Papers Amer. Chem. Soc. Meeting*, 156 (1968) CARB-21.
10 P. SINAŸ AND R. W. JEANLOZ, unpublished data.
11 Y. MATSUSHIMA AND J. T. PARK, *J. Org. Chem.*, 27 (1962) 3581.
12 P. J. STOFFYN AND R. W. JEANLOZ, *Arch. Biochem. Biophys.*, 52 (1954) 373; N. SHARON AND R. W. JEANLOZ, *J. Biol. Chem.*, 235 (1960) 1.
13 N. SHARON AND S. SEIFTER, *J. Biol. Chem.*, 239 (1964) PC 2398.
14 M. TOMODA, personal communication.