975. Pneumococcus *Type V Capsular Polysaccharide: Characterisation of Pneumosamine as 2-Amino-2,6-dideoxy-L-talopyranose.*

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2-Amino-2,6-dideoxy-D-talopyranose has been synthesised and found to be enantiomorphous with an amino-sugar present in *Pneumococcus* type V capsular polysaccharide.

Pneumococcus type V was first described by Cooper, Edwards, and Rosenstein [1] and was identified with the sub-group IIA of Avery [2] and with a strain of group IVE of Robinson.[3] Although few structural investigations have been carried out on type V *Pneumococcus* polysaccharide its alkali lability was recognised by Brown [4] who also suggested the presence of amino-sugar(s). An electrophoretically homogeneous polysaccharide, $[\alpha]_D$ −83° and containing 4% of nitrogen, was obtained in our laboratory by purification with Cetavlon.[5]

With N-hydrochloric acid at 100° for 1 hr., the purified polysaccharide gave glucose (19·5%), glucuronic acid (24%), a disaccharide, a trisaccharide, and an amino-sugar " pneumosamine " (30%). A second amino-sugar, 2-amino-2,6-dideoxy-L-galactopyranose, was a component of both the di- and the tri-saccharide.[5,6] These amino-sugars are probably present in the polysaccharide as their *N*-acetyl derivatives. Structural investigations [5,6] indicated that pneumosamine was probably a 2-amino-2,6-dideoxyhexose. Epimerisation of 2-acetamido-2,6-dideoxy-α-L-galactopyranose in the presence of nickel acetate and pyridine [6] gave, *inter alia*, a component which had chromatographic properties indistinguishable from those of *N*-acetylpneumosamine. This suggested that pneumosamine has the *talo*-configuration. We now report syntheses which establish that pneumosamine has the L-*talo*-configuration.

Displacement of secondary toluene-*p*-sulphonyloxy-groups with ammonia,[7] dimethylamine,[8] or hydrazine [9,10] is believed to be an S_N2 process with consequent inversion of configuration at the relevant carbon atom. Thus, 1,2:5,6-di-*O*-isopropylidene-3-*O*-tosyl-α-D-glucofuranose is converted by hydrazine into 3-amino-3-deoxy-D-allofuranose derivatives.[10] By analogy, a similar reaction of methyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-tosyl-α-D-galactopyranoside should lead to derivatives of 2-amino-2-deoxy-D-talose. Methyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-tosyl-α-D-galactopyranoside reacted sluggishly with hydrazine and several treatments were necessary to produce a workable amount of the hydrazino-derivative.

Several workers [9,11,12] have shown that hydrazinolysis of toluene-*p*-sulphonyloxy-groups is subject to serious steric restrictions. Horton, Wolfrom, and Thompson [12] attribute the lack of reactivity of methyl 3,5-*O*-isopropylidene-2-*O*-tosyl-β-L-xylofuranoside towards hydrazine to steric hindrance resulting from the methoxy- and the isopropylidene group's lying on the same side of the ring. Examination of molecular models

[1] Cooper, Edwards, and Rosenstein, *J. Exp. Med.*, 1929, **49**, 461.
[2] Avery, *J. Exp. Med.*, 1915, **22**, 804.
[3] Robinson, *J. Infect. Dis.*, 1927, **41**, 417.
[4] Brown, *J. Immunol.*, 1939, **37**, 445.
[5] Barker, Stacey, and Williams, *Bull. Soc. Chim. biol.*, 1960, **12**, 1611.
[6] Barker, Brimacombe, How, Stacey, and Williams, *Nature*, 1961, **189**, 303.
[7] Tipson, *Adv. Carbohydrate Chem.*, 1953, **8**, 107; Freudenberg, Burkhart, and Braun, *Ber.*, 1926, **59**, 714.
[8] Freudenberg and Smeykal, *Ber.*, 1926, **59**, 100.
[9] Lemieux and Chu, *J. Amer. Chem. Soc.*, 1958, **80**, 4745; Cope and Shen, *ibid.*, 1956, **78**, 3177; Wolfrom, Shafizadeh, and Armstrong, *ibid.*, 1958, **80**, 4885; Wolfrom, Shafizadeh, Armstrong, and Shen Han, *ibid.*, 1959, **81**, 3716.
[10] Coxon and Hough, *J.*, 1961, 1643.
[11] Roth and Pigman, *J. Org. Chem.*, 1961, **26**, 2455.
[12] Horton, Wolfrom, and Thompson, *J. Org. Chem.*, 1961, **26**, 5069.

revealed little hindrance to the approach of the nucleophile to C-2 of methyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-tosyl-α-D-galactopyranoside although there was considerable interference by the methoxy-group in the β-anomer. Steric effects cannot therefore be invoked in our case. It is known [9,11,12] that toluene-*p*-sulphonyloxy-groups attached to furanose rings are more readily displaced by hydrazine than are those in pyranose analogues. The facility of such nucleophilic displacements in the furanose compounds may be due to the relative ease of attainment of the planar transition state.

Reduction of the hydrazino-derivative derived from methyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-tosyl-α-D-galactopyranoside, followed by *N*-acetylation and acid hydrolysis, yielded, *inter alia*, a product which had chromatographic properties indistinguishable from those of pneumosamine. Fractionation of the product mixture on Dowex 50 (H^+) gave 2-amino-2,6-dideoxy-D-talopyranose hydrochloride, m. p. 161·5—163·5°, $[\alpha]_D$ +8° (4 min.) ⟶ −6° (10 min.) ⟶ −10° (final, *c* 1·71 in water). The direction of mutarotation is suggestive of the α-configuration. A similar mutarotation, $[\alpha]_D$ +3·4° (3 min.)⟶ −5·7° (final), was quoted by Kuhn and Fischer [13] for 2-amino-2-deoxy-α-D-talopyranose hydrochloride.

Confirmation that the synthetic sugar had the *talo*-configuration was afforded by its physical constants which were different from those {m. p. 192—193°, $[\alpha]_D^{27}$ −119° (2 min.) ⟶ −92° (final)} of the *galacto*-derivative [14] and by oxidative deamination with ninhydrin [15] which yielded a product indistinguishable in chromatographic properties from 5-deoxylyxose.

Since pneumosamine hydrochloride has m. p. 162—163°, $[\alpha]_D$ +7° (10 min.) ⟶ +10° (final, *c* 2·3 in water), and chromatographic and electrophoretic properties indistinguishable from those of the synthetic sugar, it may be assigned the structure 2-amino-2,6-dideoxy-L-talopyranose.

Since pneumosamine and 2-amino-2,6-dideoxy-L-galactopyranose are epimeric at C-2, one of them might have arisen by epimerisation during hydrolysis of type V capsular polysaccharide. That this is probably not the case is suggested by the following observations. Only 2-amino-2,6-dideoxy-L-talose (pneumosamine) is liberated on mild acid hydrolysis, together with a di- and a tri-saccharide, both of which contain 2-amino-2,6-dideoxy-L-galactopyranose.[5] Hydrolysis of the di- and the tri-saccharide required more vigorous conditions and yielded a single amino-sugar. Secondly, the conditions of hydrolysis do not normally cause epimerisation when other polysaccharides containing amino-sugars are degraded, although Muir [16] has detected both 2-amino-2-deoxy-D-galactose and 2-amino-2-deoxytalose in hydrolysates of chondroitin sulphate complexes after prolonged hydrolysis with 2–6N-hydrochloric acid.

Experimental

M. p.s are corrected. Paper chromatograms were run on Whatman No. 1 paper by downward irrigation with the organic phase of one of the following solvent systems: (*a*) butan-1-ol–ethanol–water (4 : 1 : 5); (*b*) butan-1-ol–acetic acid–water (4 : 1 : 5); (*c*) butan-1-ol–ethanol–water–ammonia (*d* 0·88) (40 : 10 : 49 : 1); (*d*) ethyl acetate–acetic acid–water (3 : 1 : 3).

Methyl 3,4-O-*Isopropylidene*-2,6-*di*-O-*tosyl*-α-D-*galactopyranoside*.—This compound, m. p. 150—151°, was prepared essentially by the method described by Iselin and Reichstein [17] who quote m. p. 149—150°.

Methyl 6-*Deoxy*-3,4-O-*isopropylidene*-2-O-*tosyl*-α-D-*galactopyranoside*.—A solution of the foregoing glycoside (8·9 g.) in dry teterahydrofuran (45 ml.) was added to a stirred suspension of lithium aluminium hydride (4 g.) in tetrahydrofuran (45 ml.) and the mixture refluxed for 1 hr.

[13] Kuhn and Fischer, *Annalen*, 1958, **612**, 65.
[14] Kuhn, Bister, and Dafeldecker, *Annalen*, 1959, **628**, 186.
[15] Stoffyn and Jeanloz, *Arch. Biochem. Biophys.*, 1954, **52**, 373.
[16] Muir, *Biochem. J.*, 1957, **65**, 33P.
[17] Iselin and Reichstein, *Helv. Chim. Acta*, 1946, **29**, 508.

The mixture was cooled (0°), the excess of reagent destroyed by dropwise addition of ethyl acetate, and the solution brought to pH 7 by addition of equal parts of M-sodium potassium tartrate and M-tartaric acid. The neutral solution was extracted with chloroform (6 × 50 ml.), and the combined extracts were washed with water, and dried (Na_2SO_4). Removal of the solvent gave the *product* (3·3 g., 54%), m. p. 184—185° (from acetone–ether), $[\alpha]_D^{23}$ +156° (*c* 2·64 in chloroform) (Found: C, 54·6; H, 6·5; S, 8·7. $C_{17}H_{24}O_7S$ requires C, 54·8; H, 6·5; S, 8·6%). Iselin and Reichstein [17] quote m. p. 186—188°, $[\alpha]_D^{20}$ +158° (*c* 1 in chloroform) for this compound prepared by another route.

2-*Amino*-2,6-*dideoxy*-α-D-*talopyranose Hydrochloride.*—(*a*) *Displacement with hydrazine.* Methyl 6-deoxy-3,4-*O*-isopropylidene-2-*O*-tosyl-α-D-galactopyranoside (22·7 g.) and anhydrous hydrazine (145 ml.) were heated under reflux at 140—150° for 67 hr. in a stream of oxygen-free nitrogen. The cooled solution was filtered, unchanged material (14·3 g.) was washed with distilled water, and the combined filtrate and washings were extracted with chloroform (4 × 200 ml.). The dried (Na_2SO_4) extracts were concentrated at 35° under reduced pressure to a syrup which was dissolved in 75% ethanol and stirred with Raney nickel for 3 hr. at room temperature. After addition of more Raney nickel the mixture was hydrogenated at 45—50 lb. per sq. in. for 18 hr. at room temperature in the Parr apparatus. The filtered solution was concentrated at 30—40° to give a syrup (0·89 g.); paper chromatography (solvent *b*) indicated the presence of two major components, $R_{glucose}$ 5·2 and 6·8, and several minor components which reacted with ninhydrin reagent.[18] Essentially the above reaction sequence was repeated three times with the unchanged starting material to yield 1·2 g., 1·8 g., and 2.1 g. of syrup, each of which was treated severally as described below in (*b*).

(*b*) N-*Acetylation and hydrolysis.* To a stirred and cooled (0°) solution of the foregoing syrup (0·89 g.) in water (40 ml.) and methanol (2·8 ml.) was added Dowex-1 (CO_3^{2-}) (80 ml.) and redistilled acetic anhydride (1·4 ml.), and reaction was allowed to proceed for 90 min. The combined filtrate and washings were stirred for 10 min. with Amberlite IR-120 (H^+), the solution was filtered, and the resin thoroughly washed with water. The combined filtrate and washings were freeze-dried to give a residue (0·56 g.) which was hydrolysed with 2N-hydrochloric acid (65 ml.) at 95—100° for 8 hr. Paper chromatography of the hydrolysate in solvent *b* showed the presence of three components with $R_{glucose}$ 1·28, 1·49, 2·0, which reacted with ninhydrin reagent; [18] the first two components also reacted with the Elson–Morgan reagents.[19]

(*c*) *Fractionation on Dowex*-50 (H^+). D-Glucosamine hydrochloride (20 mg.) was added as a marker to the combined hydrolysates from the previous experiment. The solution was neutralised with silver carbonate, the insoluble silver salts were removed by centrifugation and washed with water (3 × 100 ml.), and hydrogen sulphide was bubbled through the combined supernatant liquid and washings. The filtered solution was concentrated to *ca.* 250 ml. and applied to a freshly regenerated column of Dowex-50 (H^+) (33 × 6·5 cm.; 200—400 mesh). The column was washed with water (1 l.) and eluted with 0·3N-hydrochloric acid. Fractions (20 ml.) were collected automatically. A portion (1 ml.) of each of the fractions was analysed for amino-sugars with the Elson–Morgan reagent.[19] The fraction eluted from the column between 6·8—8·6 l. ($R_{glucosamine}$ 2·4) was neutralised with De-Acidite-FF (CO_3^{2-}), filtered, and freeze-dried. The residue (0·78 g.) was treated with an equivalent amount of 0·3N-hydrochloric acid and freeze-dried, the resulting pale yellow syrup was triturated with a small volume of 0·3N-hydrochloric acid, and residual acid was removed *in vacuo* (over P_2O_5). The crystalline material deposited was filtered off, washed with cold dry methanol, and dried. 2-*Amino*-2,6-*dideoxy*-α-D-*talopyranose hydrochloride* had m. p. 161·5—163·5°, $[\alpha]_D^{18\cdot5}$ +8° (4 min.) ⟶ −6° (10 min.) ⟶ −10° (final, *c* 1·71 in water) (Found: C, 36·2; H, 6·9; N, 6·8. $C_6H_{14}ClNO_4$ requires C, 36·1; H, 7·1; N, 7·0%). The chromatographic (solvents *a*—*d*) and electrophoretic properties (acetate buffer, pH 5·5) of the synthetic sugar were indistinguishable from those of pneumosamine hydrochloride. The infrared spectrum (Nujol mull) of the synthetic sugar was consistent with the proposed structure, and, in particular, showed NH_4^+ stretching bands [20] at 1985, 2630, and 2765 cm.$^{-1}$. The infrared spectrum previously recorded for pneumosamine hydrochloride was not sufficiently detailed to justify direct comparison of the two spectra.

Oxidative Deamination Experiments.—A solution of 2-amino-2,6-dideoxy-α-D-talopyranose

[18] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.
[19] Partridge, *Biochem. J.*, 1948, **42**, 238.
[20] Jones and Sandorfy, "Chemical Applications of Spectroscopy," Interscience Publ. Inc., New York, 1956, p. 515.

hydrochloride (25 mg.) in water was treated with a 2% aqueous solution of ninhydrin containing 4% of pyridine for 1 hr. by essentially the procedure described by Stoffyn and Jeanloz [15] for D-glucosamine hydrochloride. Solutions of pneumosamine and 2-amino-2,6-dideoxy-L-galactose were treated in the same manner. Paper chromatography (solvent *b*) of the solutions showed one component ($R_{glucose}$ 5·7) which was common to all reaction solutions and was indistinguishable from 5-deoxy-L-lyxose. The latter sugar can be distinguished in this solvent system from the other 5-deoxypentoses.

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