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Note

Methyl 2-amino-2-deoxy-D-glucoside 6-phosphates

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2-Acetamido-2-deoxy-D-hexose phosphates are components of many biochemically important polysaccharides[1–4]. Although hexosamine phosphates and their derivatives are essential for the complete characterisation of the structure of these polysaccharides, these compounds have not been fully described[5]. We now report on the preparation and characterisation of the methyl 2-amino-2-deoxy-α- and -β-D-glucoside 6-phosphates. Methyl 2-amino-2-deoxy-α,β-D-glucoside 6-phos-

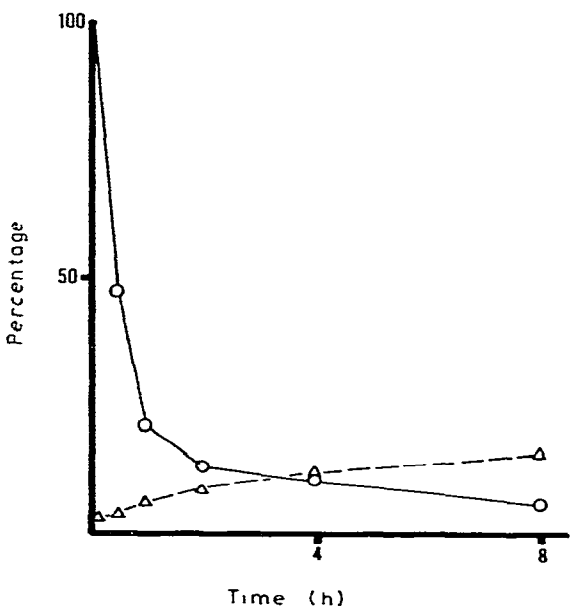

Fig. 1. Methyl glycosidation of 2-amino-2-deoxy-D-glucose 6-phosphate —○—, reducing power, --△--, released inorganic phosphorus.

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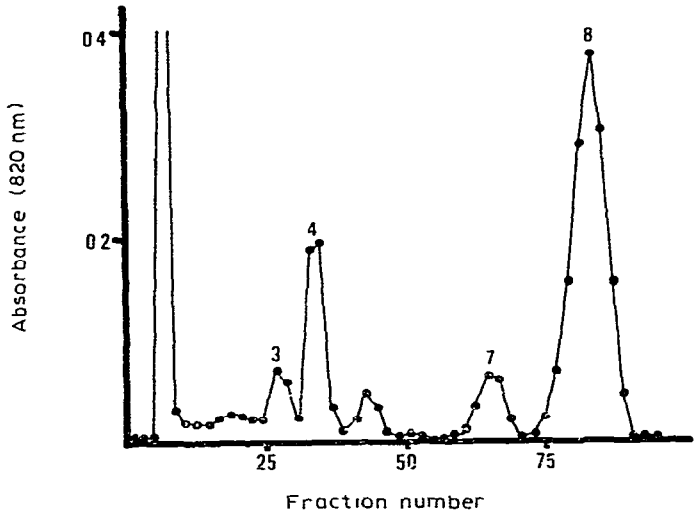

Fig 2 Elution profile on Dowex 50W-X2 (H^+) resin of the product mixture obtained on methyl glycosidation of 2-amino-2-deoxy-D-glucose 6-phosphate

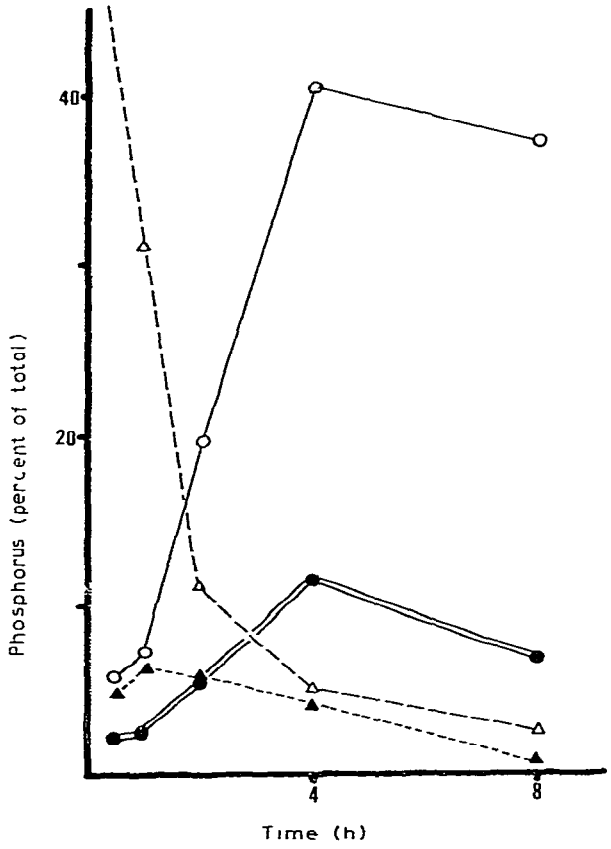

Fig 3 Rates of production and decomposition of 2-amino-2-deoxy-D-glucose 6-phosphate derivatives during methyl glycosidation at 100° —△—, 2-amino-2-deoxy-D-glucose 6-phosphate —○—, methyl 2-amino-2-deoxy-D-glucoside 6-phosphate, =●=, methyl 2-amino-2-deoxy-D-glucoside 6-(methyl phosphate), ---▲---, 2-amino-2-deoxy-D-glucose 6-(methyl phosphate)

TABLE I

PHYSICAL CONSTANTS OF SEPARATED GLYCOSIDES

Fractions[a]	$R_{Glc\backslash 61}$	*Periodate consumption (mol/PO4)*	$[\alpha]_D$ (c) *(degrees)*
3	1 27	3 90	—
4a	1 80	1 85	−38 (0 08)
4b			−116 (0 16)
8a	1 33	2 08	−39 (0 06)
8b			+120 (0 13)

[a]Key 3, 2-amino-2-deoxy-D-glucose 6-(methyl phosphate), 4a, methyl 2-amino-2-deoxy-β-D-glucoside 6-(methyl phosphate) 4b, methyl 2-amino-2-deoxy-α-D-glucoside 6-(methyl phosphate), 8a, methyl 2-amino-2-deoxy-β-D-glucoside 6-phosphate, 8b, methyl 2-amino-2-deoxy-α-D-glucoside 6-phosphate

TABLE II

^{13}C-CHEMICAL SHIFTS AND CARBON–PHOSPHORUS COUPLINGS (Hz, IN PARENTHESES) OF 2-AMINO-2-DEOXY-D-GLUCOSE 6-PHOSPHATE DERIVATIVES

Fractions[a]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*MeO-1*	*P–OMe*
3(α)	90 1	55 1	70 4	70 1	71 4(7 4)	65 0(4 9)	—	53 8(6 1)
(β)	93 7	57 6	72 7	70 2	75 8(7 3)	65 0(4 9)	—	53 8(6 1)
4a	101 1	58 4	73 0	70 1	75 8(8 6)	64 8(4 9)	56 7	53 8(6 1)
4b	97 0	54 9	70 7	70 0	71 8(8 6)	64 8(4 9)	56 2	53 8(4 9)
8a	100 8	58 4	72 7	70 1	75 9(7 3)	64 3(4 9)	56 6	—
8b	97 0	54 8	70 6	69 8	71 8(8 6)	64 4(4 9)	56 2	—

[a]For key see Table I

phate was prepared by treatment of the parent sugar with methanol in the presence of Dowex 50 (H^-) resin at 100° (Fig 1) The reaction product was fractionated[6] on Dowex 50W-X2 resin (Fig 2), which revealed by-products as well as the methyl 2-amino-2-deoxy-α,β-D-glucoside 6-phosphates, the rates of formation are shown in Fig 3

Fractions 3 and 4 contained the methyl ester of 2-amino-2-deoxy-D-glucose 6-phosphate and methyl 2-amino-2-deoxy-α β-D-glucoside 6-phosphate, respectively, as indicated by the results of periodate oxidation, phosphomonoesterase treatment, and ^{13}C-n.m r. spectroscopy

Chromatography on Dowex 50W-X8 (H^+) resin separated the anomers as shown in Fig. 4 Data on the mono- and di-esters are given in Tables I and II

It should be noted in structural studies of phosphate-containing polysaccharides by ^{13}C-n m r spectroscopy that the chemical shifts of mono- and di-esters are different

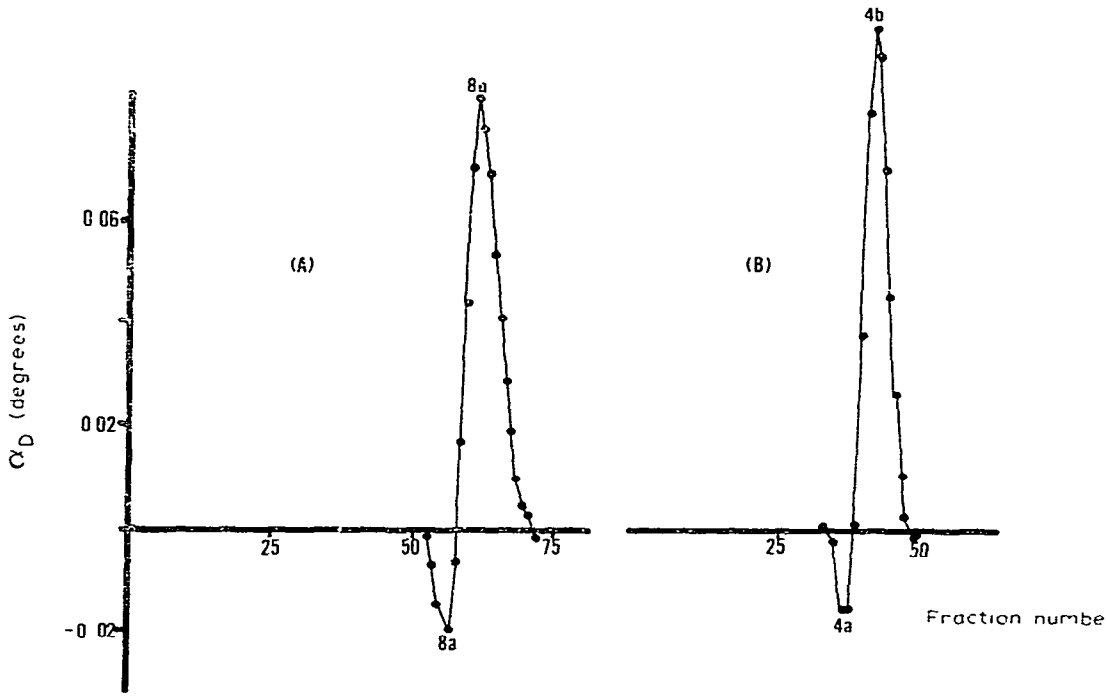

Fig 4 Elution profiles on Dowex 50W-X8 (H^+) resin of methyl 2-amino-2-deoxy-α,β-D-glucoside 6-phosphate (A, 7 5-ml fractions) and methyl 2-amino-2-deoxy-α,β-D-glucoside 6-(methyl phosphate) (B, 4 6-ml fractions) Optical rotations recorded in a 0 5-dm cell

EXPERIMENTAL

General — T l c was performed on cellulose (Merck 5577) at room temperature with ethyl acetate–pyridine–acetic acid–water (5 5 2 4) and detection with ninhydrin[6] ^{13}C-N m r spectra were recorded at room temperature on a JEOL-FX 100 spectrometer at 25 0 MHz, in the pulsed, Fourier-transform mode with complete proton-decoupling Chemical shifts are expressed as p p m downfield from the signal (49 8 p p m) of MeOH Optical rotations were measured for solutions in 0 1- or 0 5-dm tubes with a JASCO DIP-4 Digital polarimeter Melting points were determined on a Micro Melting Point Apparatus (Yanagimoto) Determinations of phosphorus content[6] and periodate consumption[7] were performed by literature methods Periodate oxidation was conducted in the dark in 40mM acetate buffer (pH 5.5) at room temperature

2-Amino-2-deoxy-D-glucose 6-phosphate, prepared by the literature methods[6], had $[\alpha]_D$ +63° (equil , *c* 0 4, water)

Anal Calc for $C_6H_{14}NO_8P$ C, 27 81, H, 5 45 N, 5 41, P, 11 95 Found C, 27 78, H, 5 64, N, 5 58, P, 12 05

Glycosidation of 2-amino-2-deoxy-D-glucose 6-phosphate — To a solution of 2-amino-2-deoxy-D-glucose 6-phosphate (100 mg) in water (250 μl) was added drop-

wise with constant stirring, a suspension of Dowex 50W-X4 (H^+) resin (20–50 mesh) (10 g) in methanol (75 ml) The mixture was then heated for 8 h at 100°, cooled, and filtered, and the resin was washed with water (~10 vol) The combined filtrate and washings were concentrated to dryness, and the residue was applied to a column (1 5 × 28 cm) of Dowex 50W-X2 (H^+) resin (200–400 mesh) and eluted with water at 4° (Fig 2) Each phosphorus-containing fraction was concentrated to dryness

Fraction 3 contained 2-amino-2-deoxy-D-glucose 6-(methyl phosphate), which could not be crystallised

Anal Calc for $C_7H_{16}NO_8P$ C, 30 77, H, 5 90 N, 5 13, P, 11 34 Found C, 30 78; H 6 21, N, 5 13, P, 11 18

Fraction 4 contained methyl 2-amino-2-deoxy-α,β-D-glucoside 6-(methyl phosphate) Separation of the anomers was performed on a column (1 2 × 60 cm) of Dowex 50W-X8 (H^+) resin (200–400 mesh) by elution with water at 4° (Fig 4) The β anomer was isolated as a colorless syrup

Anal Calc for $C_8H_{18}NO_8P$ H_2O C, 31 48 H, 6 61 N, 4 59, P, 10 15 Found C, 31 45, H, 6 44, N, 4 94, P, 10 16

The α anomer was isolated as a colorless syrup

Fraction 8 contained methyl 2-amino-2-deoxy-α,β-D-glucoside 6-phosphate Separation of the anomers was performed by the procedure described above (Fig 4) The β anomer was obtained as an amorphous powder from water–methanol–acetone, which decomposed at 140°

Anal. Calc for $C_7H_{14}NO_8P$ 2 H_2O C, 27 02, H, 6 52, N 4 53, P, 10 02 Found C, 27 38, H, 6 50, N, 4 96 P, 9 99

The α anomer was a colorless syrup

Anal Calc for $C_7H_{16}NO_8P$ C, 30 77, H, 5 90, N, 5 13, P, 11 34 Found C, 30 75, H, 5 90, N, 5 00 P, 11 52

Data on these compounds are recorded in Tables I and II

ACKNOWLEDGMENTS

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