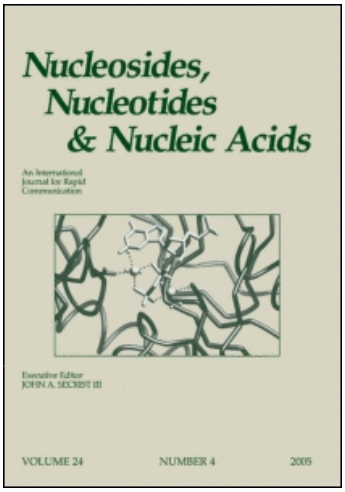

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Robert W. Humble[a]; Graham Mackenzie[a]; Gordon Shaw[b]
[a] School of Science, Humberside College of Higher Education, Hull [b] School of Chemistry. University of Bradford, Bradford, W. Yorkshire, (U.K.)

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PHOSPHORYLATION OF SOME 5-AMINOIMIDAZOLE NUCLEOSIDES TO THE 5'-PHOSPHATES USING A PHOSPHOTRANSFERASE FROM WHEAT

Robert W. Humble[+], Graham Mackenzie[+] and Gordon Shaw[*]

[+]School of Science, Humberside College of Higher Education, Hull, HU6 7RT and [*]School of Chemistry. University of Bradford, Bradford, W. Yorkshire, BD7 1DP. (U.K.)

Abstract: Several D-ribofuranosyl, D-xylofuranosyl and D-arabinofuranosyl 5-aminoimidazoles have been successfully phosphorylated to 5'-phosphates using a phosphotransferase from wheat shoots and p-nitrophenylphosphate as a phosphate donor.

Phosphotransferases from carrots[1] or wheat[2,3] shoots and a suitable phosphate donor have been used by several workers for the specific conversion of unprotected pyrimidine and purine nucleosides and some analogues into nucleoside 5'-phosphates. We have been interested to investigate the application of this technique to the phosphorylation of 5-aminoimidazole nucleosides to afford corresponding 5'-phosphates related to intermediates in purine nucleotide de novo biosynthesis.

Results and Discussion: Wheat shoot phosphotransferase was chosen for study since highly purified carrot enzyme has been reported[4] to phosphorylate nucleoside 2'- and 3'-hydroxyl groups. p-Nitrophenylphosphate was used as the phosphate donor. The unprotected ethyl 5-amino-1-β-D-ribofuranosyl[5] (α-D-xylofuranosyl[6,7] or β-D-arabinofuranosyl[8]) imidazole-4-carboxylates (1), (2) and (3) respectively gave in a single phosphorylation cycle, yields of 5'-phosphates varying from 15-41% and tending to be higher for the arabinose

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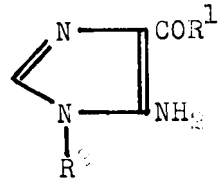
derivative. (Table). Unchanged nucleosides were readily isolated and total nucleoside yields could be increased substantially by recycling.



(1) R^1 = OEt, R^2 = β-D-ribofuranosyl
(2) R^1 = OEt, R^2 = α-D-xylofuranosyl
(3) R^1 = OEt, R^2 = β-D-arabinofuranosyl
(4) R^1 = OEt, R^2 = 2,3-O-isopropylidene-β-D-ribofuranosyl
(5) R^1 = NH_2, R^2 = 2,3-O-isopropylidene-β-D-ribofuranosyl
(6) R^1 = NH_2, R^2 = α-D-ribofuranosyl
(7) R^1 = OEt, R^2 = 2,3-O-isopropylidene-α-D-ribofuranosyl
(8) R^1 = OEt, R^2 = D-arabinityl
(9) R^1 = OCH_2Ph, R^2 = β-D-ribofuranosyl
(10) R^1 = OCH_2Ph, R^2 = 2,3-O-isopropylidene-β-D-ribofuranosyl

A single cycle phosphorylation of the 2,3-O-isopropylidene-α-D-ribose ester[5] (4) gave a slightly better yield (25-28%) than the unprotected nucleoside and this was further improved (37%) in the corresponding carboxamide[5](5). In contrast to these results both the α-D-ribose carboxamide[5] (6) and ester[5] (7) and especially the acyclic D-arabinitylimidazole[9] (8) furnished only small (2-9%)yields of nucleotides whereas both the benzyl ester (9) and its 2,3-O-isopropylidene derivative (10)[10] gave exceptionally high (72-80%) yields of phosphates after a single cycle and this might have some relevance in considerations of the mechanism of action of the phosphotransferase.

EXPERIMENTAL

Preparation of Wheat Phosphotransferase

Wheat was grown in the dark at 21°C on Perlite (inert inorganic horticultural medium supplied by Silvaperl Products Ltd., P.O. Box 8, Department 38, Harrogate, HG2 8JW. W. Yorkshire, U.K.) saturated with

TABLE

Nucleoside	Weight of Nucleoside(mg)	Yield of Nucleotide+(%)	R_F (Nucleoside)	R_F (Nucleotide)ø
(1)	50	19	0.63	0.34
	25	23		
	25	15		
	50	17		
Recycle x 3	50	55		
(4)	50	25	0.74	0.58
	20	28		
	20	25		
(9)	22	72	0.68	0.25
	26	80		
(10)	15	74	0.78	0.48
(3)	13	35	0.67	0.31
	25	41		
(7)	25	18	0.68	0.48
(2)	13	24	0.56	0.30
(5)	25	37	0.68	0.51
(6)	10	9	0.52	0.27
(8)	13	2 approx.	0.50	0.28
	13	3 approx.		

+ Yields were determined by direct weighing of purified solid nucleotides except for compounds (6) and (8) where the yields were estimated by uv absorption spectra, λ_{max} 260 nm (ε13,000).

ø See experimental section for conditions of chromatography.

distilled water over 7 days. The shoots should grow rapidly and almost double in height within 24 hours during the early stages. The shoots were ground in a mortar cooled to 4°C with 5 volumes (per gram of fresh shoots) of an ice-cold sodium acetate buffer (pH 6.0 and fine, acid-purified sand (40-100 mesh) (supplied by B.D.H. Ltd., U.K.) The mixture was filtered through several thicknesses of muslin and the filtrate centrifuged at 5000 g for 15 min. The supernatant solution was diluted with water to 10 volumes then adjusted to pH 5.0 with 50% (v/v) aqueous acetic acid. The solution was divided into small portions and kept at -20°C. The enzyme remained stable under these conditions for at least a month. The protein content of the solution, determined by the $A_{224-236}$ Isoabsorbance method[11], was 0.52 mg cm^{-3}.

Phosphorylation Conditions

A solution of the aminoimidazole nucleoside (0.04M) and p-nitrophenyl phosphate (0.6M) in water was adjusted to pH 5.0 with 50% aqueous acetic acid and treated with an equal volume of the wheat shoot extract. The mixture was incubated at 37°C for 16 hours and the reaction followed by t.l.c. on Merck F_{254} silica gel aluminium sheets using butan-1-ol:acetone:acetic acid:5% aqueous ammonia:water (35:25:15:15:10) as the solvent system. The products were visualised under a uv lamp (254 nm) and by the Bratton-Marshall[12] spray reagents.

Isolation of Products

The reaction was terminated by heating the solution to the boiling point for a few seconds then cooling to room temperature. The solution was extracted with ether or dichloromethane to remove p-nitrophenol and residual solvent removed *in vacuo*. The mixture was diluted two-fold with water, adjusted to pH 8 with aqueous ammonia and applied to a column (1.6 x 30 cm) of Bio-Rad AG1-X2, 200-400 mesh (formate form) ion-exchange resin. The column was first washed with water when unreacted nucleoside was removed quantitatively, then developed with a linear 0.0-1.0M formic acid gradient, total volume 500 cm^3. Fractions were collected at 20 min intervals with a flow rate of 0.6 cm^3 min^{-1}. Fractions containing the nucleotide (eluted at 0.5M formic acid) were combined and evaporated *in vacuo*; the residue was re-evaporated with water and finally ethanol to give the nucleotide as a white solid foam. The yields obtained are recorded in the Table.

Unreacted p-nitrophenyl phosphate was not eluted from the column even with 2M-formic acid.

Conversion of Nucleotides to Nucleosides using Calf Intestine Alkaline Phosphatase (EC. 3.1.3.1.)

Alkaline phosphatase (1 unit cm^{-3}, Sigma Chemical Co.) was prepared in a 0.1M sodium hydrogen carbonate, sodium carbonate buffer (pH 10.0). A sample of the nucleotide was incubated with the solution at 37°C overnight when quantitative conversion to the corresponding nucleoside was observed. The identity of (1) and (3) were also confirmed by comparison with authentic specimens prepared by a chemical phosphorylation method.

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