N-Acetylmuramic Acid [2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose]

By **Roy Gigg** * and **C. D. Warren,** National Institute for Medical Research, Mill Hill, London N.W.7

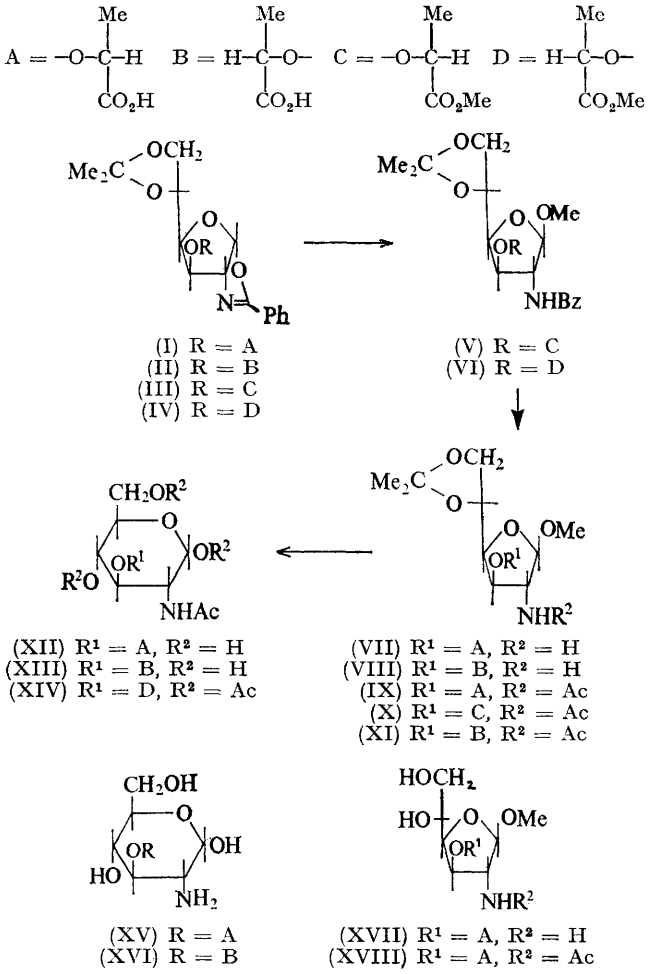
N-Acetylmuramic acid was prepared by the direct acetylation of muramic acid with acetic anhydride in aqueous methanol and by acid hydrolysis of methyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside. 2-Acetamido-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-glucose, an isomer of *N*-acetylmuramic acid, was also prepared.

THE β-(1 ⟶ 4)-linkage between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine in the glycoaminopeptide component of the bacterial cell wall [1] is cleaved by lysozyme: *N*-acetylmuramic acid and its derivatives are therefore of interest in investigating the mode of action of lysozyme.

N-Acetylmuramic acid was first prepared by Carroll [2] as a non-crystalline solid by the action of *N*-acetoxyphthalimide on muramic acid; reinvestigation of this reaction by Osawa and Jeanloz [3] showed that crystalline material could be obtained under modified conditions. Methyl or ethyl glycopyranoside derivatives of *N*-acetylmuramic acid have been intermediates in several syntheses of muramic acid [4] but these methods were not suitable for the preparation of the free *N*-acetyl sugar owing to the lability of the acetamido-group during the acid hydrolysis of the glycoside linkage. Therefore benzyl glycosides have been used as intermediates so that *N*-acetylmuramic acid could be obtained by

[1] H. J. Rogers and H. R. Perkins, 'Cell Walls and Membranes,' E. and F. N. Spon, London, 1968.
[2] P. M. Carroll, *Nature*, 1963, **197**, 694.
[3] T. Osawa and R. W. Jeanloz, *J. Org. Chem.*, 1965, **30**, 448.
[4] R. E. Strange and L. H. Kent, *Biochem. J.*, 1959, **71**, 333; R. Lambert and F. Zilliken, *Chem. Ber.*, 1960, **93**, 2915; Y. Matsushima and J. T. Park, *J. Org. Chem.*, 1962, **27**, 3581; H. M. Flowers and R. W. Jeanloz, *ibid.*, 1963, **28**, 1564; R. W. Jeanloz, E. Walker, and P. Sinaÿ, *Carbohydrate Res.*, 1968, **6**, 184.

hydrogenolysis.[3,5] Our synthesis of muramic acid [6] proceeded through the oxazoline (1). Compounds of this type are readily converted into β-methyl furanosides of 2-benzamido-2-deoxy-D-glucose under very mildly acidic conditions [7] and it was realised that if the benzamido-group was exchanged for an acetamido-group then acid hydrolysis of the intermediate should give *N*-acetylmuramic acid directly, since furanosides are more readily hydrolysed than pyranosides and the acetamido group should remain unhydrolysed.



Compound (I) [6b] was therefore converted into the methyl ester (III) with diazomethane, and this compound with methanolic hydrogen chloride gave the crystalline furanoside (V) in high yield. Alkaline hydrolysis of compound (V) gave the crystalline amino-acid (VII), which on mild acid hydrolysis gave the crystalline amino-acid (XVII). The amino-acid (VII) was most readily converted into the *N*-acetyl derivative (IX) with acetic anhydride in aqueous methanol. The amide (IX) was obtained as a syrup which gave a crystalline methyl ester (X). Very little 'lactam' formation was observed during the acetylation reaction although acetylation of muramic acid derivatives with acetic anhydride in the presence of pyridine leads to extensive 'lactam' formation.[2] The hydrolysis of acetate (IX) to *N*-acetylmuramic acid (XII) proceeded in two stages. The isopropylidene group was removed rapidly to give the amide (XVIII) and further hydrolysis of this material gave *N*-acetylmuramic acid.

N-Acetylmuramic acid was also readily formed by the action of acetic anhydride on an aqueous methanolic solution of muramic acid (XV). The product in both cases had properties similar to those reported,[3,5] but the material crystallised from ethyl acetate–methanol contained one mol. of methanol of crystallisation.

The preparation of the isomer (XIII) of *N*-acetylmuramic acid was investigated, since other workers were interested in this compound as a possible inhibitor of lysozyme. The corresponding isomer (XVI) of muramic acid has not been obtained crystalline and the hydrolysis of the intermediate (II) gave only the crude product (XVI). A direct acetylation of this crude material did not seem useful for the preparation of the pure *N*-acetyl derivative (XIII) and therefore the route through the furanoside derivatives was investigated.

The crystalline methyl ester (IV) [6b] was converted into the crystalline amino-acid (VIII) as described for the isomer and this compound was acetylated and hydrolysed to give the isomer (XIII) of *N*-acetylmuramic acid as a non-crystalline solid. For characterisation this compound was converted into the methyl ester and acetylated to give a crystalline mixture of the anomers (XIV).

EXPERIMENTAL

Solvents were evaporated off under reduced pressure. Optical rotations were measured at 22—24° with a Bendix Automatic Polarimeter. T.l.c. was carried out with microscope slides coated with silica gel G.

Methyl 2-Benzamido-3-O[D-1-(*methoxycarbonyl*)*ethyl*]-2-*deoxy*-5,6-O-*isopropylidene*-β-D-*glucofuranoside* (V).—A suspension of the acid (I) [6b] (10 g.) in ether (100 ml.) was treated with ethereal diazomethane until the solid dissolved and t.l.c. (ether) showed complete conversion of the starting material (R_F 0·2) into the ester (III) (R_F 0·8). Evaporation of the ether left the ester (III) as a syrup which was dissolved in methanolic hydrogen chloride (0·0005N; 1 l.). The solution was kept at 20° and the extent of the methanolysis was followed by t.l.c. (ether) of portions of the solution after neutralisation of the acid with silver carbonate. After 5 hr., the ester (III) had been converted into a single product (R_F 0·6) and the acid was neutralised with sodium hydrogen carbonate. A portion of the solution was evaporated to dryness and the solid residue gave *compound* (V) as needles, m.p. 109—110° (from cyclohexane), $[\alpha]_D$ +19° (*c* 1 in $CHCl_3$) (Found: C, 59·2; H, 6·7; N, 3·2. $C_{21}H_{29}NO_8$ requires C, 59·6; H, 6·9; N, 3·3%).

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

[6] (*a*) R. Gigg and P. M. Carroll, *Nature*, 1961, **191**, 495; (*b*) R. Gigg, P. M. Carroll, and C. D. Warren, *J. Chem. Soc.*, 1965, 2975; (*c*) B. Lindberg and H. Agback, *Acta Chem. Scand.*, 1964, **18**, 185.

[7] S. Konstas, I. Photaki, and L. Zervas, *Chem. Ber.*, 1959, **92**, 1288; R. Gigg and C. D. Warren, *J. Chem. Soc.*, 1965, 1351.

In the same way the crystalline ester (IV) [6b] (10 g.) was converted into the ester (VI), which was obtained as a syrup after evaporation of a portion of the solution.

Amino-acids (VII) *and* (VIII).—The methanolic solution of the ester (V) obtained above was evaporated to *ca.* 200 ml., sodium hydroxide (32 g.) was added, and the solution was heated under reflux for 72 hr. It was then cooled to 0°, 3N-hydrochloric acid (200 ml.) was added slowly with stirring, and then N-hydrochloric acid was added carefully to pH 4. Amberlite IR4B(OH^-) resin was then added to pH 5—6 and the solution was filtered and evaporated to dryness. The residue was extracted with acetone (3 × 200 ml.) and the extract evaporated to dryness. The product gave *methyl* 2-*amino*-3-O-(D-1-*carboxyethyl*)-2-*deoxy*-5,6-O-*isopropylidene*-β-D-*glucofuranoside* (VII) (5 g.) as needles, m.p. 184—186° (from methanol) $[\alpha]_D$ +18·3° (*c* 0·3 in H_2O) (Found: C, 48·4; H, 7·6; N, 4·3. $C_{13}H_{23}NO_7,H_2O$ requires C, 48·3; H, 7·8; N, 4·3%).

In the same way the solution of the ester (VI) obtained in the previous experiment was converted into the monohydrate of *methyl* 2-*amino*-3-O-(L-1-*carboxyethyl*)-2-*deoxy*-5,6-O-*isopropylidene*-β-D-*glucofuranoside* (VIII) (5·1 g.), which was obtained as needles, m.p. 178—181° (from methanol), $[\alpha]_D$ −20·9° (*c* 0·3 in H_2O) (Found: C, 48·5; H, 7·6; N, 4·4%).

Methyl 2-*Amino*-3-O-(D-1-*carboxyethyl*)-2-*deoxy*-β-D-*glucofuranoside* (XVII).—The amino-acid (VII) (0·3 g.) in 0·01N-hydrochloric acid was heated on a steam-bath for 1 hr. and the solution was cooled and treated with Amberlite IR4B(OH^-) resin to pH 5—6. It was then filtered, evaporated, and the residue gave *compound* (XVII) (0·16 g.) as needles, m.p. 205—208° (decomp.) (from methanol), $[\alpha]_D$ −6°, (*c* 0·65 in H_2O) (Found: C, 45·5; H, 7·2; N, 5·1. $C_{10}H_{19}NO_7$ requires C, 45·3; H, 7·2; N, 5·3%).

Methyl 2-*Acetamido*-3-O-[D-1-(*methoxycarbonyl*)*ethyl*]-2-*deoxy*-5,6-O-*isopropylidene*-β-D-*glucofuranoside* (X).—Acetic anhydride (5 ml.) was added dropwise during 15 min. to a stirred solution of the amino-acid (VII) (5 g.) in aqueous methanol (25 ml.) at 20°; after this time t.l.c. (ether–methanol–acetic acid 30 : 20 : 1) showed complete conversion of the starting material (R_F 0·1) into a product (R_F 0·8). The solvents were evaporated off and toluene was evaporated from the residue to remove the last traces of acetic acid, to give the amide (IX) as a syrup (5 g.).

For characterisation, a solution of the amide (IX) (0·1 g.) in methanol was treated with diazomethane in ether. The solvents were evaporated off and the residue gave *compound* (*X*) (60 mg.) as needles, m.p. 96—97° [from ether–light petroleum (b.p. 40—60°)], $[\alpha]_D$ −17·3° (*c* 0·71 in H_2O) (Found: C, 53·35; H, 7·5; N, 3·85. $C_{16}H_{27}NO_8$ requires C, 53·2; H, 7·5; N, 3·9%).

N-Acetylmuramic Acid (XII).—(*a*) Acetic anhydride (0·3 ml.) was added to a solution of muramic acid [6b] (0·3 g.) in aqueous methanol (10 ml.) at 20°. After 15 min., the solution was evaporated to dryness, the residue was dissolved in methanol, and ethyl acetate was added until a slight cloudiness appeared. The solution was then kept at 20° for 20 hr. The *N*-acetylmuramic acid (160 mg.) crystallised as prisms, m.p. 126—128° (with evolution of gas) (from ethyl acetate–methanol), $[\alpha]_D$ +73·1° (after 4 min.) ⟶ +48·3° (after 4 hr.) (*c* 0·3 in H_2O) (Found: C, 44·2; H, 7·2; N, 4·4. $C_{11}H_{19}NO_8,CH_3OH$ requires C, 44·3; H, 7·1; N, 4·3%) [lit.,[5] m.p. 122—124°, $[\alpha]_D^{22}$ +59° (8 min.) ⟶ +39° (6 hr.) (*c* 1·58 in H_2O); lit.,[3] m.p. 119—121° $[\alpha]_D^{20}$ +56° (10 min.) ⟶ +40° (24 hr.) (*c* 0·68 in H_2O)].

(*b*) A solution of the amide (IX) (1 g.) in 0·025N-hydrochloric acid (50 ml.) was heated on a steam-bath and the hydrolysis was followed by t.l.c. (ether–methanol–acetic acid 30 : 20 : 1). After 10 min. the starting material (R_F 0·8) was converted into a product (R_F 0·4) [which is assumed to be the amide (XVIII) since it was also obtained by direct acetylation of the amino-acid (XVII) with acetic anhydride in water] and after 2·25 hr. this was converted into a new product (R_F 0·5) with only traces of other products present. Sodium acetate trihydrate (170 mg., 1 equiv.) was added and the solution was evaporated to dryness. The residue was extracted with methanol and the solution diluted with ethyl acetate. The precipitate was filtered off and the solution was kept at 20° for 20 hr. The crystalline product (370 mg.) was recrystallised from methanol–ethyl acetate; m.p. and mixed m.p. with material prepared in (*a*) 126—128°.

Anomeric Mixture of the Acetates (XIV).—The amino-acid (VIII) (5 g.) was converted into the *N*-acetyl derivative (XI) as described for the preparation of the isomer (IX). The product was obtained as a syrup (5 g.) which was hydrolysed with 0·025N-hydrochloric acid as described above to give the isomer (XIII) of *N*-acetylmuramic acid (4·5 g.) as a glassy solid which could not be crystallised; $[\alpha]_D$ −18·5° (10 min.) ⟶ −28·7° (3 hr.) (*c* 0·9 in H_2O). For characterisation a solution of the compound (0·2 g.) in methanol was treated with diazomethane in ether until t.l.c. (ethyl acetate–methanol–acetic acid, 30 : 30 : 1) showed complete conversion of the starting material (R_F 0·5) into the ester (R_F 0·8). The excess of diazomethane was decomposed with acetic acid and the solvents were evaporated off to leave the methyl ester as a glass which was acetylated with acetic anhydride in pyridine. The product gave the acetates (XIV) as needles (0·1 g.), softening at 135° and forming a meniscus at 150° [from ethyl acetate–light petroleum (b.p. 40—60°)] (Found: C, 49·8; H, 6·4; N, 3·4. Calc. for $C_{18}H_{27}NO_{11}$: C, 49·9; H, 6·3; N, 3·2%).

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