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Note

2-Amino-2,6-dideoxyhexose-6-sulphonic acid: a constituent of the cell wall of *Halococcus* sp., strain 24

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Cell-wall hydrolysates of *Halococcus* sp., strain 24, an extremely halophilic bacterium, contain amino acids, amino sugars, and amino sugar-like compounds[1]. We now report the finding of a new hexosamine-sulphonic acid. Sulphonated 6-deoxyhexoses are constituents of sulpholipids isolated from photosynthetic tissues of various organisms[2]; sulphonated amino sugars have been identified in hydrolysates of sulphite-treated glycoproteins[3], but they were not original constituents of the glycoproteins.

EXPERIMENTAL

Materials and methods — The acidic amino sugar (previously[1] designated X_1) was isolated from cell walls of *Halococcus* sp., strain 24. The purified walls (15 mg) were hydrolyzed in 6M HCl (2 mg/ml) for 6 h at 105°, and the ninhydrin-positive products were separated on a Technicon amino acid analyzer column[4]. The acidic amino sugar (~200 μg, measured as 2-amino-2-deoxyglucose) was separated from the buffer salts on a column of Dowex-2 (HO^-) resin[4].

P.c. was carried out on Whatman No. 1 paper by the descending technique with *A*, ethyl acetate–pyridine–water–acetic acid[5] (5.5:3:1) for 24 h, and *B*, 1-pentanol–pyridine–water[6] (7.7:6) for 20 h. Paper electrophoresis was carried out with a Shandon High Voltage Electrophoresis Apparatus SAE 2550 on Whatman No. 4 paper at 50 V/cm for 1 h, with the following buffers, each diluted to 1 litre with distilled water: (*1*) acetic acid (87 ml)–formic acid (22 ml), pH 1.9; (*2*) acetic acid (50 ml)–pyridine (5 ml), pH 3.5, (*3*) acetic acid (25 ml)–pyridine (25 ml), pH 4.7, (*4*) acetic acid (4 ml)–pyridine (100 ml), pH 6.5. Detection was effected with silver nitrate–sodium hydroxide[7] or 0.2% ninhydrin in acetone.

Deamination[8] and determination of sulphate[9] were carried out by the literature procedures. Sulphur was detected by backscattering spectrometry[10,11].

2-Amino-2,3-dideoxyhexose-3-sulphonic acid was synthesised according to the method of Weber and Winzler[3].

Periodate oxidation was carried out[3] on the *N*-acetylated[12] acidic amino sugar and on its methyl glycoside[13]. 1 μmol of material (measured as 2-amino-2-deoxy-glucose[15]) was treated with 5 μmol of sodium metaperiodate in 0.5 ml of aqueous solution at room temperature protected from light. Oxidant uptake was monitored on aliquots (20 μl) which were diluted with 2 ml of water, and the absorbance at 225 nm was determined. The reaction was stopped by the addition of 50 μmol of sodium borohydride. After 16 h at room temperature, sufficient Dowex-50W(H^+) resin was added to make the reaction mixture acidic. The mixture was filtered, the resin was washed with water, and the combined filtrate and washings were concentrated to dryness. Borate was removed by distillation of methanol from the residue which was then treated with 4*M* HCl for 1 h at 100°. The hydrolysate was concentrated to dryness and the residue was analyzed on the amino acid analyzer.

RESULTS AND DISCUSSION

The acidic amino sugar isolated from *Halococcus* sp., strain 24, reacted[14,15] like a 2-amino-2-deoxy sugar unsubstituted at positions 3 and 4. With ninhydrin at 105°, it gave a yellow-brown colour which turned violet as reported for hexosaminuronic acids[16] and certain 6-deoxyhexosamines[17].

The acidic amino sugar was eluted from the column of the amino acid analyzer as a strongly negatively charged compound (Table I), and paper electrophoresis showed no movement of the acidic amino sugar in buffers of pH 1.9–6.5 due to inner salt formation. Deamination followed by electrophoresis showed a negatively charged compound with an equal mobility at pH 1.9 and 6.5, indicating groups more acidic than carboxyl (Table I).

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF THE ACIDIC AMINO SUGAR AND ITS DEAMINATION PRODUCT

Sugar	*Relative mobility in solvent*		*Electrophoretic mobility (cm/h) in buffers*				*Relative mobility in amino acid analyzer*
	A	B	1	2	3	4	
Acidic amino sugar	0.47	0.49	0	0	0	0	0.07
Acidic amino sugar, deaminated			+12[a]			+12	
2-Amino-2-deoxyglucose	1.00	1.00					1.00

[a] + denotes anodic mobility.

The presence of phosphate or sulphate groups was ruled out on the basis of a negative neutron-activation test for phosphorus, and a negative sulphate test[9]. However, backscattering spectrometry showed sulphur to be present[11], suggesting a sulphonate group, a sulphonated amino sugar would be stable toward acid hydrolysis[3].

The products of periodate oxidation of a 2-acetamido-2,3-dideoxyhexose-3-sulphonic acid included[3] a component containing both a sulphonate and an amino group; the methyl glycoside was not attacked by periodate. On periodate oxidation (100 h), the new *N*-acetylated amino sugar consumed twice as much periodate as its methyl glycoside. Oxidation was slow (maximum uptake after 80 h), but the amount consumed on a molar basis could not be accurately determined because of insufficient amounts of the substrates. The reaction mixture from the amino sugar contained no product possessing both an amino and a sulphonic acid group, whereas such a product was present in the reaction mixture of the methyl glycoside. These data are consistent with a 2-amino-2,6-dideoxyhexose-6-sulphonic acid.

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