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RIBONUCLEOSIDES AND RIBONUCLEOTIDES DERIVED FROM 5-ACETYLURACIL

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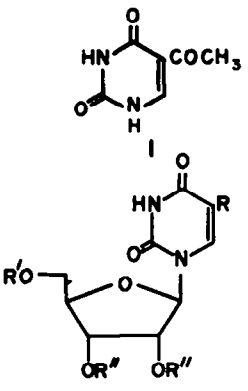
Abstract—5-Acetyluridine has been obtained by condensing the trimethylsilyl derivative of 5-acetyluracil with 2,3,5-tri-*O*-benzoylribofuranosyl chloride and removing the blocking groups with sodium methoxide. Removal of the benzoyl groups was relatively slow; by the use of 1 molecular proportion of sodium methoxide at 20°, 5-acetyl-5'-*O*-benzoyluridine was obtained. Reduction of 5-acetyluridine with sodium borohydride gave 5-(1-hydroxyethyl)uridine. 5-Acetyl-2',3',-*O*-isopropylideneuridine upon phosphorylation and removal of the blocking group gave 5-acetyluridine 5'-phosphate which was converted via its morpholidate into 5-acetyluridine 5'-diphosphate. Attempts to polymerise this compound by means of polynucleotide phosphorylase were unsuccessful. The diphosphate acted as a weak inhibitor of the enzyme.

There has been considerable interest in the synthesis of 5-substituted uridines and their conversion into 5'-diphosphates. The latter compounds often can be polymerised by the action of polynucleotide phosphorylase to give 5-substituted polyuridylic acids.[1-3] These interact with polyadenylic acid to give triple-stranded structures, the stability of which, as determined from their T_m values, gives an indication of the effect of the various substituents at the 5-position. Thus, with polyadenylic 5-chloro-, bromo- and iodo-derivatives form more stable complexes than does polyuridylic acid, whereas the 5-fluoro-derivative forms a less stable complex,[4] and both the 5-methyl- and 5-ethyl-derivatives of polyuridylic acid form more stable complexes than does polyuridylic acid itself.[3] Our interest in derivatives of 5-acetyluracil has led us to attempt the synthesis of poly-5-acetyluridylic acid in order to determine the effect of the 5-acetyl group on the stability of complexes with polyadenylic acid.

Initially the synthesis of 5-acetyluridine was carried out. This compound has been synthesised by Cusack *et al.*[5] by treating 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate with ethyl *N*-(α-acetyl-β-ethoxyacryloyl)carbamate and then removing the isopropylidene group. The overall yield obtained by this route was only 17% (from the ribofuranosylamine). This was partly due to the fact that a mixture of α-and β-anomers was obtained. It appeared that a better yield might be obtained by using the more common method of synthesising pyrimidine ribonucleosides, namely condensing the trimethylsilyl derivative of the pyrimidine with a suitably protected ribose derivative in which steric control by a 2-acyloxy group should lead to the almost exclusive production of the required β-anomer. Accordingly the trimethylsilyl derivative of 5-acetyluracil (1) was condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride to give 5-acetyl-2', 3',5-tri-*O*-benzoyluridine (2) in 62% yield. Removal of the benzoyl groups with sodium methoxide in methanol gave 5-acetyluridine in 86% yield. The assignment of the β-configuration to 2 and 3 was based on the ORD spectrum of 3 which showed a positive Cotton effect. The assignment could not be made unequivocally from the NMR spectra of 2 and 3 but the coupling constant for the anomeric proton in each case fell within the expected limits. The 5-acetyluridine obtained by this procedure appeared to be identical with that obtained by Cusack *et al.*[5] These workers assigned the β-configuration to their product on the basis of the comparison of its NMR spectrum with that of the corresponding α-nucleoside.

The removal of the benzoyl groups from 2 was slower than is usually the case; when one molecular proportion of sodium methoxide was used, treatment in boiling methanol for 8 hr was required. Treatment at room temperature for 48 hr gave a partially debenzoylated product. Comparison of the NMR spectrum of this compound with those of compounds 2 and 3 showed it to be 5-acetyl-5'-*O*-benzoyluridine (5). Thus the signal for the 5'-protons of 5 was at δ 4.5 whereas the signals for the corresponding protons of 2 and 3 were at δ 4.75 and 3.6 respectively and the signals for the 2' and 3' protons of 5 were at δ 4.0–4.3 whereas those for 2 and 3 were at δ 5.9 and 3.8–4.1 respectively. This assignment of structure was supported by the fact that 5 reacted with sodium meta periodate.

The 5-acetyluridine was characterised further by con-



2: R = —$COCH_3$; R', R'' = —COPh
3: R = —$COCH_3$; R', R'' = H
4: R = —$COCH_3$; R' = H; R'' = isopropylidene
5: R = —$COCH_3$; R' = —COPh; R'' = H
6: R = —$CHOHCH_3$; R', R'' = H
7: R = —$COCH_3$; R' = —$PO(OH)_2$; R'' = H
8: R = —$COCH_3$; R' = —$PO(OH)OPO(OH)_2$; R'' = H

Table 1. Action of polynucleotide phosphorylase on nucleoside 5'-diphosphates

Enzyme system	5-Acetyluridine 5'-diphosphate		Uridine 5'-diphosphate		5-Acetyluridine 5'-diphosphate + uridine 5'-diphosphate	
	Inorganic phosphate released in 5h(%)	Polymer formed (%)	Inorganic Phosphate released in 5h (%)	Polymer formed (%)	Inorganic phosphate released in 5h (%)	Polymer formed (%)
M. luteus polynucleotide phosphorylase. Soln. (a)†	14	0.0	80	73	25	25‡
Soln. (a)† control, no enzyme	2	—	2	—	—	—
M. luteus polynucleotide phosphorylase. Soln. (b)†	5	0.0	80	63	—	—
E. coli polynucleotide phosphorylase. Soln. (c)†	28	0.0	50	43	—	—

†See text.
‡Based on uridine 5'-diphosphate.

version into its 2',3'-*O*-isopropylidene derivative (4), which was identical to the compound obtained by Cusack *et al.*[5] and by reduction with sodium borohydride to give 5-(1-hydroxyethyl)uridine (6), which was presumably a mixture of diastereomers. Compound 6 was also obtained by the action of dilute acid on 5-vinyluridine.[6]

5-Acetyluridine 5'-phosphate (7) has been obtained by Carrington *et al.*[7] in about 5% yield from 2,3-*O*-isopropylideneribofuranosylamine 5-phosphate by synthesising the pyrimidine ring from acyclic precursors. We have obtained 7 by phosphorylating 5-acetyl-2'-3'-*O*-isopropylideneuridine (4) with phosphoryl chloride in the presence of *N*-methylmorpholine. The isopropylidene group was removed during the work up procedure to give the required product in 33% yield. The structure of the compound was established by NMR and UV spectroscopy and by the facts that it migrated on paper electrophoresis in a similar manner to uridine 5'-phosphate and that it reacted with periodate, thus showing that the 2' and 3' hydroxyl groups were free.

The selective phosphorylation of unprotected ribonucleosides on the 5'-*O*-position by the use of phosphoryl chloride in triethyl phosphate has been described.[8] Although we have applied this procedure successfully to the phosphorylation of uridine, it was found that the 5'-hydroxyl group of 5-acetyluridine was much less reactive. Attempts to obtain adequate phosphorylation at this position lead to phosphorylation at the 2'-*O*-and 3'-*O* positions and decomposition of the nucleoside. It is somewhat surprising that the presence of the acetyl group at position-5 should so markedly affect the reactivity of the 5'-hydroxyl group but it may be noted that whereas a number of ribonucleosides can be selectively phosphorylated at the 5'-*O*-position, with pyrophosphoryl chloride in *m*-cresol, orotidine gives exclusively a mixture of 2' and 3'-phosphates thus showing that the carboxyl group at the 6-position similarly modifies the reactivity of the 5'-hydroxyl group.[9]

5-Acetyluridine 5'-diphosphate (8) was obtained from 7 by standard procedures via the morpholidate.[10] The structure of 8 followed from its method of synthesis, UV adsorption spectra, phosphorus analysis and behaviour on paper electrophoresis.

Attempts were made to polymerise 8 by the action of polynucleotide phosphorylase form *Micrococcus luteus* in the presence of Mg^{2+} or Mn^{2+},[11] but without success. To obtain polymers from nucleoside 5'-diphosphates which are resistant to the *M. luteus* enzyme, the enzyme from *Escherichia coli* has been used.[3] This also gave no polymer from 5-acetyluridine 5'-diphosphate. Control experiments in which uridine 5'-diphosphate was used gave satisfactory yields of polyuridylic acid showing that the enzymes were active. When a mixture of 8 and uridine 5'-diphosphate was treated with the *M. luteus* enzyme, a polymer was formed but this had an identical UV absorption spectrum to that of polyuridylic acid. This showed that very little, if any, 5-acetyluridine residues had been incorporated into the polymer. The fact that the yield of polyuridylic acid was lower than in the absence of the analogue showed that the latter is a weak inhibitor of the enzyme.

EXPERIMENTAL

NMR spectra were recorded at 100 MHz in $(CD_3)_2SO$ unless otherwise stated. Tlc was carried out on silica gel (MN Kieselgel G/UV_{254}) or cellulose (MN300 G/UV_{254}) (Machery, Nagel & Co., W. Germany) and paper chromatography on Whatman No. 2

paper. Column chromatography was carried out on Kieselgel 60 (70–120 mesh ASTM) (type 7734) (E. Merck AG., W. Germany). The following chromatography solvents were used: (1) chloroform–ethanol (4:1); (2) butan - 1 - ol–ethanol–water (4:1:5, organic phase); (3) acetonitrile–water (17:3); (4) propan - 2 - ol–ammonia (Sp. g. 0.88)–water (7:1:2); (5) propan - 1 - ol–ammonia (Sp. g. 0.88)–water (20:20:3); (6) butan - 1 - ol–acetic acid–water (2:1:1); (7) M ammonium acetate–ethanol (1:1); (8) methylpropanoic acid–M ammonia–0.1 M EDTA (50:30:0.8).

Enzymes

Micrococcus luteus polynucleotide phosphorylase (nucleoside diphosphate, polynucleotide nucleotidyltransferase, E.C. 2.7.7.8) was obtained from P–L Biochemicals Inc., Wisconsin, U.S.A. and the corresponding enzyme from *Escherichia coli* was a gift from Dr. M. A. W. Eaton.

5 - *Acetyl* - 2',3',5' - *tri* - O - *benzoyluridine*. A mixture of 5-acetyluracil (3.08 g, 22 m mole) and hexamethyldisilazane (20 ml) was boiled under reflux until complete solution was obtained (24 h). The excess of hexamethyldisilazane was removed by distillation under reduced pressure at 40° and the resulting oil of the trimethylsilyl derivative dissolved in dry acetonitrile (60 ml). This solution was added to a solution of freshly-prepared 2,3,5 - tri - *O* - benzoyl - D - ribofuranosyl chloride (10.1 g, 20 mmole) in dry acetonitrile (60 ml) and the mixture kept at 20° for 5 days. The solution was evaporated to dryness, ethanol–water (1:1, 70 ml) was added and the mixture evaporated to dryness again. The residue was extracted with chloroform (3 × 50 ml) which left undissolved unreacted 5 - acetyluracil (577 mg). The chloroform solution was evaporated to dryness in the presence of silica gel (2 g) and the resulting powder applied to the top of a column of silica gel (100 g, 40 cm × 2.8 cm). Impurities were eluted with benzene–ethyl acetate (19:1) and the required product with benzene–ethyl acetate (4:1). The solvent was evaporated off and the residue crystallised from methanol to give 5 - acetyl - 2' - 3',5' - tri - *O* - benzoyluridine (7.37 g, 62% yield), m.p. 147–149° (Found: C, 63.9; H, 4.6; N, 4.4. $C_{32}H_{26}N_2O_{10}$ requires: C, 64.2; H, 4.4; N, 4.7%); λ_{max} 284 nm (ϵ, 14,640), λ_{min} 255 nm (ϵ, 5800) in acidic ethanol; λ_{max} 284 nm (ϵ, 11,000), λ_{min} 264 nm (ϵ, 8000) in alkaline ethanol; δ ($CDCl_3$) 2.65 (3H, s, CH_3CO), 4.75 (3H, m, H-4', H-5'), 5.90 (2H, m, H-2', H-3'), 6.20 (1H, d, H-1', $J_{1',2'}$ = 2.5 Hz), 7.1–8.2 (15H, m, benzoyl protons), 8.5 ppm (1H, s, H-6).

5 - *Acetyluridine*. 5 - Acetyl - 2',3',5' - tri - *O* - benzoyluridine (3 g, 5 mmole) and sodium methoxide (270 mg, 5.0 mmole) were dissolved in dry methanol (50 ml) and the solution boiled under reflux for 8 h. It was then neutralised by passing it through a column of Dowex 50 (H^+) ion exchange resin (50 × 1.0 cm) and the eluate and washings evaporated to dryness. The residue was crystallised from propan - 2 - ol to give 5 - acetyluridine (1.24 g, 86%), m.p. 163–165° (Found: C, 45.6; H, 4.9; N, 9.5. Calc. for $C_{11}H_{14}N_2O_7$: C, 46.2; H, 4.9; N, 9.8%); λ_{max} 230 nm (ϵ, 9700), 284 nm (ϵ, 11,400), λ_{min} 251 nm (ϵ, 2670) at pH 1; λ_{max} 287 nm (ϵ, 8750), λ_{min} 262 nm (ϵ, 4000) at pH 13. ORD, + VE Cotton effect, peak at 239 nm, crossover at 224 nm, trough at 204 nm; δ 2.4 (singlet, partially obscured by solvent, CH_3CO), 3.6 (2H, s, H-5'), 3.8–4.1 (3H, m, H-2', H-3', H-4'), 5.08 (2H, s, OH-2', OH-3'), 5.4 (1H, s, OH-5'), 5.76 (1H, d, H-1', $J_{1',2'}$ = 4.3 Hz), 8.67 ppm (1H, s, H-6); δ (D_2O) 2.5 (3H, s, CH_3CO), 5.9 (1H, d, H-1'), 8.85 ppm (1H, s, H-6); other signals as in $(CD_3)_2SO$.

5 - *Acetyl* - 5' - O - *benzoyluridine*. 5 - Acetyl - 2',3',5', - tri - *O* - benzoyluridine (598 mg, 1.0 mmole) and sodium methoxide (54.0 mg, 1.0 mmole) were dissolved in dry methanol (25 ml) and kept at 20° for 48 h. The solution was neutralised with Dowex 50 (H^+) ion exchange resin, evaporated to dryness and the residue fractionated by chromatography on silica gel. The product was eluted from the column with solvent (1) and crystallised from methanol to give 5 - acetyl - 5' - *O* - benzoyluridine (220 mg, 57% yield), λ_{max} 231 nm (ϵ, 18,200), 287 nm (ϵ, 9450), λ_{min} 255 nm (ϵ, 2430) in ethanol; δ 2.35 (singlet, partially obscured by solvent, CH_3CO), 4.0–4.3 (3H, m, H-2', H-3', H-4'), 4.5 (2H, d, H-5'), 5.5 (2H, s, OH-2', OH-3'), 5.75 (1H, d, H-1'), 7.3–8.1 (5H, m, benzoyl group), 8.3 ppm (1H, s, H-6). The compound was homogeneous by tlc on silica gel in solvent (1) (R_f 0.85) and in solvent (2) (R_f 0.60) and it gave a positive reaction with sodium metaperiodate.

5 - *Acetyl* - 2',3' - O - *isopropylideneuridine*. 5 - Acetyluridine (286 mg) and toluene - *p* - sulphonic acid (1.9 g) were dissolved in dry acetone (20 ml) and kept at room temperature for 30 min. Sodium hydrogen carbonate (2.2 g) was added, the mixture stirred for 1h, filtered and the residue washed with hot acetone (2 × 20 ml). The filtrate and washings were evaporated to dryness and crystallised from water to give 5 - acetyl - 2',3' - *O* - isopropylideneuridine (267 mg, 82% yield), m.p. 179–183° (Found: C, 51.7; H, 5.7; N, 8.4. Calc. for $C_{14}H_{18}N_2O_7$: C, 51.5; H, 5.6; N, 8.6%); λ_{max} 227 nm (ϵ, 10,300), 285 nm (ϵ, 11,700), λ_{min} 248 nm (ϵ, 1715) in acidic ethanol; λ_{max} 285 nm (ϵ, 9750), λ_{min} 256 nm (ϵ, 4460) in alkaline ethanol; δ 1.30 and 1.49 (6H, 2s, isopropylidene), 2.45 (singlet, partially obscured by solvent, CH_3CO), 3.58 (2H, d, H-5'), 4.19 (1H, m, H-4'), 4.73 (1H, dd, H-3'), 4.91 (1H, dd, H-2'), 5.84 (1H, d, H-1', $J_{1',2'}$ = 2.5 Hz), 8.56 (1H, s, H-6), 11.5 ppm (1H, s, -NH).

5 - (1 - *Hydroxyethyl*)*uridine*. 5 - Acetyluridine (286 mg, 1 mmole) was dissolved in 0.1 M sodium hydroxide (20 ml), sodium borohydride (152 mg, 4 mmole) was added and the solution kept at room temperature in the dark for 4 h. The excess of sodium borohydride was destroyed by the addition of acetone and the solution neutralised with Dowex 50 (H^+) ion exchange resin. The resin was filtered off, washed with water and the filtrate and washings evaporated to dryness. The residue was repeatedly co-evaporated with methanol to remove borate and the residue crystallised from propan - 2 - ol to give 5 - (1 - hydroxyethyl)uridine (218 mg, 76% yield), m.p. 182–184° (Found: C, 45.2; H, 5.6; N, 9.1. $C_{11}H_{16}N_2O_7$ requires: C, 45.8; H, 5.6; N, 9.7%); λ_{max} 266 nm (ϵ, 7520), λ_{min} 235 nm (ϵ, 2220) at pH 1; λ_{max} 266 nm (ϵ, 5540), λ_{min} 247 nm (ϵ, 4180) at pH 13; δ 1.27 (3H, d, $-CH_3$, J = 8 Hz, 3.62 (2H, s, H-5'), 3.8–4.2 (3H, m, H-2', H-3', H-4'), 4.57 (1H, d, H-1 of hydroxyethyl, J = 8 Hz), 5.85 (1H, d, H-1', $J_{1',2'}$ = 4.3 Hz), 7.79 ppm (1H, s, H-6).

5 - Vinyluridine[6] upon treatment with 0.1 M hydrochloric acid at 100° for 1 h or at 20° for 10 days gave a product which had an identical UV absorption spectrum to that of 5 - (1 - hydroxyethyl)uridine (**6**) and which ran upon tlc on silica gel in solvent 3 (R_f 0.57) and paper chromatography in solvent (4) (R_f 0.52) with identical R_f values to those of **6**.

5 - *Acetyluridine* 5' - *phosphate*. 5 - Acetyl - 2',3' - *O* - isoproylideneuridine (1.47 g) was dissolved in glyme (50 ml) *N*-methylmorpholine (5.4 ml) was added, the solution cooled to 0° and a solution of phosphoryl chloride (1.25 ml) in glyme (20 ml) slowly added. The reaction mixture was allowed to warm up to room temp. and then after 18 h the solvent was evaporated off. The residue was co-evaporated with benzene (2 × 20 ml), dissolved in M ammonia (100 ml) and solution heated at 90° for 30 min. It was evaporated to a small volume, adjusted to pH 7.5 and M barium acetate (10 ml) added. The resulting suspension was stored at 0° for 1 h and then the precipitate of barium phosphate centrifuged off and washed with cold aqueous ammonia (pH 7.5). The volume of the solution was reduced to about 25 ml and applied to a column of Dowex 50 (H^+) ion exchange resin (50 × 1.0 cm) and the column eluted with water until no more UV absorbing material was removed. The eluate was evaporated to dryness, co-evaporated with ethanol (3 × 20 ml), dissolved in water (100 ml) and the solution adjusted to pH 7.5 with ammonia. The solution was then applied to a column of DEAE cellulose (35 × 2.5 cm) which was washed with water to elute unreacted 5 - acetyluridine (absorbance at 284 nm, 9000; 18% recovery) and then with a linear gradient of triethylammonium bicarbonate (0.00–0.1 M, pH 7.5) to elute the nucleotide (Absorbance at 284 nm, 17,00, 33% yield). The solution of the nucleotide was evaporated to dryness and the residue co-evaporated with methanol until all of the triethylammonium bicarbonate had been removed and then dissolved in water. Cations were removed from the solution by treatment with Dowex 50 (H^+) ion exchange resin in the usual way, the solution neutralised with barium hydroxide and freeze dried to give the barium salt of 5 - acetyluridine 5' - phosphate. The product was homogeneous by tlc on cellulose in solvents 7 and 8 and by paper chromatography in solvents 4, 5 and 6. It moved on paper electrophoresis at pH 7.5 and 3.6 at 0.88 times the mobility

of uridine 5' - phosphate. δ (D_2O) 2.54 (3H, s, CH_3CO), 3.38 (2H, s, H-5'), 5.92 (1H, d, H-1'), 8.59 ppm. (1H, s, H-6). The UV absorption spectrum was similar to that of 5 - acetyluridine but the apparent extinction coefficient was low. The compound gave positive reactions for organic phosphate and for a vicinal diol.

5 - Acetyluridine 5' - diphosphate. A solution of 5 - acetyluridine 5' - phosphate (1.4 mmole, free acid) in a mixture of water (14 ml), *t* - butanol (14 ml) and morpholine (0.48 ml) was boiled under reflux. A solution of dicyclohexylcarbodiimide (1.15 g) in *t* - butanol (21 ml) was added dropwise over a period of 2 h and the mixture boiled for a further 3 h. Then morpholine (0.24 ml) was added and dicyclohexylcarbodiimide (0.58 g) in a mixture of water (2 ml) and *t* - butanol (5 ml) added dropwise and the mixture boiled under reflux for 3 h. The reaction mixture was cooled, crystalline material filtered off, the filtrate evaporated to a small volume to remove *t* - butanol and the resulting aqueous solution evaporated to dryness and the residue dissolved in methanol (4 ml). To this solution there was added ether (50 ml) to give a gummy precipitate of the required phosphoromorpholidate (this compound ran as a single spot upon paper electrophoresis at pH 7.5 with a mobility of 0.47 of that of uridine 5' - phosphate, 62% yield). This was dried by repeated co-evaporation with dry pyridine, dissolved in dry pyridine (20 ml) and allowed to react with tri - n - butylammonium phosphate (4 mmole) under rigorously dry conditions for 4 days at room temp. The pyridine was removed by evaporation and the residue dissolved in water (20 ml) containing lithium acetate (510 mg). The solution was extracted with ether (20 ml) and the aqueous layer adjusted to pH 12 with lithium hydroxide. The mixture was kept at 0° for 30 min and the precipitate of trilithium phosphate filtered off. The filtrate was adjusted to pH 7.5 by the use of Dowex 50 (H^+) ion exchange resin and applied to a column of DEAE cellulose (25 × 2.0 cm). After washing the column with water, the products were eluted with a linear gradient of triethylammonium bicarbonate (0.0 → 0.5 M, pH 7.5). A small amount of 5 - acetyluridine 5'-phosphate (1%) was eluted off first followed by the required compound (40%, based on the morpholidate). This was isolated as the sodium salt in the usual way. It was still contaminated with inorganic phosphate so it was purified further as follows. Impure 5 - acetyluridine 5' - diphosphate (15 mg, $A_{281\,nm}$ 250) was dissolved in water and applied to a Biogel P2 column (48 × 2.0 cm). The column was eluted with water (0.4 ml/min) and fractions (3 ml) collected. Fractions 12–23 contained the diphosphate with a maximum in fractions 17 and 18 whereas only fractions 19 onward contained the inorganic phosphate. Fractions 16–18 were pooled and evaporated to dryness to give the sodium salt of 5 - acetyluridine 5'-diphosphate (5.5 mg) (Found: P, 2.01 g atoms/mole); λ_{max} 228 nm (ε, 10,900), 281 nm (ε, 11,400), λ_{min} 249 nm (ε, 2500) at pH 1; λ_{max} 283 nm (ε, 8700), λ_{min} 259 nm ε, 4100) at pH 13. The compound was homogeneous by tlc on cellulose in solvents 7 and 8 and by paper chromatography in solvents 4, 5 and 6. Upon paper electrophoresis at 3.6 it moved at 1.02 times the mobility of uridine 5' - phosphate and 1.15 times the mobility of 5 - acetyluridine 5' - phosphate.

Attempted enzymic polymerisation of 5 - acetyluridine 5' - diphosphate. The following solutions were used: (a) 0.01 M EDTA (0.50 ml), 1.0 M tris-chloride buffer, pH 9.0 (0.15 ml), *Micrococcus luteus* polynucleotide phosphorylase (10 units), 0.01 M magnesium chloride (0.10 ml), nucleoside 5' - diphosphate (20 μmole) and water to a total volume of 1.0 ml; (b) 0.01 M EDTA (0.05 ml), 1.0 M Tris-chloride buffer, pH 9.0 (0.35 ml), *M. luteus* polynucleotide phosphorylase (10 units), 0.10 M manganese chloride (0.20 ml), nucleoside 5' - diphosphate (20 μmole) and water to a total volume of 1.0 ml; (c) 0.01 M EDTA (0.15 ml), 1.0 M Tris-chloride buffer, pH 9.0 (0.15 ml), *Escherichia coli* polynucleotide phosphorylase (40 units), 0.10 M magnesium chloride (0.15 ml), nucleoside 5' - diphosphate (30 μmole) and water to a total volume of 1.0 ml. The enzyme reactions were carried out at 37° and the amount of inorganic phosphate released measured by Fiske and Subbarow's method.[12] A 100% release was equivalent to each diphosphate molecule losing one phosphate group. When the release of inorganic phosphate had levelled off, the reaction was terminated by the addition of phenol, buffered to pH 7.5 with Tris chloride, and the aqueous layer separated. A sample of this was examined by tlc on cellulose in solvents (7) and (8) to detect the presence of polymer or short-chain oligomers which would run with low R_f values. The bulk of the aqueous layer was dialysed at 5° for a total of 40 hr vs 100 mM NaCl–1 mM EDTA, 10 mM NaCl–0.1 mM EDTA and then twice against water. The amount of polymer remaining in the dialysis bag was determined by measuring the UV absorption of the solution. Experiments were carried out with 5 - acetyluridine 5' - diphosphate, uridine 5' - diphosphate and a mixture of the two in equimolar amounts. The results are shown in the table. In the experiments with 5 - acetyluridine 5' - diphosphate no oligomeric material was detected upon examination by tlc. The polymer obtained from the action of the enzyme on the mixture of the two diphosphates had λ_{max} 262 nm, λ_{min} 233 nm, $A_{260}/A_{280} = 2.4$ at pH 7. This was identical to the values given by the polymer obtained from uridine 5' - diphosphate alone.

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