533. *A Synthesis of Muramic Acid*

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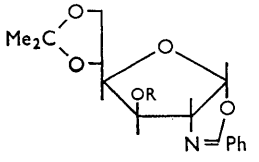
The condensation of ethyl DL-2-bromopropionate with the sodio-derivative of the oxazoline (I) derived from 2-benzamido-2-deoxy-D-glucose, gave a mixture of the esters (II) which were hydrolysed by alkali to the mixed acids (III) and (IV). These were separated by preferential crystallisation of the *S*-benzylthiouronium salt of the acid (III) from aqueous methanol. The condensation showed some stereospecificity and the acid (III) was obtained in 70% yield from the mixed acids. Hydrolysis of the acid (III) gave muramic acid.

2-Amino-3-*O*-(D-1′-carboxyethyl)-2-deoxy-D-glucose (muramic acid) is an amino-sugar found in the cell walls of bacteria,[1] and was first characterised by Strange and Kent,[2] who also synthesised it by the condensation of ethyl DL-2-iodopropionate with the sodio-derivative of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside followed

[1] M. R. J. Salton, " The Bacterial Cell Wall," Elsevier, Amsterdam, 1964.
[2] R. E. Strange and L. H. Kent, *Biochem. J.*, 1959, **71**, 333; " Methods in Carbohydrate Chemistry," Academic Press, New York, 1962, vol. 1, p. 250.

by acid hydrolysis and separation of the two isomers by ion-exchange chromatography. A similar synthetic route was adopted by Lambert and Zilliken [3] who used ethyl DL-2-bromopropionate and the corresponding β-ethyl glycoside. After partial hydrolysis of the condensation product to ethyl 2-acetamido-3-*O*-(DL-1′-carboxyethyl)-2-deoxy-β-D-glucopyranoside, the two isomers were again separated by ion-exchange chromatography. To avoid the chromatographic separation of the isomers, Matsushima and Park [4] used L-2-chloropropionic acid in the condensation and this method was also used with some modifications by Flowers and Jeanloz,[5] for the preparation of glycosides of muramic acid. In seeking a convenient synthesis of muramic acid which would not require the preparation of optically active halogenopropionic acid derivatives, we investigated the use of the



(I) R = H
(II) R = $CH(CH_3)CO_2C_2H_5$(DL)
(III) R = $CH(CH_3)CO_2H$(D)
(IV) R = $CH(CH_3)CO_2H$(L)

oxazoline (I) which was first described by Konstas, Photaki, and Zervas [6] and in a preliminary Communication [7] we reported the preparation of muramic acid and other 3-*O*-ethers of 2-amino-2-deoxy-D-glucose *via* this intermediate. Alkylation of the oxazoline (I) with ethyl DL-2-bromopropionate gave a mixture of the esters (II) which were hydrolysed by alkali to a mixture of the acids (III) and (IV) and these were separated by crystallisation from benzene. We now record the synthesis in detail, and also a more convenient method for the separation of the isomeric acids (III) and (IV). When the mixed acids were converted into the *S*-benzylthiouronium salts in aqueous methanol, only the salt of the acid (III) separated. By this method the recovery of the acid (III) was improved and the yield was greater than 50% of the total mixed acids. The conditions of the condensation of the bromo-ester with the oxazoline (I) were therefore modified to profit from this stereospecificity. By keeping the temperature low and using a large excess of the bromo-ester, the required acid (III) was obtained in a yield of 70% from the mixed acids. A similar stereospecificity in the alkylation of benzyl and other glycosides of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose with DL-2-chloropropionic acid has also been observed.[8] Several analogues of muramic acid have recently been prepared [9] using the oxazoline (I) as an intermediate.

Experimental

Infrared spectra were measured in potassium chloride discs.

Mixed Acids (III) *and* (IV).—The oxazoline (I) [6,10] (10 g., 33 mmole) was added in portions to a suspension of sodium hydride (2·5 g. of a " 50% dispersion in oil," L. Light and Co. Ltd., 50 mmole) in dry tetrahydrofuran (100 ml.) under dry nitrogen. When the initial vigorous reaction had subsided, the mixture was heated under reflux with stirring for 2 hr. The solution was cooled to 10°, ethyl DL-2-bromopropionate (30 g., 165 mmole) was added, and the solution was stirred at 10° for 2 hr. and then at room temperature for 2 hr. Methanol was added to decompose the excess of sodium hydride and the tetrahydrofuran and excess of bromo-ester were distilled off under reduced pressure. Methanol (12 ml.) and a solution of sodium hydroxide (2 g.) in water (5 ml.) were added to the residue and the solution was heated on a steam-bath for

[3] R. Lambert and F. Zilliken, *Chem. Ber.*, 1960, **93**, 2915.
[4] Y. Matsushima and J. T. Park, *J. Org. Chem.*, 1962, **27**, 3581; *Biochem. Prep.*, 1963, **10**, 109.
[5] H. M. Flowers and R. W. Jeanloz, *J. Org. Chem.*, 1963, **28**, 1564, 2983.
[6] S. Konstas, I. Photaki, and L. Zervas, *Chem. Ber.*, 1959, **92**, 1288.
[7] R. Gigg and P. M. Carroll, *Nature*, 1961, **191**, 495.
[8] T. Osawa, E. Walker, and R. W. Jeanloz, *Fed. Proc.*, 1964, **23**, 379.
[9] B. Lindberg and H. Agback, *Acta Chem. Scand.*, 1964, **18**, 185.
[10] R. Gigg and C. D. Warren, *J.*, 1965, 2205.

15 min. Water (150 ml.) was added and the solution was extracted with ether (3 × 50 ml.) to remove the oil and any unchanged oxazoline (I). The dissolved ether was removed from the aqueous solution by heating under vacuum, and the solution was then acidified to pH 4 by the careful addition of N-hydrochloric acid with stirring. The mixed acids (11·5 g., 93%) (III) and (IV) which precipitated were filtered off, washed thoroughly with water, and dried.

S-Benzylthiouronium Salt of the Acid (III).—The mixed acids (11·5 g.) were dissolved in methanol (115 ml.) and N-sodium hydroxide solution (~31 ml.) was added to an end-point with phenolphthalein as indicator. Water (115 ml.) was added and the solution was warmed to 40° and mixed with a warm (40°) solution of *S*-benzylthiouronium chloride (6·3 g.) in water (310 ml.). The solution was kept at room temperature for 5 hr. and the crystalline salt (10·1 g., 61%) filtered off. The filtrate was acidified carefully to pH 4 and the precipitated acids (4·3 g.) were extracted with chloroform. The acids were reconverted into the *S*-benzylthiouronium salts as described above and the crystalline salt (1·4 g.) of the acid (III) was filtered off. The *salt* (11·5 g., 70%) of the acid (III) was recrystallised from 20% aqueous methanol giving needles, m. p. 134—136°, $[\alpha]_D^{23}$ +32° (*c* 1·4 in methanol) (Found: C, 59·3; H, 6·5; N, 7·4; S, 5·5. $C_8H_{10}N_2S,C_{19}H_{23}NO_7$ requires C, 59·6; H, 6·1; N, 7·7; S, 5·9%).

Acid (III).—The *S*-benzylthiouronium salt (11·5 g.) of the acid (III) was suspended in water (25 ml.) and ether (200 ml.) and then N-hydrochloric acid (21 ml.) was added with stirring. The ether solution was separated, washed with water, and dried ($MgSO_4$). After evaporation of the ether the residual crystalline solid (7·8 g., 98%) was recrystallised from ethyl acetate to give the *acid* (III), m. p. 163—164°, $[\alpha]_D^{20}$ +65° (*c* 2 in chloroform), $\nu_{max.}$ 1635 (C=N) and 1725 cm.$^{-1}$ (C=O) (Found: C, 60·3; H, 6·2; N, 3·8. $C_{19}H_{23}NO_7$ requires C, 60·5; H, 6·1; N, 3·7%).

Acid (IV).—The mother-liquors from the preparation of the crystalline *S*-benzylthiouronium salt of the acid (III) were carefully acidified to pH 4 by the addition of N-hydrochloric acid and the precipitated acids were extracted with chloroform and recrystallised from benzene to give the *acid* (IV) (1·8 g.), m. p. 208—210°, $[\alpha]_D^{24}$ −34° (*c* 2 in chloroform), $\nu_{max.}$ 1635 (C=N) and 1705 cm.$^{-1}$ (C=O) (Found: C, 60·6; H, 6·0; N, 3·8. $C_{19}H_{23}NO_7$ requires C, 60·5; H, 6·1; N, 3·7%). The *methyl ester* of the acid (IV) was prepared by the action of diazomethane and was recrystallised from light petroleum (b. p. 60—80°), m. p. 88—90°, $[\alpha]_D^{22}$ −31° (*c* 1 in chloroform), $\nu_{max.}$ 1645 (C=N) and 1730 cm.$^{-1}$ (C=O) (Found: C, 61·4; H, 6·5; N, 3·6. $C_{20}H_{25}NO_7$ requires C, 61·4; H, 6·4; N, 3·6%).

Muramic Acid.—The acid (III) (1 g.) and hydrochloric acid (10 ml., 2·6N) were heated under reflux for 4 hr. After cooling, the benzoic acid was extracted with ether and the aqueous solution was evaporated under reduced pressure. The residue was diluted with water and the hydrogen chloride was removed by the addition of Amberlite IR4B (OH) resin. After filtration, the solution was decolourised with charcoal and the volume was reduced (to ~10 ml.) by evaporation under reduced pressure. Acetone (~10 ml.) was added until a slight cloudiness appeared and the solution was cooled to 4° and more acetone was added after the crystallisation had started. After 24 hr. at 4° the crystals (0·3 g.) were collected and recrystallised from acetone–water to give needles of hydrated muramic acid (0·25 g., 31%), m. p. 150°, $[\alpha]_D^{22}$ +114° (*c* 1 in water; final value) (Found: C, 37·6; H, 7·3; N, 4·8. Calc. for $C_9H_{17}NO_7,2H_2O$: C, 37·3; H, 7·1; N, 4·9%) {lit.,[2] $[\alpha]_D^{20}$ +109° (*c* 2 in water); lit.,[3] $[\alpha]_D^{25}$ +123° (*c* 3 in water); lit.,[4] m. p. 152—154°, $[\alpha]_D^{25}$ +103° (*c* 2·6 in water)}. The muramic acid was dehydrated by heating at 100° for 12 hr. over phosphoric oxide and after correction for a 1·4% ash content the analytical figures were: Found: C, 43·0; H, 6·8; N, 5·6; Calc. for $C_9H_{17}NO_7$: C, 43·0; H, 6·8; N, 5·6%.

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