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Note

The identification of 2-amino-2-deoxy-L-altruronic acid as a constituent of *Shigella sonnei* phase I lipopolysaccharide

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Immunochemical studies of the serologically active O-side chain of the *Shigella sonnei* phase I lipopolysaccharide have revealed a structure consisting of some carbohydrate components[1–3] that were hitherto unknown Recently, an amino sugar has been isolated[2] which was tentatively characterized as a 2-amino-2-deoxyhexuronic acid having a configuration other than *gluco*, *galacto*, or *manno*

We now report the identification of this sugar as 2-amino-2-deoxy-L-altruronic acid, apparently hitherto unknown in Nature

In contrast to the usual hydrolytic methods involving mineral acids at elevated temperature, methanolysis of the lipopolysaccharide and subsequent brief hydrolysis of the products gave the new aminouronic acid (*X*) in good yield No humin was formed during methanolysis, but darkening of the solution occurred during hydrolysis

On elution of the products of hydrolysis from Dowex 50 x8 (H^+) resin, *X* emerged before 2-amino-2-deoxyglucose When the methanolysate, or the product obtained by treatment of *X* with methanolic hydrogen chloride, was subjected to *N*-acetylation, carboxyl reduction, and acid hydrolysis, a new amino sugar (*Y*) was formed (as indicated by p c , t l c , and paper electrophoresis), which was eluted from Dowex 50 x8 (H^+) resin after 2-amino-2-deoxyglucose

Degradation of *Y* with ninhydrin gave a product having R_{Rib} 1 00 (solvents *C* and *D*, *cf* arabinose 0 86, 0 53, lyxose 0 94, 0 66, and xylose 0 89, 0 60), thereby indicating *Y* to be either 2-amino-2-deoxyallose or 2-amino-2-deoxyaltrose *Y* had R_{GlcN} 1 03 (solvent *A*) and M_{GlcN} 1 04 (buffer *A*), values which were identical to those of 2-amino-2-deoxyaltrose (*cf* 0 95 and 1 04 for the *allo* compound), and its *N*-acetyl derivative had R_{Gal} 1 93 and M_{Gal} 1 25, values which were identical to those of 2-acetamido-2-deoxyaltrose (*cf* 1 67 and 1 13 for the *allo* compound) The g l c retention time (29 27 min, column *A*) of the acetylated alditol derived from *Y* was similar to that (29 40) of peracetylated 2-amino-2-deoxyaltritol (*cf* 23 47 for the *allo* compound) Also, the retention times [16 27 (column *B*), 7 46 (column *C*)] of the main product formed on deamination of *Y* with nitrous acid were similar to those (16 30, 7 47) of the product similarly derived from 2-amino-2-deoxyaltrose (*cf* 18 16 and 7 77 for the main product derived from the *allo* compound)

Because of the small amount of material available, *Y* could not be satisfactorily crystallised, but the approximate $[\alpha]_D^{25}$ value of $+10°$ (*c* 0 45, water) is indicative of the L configuration, as the corresponding value for 2-amino-2-deoxy-D-altrose[4] is $-17\ 3°$

The foregoing data indicate *Y* to be 2-amino-2-deoxy-L-altrose, and it follows that *X* is 2-amino-2-deoxy-L-altruronic acid

EXPERIMENTAL

2-Amino-2-deoxy-D-allose and 2-amino-2-deoxy-D-altrose were synthesised by the method of Perry and Furdova[4]

Isolation and reduction of 2-amino-2-deoxy-L-altruronic acid — Purified lipopolysaccharide (0 5 g) from *Shigella sonnei* phase I strain[1] was treated with M HCl in dry methanol (50 ml) at 80° for 20 h The solution was then treated as follows

(*a*) The methanolysate was neutralized (Ag_2CO_3), and insoluble material was collected by centrifugation and washed twice with dry methanol The combined supernatant and washings were concentrated under reduced pressure, and a solution of the residue in 2M HCl (50 ml) was kept at 100° for 30 min and then concentrated under reduced pressure A solution of the residue in water was decolorized with activated charcoal, concentrated to ~2 ml, and then eluted from a column (0 9 × 58 cm) of Dowex 50 x8 (H^+) (−400 mesh) resin, first with water (20 ml) and then with 0 33M HCl Fractions (5 ml) were tested by the Elson–Morgan procedure[5] The new aminouronic acid emerged before 2-amino-2-deoxyglucose, and the appropriate fractions were combined and concentrated to dryness *in vacuo*

(*b*) The methanolysate (100 ml), after neutralization (Ag_2CO_3), was treated[6] with acetic anhydride (15 ml) overnight at room temperature The silver salts were removed by centrifugation, the supernatant was concentrated to dryness, and traces of acetic anhydride were removed from the residue by repeated dissolution in methanol and concentration to dryness A solution of the residue in water (100 ml) was treated with sodium borohydride (1 g) overnight at room temperature, and sodium ions were then removed by using Amberlite CG-120 (H^+) resin The solution was concentrated to dryness and boric acid was removed with methanol in the usual way

The residue was hydrolysed with 4M HCl for 2 h at 100° The solution was then concentrated to dryness, the residue was decolorised with charcoal, and the amino sugars were separated on an ion-exchange column as described above

(*c*) The aminouronic acid isolated in (*a*) was treated with methanolic hydrogen chloride, and the product was subjected to *N*-acetylation, reduction, and hydrolysis[6] as described in (*b*)

Analytical methods — P c was performed on Whatman No 1 paper by the descending technique, and Macherey–Nagel plastic sheets precoated with cellulose type Polygram Cel-300 were used for t l c The following solvents were used *A*, ethyl acetate–pyridine–water–acetic acid[7] (5 5 3 1), *B*, ethyl acetate–pyridine–water[4] (5 2 5, upper layer), *C*, 1-butanol–pyridine–water (5 4 3), and D, butanone–acetic acid–saturated, aqueous boric acid[8] (9 1 1)

Paper electrophoresis was performed on Whatman No 1 paper with the following buffer systems *A*, pyridine–formic acid–acetic acid–water (1·1 5:10 90), pH 2 8, at 42 V/cm for 60 min[9], and *B*, 0 05 M sodium tetraborate–sodium hydroxide, pH 10 0, at 50 V/cm for 120 min[10]

Detection was effected with silver nitrate–sodium hydroxide[11], 0 4% of ninhydrin in water-saturated 1-butanol, or with aniline hydrogen phthalate[12]

The degradation of hexosamines to pentoses with ninhydrin was performed according to the method of Stoffyn and Jeanloz[13]

Deamination of amino sugars with nitrous acid was performed by the method of Dmitriev *et al*[14] The products were conventionally converted[15] into alditol acetates, and analysed by g l c

G l c was performed on a Varian Aerograph Model 2740 fitted with an electronic integrator and with glass columns (0 3 × 180 cm, with 100/120 mesh Gas-Chrom Q as support) containing *A* 10% of neopentylglycol sebacate at 230° for aminoalditol acetates[16], and *B* 3% of ECNSS-M at 180° or *C* 3% of OV-225 at 180° for alditol acetate derivatives of deaminated amino sugars

Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter

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REFERENCES

1 T Kontrohr and O Westphal, *Acta Microbiol Acad Sci Hung*, 14 (1967) 205–222
2 E Romanowska and V Reinhold, *Eur J Biochem*, 36 (1973) 160–166
3 T Kontrohr and B Kocsis, *Abstr Papers, Fed Eur Biochem Soc, 9th Meeting*, (1974) 499
4 M B Perry and J Furdova, *Can J Chem*, 46 (1968) 2859–2862
5 M J Crumpton, *Biochem J*, 72 (1959) 479–486
6 R E Chambers and J R Clamp, *Biochem J*, 125 (1971) 1009–1018
7 F G Fischer and H J Nebel, *Z Physiol Chem*, 302 (1955) 10–19
8 W R Rees and T Reynolds, *Nature (London)*, 181 (1958) 767
9 H Mayer, *Eur J Biochem*, 8 (1969) 139–145
10 A B Foster, *Chem Ind (London)*, (1952) 1050–1051
11 W E Trevelyan, D P Procter, and J S Harrison, *Nature (London)*, 166 (1950) 444–445
12 S M Partridge, *Nature (London)*, 164 (1949) 443
13 P J Stoffyn and R W Jeanloz, *Arch Biochem Biophys*, 52 (1954) 373–379
14 B A Dmitriev, L V Backinowsky, V L Lvov, N K. Kochetkov, and I L Hofman, *Eur J Biochem*, 50 (1975) 539–547
15 J S Sawardeker, J H Sloneker, and A Jeanes *Anal Chem*, 37 (1965) 1602–1604
16 M B Perry and A C Webb, *Can J Biochem*, 46 (1968) 1163–1165