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SOME SYNTHETIC ANALOGUES OF URIDINE DIPHOSPHATE GLUCOSE

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Abstract—In order to investigate the mechanisms of uridine diphosphate glucose enzymic reactions, a number of analogues with modified uracyl residues were synthesized by the morpholidate method. 3-N-Methyl-uridine (I), 4-thiouridine (II), cytidine (III), 2-thiouridine (IV), iso-cytidine (V) and 6-azauridine (VI) diphosphate α-D-glucopyranoses were obtained. These modified nucleotides were prepared via 2',3'-O-isopropylidene nucleosides the latter being obtained from uridine. Conditions are described for phosphorylation of nucleosides with dimorpholinochlorophosphate.

NUCLEOTIDE anhydrides are associated with important metabolic reactions of carbohydrates, alcohols and carboxylic acids but the mechanisms of these reactions are still obscure. The nucleoside diphosphate sugars (NDPS) are probably the most significant group of nucleotide anhydrides and are represented by compounds with a wide variety of structures and biochemical functions.

In naturally occurring NDPS, there is a close structural relationship between the nucleotide and sugar moieties, and recently, it has been suggested that in NDPS the heterocyclic base residue may serve as a template, fitting a limited number of sugars with common structural features, the two parts of the molecule being bound by intramolecular hydrogen bonding.[1]

Experimental evidence in favour of this view may be provided by the investigation, of NDPS analogues with modified heterocyclic nuclei, now in progress in this laboratory. The present communication describes the synthesis of a number of uridine diphosphate glucose (UDPG) analogues and is probably the first systematic investigation in this field although Ueda has recently described the synthesis of 5-bromo-uridine diphosphate glucose.[2]

If the hydrogen bonding in the uracil residue is associated with the amide system —C_2 and C_4 carbonyls and the hydrogen atom at N_3—then a modification of these positions will change the affinity of the group in hydrogen bonding. Consequently, the analogues 3-N-methyluridine- (I),[3] 4-thiouridine- (II),[4] cytidine- (III),[5] 2-thiouridine- (IV) and iso-cytidine-(V) diphosphate glucoses were synthesized. One of the analogues—6-azauridine diphosphate glucose—has an unmodified uracil amide system;

[1] N. K. Kochetkov, E. I. Budowsky and V. N. Shibaev, *Biokhimia. (U.S.S.R)*, **28**, N4 (1963).

[2] T. Ueda, *Chem. Pharm. Bull., Japan* **8**, 455 (1960).

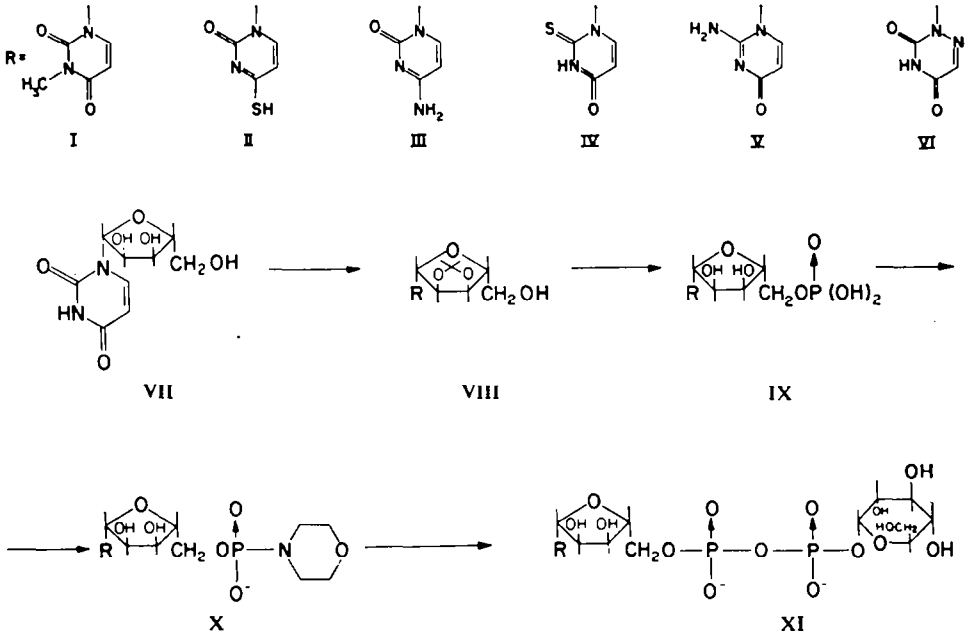
[3] N. K. Kochetkov, E. I. Budowsky and V. N. Shibaev, *Izv. Akad. Nauk SSSR, Otdel, Khim. Nauk* 1035 (1962).

[4] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev and M. A. Grachev, *Biochim. Biophys. Acta* **59**, 747 (1962).

[5] E. I. Budowsky, V. N. Shibaev, G. I. Yeliseeva and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1491 (1962).

this compound may become of additional interest as an inhibitor of cell wall synthesis by bacteria (cf. bacteriostatic activity of 6-azauracil[6]).

The syntheses of compounds I–VI were based on the following general scheme:



The scheme involves the following four syntheses: (a) 2′,3′-O-isopropylidene nucleosides with modified heterocyclic nuclei (VIII), (b) the corresponding nucleoside-5′-phosphates (IX), (c) the nucleoside-5′-phosphomorpholidates (X) and (d) the pyrophosphates (XI). These four main steps though routine in the syntheses of naturally occurring pyrophosphates often need considerable modification when applied to the analogues. Hence, the synthesis of UDPG analogues is a further development of the general methods in synthetic nucleotide and nucleoside pyrophosphate chemistry.

The synthesis of 2′-3′-O-*isopropylidene nucleosides with modified uracil nuclei*

The lack of a convenient method for the ribosylation of pyrimidines suggested uridine (VII) as the starting compound for the synthesis of the UDPG analogues. In the case of the cytidine and 6-azauridine analogues, the synthesis was achieved via the corresponding nucleotide and nucleoside both of which are available commercially.

4-Thiouridine, synthesized according to the method of Fox *et al.*,[7] was isolated from the reaction mixture after alcoholysis of 2′-3′-5′-tri-O-benzoyl-4-thiouridine by an improved method. Cellulose partition chromatography made possible the removal of the by-products and the 4-thiouridine was obtained in a pure crystalline form, the three-step synthesis resulting in a 51 per cent yield. Both cellulose and

[6] J. Tokagi and N. Otsuji, *Biochim. Biophys. Acta* **29**, 227 (1958).

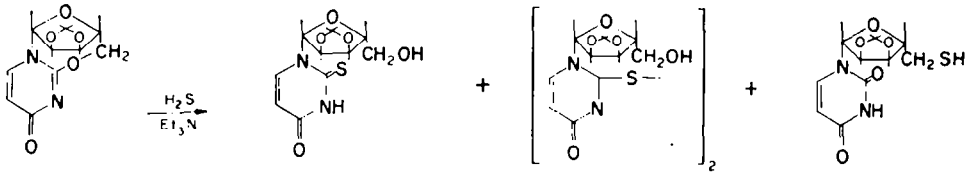
[7] J. Fox, D. Praag, J. Wempen, L. Doerr, L. Choeng, J. Knoll, M. Eidinoff, A. Bendich and G. Brown, *J. Amer. Chem. Soc.* **81**, 178 (1958).

silica gel partition chromatography are convenient methods for the final purification of nucleosides.

Condensation of 4-thiouridine with acetone by the usual method (in the presence of sulphuric and *p*-toluene sulphonic acids etc.) failed due to fission of the mercapto group and formation of uridine. A good yield was obtained, however when di-(*p*-nitrophenyl)phosphate and 2,2-diethoxypropane were used as reagents.[8] No side reactions were observed and 2′,3′-O-isopropylidene-4-thiouridine (VIII, R = 4-thiouracil) was isolated from the reaction mixture by ion exchange chromatography in a yield of 80 per cent. This method for introduction of the isopropylidene protective group proved equally effective in the syntheses of 2′,3′-O-isopropylidine-6-azauridine (VIII, R = 6-azauracil) and 2′,3′-O-isopropylidene-5-bromouridine (VIII, R = 5-bromouracil).

3-N-methyl-2′,3′-O-isopropylidene uridine (VIII, R = 3-N-methyluracil) was obtained in a 98 per cent yield by methylation of 2′,3′-O-isopropylidene uridine with diazomethane in an ethereal-methanolic solution.

The synthesis of analogues, modified in position 2, (VIII, R = iso-cytosine, 2-thiouracil) was performed via 2′,3′-O-isopropylidene-O[2], 5′-cyclouridine (XII), obtained by the method of Todd *et al.*[9] This compound reacts with methanolic ammonia to give 2′,3′,O-isopropylidene-iso-cytosine (VIII, R = iso-cytosine).[9] The reaction with hydrogen sulphide in the presence of triethylamine[10] results in a mixture which may be separated by adsorption chromatography on alumina, yielding 2′,3′-O-isopropylidene-2-thiouridine (50 per cent) as the main product. In addition to the corresponding disulphide (XIII), a compound with properties corresponding to 2′,3′-O-isopropylidene-5′-mercapto-5′-deoxyuridine (XIV) was isolated. The possibility of a ring opening in this manner has not previously been reported.



XII XIII XIV

The structures of the isopropylidene modified nucleoside derivatives were confirmed by the close similarity of their U.V.-spectra to those of the corresponding unprotected nucleosides and also by the characteristic shifts of the U.V.-spectra at different pH values.

The thin non-fixed layer chromatography, previously employed for protected carbohydrates,[11] was successfully applied to the protected nucleosides, the method proving very useful in controlling the reactions, assaying the purity of the products and modelling preparative column adsorption chromatography. In addition, the reactions were also checked by U.V.-spectroscopy.

[8] A. Hampton, *J. Amer. Chem. Soc.* **83**, 3460 (1961).
[9] D. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).
[10] D. Brown, D. Parihar, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).
[11] N. K. Kochetkov, B. A. Dmitriev and A. I. Usov, *Dokl. Akad. Nauk. SSSR* **143**, 863 (1962).

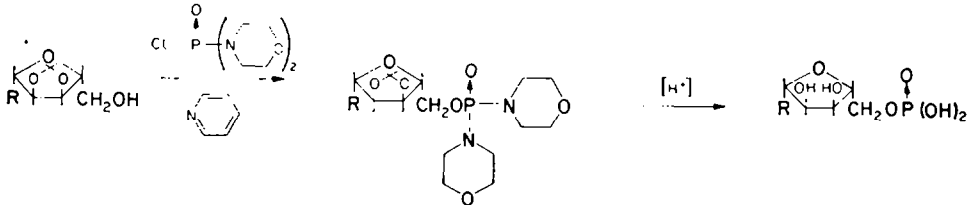
The synthesis of nucleoside phosphates

The isopropylidene derivatives VIII (R = 2- and 4-thiouracil, iso-cytosine, 6-azauracil) were converted into the corresponding phosphates according to Tener[12] by reaction with 2-cyanoethylphosphate and dicyclohexylcarbodiimide followed by alkaline hydrolysis for removal of the cyanoethyl protective group.

This method is probably the only possible route to 4-thiouridine 5′-phosphate as the labile mercapto group is sensitive to the acid hydrolysis and hydrogenolysis used in other known methods. A modification of Tener's procedure by performing the reaction at 60°, results in acceleration of the reaction rate without a marked decrease in yield. The mercapto group of thionucleotides is inert under the conditions of alkaline hydrolysis, employed by Tener for removal of the cyanoethyl group (1N KOH at 100° for 15 min.) and the acid hydrolysis (70% acetic acid at 100° for 30 minutes) necessary for removal of the isopropylidene group affects the mercapto group to only a slight extent. The thiouridine phosphates were isolated by ion-exchange chromatography. Phosphorylation of 6-azauridine performed under analogous conditions resulted in high yields of 6-azauridine-5′-phosphate.

The synthesis of iso-cytidine-5′-phosphate was complicated by polymerization reactions due to the presence of a reactive amidine group and during the hydrolysis for removal of the isopropylidene group, cleavage of the N-glycosidic bond took place. These difficulties were overcome if the time of phosphorylation was reduced and if cold 85 per cent formic acid was used instead of 70 per cent acetic acid for hydrolysis of the isopropylidene group. Isocytidine 5′-phosphate was then obtained in a 15–25 per cent yield.

Phosphorylation of 2′,3′-O-isopropylidene-3-N-methyluridine with cyanoethyl phosphate was unsuccessful on account of the ease with which the 3-N-methyluracil ring is opened during alkaline hydrolysis. A new method of phosphorylation, requiring only mild acid hydrolysis for removal of the protective group, has been specially developed for the nucleotide analogues unstable in alkali. It is based on the reaction with dimorpholinophosphochloridate previously used in the synthesis of phosphates of simple alcohols.[13] Reaction of 2′,3′-O-isopropylidene nucleosides with a large excess dimorpholinophosphochloridate in dry pyridine, followed by mild acid hydrolysis (0·1 NHCl at 100° for 2 hr) and ion-exchange chromatography afforded nucleotides in a 30–40 per cent yield.



Uridine-, cytidine- and 5-bromouridine-5′-phosphates were obtained by this procedure and the method was also successfully applied to the synthesis of 3-N-methyluridine-5′-phosphate.

[12] G. Tener, *J. Amer. Chem. Soc.* **83**, 159 (1961).
[13] H. Montgomery and J. Turnbull, *J. Chem. Soc.* 1963 (1958).

TABLE 1. THE PROPERTIES OF NUCLEOSIDE 5′-PHOSPHATES

Substance	R_f in paper chromatography		R_{UMP} in paper electrophoresis		U.V.-spectrum					
					Nucleoside			Nucleotide		
	Ethanol–0·5M CH_3COONH_4 pH 7·5, 5:2	Ethanol–0·5M CH_3COONH_4 satd. with borax 5:2	pH 7·5	pH 4·0	λ_{max}, mμ	λ_{min}, mμ	ε_{max}	λ_{max}, mμ	λ_{min}, mμ	ε_{max}
Uridine 5′-phosphate	0·21	0·05	1·0	1·0	—	—	—	—	—	—
Uridine 2′(3′)-phosphate	0·22	0·26	1·0	1·0	—	—	—	—	—	—
4-Thiouridine 5′-phosphate	0·26	0·05	1·25	1·0	331	274	21200	331	274	20600
					245	225	(331)	245	225	(331)
3-N-Methyluridine 5′-phosphate	0·37	0·05	1·0	1·0	260	232	9200	262	232	8800
2-Thiouridine-5′-phosphate	0·25	0·05	1·20	1·0	272	243	11300	272	243	—
Isocytidine-5′-phosphate	0·18	0·05	1·0	0·63	256*	235*	7100*	256*	240*	—
					220		(256)			
6-Azauridine-5′-phosphate	0·24	0·05	1·3	1·0	263	—	5500	262	—	5800
5-Bromouridine-5′-phosphate	0·21	0·03	0·86	0·87	276	250	9000	278	250	—

* pH 1

The structures of the nucleosides-5′-phosphates were confirmed by a comparison of their U.V.spectra with those of the corresponding nucleosides and their behaviour during paper chromatography and electrophoresis. The decrease in the R_f value of the substances after addition of borate indicated the presence of an unsubstituted *cis*-glycol group; the mobility of the substances during paper electrophoresis at pH 4·0 and 7·5 correspond to that expected for a monophosphate; the homogenity of the phosphates during chromatography and their molar extinction values being close to those observed for the corresponding nucleosides indicated their high purity. The data for nucleoside 5′-phosphates are listed in Table 1.

The synthesis of nucleoside 5′-phosphomorpholidates

Nucleoside 5′-phosphomorpholidates were obtained by condensation of the nucleotides with morpholine in the presence of dicyclohexylcarbodiimide[14] and good yields resulted for almost all the analogues in spite of the presence of reactive groups in the heterocyclic nuclei. Only in the case of 4-thiouridine 5′-phosphate did the mercapto group enter into a side reaction with morpholine but the amount of the by-product could be considerably reduced by using shorter reaction periods. With 2-thiouridine 5′-phosphate no side reactions were observed.

The nucleoside-5′-phosphomorpholidates, isolated as 4-morpholino-N,N- dicyclohexylcarboxamidinium salts, were characterized by U.V.-spectra, paper chromatography and electrophoresis. The yields and properties of the compounds are listed in Table 2. Minor contamination of the nucleoside-5′-phosphomorpholidate preparations with the starting nucleosides does not effect the pyrophosphate synthesis and the compounds were, therefore, used without further purification.

TABLE 2. NUCLEOSIDE 5′-PHOSPHOMORPHOLIDATES (X)

Substance	Yield %	Ethanol–0·5M CH_3COONH_4 pH 7·5, 5:2	Electrophoresis		λ_{max}
			pH 7·5 R_{UMP}	pH 4·0 R_{UMP}	
3-N-Methyluridine-5′-phosphomorpholidate	62	0·72	0·45	0·91	262
4-Thiouridine-5′-phosphomorpholidate*	67	0·79	0·90	1·00	331
2-Thiouridine-5′-phosphomorpholidate	68	0·58	0·72	0·98	272
Isocytidine-5′-phosphomorpholidate	94	0·50	0·48	0·63	256, 220 (pH 1)
6-Azauridine-5′-phosphomorpholidate	95	0·60	0·90	1·0	262

* The preparation was contaminated with a minor quantity of a substance with R_{UMP} 0·32 (pH 7·5) and U.V.-spectrum similar to that of cytidine.

The synthesis of nucleoside 5′-diphosphate α-D-glucopyranoses

The pyrophosphate synthesis according to the method of Khorana *et al.*[15], proceeds much faster and without decrease in the yield if the reaction temperature is raised to 60°. Isocytidine 5′-phosphomorpholidate reacts much slower than the other analogues, the yield of isocytidine diphosphate glucose being only 15 per cent after 24 hr heating.

UDPG analogues were isolated from the reaction mixtures by ion exchange chromatography. Initially this was performed with strong anion-exchangers (Dowex 1),

[14] J. Moffatt and H. Khorana, *J. Amer. Chem. Soc.* **83**, 649 (1961).
[15] S. Roseman, J. Distler, J. Moffatt and H. Khorana, *J. Amer. Chem. Soc.* **83**, 659 (1961).

but better results are obtained with DEAE-Sephadex A-25 as elution is possible at lower concentrations of buffer solutions and less irreversible adsorption of the pyrophosphates by the anion exchanger takes place. Good separation was observed when elution was performed with triethylammonium acetate pH 4·0 in increasing concentration (linear gradient). Triethylammonium acetate was quantitatively removed by vaccum drying over P_2O_5 at 50–60°. The triethylammonium salts of the UDPG analogues were transformed into sodium salts for analysis and biochemical assays.

The structures of the compounds followed from the U.V.-spectroscopic data similar to the corresponding nucleosides and nucleotides, behaviour during paper chromatography (comparison with uridine 5'-phosphate, UDPG and the starting nucleoside 5'-phosphate) and electrophoresis (mobility expected for a two-substituted pyrophosphate). The UDPG-analogues were subjected to acid hydrolysis and the glucose formed determined colorimetrically[16] and identified by paper chromatography. The purity of the compounds was evaluated from the molar extinction value and the glucose:base ratio. The yields and properties of the UDPG analogues are listed in Table 3.

The biochemical assays and the physical properties of the compounds in solution will be published later.

TABLE 3. NUCLEOSIDE DIPHOSPHATE SUGARS (XI)

Substance	Yield %	R_f in Ethanol–0·5M CH_3COONH_4 pH 7·5, 5:2	R_{UMP} in paper electrophoresis		Base:glucose ratio
			pH 4·0	pH 7·5	
3-N-Methyluridine diphosphate glucose	53	0·49	1·25	0·85	1:0·98
4-Thiouridine diphosphate glucose	46	0·31	1·25	1·25	1:0·98
Cytidine diphosphate glucose	40	0·18	1·0	0·82	1:0·96
2-Thiouridine diphosphate glucose	50	0·32	1·6	1·13	1:0·99
Isocytidine diphosphate glucose	15*	0·20	1·15	0·90	1:0·90
6-azauridine diphosphate glucose	50	0·28	1·75	—	1:0·97

* The time of reaction was 24 hr.

EXPERIMENTAL

Chromatography was performed with Leningrad Goznak factory "roll 12" and Chistie Soli factory chromatographic papers. Alumina of the II activity grade by Brockman was used for thin layer chromatography. Electrophoresis was performed with the same chromatographic papers in "EFA-1" apparatus; the stress gradient was equal to 20–23 V/cm; the following buffer solutions were used: pH 7·0—0·02M triethylammonium bicarbonate; pH 4·0—0·02M triethylammonium acetate; the time of electrophoresis was usually 45–60 min. The spots on paper chromatograms and electrophoregrams as well as on thin layer chromatograms were detected with ultrachemiscope UI-1. All evaporations were performed at 37° *in vacuo*.

1. *The Synthesis of Modified Nucleosides Isopropylidene Derivatives*

2',3'-O-*Isopropylidene-4-thiouridine*. 2',3',5'-Tri-O-benzoyl-4-thiouridine[7] (5·4 g; 9·4 mmoles) dissolved in 130 ml dry methanol was refluxed for 5 hr with 132 ml 0·1M sodium methylate. The hot solution was treated with CH_3COOH (1 ml), cooled and evaporated to dryness. The residue was dissolved in water (10 ml); the solution extracted 3 times with chloroform, the combined chloroform layers washed with water and the water phases evaporated to dryness. The residue was subjected to partition chromatography on a cellulose column (5 × 32 cm) with solvent system A (isoamyl alcohol–acetone–water 3:2:1). Fractions containing pure 4-thiouridine as revealed by paper chromatography with the same system were pooled together and evaporated to dryness (water was added

[16] N. Nelson, *J. Biol. Chem.* **153**, 375 (1944).

in course of evaporation for removal of isoamyl alcohol). The residue was recrystallized from ethanol–ether to give 4-thiouridine as long yellow needles; yield 1·7 g (69%) m.p. 135–138°(dec). U.V.-spectrum (water): pH 6·5—λ_{max} 331, 245 mμ; ε_{max} 21200, 4000; λ_{min} 274, 225 mμ; ε_{min} 2400, 1600; pH 12—λ_{max} 316, ε_{max} 19700, λ_{min} 268, ε_{min} 2400. (Found: C, 41·36, H, 4·74, S, 12·32; Calc. for $C_9H_{12}O_5SN_2$: C 41·43, H 4·65, S 12·33%). The substance was homogeneous in paper chromatography, R_f 0·34 (A) and 0·25 (n-butanol–water 86:14, Syst. B).

4-*Thiouridine* (550 mg; 1·82 mmoles), 800 mg (2·35 mmoles) di-(*p*-nitrophenyl)-phosphate[17] and 8 ml (16 mmoles) 2,2-diethoxypropane[18] in dry acetone (25 ml) were stirred for 45 min at room temp with special precautions to exclude moisture. The solution was poured into 1% solution NH_4OH in 50% aq. methanol (500 ml) and 2′-3′-O-isopropylidene-4-thiouridine was isolated from the mixture by ion-exchange chromatography on a column with Dowex 1 × 4 HCO_3^- (2 × 14 cm); linear gradient, mixer—500 ml 50% aq. methanol, reservoir—500 ml of 0·25M NH_4HCO_3 in 50% aq. methanol, yield 30000 $OU_{316\ m\mu}$ (80%). The sample for analysis was purified by partition chromatography on Al_2O_3 with Syst. A and recrystallized from ethanol–n-heptane, m.p. 170–172°. The U.V.-spectrum closely resembles that of 4-thiouridine. (Found: C, 47·98, H, 5·58, S, 10·72; $C_{12}H_{16}O_5SN_2$ requires: C, 47·98, H, 5·37, S, 10·64%). The substance was homogeneous in paper chromatography, R_f 0·90 (Syst. A).

3-*N-Methyl*-2′,3′-*O-isopropylideneuridine*[3]. A solution of 570 mg (2 mmoles) 2,′3′-O-isopropylidene uridine[19] in 15 ml methanol was treated at 0° with dry ethereal diazomethane; the reaction was followed spectrophotometrically (the reaction product exhibits equal adsorpton at 260 mμ at pH 7·0 and 12). The solution was evaporated to dryness to give 3-N-methyl-2′,3′-O-isopropylideneuridine, 581 mg (98%), m.p. of analytical sample 133·5–134° (from tetrahydrofuran–cyclohexane). (Found: C, 52·39, H, 6·04, N, 9·19, $C_{13}H_{18}O_6N_2$ requires: C, 52·34, H, 6·08, N, 9·40%). U.V.-spectrum: λ_{max} 260 mμ, ε_{max} = 9200; λ_{min} 232 mμ, ε_{min} = 2900. The substance was homogeneous in thin-layer chromatography, R_f 0·40 (chloroform–ethanol 30:1).

5′-O-Tosyl-2′,3′-O-isopropylidene uridine was obtained by the prodecure of Levene and Tipson[19]. The substance, precipitated from the reaction mixture with water, was homogeneous in paper chromatography, R_f 0·88 (Syst. B) and thin layer chromatography on Al_2O_3, R_f 0·78 (chloroform–methanol 1:1) and was used in the next step without further purification.

5′-*Iodo*-5′-*deoxy*-2′,3′-O-*isopropylideneuridine* was obtained from the tosylate by reaction with sodium iodide in acetone.[19] The product was isolated by adsorption chromatography on alumina (acetone:chloroform 1:1). The substance was homogeneous in paper chromatography, R_f 0·95 (Syst. B) and thin layer chromatography on alumina, R_f 0·36 (chloroform).

2′,3′-O-*isopropylidene*-O²,5′-*cyclouridine* was obtained by the procedure of Todd *et al.*[8] and purified by crystallization from ethanol. U.V.-spectrum (in methanol): λ_{max} 237 mμ, λ_{min} 212 mμ coincides with the previously published. The substance is homogeneous in paper chromatography, R_f 0·53 (butanol–water).

2′,3′-O-*isopropylidene-isocytidine* was obtained by the method of Todd *et al.*[10]. The U.V.-spectrum of the compound closely resembled that observed by previous investigators: 0·1N HCl—λ_{max} 256, 220 mμ, λ_{min} 239 mμ; 0·1N NaOH—λ_{max} 224 mμ. R_f 0·47 (Syst. B), 0·17 (chloroform–methanol 10:1, thin layer chromatography).

2′,3′-O-*isopropylidene*-2-*thiouridine*. Hydrogen sulphide was passed for 1·5 hr through a solution of 199·5 mg (0·75 mmoles) 2′,3′-O-isopropylidene-O²,5′-cyclouridine in a 5% dimethylformamide solution of triethylamine (2·8 ml) and the reaction mixture left for 24 hr at room temp. The solution was evaporated, the residue dissolved in acetone and chromatographed on a column with 30 g of Al_2O_3. Acetone eluted 2′,3′-O′-isopropylidene-2-thiouridine disulphide, white crystals from ethanol, yield 49·6 mg, m.p. 197–200°(dec), R_f 0·47 (Syst. B), 0·54 (chloroform–ethanol 30:1, thin layer chromatography). U.V.-spectrum contains no maximuma or minimuma in the 220–280 mμ region. Todd *et al.* give m.p. 205–206°, U.V.-spectrum—inflection at 210–212 mμ.

* OU—optical unit—amount of a substance, giving an optical density equal to 1 at a given wavelength value when dissolved in 1 ml. solvent measured in a cell with light path equal to 1·00 cm TOD—total optical density—optical density of solution, multiplied by its volume.

[17] H. Khorana and J. Moffatt, *J. Amer. Chem. Soc.* **79**, 3741 (1958).

[18] A. E. Tschitschibabin and S. A. Elgasin, *Ber. Dtsch. Chem. Ges.* **47**, 1851 (1914).

[19] P. Levene and R. Tipson, *J. Biol. Chem.* **106**, 113 (1934).

Continued elution with acetone and then with acetone–methanol 1:1 afforded 2',3'-O-isopropylidene-2-thiouridine, 95 mg, syrup, R_f 0·86 (Syst. B), 0·30 (chloroform–ethanol 30:1, thin layer chromatography), 0·85 (acetone:methanol 1:1, thin layer chromatography). U.V.-spectrum closely resembles that observed by previous investigators: λ_{max} 272 mμ, λ_{min} 243 mμ.

Elution with methanol afforded 5'-mercapto-5'-deoxy-2',3'-O-isopropylideneuridine, white crystals from benzene, yield 36 mg, m.p. 185–187° (dec), R_f 0·86 (Syst B); in thin layer chromatography R_f 0·25 (chloroform–ethanol 30:1) and 0·20 (acetone–methanol 1:1). U.V.-spectrum closely resembles that of uridine, λ_{max} 260 mμ, λ_{min} 230 mμ. (Found: S, 10·90. Calc. for $C_{12}H_{16}O_5SN_2$: S, 10·72%).

2',3'-O-*Isopropylidene-6-azauridine*. Dry acetone (60 ml) and 2,2-diethoxypropane (8 ml) were added to 0·984 g (4 mmoles) 6-azauridine and 1·4 g (4 mmoles) di-(*p*-nitrophenyl phosphate and the solution stirred for 3 hr at room temp. The reaction mixture was poured into 200 ml 1% NH_4OH in 50% aq. methanol. The 2',3'-O-isopropylidene-6-azauridine was isolated by ion-exchange chromatography on a column with Dowex 1 × 4 HCO_3^- (2 × 9 cm; linear gradient, 50% aq. methanol–0·3M NH_4HCO_3 in 50% aq. methanol, 1000 ml). Fractions, containing 2',3'-O-isopropylidene-6-azauridine, contaminated with 6-azauridine, were pooled together and evaporated to dryness. The product was purified by cellulose partition chromatography in Syst. B, yield 0·930 g (82%), m.p. 138–139° (from acetone–cyclohexane). The substance was homogeneous by paper chromatography. (Cf. ref.[20], m.p. 142–143°).

2',3',-O-*Isopropylidene-5-bromouridine*. This was obtained by a procedure similar to that described, yield 65%, m.p. 207–208° (dec), U.V.-spectrum: λ_{max} 278 mμ, ε_{max} 9000, λ_{min} 248 mμ, ε_{min} 1740. (Found: C, 39·55, H, 4·08, Br, 22·07; calc. for $C_{12}H_{14}N_2O_6$ Br: C, 39·66, H, 4·15, Br, 22·05%) Cf. ref.[21]: m.p. 231–232°, λ_{max} 278 mμ.

II. *Nucleoside 5'-Phosphates* (IX)

A *Phosphorylation with 2-cyanoethyl phosphate*

4-*Thiouridine-5'-phosphate*. 2',3'-O-Isopropylidene-4-thiouridine (1·52 mmoles) were dissolved in dry pyridine (30 ml); 6·6 ml of 1M aq. pyridine solution of 2-cyanoethyl phosphate[18] were added and the mixture evaporated to dryness. Dry pyridine (30 ml) was added and the solution again evaporated to dryness. The operation was repeated twice for removal of water. The residue was dissolved in 66 ml dry pyridine, 6·7 g dicyclohexylcarbodiimide were added and the solution heated for 5 hr at 60°. The solution was evaporated to dryness after addition of water and the residue evaporated with water for removal of pyridine. The residue was dissolved in 66 ml 70% CH_3COOH, heated for 30 min at 100° and acetic acid removed by evaporation with water. The residue was dissolved in 66 ml 1N KOH and heated for 15 min at 100°. The solution was passed through a column (4 × 6 cm) with Dowex 50 H^+, the effluent brought to pH 9 and passed through a column with Dowex-1 × 4 Cl^- (1·7 × 17 cm), 0·01N HCl eluted 4-thiouridine, $TOD_{330\ m\mu}$ 2470; elution with 0·015N HCl afforded uridine-5'-phosphate, $TOD_{260\ m\mu}$ 660; 0·02N HCl eluted 4-thiouridine 5'-phosphate, $TOD_{330\ m\mu}$ 16200 (yield 51%). Fractions, containing 4-thiouridine-5'-phosphate, were diluted to 500 ml, brought to pH 9 and passed through a column (1·5 × 10 cm) with Dowex 1 × 4 HCO_3^-. 4-thiouridine-5'-phosphate was recovered by gradient elution with NH_4HCO_3 (water–0·5M NH_4HCO_3, 700 ml) after the column was washed with 0·1M Na_2CO_3 and water. Ammonium bicarbonate was partially removed from the solution by evaporation to ½ the initial volume and the nucleotide transformed into a morpholinium salt by passing through a funnel with Dowex 50 H^+ and then through a column with the same resin in the morpholinium form, yield 300 mg (38%). (Found: P 6·46; $C_{17}H_{31}O_{10}N_4P$ requires: P 6·05%). For paper chromatographic data see Table 1.

2-*Thiouridine-5'-phosphate*. Phosphorylation, acid and alkaline hydrolysis were performed as described above, starting with 0·84 mmoles 2',3'-O-isopropylidene-2-thiouridine. The product was isolated by ion exchange chromatography on a column with DEAE-Sephadex A-25 "medium" (1·4 × 12·5 cm), linear gradient, water (500 ml)–0·4M triethylammonium bicarbonate pH 7·5 (500 ml). 215–440 ml of the effluent contained 2'-thiouridine-5'-phosphate, yield 50% by TOD. The solution was evaporated to dryness, triethylammonium bicarbonate partially removed by

[20] J. Smrt and F. Šorm, *Coll. Chechosl. Chem. Comm.* **25**, 130 (1960).
[21] J. Smrt and F. Šorm, *Coll. Chechosl. Chem. Comm.* **25**, 553 (1960).

evaporation with water (× 2) and the residue dissolved in water. The solution was passed through a column with Dowex 50H$^+$. The eluate was evaporated to dryness, the residue dried *in vacuo* and the resulting syrup extracted with ether in a Soxlet apparatus for removal of H_3PO_4. The 2-thiouridine-5′-phosphate was obtained in a yield of 35%; for properties see Table 1.

6-*Azauridine-5′-phosphate*. Phosphorylation and acid hydrolysis were performed as described above for 4-thiouridine, starting from 2·8 mmoles 2′,3′-O-isopropylidene-6-azauridine. The residue after removal of acetic acid was heated for 3 hr at 100° with 50 ml of 9N NH_4OH. The filtered solution was evaporated to dryness for removal of excess ammonia and barium phosphate precipitated with a saturated solution of barium acetate. The precipitate was washed several times with water and 6-azauridine-5′-phosphate barium salt precipitated from the combined filtrate and washings with two vols ethanol. It was washed with ethanol, dissolved in water and the solution passed through a column with Dowex 50 H$^+$. Evaporation of the solution and extraction with ether for removal of traces of H_3PO_4 afforded 6-azauridine-5′-phosphate, yield 67%; for properties see Table 1.

Isocytidine-5′-phosphate. 2′,3′-O-Isopropylidene isocytidine (66·2 mg; 0·23 mmoles) and 0·92 ml of a 1M aq. pyridine solution of 2-cyanoethyl phosphate were treated as described for removal of water and dissolved in 5 ml dry pyridine and left for 1 hr at 60° after addition of dicyclohexylcarbodiimide (585 mg). The reaction mixture was evaporated to dryness after addition of several drops water. The dry residue was dissolved in 15 ml 9N NH_4OH and heated for 1·5 hr at 100°. The solution was evaporated to dryness and freed from ammonia by evaporation with water. The residue was dissolved in 10 ml 85% HCOOH and left for 12 hr at room temp. Formic acid was removed by evaporation with ethanol and the residue dissolved in water. The solution was brought to pH 9 with NH_4OH and treated with 100 mg barium acetate and 3 vol ethanol. The precipitate was filtered off and washed with hot water. The filtrate contained isocytidine-5′-phosphate barium salt, $TOD_{246\,m\mu}$ 340 (yield 28%). The solution was passed through a column with Dowex 50 (morpholinium form) and evaporated to give isocytidine-5′-phosphate morpholinium salt.

B. *Phosphorylation with dimorpholinophosphochloridate*

3-N-*methyluridine-5′-phosphate*. 2′,3′-O-Isopropylidene-3-N-methyluridine (335 mg; 1·12 mmoles) was treated with 560 mg dimorpholinophosphochloridate in pyridine solution (33 ml) and the mixture refluxed for 30 min with special protection from moisture. The residue after evaporation was dried *in vacuo*, treated with 0·1N HCl (30 ml) at 100° for 2 hr and the solution passed through a column with Dowex 1 × 4 HCO_3^- (15 × 1·8 cm) after adjusting to pH 9. The column was washed with water, 0·05M triethylammonium bicarbonate and 0·1M solution of the same buffer. The latter eluted N-methyluridine-5′-phosphate, TOD_{262} 4000 (yield 40%). The solution was evaporated to dryness for partial removal of triethylammonium bicarbonate and the residue, dissolved in water, was passed through a column with Dowex 50 H$^+$. Evaporation of the eluate afforded chromatographically pure N-methyluridine-5′-phosphate.

Uridine-5′-phosphate was obtained by a similar procedure. Gradient elution was used for ion exchange chromatography (water–0·25M ammonium bicarbonate). Uridine-5′-phosphate ammonium salt was obtained, yield 32%. The substance was identified with an authentic sample of uridine-5′-phosphate by paper chromatography in several solvent systems.

Cytidine-5′-phosphate was obtained by a similar procedure, yield 31%; the substance was identified with an authentic sample by paper chromatography.

5-*Bromouridine-5′-phosphate* was obtained by a similar procedure, yield 31%. The properties of the nucleoside 5′-phosphates obtained are listed in Table 1.

III. *Nucleoside 5′-Phosphomorpholidates* (X)

3-N-*methyluridine-5′-phosphomorpholidate*. To a boiling solution of 87·0 mg (0·292 mmoles) 3-N-methyluridine-5′-phosphate in a mixture of water (3 ml), t-butanol (3 ml) and 0·1 ml morpholine, 240 mg dicyclohexylcarbondiimide in 5 ml of t-butanol was added dropwise during 1 hr; after refluxing for 2 more hr, the dicyclohexylurea was filtered off, the solution evaporated to 2/3 the initial volume and extracted with ether. The aqueous phase was evaporated to dryness and the residue dried *in vacuo*. The product was dissolved in methanol and 3-N-methyluridine-5′-phosphomorpholidate-4-morpholino-N,N-dicyclohexylcarboxamidinium salt precipitated with ether, yield 120 mg (62%). (Found, after 6 hr drying at 100°/1 mm: N 11·70; $C_{31}H_{53}N_6O_{10}P$ requires: N, 11·99%). The substance was homogeneous in paper chromatography, R_f 0·72 (ethanol-0·5M NH_4CH_3

TABLE 4. ION EXCHANGE CHROMATOGRAPHY OF THE UDGP ANALOGUES

Compound	Mmoles of X	Anion exchanger	Dimensions of the column (cm)	Conditions of linear gradient			Volume of effluent (ml), Containing	
				Vessel volume (ml)	Molarity of buffer in mixing vessel	Molarity of buffer in reservoir	Nucleoside 5′-phosphate	Nucleoside diphosphate glucose
II	0·34	Dowex I × 4 200–400 mesh	15·5 × 1·0	1000	0	2	740–940	1400–1900
III	0·153	Dowex 1 × 8 200–400 mesh	7·5 × 1·2	300 300	0 0·075*	0·1 1·0*	180–270	470–540
IV	0·13	DEAE-Sephadex A-25 medium	8 × 0·9	300	0	0·5	90–250	380–550
V	0·06	DEAE-Sephadex .. A-25 medium	8 × 0·9	200	0·01	0·2	20–80	170–320
VI	0·91	DEAE-Sephadex A-25 medium	20 × 2·0	750	0·01	0·2	300–450	790–1150

* Two-step linear gradient. The second gradient was applied after 360 ml of the first one have passed through the column.

COO). Other nucleoside 5′-phosphomorpholidates were obtained similarly and the data for the compounds are listed in Table 2.

IV. *Nucleoside Diphosphate Glucoses*

3-N-*Methyluridine diphosphate glucose.* A 0·1M aq. pyridine solution of trioctylammonium α-D-glucose-1-phosphate (2·9 ml) was evaporated and the residue dried by evaporation of pyridine. 3-N-Methyluridine-5′-phosphate 4-morpholino-N,N-dicyclohexylcarboxamidinium salt (50·1 mg; 0·0715 mmole) was prepared in a similar manner. The latter was dissolved in dry pyridine (5 ml) and added to the glucose-1-phosphate. The mixture was dried by twofold evaporation of pyridine. The residue was dissolved in dry pyridine (5 ml) and the solution left for 4 hr at 60°. The reaction mixture was diluted with water, 0·5 ml 4M sodium acetate added and the trioctylamine extracted with ether. The ethereal solution was washed with water. The combined water layers were diluted to 100 ml and the resultant solution passed through a column with Dowex 1 × 4 CH_3COO^- (10 × 1·5 cm). The column was washed with water and eluted with increasing concentration of triethylammonium acetate pH 4·0 (linear gradient, 300 ml of 0·1M buffer—300 ml of 1·0M buffer); 70–170 ml contain 3-N-methyluridine-5′-phosphate; 350–539 ml contained 3-N-methyluridine diphosphate glucose, yield 53% ($TOD_{262m\mu}$ 340). The fractions with the desired product were pooled together, evaporated to dryness and the residue dried for 1 hr at 50°/0·01 mm over P_2O_5. The product was dissolved in water, the solution passed through a column with Dowex 50 Na^+ and evaporated to dryness. The N-methyluridine diphosphate α-D-glucopyranoside was homogeneous by paper chromatography, R_f 0·49 (ethanol–0·5M ammonium acetate pH 7·5). The mobility of the substance by paper electrophoresis corresponded to the structure proposed—R_{UMP} 1·25 (pH 4·0), 0·84 (pH 7·5). Acid hydrolysis (0·1N HCl, 100°, 15 min) afforded glucose, identified by paper chromatography, R_f 0·10 (butanol–water). The glucose:base ratio[16] was found equal to 0·98:1·0.

The procedures for other UDPG analogues differed only in conditions of ion exchange chromatography; the data, concerning ion exchange chromatography, are listed in Table 4. With isocytidine diphosphate glucose the reaction mixture was left for 24 hr at 60°. The properties of the UDPG analogues obtained are listed in Table 3.