THE ISOLATION AND CHARACTERIZATION OF 2-AMINO-2:6-DIDEOXY D-GLUCOSE (D-QUINOVOSAMINE) FROM A BACTERIAL POLYSACCHARIDE

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During an investigation of bacterial mucopolysaccharidases, a gram-negative micro-organism designated COC-21 was isolated from the Chesapeake and Ohio Canal. This organism was subsequently used in a study on the biosynthesis and distribution of methyl pentoses and amino sugars in bacterial polysaccharides. Attempts to identify the hexosamine components in acid hydrolysates of polysaccharides extracted from COC-21 revealed, in addition to glucosamine and D-fucosamine, the presence of D-quinovosamine (2-amino-2:6-dideoxy D-glucose).

Materials and Methods

COC-21 was grown at room temperature on: 1% glucose 0.3% yeast extract; 0.15% ammonium sulfate; 0.5% NaCl; 0.005% $MgSO_4$ $7H_2O$; 0.025 M potassium phosphate buffer; final pH 6.8. The culture was aerated vigorously for approximately 20 hours and harvested with a continuous flow Servall centrifuge. Cells were dessicated with 8 volumes of acetone immediately after collection.

Authentic D-galactosamine HCl and D-fucosamine HCl were gifts from Dr. R. W. Wheat. Dr. R. Kuhn kindly provided synthetic D-quinovosamine. A column of Dowex 50-H, 200 X 400 mesh, previously

calibrated with glucosamine, galactosamine and fucosamine aided in the tentative identification of amino sugar peaks.

Descending chromatography on Whatman # 1 filter paper was conducted with the following solvents: (A) n-butanol-pyridine-water 6:4:3, (B) 70 percent phenol-H_2O, (C) pyridine-ethyl acetate-acetic acid-water 5:5:1:4. Amino sugars were routinely detected on paper with a ninhydrin spray. The Dische and Shettles (1948) test for methyl pentoses was used after nitrous acid deamination. Periodate consumption was measured spectrophotometrically Dixon and Lipkin (1954). Formaldehyde was analyzed by the chromotropic acid method of MacFayden (1945). Acetaldehyde was determined colorimetrically after periodate oxidation according to the procedure of Barker and Summerson (1941).

Infra-red spectra were obtained with pressed KBr pellets and a Beckman IR-5 spectrophotometer. Optical rotation data were determined through the courtesy of Dr. G. Ashwell. Elemental analyses were performed by Chemco Inc., Washington 9, D. C.

Twelve and one-half grams of acetone powder were extracted with hot 45% phenol according to the procedure described by Westphal et al. (1952). After overnight dialysis, the polysaccharide in the aqueous phase, was precipitated with four volumes of ethanol and represented approximately 14 percent of the initial cell dry weight. A 1 percent solution of the polysaccharide in 2N HCl was hydrolyzed for four hours in sealed tubes at 100°C.

The hydrolysate was decolorized with a small amount of Darco G-60, evaporated to dryness at 40°C, in vacuo and placed on a 1 X 49 cm. column of Dowex 50-H. Amino sugars were eluted with 0.33 N HCl and fractions were analyzed for 2-amino sugars by the method of Rondle and Morgan (1955).

Results and Discussion

Fractions from each of the three hexosamine peaks which emerged from the column were pooled, taken to dryness *in vacuo* and crystallized from water-acetone mixtures. The elution volume relative to glucosamine, and recoveries after crystallization respectively were: Compound 1, glucosamine (1.00, 16 mgms); Compound 2, (1.43, 10 mgms); and Compound 3, D-fucosamine (1.75, 11 mgms). The chromophore produced by Compound 2 in the hexosamine test showed maximum absorption at 532 mu, a characteristic of 2-amino sugars. However, chromatographic mobility of Compound 2 relative to glucosamine in solvents A, B, and C was: 1.85, 2.53, and 1.30 respectively and the fast mobility suggested a nonpolar substituent. Subsequently, Compound 2 was shown to migrate the same distance as authentic quinovosamine in these solvents, whereas fucosamine clearly migrated in an intermediate position i.e., between glucosamine and quinovosamine.

Upon nitrous acid deamination, and treatment with cysteine and sulfuric acid, Compound 2 produced a chromophore with peak absorption at 405 mu, identical with that produced by fucose. After 22 hours, periodate oxidation of Compound 2 and authentic D-quinovosamine hydrochloride revealed a respective consumption Of 3.9 and 3.8 moles of periodate per mole of amino sugar. These results are in close agreement with the theoretical periodate consumption value of four moles for a 6-deoxy hexosamine. Additional evidence that Compound 2 was a 6-deoxy hexosamine was provied by periodic acid oxidation using the Conway diffusion cup technique. After 18 hours, at neutral pH, 0.85 moles acetaldehyde were produced per mole of Compound 2. No formaldehyde was detectable among the oxidation products.

The average of duplicate elemental analyses of Compound 2 calculated as the hydrochloride salt was:

Found	C 36.67	H 6.59	N 6.66
Theory	C 36.10	H 7.07	N 7.02

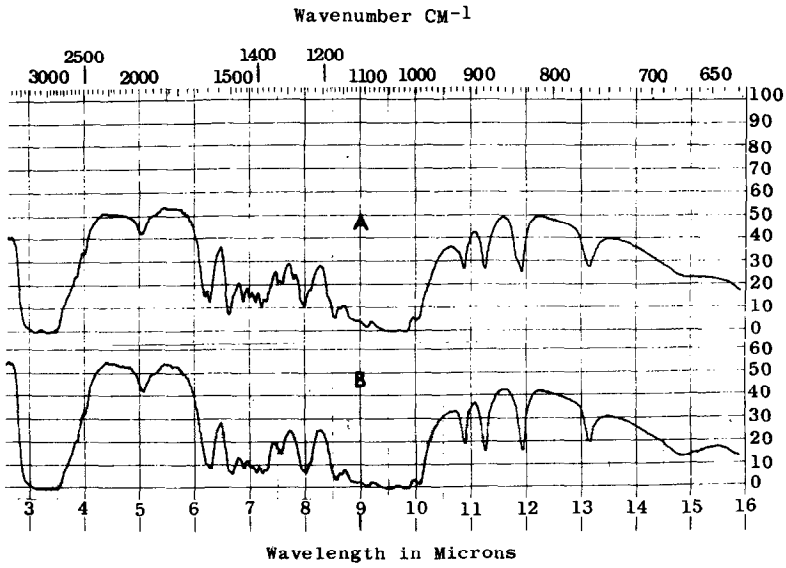

Fig. 1 Infra-red spectra obtained in KBr with (A) authentic D-quinovosamine hydrochloride and (B) Compound 2.

At equilibrium, the respective optical rotations of authentic D-quinovosamine HCl and Compound 2 were $[\alpha]_D$ + 51.5° (0.33 in water); and $[\alpha]_D$ + 55.5° (0.53 in water). Kuhn et al. (1958) report $[\alpha]_D$ + 53° for D-quinovosamine HCl. Evidence that the isolated fucosamine was of the D-configuration was provided by the high positive rotation $[\alpha]_D$ + 92.5°.

The melting point of Compound 2 was determined to be 165-170°C, in close agreement with the reported 163-170°C for D-quinovosamine, Kuhn et al. (1958).

Over a wide concentration range, the colorimetric hexosamine assay produced equivalent optical densities at 530 mu

with both quinovosamine and Compound 2. This value corresponds to 85 percent of that obtained with equimolar concentrations of glucosamine.

Previously, Crumpton and Davies (1958) isolated D-fucosamine in C. violaceum, and, more recently, Barker et al. (1961) reported the occurrence of 2-amino-2:6-dideoxytalose "pneumosamine" and L-fucosamine in Pneumococcus Type V polysaccharide. Thus, D-quinovosamine represents the fourth 2-amino-2:6-dideoxy hexose isolated from Nature.

Further studies on the biosynthesis of D-quinovosamine and the structure of the polysaccharide are in progress.

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