Ester and Lactone Linkages in Acidic Polysaccharides. Part II. Lactones of* D-*Glucosaminic Acid.*

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Methylation and hydrolysis of ethyl *N*-benzoyl-4 : 6-*O*-benzylidene-D-glucosaminate leads to the crystalline 2-benzamido-2-deoxy-3 : 5-di-*O*-methyl-D-glucono-1 : 4-lactone. Evidence has been found for the lactonisation of anionic salts ($^-NH_2^+$) of D-glucosaminic acid and of its *N*-acetyl, -toluene-*p*-sulphonyl, and -2 : 4-dinitrophenyl derivatives. *N*-Benzoyl-D-glucosaminic acid has been characterised as a 1 : 5-lactone.

In Part I* the susceptibility of certain acidic polysaccharides to lactonisation was correlated with the positions of the glycosidic linkages. We have now investigated the influence, on the course of lactonisation, of a 2 amino-group, as exemplified by D-glucosaminic acid (2-amino-2-deoxy-D-gluconic acid) and its derivatives.

In general, attempts to isolate D-gluconic acid from solution have resulted in the formation of a 1 : 4- or 1 : 5-lactone, whereas by the same processes D-glucosaminic acid is invariably obtained as the free acid. The results of Fischer and Leuchs (*Ber.*, 1903, **36**, 24) suggest however that conditions exist in which derivatives of glucosaminic acid lactonise, as in the reduction of D-glucosaminic acid to D-glucosamine. Lactones of *N*-acetyl-D-galactosaminic acid (Karrer and Meyer, *Helv. Chim. Acta*, 1937, **20**, 407) and *N*-methyl-L-glucosaminic acid (Wolfrom, Thompson, and Hooper, *Science*, 1946, **104**, 276) have also been described although their ring form has not been characterised : in these instances, the amino-group was substituted. In order to study the unambiguous behaviour of a 1 : 4-lactone, 2-benzamido-2-deoxy-3 : 5-di-*O*-methyl-D-glucono-1 : 4-lactone was synthesised by the following reactions. D-Glucosaminic acid with benzaldehyde in acidic conditions afforded a compound (m. p. 129°) having the analytical composition of ethyl 4 : 6-*O*-benzylidene-D-glucosaminate hydrochloride. The compound failed to form a hydroxamic acid, as other α-amino-esters do, and when recrystallised from pryidine–methanol gave 4 : 6-*O*-benzylidene-D-glucosaminic acid. It was therefore concluded that the compound was 4 : 6-*O*-benzylidene-D-glucosaminic acid hydrochloride with one molecule of ethanol of crystallisation. The existence of this compound may explain the divergent melting points (200°, Levene, *J. Biol. Chem.*, 1922, **53**, 449; 167—168°, Levene and La Forge, *ibid.*, 1915, **21**, 345; 126°, Levene, "Hexosamines and Mucoproteins," London, Longmans, 1926) reported for ethyl 4 : 6-*O*-benzylidene-D-glucosaminate.

The true ester hydrochloride, obtained from the above benzylidene compound by reaction with hot ethanolic hydrogen chloride, was converted into crystalline *N*-benzyloxycarbonyl and *N*-benzoyl esters. Methylation of the latter by Purdie's reagents resulted in a

* Part I, *J.*, 1953, 79.

liquid di-*O*-methyl derivative which after mild hydrolysis gave crystalline 2-benzamido-2-deoxy-3 : 5-di-*O*-methyl-D-glucono-1 : 4-lactone. The 1 : 4-lactone structure is supported by (i) the elementary composition, (ii) the neutrality of the compound and its reaction with one mol. of alkali, (iii) the formation of a hydroxamic acid under the same conditions as D-glucono-1 : 4-lactone gives one, and (iv) the resemblance of the rate of mutarotation ($[\alpha]_D^{20}$ $+77° \rightarrow +70°$ in 10 days) to that of 1 : 4-lactones.

Benzoylation of D-glucosaminic acid yielded a crystalline derivative having the properties of 2-benzamido-2-deoxy-D-glucono-1 : 5-lactone. This substance readily formed a hydroxamic acid and, in contrast to the 1 : 4-lactone, mutarotated rapidly ($[\alpha]_D^{21}$ $+118° \rightarrow$ 42° in 36 hr.) in common with other 1 : 5-lactones. It reacted readily with hydrazine and phenylhydrazine, giving hydrazide and phenylhydrazide.

Acetylation of D-glucosaminic acid with keten gave a non-crystalline product which nevertheless exhibited typical relations of a lactone. The *N*-2 : 4-dinitrophenyl derivative of the derived acid crystallised from aqueous solution as a lactone, as also did the *N*-toluene-*p*-sulphonyl derivative.

D-Glucosaminic acid, on the other hand, failed to form a hydroxamic acid even after being heated in the dry state at temperatures between 25° and 200° for 0·5—3 hr. The acid, heated or otherwise, had zero optical rotation in aqueous solution when examined in sodium-D light or at 5461 Å. In dilute hydrochloric acid (2·5% w/v), D-glucosaminic acid exhibited mutarotation ($[\alpha]_D^{21}$ $-16·5° \rightarrow -14°$ in 48 hr.).

It is consistent with these results to ascribe the failure of D-glucosaminic acid to lactonise to zwitterion formation (Sidgwick, "Organic Chemistry of Nitrogen," Oxford, 1937). This is known to be an exothermic reaction: the reaction $NH_2 \cdot CH_2 \cdot CO_2H \longrightarrow {}^+NH_3 \cdot CH_2 \cdot CO_2^-$ has ΔH $-11·5 \pm 1$ kcal./mole. It appears likely therefore that similar ionization stabilises D-glucosaminic acid. In acidic solution or other conditions for *N*-substituted glucosaminic acids where zwitterion formation is impossible, lactonisation appears to take the course usual for hexonic acids.

Experimental.

D-Glucosaminic Acid.—D-Glucosamine hydrochloride (10 g.) in water (100 ml.), mixed with freshly precipitated yellow mercuric oxide (30 g.) suspended in water (400 ml.), was heated with stirring at 95—100° for 25 min. The filtered solution was treated with hydrogen sulphide and, after separation of the precipitate, was concentrated at 50° under reduced pressure until crystallisation was observed. An equal volume of hot methanol was added. The product (6·7 g.), recrystallised from aqueous methanol, decomposed at 250° and had $[\alpha]_D^{23}$ 0° ± 2° in H_2O (*c* 0·83), $[\alpha]_D^{21}$ $-16·5°$ $\longrightarrow$ $-14°$ in 2·5% HCl (*c* 1·64) (Found: N, 7·1. Calc. for $C_6H_{13}O_6N$: N, 7·2%).

4 : 6-O-*Benzylidene*-D-*glucosaminic Acid Hydrochloride.*—The preceding acid (2 g.) in dry ethanol (15 ml.) and freshly distilled benzaldehyde (3 ml.) was saturated with dry hydrogen chloride at room temperature. The mixture which rapidly solidified was washed with ether (100 ml.), and the residue recrystallised from dry ethanol. The solvated 4 : 6-O-*benzylidene*-D-*glucosaminic acid hydrochloride* (2·9 g.) had m. p. 129°, $[\alpha]_D^{25}$ $-54·4°$ (*c* 0·65 in MeOH), $[\alpha]_D^{25}$ $-30·2°$ (*c* 0·86 in H_2O) (Found: C, 49·4; H, 6·3; N, 4·0; OEt, 13·3. $C_{15}H_{24}O_7NCl$ requires C, 49·1; H, 6·55; N, 3·8; OEt, 13·0%). When recrystallised from methanol containing 5% of pyridine (or an equivalent amount of ammonia or hydroxylamine) this yielded 4 : 6-*O* benzylidene-D-glucosaminic acid (Levene, *J. Biol. Chem.*, 1922, **53**, 449), m. p. 214—220 (decomp.) (Found: C, 55·0; H, 5·9; N, 4·7. Calc. for $C_{13}H_{17}O_6N$: C, 55·1; H, 6·05; N, 4·9%).

Ethyl 4 : 6-O-*Benzylidene*-D-*glucosaminate Hydrochloride.*—The above benzylidene derivative (3 g.) was dissolved in ethanol (10 ml.), and the solution saturated with dry hydrogen chloride. After 3 hr. ether (1 vol.) was added and the resulting ester (2·6 g.), recrystallised from dry ethanol, had m. p. 168°, $[\alpha]_D^{23}$ $-31·7°$ (*c* 0·98 in H_2O) (Found: C, 51·9; H, 6·3; N, 4·1; Cl, 10·5. Calc. for $C_{15}H_{22}O_6NCl$: C, 51·8; H, 6·3; N, 4·0; Cl, 10·2%).

Ethyl N-*Benzoyl*-4 : 6-O-*benzylidene*-D-*glucosaminate.*—The foregoing ester salt (3 g.) was dissolved at 0° in water (20 ml.) containing sodium hydrogen carbonate (1·8 g.), and benzoyl chloride (1·2 ml.) was added in 0·1-ml. portions, with continuous shaking, during 30 min. The resulting solid was recrystallised from ethanol, and the N-*benzoyl ester* (3·5 g.) had m. p. 167—168°, $[\alpha]_D^{23}$ $-81·3°$ (*c* 1·92 in $CHCl_3$) (Found: C, 63·7; H, 6·2; N, 3·4. $C_{22}H_{25}O_7N$ requires C, 63·6; H, 6·0; N, 3·4%).

Ethyl 4 : 6-O-*Benzylidene*-N-*benzyloxycarbonyl*-D-*glucosaminate*.—The ester salt (0·5 g.) was treated with benzoyl chloroformate (0·8 g.), water (5 ml.), and sodium hydrogen carbonate (0·3 g.). The resulting *product* (0·62 g.), recrystallised from ethanol, had m. p. 141—142°, $[\alpha]_D^{22}$ −56·4° (*c* 1·12 in $CHCl_3$) (Found: C, 62·0; H, 6·3; N, 3·1. $C_{23}H_{27}O_8N$ requires C, 62·0; H, 6·1; N, 3·1%).

2-*Benzamido*-2-*deoxy*-3 : 5-*di*-O-*methyl*-D-*glucono*-1 : 4-*lactone*.—Ethyl *N*-benzoyl-4 : 6-*O*-benzylidene-D-glucosaminate (3 g.) was boiled with dry acetone (5 ml.), methyl iodide (10 ml.), and dry silver oxide (10 g.) for 4 hr. Fresh methyl iodide (5 ml.) and silver oxide (5 g.) were added and the mixture heated for a similar period. The product obtained on evaporation of the filtered solution was remethylated in the same way. The *di*-O-*methyl* derivative, n_D^{20} 1·5480, failed to crystallise (Found: OEt, 9·7; OMe, 13·5. $C_{24}H_{29}O_7N$ requires OEt, 10·2; OMe 13·8%).

The di-*O*-methyl compound (0·5 g.) was heated at 100° for 24 hr. with acetic acid (60% v/v; 10 ml.) and then concentrated under reduced pressure at 80°. The product was made alkaline with N-sodium hydroxide and set aside at room temperature for 3 hr. The solution, neutralised (phenolphthalein) with sulphuric acid, was concentrated under reduced pressure until an oil separated, which was caused to redissolve by addition of ethanol. When cooled to 0°, the *di*-O-*methyl*-1 : 4-*lactone* (0·08 g.) separated and had m. p. 186°, $[\alpha]_D^{20}$ +76·9° → +70° in 10 days (*c* 1 in 50% aqueous ethanol) (Found: C, 57·9; H, 6·7; N, 4·2. $C_{15}H_{19}O_6N$ requires C, 58·25; H, 6·15; N, 4·5%).

2-*Benzamido*-2-*deoxy*-D-*gluconohydrazide*.—The lactone (0·05 g.) was heated with 50% aqueous hydrazine hydrate (0·5 ml.) and the minimum amount of ethanol necessary for solution. On cooling, the *hydrazide* (0·046 g.) separated and after recrystallisation from ethanol had m. p. 196—197° (Found: C, 49·6; H, 5·9; N, 13·5. $C_{13}H_{19}O_6N_3$ requires C, 49·3; H, 6·1; N, 13·4%).

2-*Deoxy*-2-*toluene*-p-*sulphonamido*-D-*gluconolactone*.—D-Glucosaminic acid (2·06 g.), dissolved in N-sodium hydroxide (30 ml.), was shaken with toluene-*p*-sulphonyl chloride (2 g.) in ether (25 ml.). After 3 hr. the aqueous layer was neutralised with 5N-sulphuric acid (6 ml.), and the resulting solid recrystallised from ethanol. The *toluene*-p-*sulphonyl* derivative (2·8 g.) had m. p. 172—173°, $[\alpha]_D^{20}$ −24·7° (*c* 0·193 in 1 : 1 aq. EtOH) (Found: C, 42·4; H, 5·6; N, 3·6. $C_{13}H_{21}O_9NS$ requires C, 42·5; H, 5·7; N, 3·8%).

2-*Deoxy*-2-*toluene*-p-*sulphonamidogluconophenylhydrazide*.—The preceding derivative (0·06 g.) was heated for 30 min. on a water-bath with phenylhydrazine (0·04 g.) in methanol (3 ml.). The *phenylhydrazide* (0·052 g.) which separated from the cooled solution crystallised from ethanol and had m. p. 178—179° (Found: C, 50·6; H, 5·8; N, 9·8. $C_{19}H_{25}O_7N_3S$ requires C, 50·6; H, 6·7; N, 9·6%).

2-*Deoxy*-2 : 4-*dinitroanilino*-2-*gluconolactone*.—D-Glucosaminic acid (1 g.) was treated with N-sodium hydroxide (10 ml.) and 1-fluoro-2 : 4-dinitrobenzene and after 2 hr. the mixture was extracted with ether. The aqueous layer was acidified with 5N-hydrochloric acid (2·5 ml.). The resulting N-2 : 4-*dinitrophenyl* derivative (0·3 g.) after being thrice recrystallised from water had m. p. 193—194° (Found: C, 41·3; H, 3·6; N, 11·8. $C_{12}H_{13}O_9N_3$ requires C, 42·0; H, 3·8; N, 12·25%).

2-*Benzamido*-2-*deoxy*-D-*glucono*-1 : 5-*lactone*.—D-Glucosaminic acid (2 g.) in 0·8N-sodium hydroxide (25 ml.) was treated at 0° with benzoyl chloride (1·2 ml.). After acidification with 5N-hydrochloric acid and filtration a solid separated from the cooled solution. The *lactone* (0·6 g.), recrystallised from ethanol, had m. p. 192—193°, $[\alpha]_D^{21}$ +118° → +42° in 36 hr. (*c* 0·57 in H_2O) (Found: C, 55·7; H, 5·6; N, 4·5. $C_{13}H_{15}O_6N$ requires C, 55·5; H, 5·3; N, 5·0%).

An aqueous solution (10 ml.) of the lactone (57·1 mg.) exhibited the following rotations at 21°: 0·69° (0 hr.), 0·51° (6 hr.), 0·40° (12 hr.), 0·33° (18 hr.), 0·28° (24 hr.), 0·24° (const., 36 hr.).

2-*Benzamido*-2-*deoxy*-N-*phenyl*-D-*gluconohydrazide*.—The preceding lactone (0·05 g.) with phenylhydrazine (0·03 g.) in hot ethanol (4 ml.) afforded after 30 min. a crystalline *phenylhydrazide* (0·052 g.) which, after recrystallisation from aqueous methanol, had m. p. 200—201° (Found: C, 58·3; H, 5·8; N, 10·8. $C_{19}H_{23}O_6N_3$ requires C, 58·6; H, 5·9; N, 10·8%).

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