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THE SYNTHESIS OF OLIGORIBONUCLEOTIDES—II*

METHOXYMETHYLIDENE DERIVATIVES OF RIBONUCLEOSIDES AND 5'-RIBONUCLEOTIDES

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Abstract—Ribonucleosides undergo acid-catalysed orthoester exchange with trimethyl orthoformate to give their 2',3'-O-methoxymethylidene derivatives. The latter are converted mainly into mixtures of 2'- and 3'-O-formyl nucleosides by aqueous acids, under very mild conditions. The formate esters are readily hydrolysed to give the parent nucleosides at, and above pH 7. 2',3'-O-Methoxymethylidene ribonucleoside 5'-phosphates, which are required as intermediates in oligoribonucleotide synthesis, have been prepared.

One of the basic requirements in our approach to the synthesis of oligoribonucleotides[1] is the development of a suitable acid-labile (base-stable) protecting group for the ribonucleoside 2',3'-*cis*-diol system. In order to minimize acid-catalysed phosphoryl migration,[2] it is essential that the protecting group should be at least as acid-labile as the acetal function† used[3] to block the 2'-OH groups, vicinal to phosphodiester linkages. It is further essential that the protecting group should be stable enough to remain intact during the building up of the oligonucleotide chain.

It seemed probable at the outset that the commonly used isopropylidene and benzylidene protecting groups, which are removed only slowly at room temperature by aqueous acids at pH 1, would prove to be too stable for the present purpose. The benzylidene group can be modified by the introduction of *para* and *ortho* electron donating substituents, and thus made more labile.[4] However, it seemed likely that the effect of such a substituent (e.g.—OMe) would be much more pronounced if it were attached directly to the acetal carbon (as in II). It has recently been shown[5] that a very effective method of preparing ribonucleoside 2',3'-cyclic acetals and ketals (and no doubt other derivatives of 1,3-dioxalan) is by an acid-catalysed exchange reaction between the ribonucleoside and an excess of the appropriate dimethyl acetal or ketal.

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* See Ref. 1 for the paper now considered to be part I of this series.

† The acetal function which has been commonly used by other workers[3] and ourselves[1] is the tetrahydropyranyl ether. We are investigating the possible use of a number of ketal functions of similar and greater lability.

[1] B. E. Griffin and C. B. Reese, *Tetrahedron Letters* 2925 (1964).

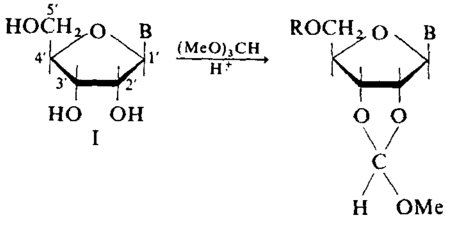
[2] D. M. Brown, D. I. Magrath, A. H. Neilson and A. R. Todd, *Nature, Lond.* **177**, 1124 (1956).

[3] J. Smrt and F. Šorm, *Coll. Czech. Chem. Comm.* **27**, 73 (1962); M. Smith, D. H. Rammler, I. H. Goldberg and H. G. Khorana, *J. Am. Chem. Soc.* **84**, 430 (1962); F. Cramer, H.-J. Rhaese, S. Rittner and K.-H. Scheit, *Liebigs Ann.* **683**, 199 (1965).

[4] F. Cramer, W. Saenger, K.-H. Scheit and J. Tennigkeit, *Liebigs. Ann.* **679**, 156 (1964).

[5] A. Hampton, *J. Am. Chem. Soc.* **83**, 3640 (1961); A. Hampton, J. C. Frantantoni, P. M. Carroll and S. Wang, *Ibid.* **87**, 5481 (1965); S. Chládek and J. Smrt, *Coll. Czech. Chem. Comm.* **28**, 1301 (1963).

We therefore investigated the acid-catalysed orthoester exchange reaction between the common ribonucleosides and trimethyl orthoformate. A preliminary account of some of this work has already been reported.[6]



I

II

a; B = uracil-1
b; B = adenine-9
c; B = cytosine-1
d; B = guanine-9
e; B = hypoxanthine-9

When a suspension of uridine (Ia) in trimethyl orthoformate, containing toluene-*p*-sulphonic acid, was stirred at room temperature, it soon dissolved and 2',3'-O-methoxymethylideneuridine (IIa; R = H) began to precipitate from the reaction mixture. Recrystallization of the precipitate gave the product in 77% yield. Paper chromatography indicated that the liquors of the original reaction mixture contained more 2',3'-O-methoxymethylideneuridine together with two other products, of which one was probably the bis-orthoester (IIa; R = $CH(OMe)_2$).

The 2',3'-O-methoxymethylidene derivatives of adenosine, cytidine, guanosine and inosine were all obtained from the corresponding nucleosides in good yield. It should be noted, however, that the actual preparative details differed for individual nucleosides (Experimental). An excess of acid-catalyst was required for the more basic nucleosides, and it was then necessary to neutralize the reaction mixtures before the methoxymethylidene derivatives (II; R = H) could be isolated. In the reactions involving adenosine and cytidine, where the reaction mixtures were heated and the products remained in solution, by-products were formed in appreciable quantities. However, the cytidine by-product appeared to decompose during the purification procedure, which involved silicic acid chromatography, to give the required 2',3'-O-methoxymethylidenecytidine (IIc; R = H). A solubility problem arose with both guanosine and inosine: in the former case, this was overcome by using mesitylenesulphonic acid as the catalyst and, in the latter case, by adding dimethyl sulphoxide to the reaction medium.

All the methoxymethylidene nucleosides (IIa–IIe; R = H) were isolated in a pure state (as indicated by elemental analysis and paper chromatographic homogeneity), and all except the cytidine derivative (IIc; R = H) were obtained crystalline.* However, the orthoester exchange reaction leads to a nucleoside derivative with an additional asymmetric carbon centre and, by analogy with the preparation of 2',3'-O-benzylidene nucleosides,[9] this reaction would not be expected to be stereospecific. This matter was carefully investigated in connection with the formation of 2',3'-O-methoxymethylideneadenosine (IIb; R = H). An analytical specimen of the latter

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* After this work had been completed, the preparation of some of the corresponding 2',3'-O-ethoxymethylidene nucleosides was reported from two laboratories,[7,8] These products were obtained by an analogous orthoester exchange reaction between the nucleosides and triethyl orthoformate.

[6] M. Jarman and C. B. Reese, *Chem. & Ind.* 1493 (1964).

[7] J. Žemlička, *Chem. & Ind.* 581 (1964).

[8] F. Eckstein and F. Cramer, *Chem. Ber.* **98**, 995 (1965).

was obtained by recrystallizing the crude products from water; it had m.p. 185–189° and $[\alpha]_D^{26} = -57°$. The complexity of its NMR spectrum suggested that it was a mixture of diastereoisomers. However, a material which had m.p. 215° decomp. and $[\alpha]_D^{26} = -76°$ was obtained after two recrystallizations from ethanol; its NMR spectrum indicated that it was a pure diasteroisomer: the signals corresponding to H(2), H(8) and the orthoformate proton appeared as singlets, and that corresponding to H(1′) as a doublet. By substracting the signals pertaining to the latter diastereoisomer from the total spectrum of the mixture, the low-field region of the spectrum of diastereoisomer (ii) could be obtained (Table 1).

TABLE 1. NMR SPECTRA OF DIASTEREOISOMERS OF 2′,3′-O-METHOXYMETHYLIDENEADENOSINE IN DIMETHYL SULPHOXIDE/DEUTERIUM OXIDE

Diastereoisomer	Chemical shift (τ scale)			
	H(2)	H(8)	H(1′)	Orthoformate proton
(i) m.p. 215°	1·52 s	1·72 s	3·65 d	3·86 s
(ii)	1·56 s	1·72 s	3·78 d	3·76 s

s = singlet, d = doublet

It can be seen from the Table that the H(2), H(1′) and orthoformate proton resonances of the two diastereoisomers all differ. However, it would seem likely that the chemical shift of the orthoformate proton resonance would be the best guide to the absolute configuration of the methoxymethylidene carbon centre of a particular diastereoisomer. For the diastereoisomers of 2′,3′-O-benzylideneadenosine, it has been claimed[9] that the benzylic proton *endo* to the ribose ring is more deshielded then the *exo*-proton. If this holds for the corresponding methoxymethylidene derivatives, then diastereoisomer (i) has its orthoformate proton *exo* to the ribose ring. Further examination of the NMR spectrum of the original mixture indicated that diastereoisomer (ii) was marginally the more abundant product.

It is unnecessary to prepare optically pure methoxymethylidene derivatives for the purpose of oligoribonucleotide synthesis. For this reason, no attempt was made to obtain pure diastereoisomers of other methoxymethylidene nucleosides. It was noteworthy, however, that the unpurified 2′,3′-O-methoxymethylideneuridine (IIa; R = H) appeared, from its NMR spectrum, to consist largely of one diastereoisomer. As a consequence of the non-stereospecificity of the orthoester exchange reaction, the m.p.'s of even analytically pure samples of methoxymethylidene derivatives are liable to vary.

The acidic hydrolysis of a methoxymethylidene nucleoside (II; R = H) could conceivably follow two courses: the first would lead to the free nucleoside (V; R = H) and methyl formate, whereas the second would lead to a mixture of the 2′- and 3′-formate esters (III and IV; R = H) and methanol. The first hydrolytic process requires initial protonation of one of the oxygen atoms of the 1,3-dioxalan system, but the second could follow the protonation of any one of the orthoester oxygen atoms. In each case examined, the actual products of acidic hydrolysis were the mixed formate esters, contaminated with a small amount (not more than 5%) of the free

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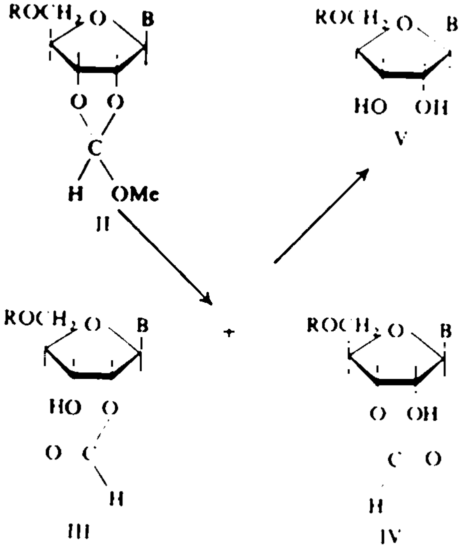
[9] N. Baggett, A. B. Foster, J. M. Webber, D. Lipkin and B. E. Phillips, *Chem. & Ind.* 136 (1965).



nucleoside.[*] Two different hydrolytic procedures were followed: treatment with (a) 98% formic acid and (b) hydrochloric acid (pH 2), both at 20°. In formic acid solution, hydrolysis appeared to occur instantaneously and the formate esters (III and IV; R = H) could be isolated by lyophilization. In hydrochloric acid solution at pH 2, the methoxymethylidene derivatives all underwent a hydrolytic reaction which was first order with respect to substrate, but the individual rates of hydrolysis differed by a factor of as much as 3·5 (Table 2). Protonation of the pyrimidine or purine base would introduce an electrostatic or inductive effect, which might be expected to have an inhibitory action on the protonation and hence on the hydrolysis of the orthoester grouping. This rationalizes the observation (Table 2) that the more basic substrates are the less easily hydrolyzable, but it is not clear why the purine derivatives are apparently more stable to acidic hydrolysis than the pyrimidine derivatives.

TABLE 2. HALF-LIVES OF HYDROLYSIS OF 2′,3′-O-METHOXYMETHYLIDENENUCLEOSIDES IN HYDROCHLORIC ACID AT pH 2 AND 20°

Compound	$t_{1/2}$ (min)
2′,3′-O-Methoxymethylideneuridine (IIa)	10·5
2′,3′-O-Methoxymethylideneadenosine (IIb)	36
2′,3′-O-Methoxymethylidenecytidine (IIc)	25
2′,3′-O-Methoxymethylideneguanosine (IId)	25

The existence of the intermediates (III and IV; R = H) was detected by paper chromatography (in an acidic solvent system), by TLC and by NMR spectroscopy. Their formulation as mixed 2′(3′)-O-formyl nucleosides rests both on chemical and

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[*] The acidic hydrolysis of the corresponding nucleoside orthoacetates and orthobenzoates[10] gave no detectable free nucleoside, but only the appropriate mixtures of 2′- and 3′-acetates and benzoates.

[10] H. P. M. Fromageot, B. E. Griffin, C. B. Reese and J. E. Sulston, *Tetrahedron* **23**, 2315 (1967).

physical evidence. In the first place, they were found to be extremely labile to base*: at pH 9 and 20°, they were completely hydrolysed to the parent nucleosides (V; R = H) within 10 min. The half-lives of both the uridine and adenosine intermediates at pH 7 and 20° in 0·1M-phosphate buffer, were ca. 3 hr.

More direct evidence in support of the proposed structures of the intermediates was provided by NMR spectroscopy. A solution (ca. 0·5M) of 2',3'-O-methoxymethylideneuridine (IIa) in deuterium oxide was acidified with hydrochloric acid, and the NMR spectrum of the products taken immediately. A new low-field signal, of weight one, was apparent at τ 1·75; this had the chemical shift expected for a formyl proton resonance. The complexity of the signals corresponding to the resonances of H(5) and H(1') (at $\sim\tau$ 4) and of H(6) (at $\sim\tau$ 2·15) indicated a mixture of 2'- and 3'-O-acyl ribonucleosides.[13]

In an even more convincing manner, the course of the acidic hydrolysis of methoxymethylidene derivatives was demonstrated by isolating a product in a pure crystalline form, and then characterizing it in the usual way: 2', 3'-O-methoxymethylideneuridine was converted into its 5'-O-formyl derivative[11] (IIa; R = CHO), and the latter treated with 96% formic acid. Pure 3',5'-di-O-formyluridine (IVa; R = CHO) crystallized from the hydrolysis products in 68% yield. The orientation of this compound was established chemically,[14] and confirmed by NMR spectroscopy.[13] It is not uncommon for a pure 2'- or 3'-O-acyl ribonucleoside to crystallize from a mixture of isomers.[10]

The methoxymethylidene group was readily shown to be suitable for the protection of the *cis*-2',3'-diol system of ribonucleosides. A solution of 2',3'-O-methoxymethylideneuridine (IIa; R = H) in pyridine was allowed to react with acetic anhydride, and the product (IIa; R = Ac) treated with 98% formic acid. After the acidic hydrolysis products had been de-formylated by boiling with ethanol, crystalline 5'-O-acetyluridine[15] (V; R = Ac) could be isolated in 86% yield. The particular suitability of the methoxymethylidene protecting group in oligoribonucleotide synthesis was evident when it was found that it could be removed at 20° under comparatively mild hydrolytic conditions: 1–3 hr. at pH 2, followed by several hours at pH 7 (or shorter times at higher pH's). It appeared to fulfil all the requirements considered above.

Although satisfactory procedures had been found for the preparation of the four principal methoxymethylidene nucleosides, it seemed desirable[16] to protect the base residues of the cytidine and perhaps also of the adenosine derivatives, before phosphorylation. The required N^4-benzoyl-2',3'-O-methoxymethylidenecytidine (VI; R = H) was obtained crystalline, in good yield, by the action of sodium methoxide on the dibenzoyl compound (VI; R = Bz) in methanol/dioxane solution.[16] In a

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* It has been shown[11] that 5'-O-formyl nucleosides (V, R = CHO) readily undergo base-catalysed hydrolysis. Therefore, with the additional effect of a neighbouring—OH group,[12] the isomeric 2'- and 3'-esters (III and IV; R = H) would be expected to be extremely labile to base.

[11] J. Žemlička, J. Beránek and J. Smrt, *Coll. Czech. Chem. Comm.* **27**, 2784 (1962).

[12] B. E. Griffin and C. B. Reese, *Proc. Nat. Acad. Sci. U.S.A.* **51**, 440 (1964).

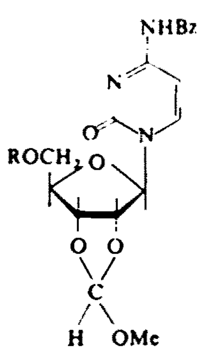
[13] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston and D. R. Trentham, *Tetrahedron* **22**, 705 (1966).

[14] C. B. Reese and D. R. Trentham, *Tetrahedron Letters* 2459 (1965).

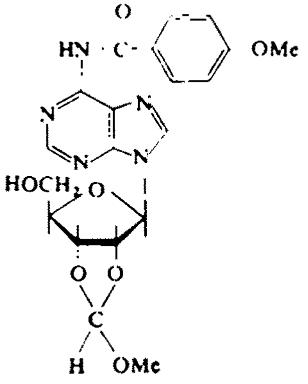
[15] D. M. Brown, Sir Alexander Todd and S. Varadarajan, *J. Chem. Soc.* 2388 (1956).

[16] D. H. Rammler and H. G. Khorana, *J. Am. Chem. Soc.* **84**, 3112 (1962).

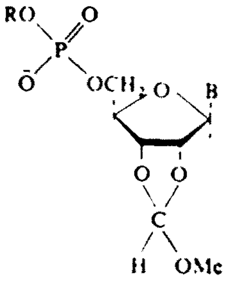
similar way, N^6-*p*-anisoyl-2′,3′-O-methoxymethylideneadenosine (VII) was obtained from (IIb), in 35% overall yield.



VI



VII



VIII

The methoxymethylidene derivatives (IIa; R = H), (VII), (VI; R = H), and (IId; R = H) were all converted into their 5′-phosphates (VIII; R = H) by Tener's method[17]. For example, 2′,3′-O-methoxymethylideneuridine (IIa) was phosphorylated by allowing it to react with an excess of β-cyanoethyl phosphate and N,N′-dicyclohexylcarbodiimide in anhydrous pyridine solution. The required 2′,3′-O-methoxymethylideneuridine 5′-β-cyanoethyl phosphate (VIIIa; R = CH_2CH_2CN) was isolated pure* after chromatography of the products on DEAE cellulose; it was then heated with aqueous ammonia and thus converted to 2′,3′-O-methoxymethylideneuridine 5′-phosphate (VIIIa; R = H), which was isolated as a solid ammonium salt in 92% yield. It should be emphasized that considerable care was taken throughout this and all other preparations of methoxymethylidene nucleotides to ensure that the pH remained *above* 7 during evaporations and other manipulations.

In the same way, the required adenosine derivative (VIII; B = N^6-*p*-anisoyladenine-9, R = H) was obtained as an analytically pure ammonium salt in 72% yield. Even greater care was taken in this preparation as it was necessary to maintain the pH *above* 7 as before. and also *below* 8 to avoid removing the *p*-anisoyl group. Similar precautions were observed in the preparation of the ammonium salt of N^4-benzoyl-2′,3′-O-methoxymethylidenecytidine 5′-phosphate (VIII; B = N^4-benzoylcytosine-1, R = H), which was isolated in 74% yield. The latter material was contaminated with ca. 1% of the debenzoylated material (VIIIc; R = H). Finally, the ammonium salt of 2′,3′-O-methoxymethylideneguanosine 5′-phosphate (VIIId; R = H) was prepared and isolated by a modification of the standard procedure (see experimental section). This material was contaminated with ca. 10% of an impurity, which was also converted to guanosine 5′-phosphate by acidic hydrolysis. As the impurity appeared to be a derivative of the main component, it was considered likely that the material could be used in synthetic reactions without further purification.

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* In order that the final product (VIIIa; R = H) should be free from inorganic phosphate, it was found necessary to carry out the DEAE chromatography purification step *before* ammoniacal treatment. This procedure was adopted in the preparation of all the methoxymethylidene 5′-nucleotides.

[17] G. M. Tener, *J. Am. Chem. Soc.* **83**, 159 (1961).

The use of methoxymethylidene 5'-nucleotides in oligoribonucleotide synthesis will be described in part IV and in subsequent papers of this series.

TABLE 3. R_f VALUES: ASCENDING PAPER CHROMATOGRAPHY OF 2',3'-O-METHOXYMETHYLIDENE DERIVATIVES

Compound	Solvent system		
	C	E	F
2',3'-O-Methoxymethylideneuridine	0·56		0·75
2',3'-O-Methoxymethylideneadenosine	0·69		
2',3'-O-Methoxymethylidenecytidine	0·64		
2',3'-O-Methoxymethylideneguanosine	0·46		
2',3'-O-Methoxymethylideneinosine	0·66		
N^6-p-Anisoyl-2',3'-O-Methoxymethylideneadenosine	0·85		
N^4,O$^{5'}$-Dibenzoyl-2',3'-O-Methoxymethylidenecytidine		0·89	
N^4-Benzoyl-2',3'-O-Methoxymethylidenecytidine		0·84	
2',3'-O-Methoxymethylideneuridine-5'-phosphate	0·14		0·30
N^6-p-Anisoyl-2',3'-O-Methoxymethylideneadenosine-5'-phosphate	0·24		0·27
N^4-Benzoyl-2',3'-O-Methoxymethylidenecytidine-5'-phosphate	0·49		
2',3'-O-Methoxymethylideneguanosine 5'-phosphate	0·08		0·31

TABLE 4. R_f VALUES: DESCENDING PAPER CHROMATOGRAPHY IN SOLVENT SYSTEM B

	Parent nucleoside	2',3'-O-Methoxymethylidene derivative	2'(3')-O-Formyl derivatives
Uridine	0·45	0·69	0·56
Adenosine	0·59	0·75	0·67
Cytidine	0·44	0·66	0·53
Guanosine	0·26	0·65	0·43
Inosine	0·42	0·66	0·51

EXPERIMENTAL

UV absorption spectra were measured with a Cary recording spectrophotometer, model 14M-50. NMR spectra were measured with a Perkin-Elmer Spectrometer, operating at 60 Mc/s. TMS and t-butanol were used as internal standards.

Paper electrophoresis on Whatman No. 4 paper (unless stated otherwise) was conducted in a CCl_4-cooled apparatus (ca. 30 v/cm) with the following buffers: 0·1M-triethylammonium bicarbonate, pH 7·5; 0·1M-phosphate, pH 8; 0·1M-borate, pH 8·5; 0·1M-borate (pH 9).

The following solvent systems were used for paper chromatography: A, butan-1-ol-AcOH-water (4:1:5); B, butan-1-ol-AcOH-water (5:2:3); C, propan-2-ol-ammonia (*d* 0·88)-water (7:1:2); D, propan-1-ol-ammonia (*d* 0·88)-water (11:2:7); E, butan-1-ol-water (86-14); F, EtOH-M-aqueous ammonium acetate (5:2). Ascending chromatograms were run on Whatman 1 paper, unless stated otherwise.

Microscope slides, coated with Merck Kieselgel GF_{254}, were used for TLC. The chromatograms were developed with solns of MeOH (1-15%) in $CHCl_3$. Mallinckrodt analytical grade silicic acid (100 mesh) was used for adsorption chromatography.

Both pyridine and trimethyl orthoformate were dried by heating with CaH_2, under reflux, and were then redistilled before use.

2',3'-O-*Methoxymethylideneuridine* (IIa; R = H)

Uridine (3 g, 12·3 mmoles) and toluene-p-sulphonic acid, monohydrate (0·3 g, 1·5 mmoles) were added to trimethyl orthoformate (15 ml), and the suspension magnetically stirred at 20°, with the exclusion of moisture. Complete soln occurred within 45 min, and shortly afterwards precipitation commenced. Paper chromatography of the liquors after 24 hr (see below) indicated that very little uridine remained.

The ppt was collected by filtration, washed with a small quantity of trimethyl orthoformate, and then dissolved in very dilute aqueous ammonia (25 ml). The soln was evaporated to dryness under red. press., and the residual 2′,3′-O-*methoxymethylideneuridine* crystallized from abs EtOH, m.p. 189–190° dec. (Found: in material dried *in vacuo* over P_2O_5 at 100°: C, 46·5; H, 5·1; N, 9·75; M.W., 280. $C_{11}H_{14}N_2O_7$ requires: C, 46·1; H, 4·7; N, 9·8%; M.W., 286), yield 2·7 g (77%). UV (95% EtOH): λ_{max} 258 (log ε 4·00), λ_{min} 229 mμ (log ε 3·36); NMR spectrum* in Me_2SO/D_2O: τ 2·17, d, weight 1, assigned to H(6); τ 3·86, s, weight 1, assigned to orthoformate proton; τ 4·14, d, weight 1, assigned to H(1′); τ 4·25, d, weight 1, assigned to H(5); τ 5·00, m, weight 2, assigned to H(2′) and H(3′).

A paper chromatographic (system C) examination of the filtrate from the original reaction mixture (see above) revealed 4 components: the two slower-running spots (R_f's 0·35, 0·54) corresponded to uridine (ca. 5%) and 2′,3′-O-methoxymethylideneuridine (ca. 70%); the two faster-running spots (R_f's 0·68, 0·79) accounted respectively for about 5 and 20% of the total UV absorption.

2′,3′-O-*Methoxymethylideneadenosine* (IIb; R = H)

Adenosine (5 g, 18·8 mmoles), toluene-*p*-sulphonic acid, monohydrate (4·25 g, 22·5 mmoles) and trimethyl orthoformate (20 ml) were heated together, under reflux (bath temp, 130°), for 30 min. The products were cooled to room temp and carefully neutralized with methanolic MeONa (2M, 11·3 ml). The precipitated sodium tosylate was removed by filtration and the filtrate concentrated, under red. press., at 30°. The resultant glass was triturated with $CHCl_3$ to extract the nucleosidic material from the remaining sodium tosylate. The $CHCl_3$ extract was collected by filtration, concentrated under reduced press., and then re-evaporated with MeOH. When a soln of the residual glass in hot water (40 ml) was cooled to 0°, it slowly deposited 2′,3′-O-*methoxymethylideneadenosine* as colourless crystals, m.p. 185–189°. (Found: C, 46·8; H, 5·15; N, 22·55. $C_{12}H_{15}N_5O_5$ requires: C, 46·6; H, 4·9; N, 22·65%), yield 3·2 g (56%). UV (95% EtOH): λ_{max} 259 (log ε 4·19), λ_{min} 226 mμ (log ε 3·46).

Earlier examination of the crude products by paper chromatograph (system C) revealed adenosine (R_f 0·50), 2′,3′-O-methoxymethylideneadenosine (R_f 0·69), and another constituent (R_f 0·84) in the approximate respective proportions of 1:5:2.

Preparation of pure diastereoisomer of 2′,3′-O-methoxymethylideneadenosine

The 2′,3′-O-methoxymethylideneadenosine with m.p. 185–189°, described above, had $[\alpha]_D^{25} = -57°$ (c = 2·3 in Me_2SO) and a complex NMR spectrum which indicated that it was a mixture of diastereoisomers. This product (1 g) was twice recrystallized from EtOH to give a material (0·12 g) with m.p. 215° dec and $[\alpha]_D^{25} = -76°$ (c = 2·3 in Me_2SO). (Found: C, 46·9; H, 5·25; N, 22·6%.) NMR spectrum in Me_2SO/D_2O: τ 1·52, s, weight 1, assigned to H(2), τ 1·72, s, weight 1, assigned to H(8); τ 3·65, d, weight 1, assigned to H(1′); τ 3·86, s, weight 1, assigned to orthoformate proton; τ 4·53, quartet, weight 1, assigned to H(2′); τ 4·93, quartet, weight 1, assigned to H(3′). The low field spectrum of the other diastereoisomer was obtained by subtraction of the latter spectrum from that of the mixture: τ 1·56, s, assigned to H(2); τ 1·72, s, assigned to H(8); τ 3·76, s, assigned to orthoformate proton; τ 3·78, d, assigned to H(1′).

2′,3′-O-*Methoxymethylidenecytidine* (IIc; R = H)

Cytidine (5 g, 20·5 mmoles), toluene-*p*-sulphonic acid, monohydrate (4·75 g, 24·5 mmoles) and trimethyl orthoformate (25 ml) were heated together, under reflux (bath temp, 130°), for 1 hr. Paper chromatography (system C) indicated very little unreacted cytidine (R_f 0·48) and two products (R_f's 0·64, 0·78) in approximately equal amounts. After neutralization with methanolic MeONa (2M, 12·25 ml), the products were worked up as in the preparation of the corresponding adenosine derivative (see above). The nucleosidic material, thus freed from sodium tosylate, was dissolved in $CHCl_3$ (100 ml) and slurried with silicic acid (20 g). The slurry was applied to a silicic acid column (20 cm × 6 cm²), which was washed with $CHCl_3$ (500 ml) and allowed to stand at 20° for 3 days. The column

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* Due to the presence of the solvent, the part of the spectrum at higher field than τ 6·0 is obscured.

was then eluted with $CHCl_3$–8% MeOH,* and the eluate evaporated to give 2′,3′-O-methoxymethylidenecytidine as a chromatographically pure (R_f, system C, 0·64) glass (Found: C, 46·0; H, 5·6; N, 14·2. Calc. for $C_{11}H_{15}N_3O_6$: C, 46·3; H, 5·3; N, 14·7%), yield 5·5 g (92%).

2′,3′-O-*Methoxymethylideneguanosine* (IId; R = H)

Guanosine (5 g, 17·7 mmoles) and anhyd mesitylenesulphonic acid (4·35 g, 21·7 mmoles) were added to trimethyl orthoformate (25 ml), and the mixture stirred magnetically at 20°. Virtually complete soln occurred within 10 min, and precipitation of product commenced after 30 min. After 12 hr, the curdled reaction mixture was carefully neutralized with methanolic MeONa (2M, 10·9 ml). MeOH (50 ml) was added and the products filtered. The residue was washed with MeOH (3 × 20 ml) and then dried *in vacuo* over KOH to give the required product (5·5 g, ca. 95%), as a white solid. Paper chromatographic (system C) examination of this material revealed a major constituent (R_f 0·46), contaminated with ca. 5% of guanosine (R_f 0·24). Recrystallization from water (100 ml) gave 4·45 g of material (containing ca. 1·5% of guanosine), which on further recrystallization from water (containing a small amount of triethylammonium bicarbonate buffer, pH 7·5) gave, in 80% recovery, chromatographically pure 2′,3′-O-*methoxymethylideneguanosine* (Found: C, 44·2; H, 5·0; N, 21·5. $C_{12}H_{15}N_5O_6$ requires: C, 44·3; H, 4·65; N, 21·5%), m.p. 265° dec. UV (95% EtOH): λ_{max} 253 (log ε 4·18), λ_{min} 222 mμ (log ε 3·60)

2′,3′-O-*Methoxymethylideneinosine* (IIe; R = H)

Inosine (1 g, 3·7 mmoles) and toluene-*p*-sulphonic acid, monohydrate (0·84 g, 4·4 mmoles) were dissolved in trimethyl orthoformate (5 ml) and dimethyl sulphoxide (5 ml), and the reaction mixture allowed to stand at 20° for 16 hr. The products were then neutralized by the careful addition of methanolic MeONa (2M, 2·2 ml). Paper chromatography (system C) revealed inosine (R_f 0·46) and two products (R_f's 0·66, 0·82) in the approximate respective proportions of 1:4:1. The soln was concentrated below 40° (first at 10 mm, and then at 0·1 mm). $CHCl_3$ (20 ml) and silicic acid (5 g) were added to the residue and the slurry, so obtained, was applied to a column of silicic acid (12 cm × 3 cm^2), which was eluted first with $CHCl_3$ (250 ml) and then with $CHCl_3$–4–8% MeOH (700 ml). The combined latter fractions were evaporated, and the residue crystallized from EtOH to give 2′,3′-O-*methoxymethylideneinosine*, m.p. 197–200°. (Found: C, 46·45; H, 4·4; N, 18·2. $C_{12}H_{14}N_4O_6$ requires: C, 46·45; H, 4·5; N, 18·1%), yield 0·735 g (63%). UV (95% EtOH): λ_{max} 249 (log ε 4·08), λ_{min} 221 mμ (log ε 3·59).

Acidic hydrolysis of 2′,3′-O-*methoxymethylidene ribonucleosides*

(a) *With* 98% *formic acid at* 20°. The methoxymethylidene derivative (0·01 g) was dissolved in 98% formic acid (1 ml), and the soln lyophilized after 1 min. Paper chromatography (system B, descending) indicated one major component with R_f intermediate between that of the starting material and the parent nucleoside, in all cases examined (uridine, adenosine, cytidine, guanosine, and inosine). In no case was any remaining starting material observed, but traces of the free nucleosides were detected.

The acidic hydrolysis products were converted to the parent nucleosides under very mildly basic conditions (see below).

(b) *With hydrochloric acid at pH* 2·0 *and* 20°. The methoxymethylidene derivative (0·005 g) was dissolved in HCl (1 ml) of the concentration required to make the pH of the resultant solution, 2·0. After suitable time intervals, aliquots were removed and treated with excesses of 0·3N aq.NH_3 (to quench the acidic hydrolysis, and convert the 2′(3′)-formate esters into the parent nucleosides), and then submitted to paper chromatography (system C, on Whatman No. 42 paper). The extent of acidic hydrolysis after a given time was determined by eluting the starting material and the free nucleoside separately from the developed chromatogram, and then estimating their proportions spectrophotometrically at λ_{max}. The half-lives for the uridine, adenosine, cytidine and guanosine derivatives were found to be 10·5, 36, 25, and 25 min, respectively.

NMR spectrum of 2′(3′)-O-*formyluridines* (IIIa *and* IVa)

2′,3′-O-Methoxymethylideneuridine (0·06 g) was dissolved in D_2O (0·45 ml) and 3N HCl (0·01 ml) was added. The NMR spectrum of the products included a signal at τ 1·75 (weight 1·0) which was

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* $CHCl_3$–x% MeOH signifies a solution containing x% of MeOH (by volume) in $CHCl_3$.

assigned to a formate proton resonance. The H(6) proton resonances of the two isomers were observed as doublets (J = 8 c/s in both cases) at τ 2·16 and τ 2·17 (total weight 1·0): the former was the more intense signal. It was not possible to analyze fully the H(1′) + H(5) signals (total weight 2·0) in the region of τ 4·1.

Hydrolysis of nucleoside 2′(3′)-formate esters

(a) *At pH* 9 *and* 20°. Methoxymethylidene derivative (0·005 g) was kept at pH 2 (HCl, 1 ml) at 20° for 150 min, after which the pH was rapidly raised to 9 by the addition of 0·3M aq.NH_3. After suitable time intervals, aliquots were removed and the hydrolysis quenched by the addition of 0·01N HCl. The relative proportions of formyl- and free nucleosides were estimated by the paper chromatographic method (using system B, descending), described above. Complete hydrolysis of the 2′(3′)-formate esters of uridine, adenosine, cytidine, guanosine and inosine had occurred within 10 min.

(b) *At pH* 7 *and* 20°. The mixture of nucleoside 2′- and 3′-formates, obtained by the acidic hydrolysis of the corresponding methoxymethylidene derivative (ca. 0·02 g) was treated with 0·1M phosphate buffer (pH 7, 2·0 ml). After suitable time intervals, aliquots were removed and the hydrolysis quenched (i.e. pH taken to 4–5) by the addition of 0·1M citric acid. The proportions of starting material and free nucleoside were again determined by the paper chromatographic elution method. The 2′(3′)-formates of both uridine and adenosine were found to have half-lives of 180 ± 10 min.

3′,5′-Di-O-*formyluridine* (IVa; R = CHO)

A soln of 2′,3′-O-methoxymethylideneuridine (6·0 g) in dry pyridine (60 ml) was cooled to −30° and treated with formic acetic anhydride (6 ml). The reaction mixture was allowed to stand at −15° for 6 hr, concentrated under red. press., and then repeatedly evaporated with EtOH. The glass, so obtained, was shaken with 96% formic acid (30 ml) until complete soln occurred. The products were lyophilized, the resulting glass taken up in EtOH (120 ml), and filtered. 3′,5′-Di-O-*formyluridine* was deposited as a colourless crystalline solid. (Found: C, 44·0; H, 4·3; N, 9·4. $C_{11}H_{12}N_2O_8$ requires: C, 44·0; H, 4·0; N, 9·3%), m.p. 144–146°, yield 4·3 g (68%); R_f (system B) 0·63. UV (dioxan): λ_{max} 257 mμ (log ε 3·98). NMR spectrum in dimethylcyanamide–D_2O (0·1M with respect to HCl): τ 1·80, s, weight 2, assigned to formyl protons; τ 2·39, d, (J = 8 c/s), weight 1, assigned to H(6); τ 4·14, d (J = 6 c/s), weight 1, assigned to H(1′); τ 4·25, d (J = 8 c/s), weight 1, assigned to H(5); τ 4·74, m, weight 1, assigned to H(3′).

This compound was orientated as pure 3′-isomer by the mesylation procedure,[14] and by comparing its NMR spectrum with that of an equilibrium mixture of 2′- and 3′-isomers.[18]

Preparation of 5′-O-acetyluridine from 2′,3′-O-methoxymethylideneuridine

A soln of 2′,3′-O-methoxymethylideneuridine (0·73 g, 2·56 mmole) and Ac_2O (1 ml, ca. 10 mmole) in anhyd pyridine (5 ml) was allowed to stand at 20°. After 12 hr, MeOH (5 ml) was added and, after a further 1 hr, the soln was evaporated under red. press. The resultant oily product was re-evaporated several times with EtOH and then dissolved in 60% formic acid (5 ml). After a short time, the aqueous formic acid was removed under red. press. (0·1 mm). The oil, so obtained, was evaporated with EtOH several times and finally dissolved in EtOH (10 ml) and cooled to 0°, when 5′-O-acetyluridine (0·56 g, 77%) precipitated in colourless crystals, m.p. 161–164° (lit[16], 162–164°). Paper chromatography (system A) of the mother liquors indicated another component (R_f 0·65) besides 5′-O-acetyluridine (R_f 0·51), but no uridine (R_f 0·33). The mother liquors were then heated on a water-bath for 30 min and cooled to 0°, when a second crop of 5′-O-acetyluridine (0·065 g; total yield: 0·625 g, 86%) was obtained. The second component (R_f 0·65) was no longer detectable.

N^6-p-*Anisoyl-2′,3′-O-methoxymethylideneadenosine* (VII)

A soln of 2′,3′-O-methoxymethylideneadenosine (1·24 g, 4 mmole) and *p*-anisoyl chloride (1·24 g, 8·4 mmole) in anhyd pyridine (10 ml) was allowed to stand at 20°. After 16 hr, water (1 ml) was added and, after a further 2 hr, the products were concentrated (to ca. 3 ml) at 20° under red. press., and then added slowly to a stirred ice–water mixture (50 g). A cream-coloured ppt (2·5 g) was collected by filtration, dried, and dissolved in THF (2·6 ml) and EtOH (4 ml). To this soln were added methanolic MeONa (2M, 5·4 ml) and water (5·4 ml). After the reaction mixture had been allowed to stand at 20° for 10 min, it was neutralized by the addition of an excess of Dowex 50 (pyridinium form) cation-exchange resin, which was then removed by filtration. The filtrate was evaporated under red. press. and the residue dissolved in $CHCl_3$ and applied to a silicic acid column (12 cm × 3 cm^2). Elution with $CHCl_3$ gave, first, a non-nucleosidic fraction (250 ml), followed by the required product. The fractions containing the latter were combined and concentrated to give a yellow glass (0·96 g) which was crystallized from MeOH, and then recrystallized from EtOH to give N^6-p-*anisoyl*-2′,3′-O-*methoxymethylideneadensine* (0·615 g, 35%) as colourless needles, m.p. 143–146° (m.p. 147–148° after two further recrystallizations). (Found: C, 53·95; H, 4·9; N, 15·8. $C_{20}H_{21}N_5O_7$ requires: C, 54·2; H, 4·8; N, 15·8%.) UV (95% EtOH: λ_{max} 289 (log ε 4·51); λ_{min} 239 mμ (log ε 3·77).

N^4,$O^{5'}$-*Dibenzoyl*-2′,3′-O-*methoxymethylidenecytidine* (VI; R = Bz)

Benzoyl chloride (5 g, 36 mmoles) was added dropwise to a cooled soln of 2′,3′-O-methoxymethylidenecytidine (5 g, 17·5 mmoles) in anhyd pyridine (50 ml), and the reaction mixture stirred magnetically at 20° for 16 hr. Water (1 ml), was added and, after 1 hr, the products were concentrated to small volume at 20° under red. press., and then partitioned between $CHCl_3$ and very dilute triethylammonium bicarbonate buffer (pH 7·5). The $CHCl_3$ layer was washed, dried (Na_2SO_4) and evaporated. The residue was crystallized from EtOH to give 6·5 g (77%) of N^4,$O^{5'}$-*dibenzoyl*-2′,3′-O-*methoxymethylidenecytidine*. Recrystallized material had m.p. 159–161°. (Found: C, 60·9; H, 4·55; N, 8·6. $C_{25}H_{23}N_3O_8$ requires: C, 60·85; H, 4·7; N, 8·5%.) UV (95% EtOH): λ_{max} 228, 261, 301 (log ε 4·37, 4·43, 3·97); λ_{min} 214, 244, 290 mμ (log ε 4·34, 4·28, 3·94).

N^4-*Benzoyl*-2′,3′-O-*methoxymethylidenecytidine* (VI; R = H)

Methanolic MeONa (2M, 6·5 ml) was added to a stirred soln of N^4,$O^{5'}$-dibenzoyl-2′,3′-O-methoxymethylidenecytidine (5 g, 10·1 mmoles) in dioxan (100 ml) and MeOH (93·5 ml). After the reaction had been allowed to stand at 20° for 25 min, it was neutralized by the addition of an excess of an aqueous suspension of Dowex 50 (pyridinium form) cation-exchange resin. The resin was removed by filtration, the fitrate concentrated under red. press. and then evaporated several times with MeOH at 20°. The residue was crystallized from EtOH to give N^4-*benzoyl*-2′,3′-O-*methoxymethylidenecytidine*, m.p. 196·5–197·5°. (Found: C, 55·9; H, 4·9; N, 10·75. $C_{18}H_{19}N_3O_7$ requires: C, 55·5; H, 4·9; N, 10·8%), yield 2·6 g (66%). UV (95% EtOH): λ_{max} 260, 303 (log ε 4·39, 4·00); λ_{min} 230, 287 mμ (log ε 4·03, 3·93).

2′,3′-O-*Methoxymethylideneuridine 5′-phosphate** (VIIIa; R = H)

To an anhyd soln of pyridinium β-cyanoethyl phosphate (4 mmoles, obtained from 1·30 g of Ba salt by ion-exchange) in pyridine (10 ml), was added 2′,3′-O-methoxymethylideneuridine (0·572 g, 2 mmoles) and N,N′-dicyclohexylcarbodiimide (4·12 g, 20 mmoles). The reaction mixture was sealed to exclude moisture, and was stirred magnetically at 20°. After 3 days, water (10 ml) was added, and the reaction mixture stirred for a further 12 hr. The precipitated N,N′-dicyclohexylurea was removed by filtration and washed with 10% aqueous pyridine (3 × 5 ml). The combined filtrate and washings were extracted with petroleum ether (b.p. 40–60°, 3 × 5 ml), treated with aqueous triethylammonium bicarbonate buffer (0·1M, 4 ml; pH 7·5), and then concentrated to about half volume. Paper electrophoresis (pH 8) indicated the presence, in the resulting solution, of a principal UV-absorbing component with a mobility ca. 0·4 that of uridine 5′-phosphate.

This soln was applied to a DEAE-cellulose anion-exchange column (HCO_3^- form, 50 cm × 5 cm^2), which was then eluted with triethylammonium bicarbonate buffer (pH 7·5, linear gradient from 0–0·05M over 2 l). 25 Ml. fractions were collected: fractions 20–40 (buffer concentration, ca. 0·02M), which contained the required β-cyanoethyl ester of 2′,3′-O-methoxymethylideneuridine 5′-phosphate, were combined and concentrated to dryness below 30°, under red. press. Paper chromatography (system C) indicated that this material contained a sole UV absorbing constituent (R_f 0·44) and no inorganic phosphate.

A soln of this material in 10% aqueous pyridine (5 ml) was passed through a Dowex 50 cation-exchange column (pyridinium form, 10 cm × 1 cm^2), which was then washed with 10% aqueous

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* Considerable care was exercised during evaporations and other procedures involved in the preparation of all methoxymethylidene derivatives of nucleotides, to ensure that the pH never fell below 7.

pyridine (5 × 10 ml). The total eluate was treated with an equal volume of 6N aqNH_4, and heated at 50° for 3 hr. The products were filtered, the filtrate concentrated to a small volume under red. press., and then dried by repeated evaporations with anhyd pyridine. A soln of the resultant glass in pyridine (5 ml) was added dropwise to anhyd ether[18] (100 ml). A colourless ppt of *diammonium* 2′,3′-O-*methoxymethylideneuridine 5′-phosphate*, so obtained, was collected by centrifugation, washed twice with ether, and then dried *in vacuo* over P_2O_5 at 20°. (Found: N, 14·0; P, 7·65. $C_{11}H_{21}N_4O_{10}P$ requires: N, 14·0; P, 7·75%), yield 0·74 g, 92%. The product was paper chromatographically homogeneous (systems C, F); its paper electrophoretic mobility, relative to that of uridine 5′-phosphate was 0·94 at pH 8 and 0·80 at pH 9 (borate buffer).

N^6-p-*Anisoyl-2′,3′-O-methoxymethylideneadenosine 5′-phosphate* (VIIIb; R = H)

To an anhyd soln of pyridinium β-cyanoethyl phosphate (1·5 mmoles, from 0·485 g of Ba salt) in pyridine (10 ml), was added N^6-*p*-anisoyl-2′,3′-O-methoxymethylideneadenosine (0·304 g, 0·75 mmole) and N,N′-dicyclohexylcarbodiimide (1·55 g, 7·5 mmoles). After the reaction had been allowed to proceed for 3 days at 20°, the products were worked up in the manner described above (in the preparation of 2′,3′-O-methoxymethylideneuridine 5′-phosphate). After extraction with petroleum ether, paper electrophoresis (pH 8) of the aqueous products indicated one principal component with a mobility ca. 0·3 that of adenosine 5′-phosphate.

The aqueous soln was not concentrated, but applied directly to a DEAE-cellulose anion-exchange column (HCO_3^- form, 46 cm × 2·2 cm^2), which was then eluted with triethylammonium bicarbonate buffer (pH 7·5, linear gradient from 0–0·05M over 2l). Fractions (each of 25 ml) 30–38 (eluted with buffer concentration ca. 0·017M), which had $\varepsilon_{280}/\varepsilon_{260}$ = 1·0, were discarded; fractions 39–70 (average buffer concentration ca. 0·027M), which contained the required β-cyanoethyl ester of N^6-*p*-anisoyl-2′,3′-O-methoxymethylideneadenosine 5′-phosphate and had the expected $\varepsilon_{280}/\varepsilon_{260}$ ratio* of ca. 2·2, were combined and concentrated carefully under red. press. below 30° to ca. 10 ml (small quantities of dry ice were added at intervals to prevent the pH rising above 7. 2N NaOH (10 ml) was added to the the concentrate and, after *precisely* 2 min, the products were neutralized by the addition of an excess of Dowex 50 (pyridinium form) cation-exchange resin, which was then recovered by filtration and washed with water (20 ml). The combined filtrate and washings were passed successively through two Dowex 50 cation exchange columns: (a) pyridinium form, 4 cm × 1 cm^2; (b) ammonium form, 5 cm × 2·5 cm^2. The latter column was washed with water until the eluate was no longer detectably UV-absorbing. The eluate and washings were combined, concentrated to small volume under red. press. below 30°, and finally evaporated to dryness with anhyd pyridine (3 × 10 ml). The residue was dissolved in anhyd pyridine (10 ml), filtered, and added dropwise with stirring to dry ether (100 ml). The resulting ppt of *monoammonium* N^6-p-*anisoyl-2′,3′-O-methoxymethylideneadenosine 5′-phosphate* was collected by centrifugation, washed with ether, and dried *in vacuo* over P_2O_5 at 20°. (Found: C, 42·0; H, 5·0; N, 14·7; P, 5·3. $C_{20}H_{25}N_6O_{10}P$, $2H_2O$ requires: C, 41·7; H, 5·1; N, 14·6; P, 5·4%); yield of colourless material 0·43 g (72%). UV (water at pH 7): λ_{max} 291 (log ε 4·46), λ_{min} 240 mμ (log ε 3·77). The extinction coefficients are based on mol. wt. = 576. Paper electrophoretic mobility (borate buffer, pH 9): 0·58 that of adenosine 5′-phosphate. R_f: 0·24 (system C); 0·27 (system F).

N^4-*Benzoyl-2′,3′-O-methoxymethylidenecytidine 5′-phosphate* (VI; R = PO_3^{2-})

N^4-Benzoyl-2′,3′-O-methoxymethylidenecytidine (0·25 g, 0·625 mmole) was phosphorylated with pyridinium β-cyanoethyl phosphate (1·25 mmoles, from 0·40 g of Ba salt) and N,N′-dicyclohexylcarbodiimide (1·30 g, 6·25 mmole) in pyridine (7·5 ml) soln. The reacton was conducted, and the products were worked up in the manner described above for the phosphorylation of N^6-p-anisoyl-2′,3′-O-methoxymethylideneadenosine. The aqueous pyridine soln of crude products, so obtained, contained one principal component with R_f (system F), 0·69 and with electrophoretic mobility (phosphate buffer, pH 8) 0·3 that of cytidine 5′-phosphate.

The latter soln was applied to a DEAE-cellulose anion-exchange column (HCO_3^- form, 35 cm ×

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* UV absorption of N^6-*p*-anisoyl-2′,3′-O-methoxymethylideneadenosine in 0·05M triethylammonium bicarbonate (pH 7·5): λ_{max} 291, λ_{min} 243 mμ. $\varepsilon_{280}/\varepsilon_{260}$ = 2·2.

[18] Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.* 85, 3852 (1963).

2·5 cm^2), which was eluted first with water (500 ml) and then with triethylammonium bicarbonate buffer (pH 7·5, linear gradient from 0–0·05M over 2 l). The required β-cyanoethyl ester of N^4-benzoyl-2′,3′-O-methoxymethylidenecytidine 5′-phosphate was collected in fractions (each of 25 ml) 25–54 [average buffer concentration, ca. 0·025M]. The combined fractions (which contained 10·5 × 10^3 O.D. units of material at 260 mμ; estimated yield, 75%), were concentrated under red. press. below 30° with the usual caution: it was necessary to add small portions of dry ice at intervals to keep the pH from rising above 8. 2N NaOH (5 ml) was added to the concentrate (ca. 5 ml), and the alkaline soln allowed to stand at 20° for *precisely* 2 min, after which it was neutralized with an excess of Dowex 50 (pyridinium form) cation-exchange resin. The resin was collected by filtration, and washed thoroughly with water (ca. 20 ml). The combined filtrate and washings were passed through a Dowex 50 (NH_4^+ form, 4 cm × 2·8 cm^2) cation-exchange column, which was then washed with water until the eluate no longer showed detectable absorption at 260 mμ. The total eluate was concentrated to small volume under red. press., and finally dried by repeated evaporation with anhydrous pyridine.

The solid residue was taken up in pyridine (10 ml), filtered, and the filtrate added dropwise, with stirring, to anhydrous ether (100 ml). The colourless ammonium salt of N^4-*benzoyl-2′,3′-O-methoxymethylidenecytidine 5′-phosphate* was collected by centrifugation, washed with ether (2 × 25 ml), and dried *in vacuo* over P_2O_5 at 20°. (Found: N, 10·4; P, 6·1. $C_{18}H_{23}N_4O_{10}P, 2H_2O$ requires: N, 10·7; P, 5·9%.) UV (water): λ_{max} 259, 301 (log ε 4·30, 4·00); λ_{min} 230, 285 mμ (log ε 3·92, 3·93); yield 0·245 g, 74%. Paper chromatography (system F) indicated that the product (R_f 0·49) was free from inorganic phosphate and was not less than 99% pure. Its paper electrophoretic mobility (phosphate buffer, pH 8) was ca. 0·75 that of cytidine 5′-phosphate.

2′,3′-O-*Methoxymethylideneguanosine 5′-phosphate* (VIIId; R = H)

2′,3′-O-Methoxymethylideneguanosine (0·325 g, 1·0 mmole) was phosphorylated with pyridinium β-cyanoethyl phosphate (1·5 mmole, from 0·48 g of Ba salt) and N,N′-dicyclohexylcarbodiimide (2·06 g, 10 mmoles) in pyridine (2·5 ml) and dimethylformamide (2·5 ml) soln. After the reaction had proceeded at 20° for 24 hr, water (1 ml) was added and after a further 16 hr, the precipitated N,N′-dicyclohexylurea was collected by filtration and washed with 50% aqueous pyridine (3 × 5 ml). The combined filtrate and washings were treated with triethylamine (0·2 ml) and concentrated to small volume under red. press. at 20°. After water (20 ml) had been added, the products were extracted with ether (2 × 20 ml) and then applied to a DEAE cellulose column (HCO_3^- form, 60 cm × 3 cm^2), which was eluted with triethylammonium bicarbonate buffer (pH 7·5, linear gradient from 0–0·05M over 2l). Fractions (each of 25 ml) 23–80 were combined and concentrated under red. press. at 30° to give a residue, which was dissolved in 3N aqNH_3 (25 ml). The resultant solution was heated at 50° for 5 hr, evaporated to dryness, and dissolved in 10% aqueous pyridine (10 ml). The solution was applied to a Dowex 50 cation-exchange column (pyridinium form, 6 cm × 3 cm^2), which was eluted and subsequently washed with 10% aqueous pyridine (6 × 20 ml). The combined eluate and washings were treated with 3N aqNH_3 (1 ml), and then concentrated under red. press. at 30° to give a slightly discoloured solid which was dried *in vacuo* over KOH; yield 0·31 g. Paper chromatography (system F) of this material indicated a major component (90%, R_f 0·37) with the expected properties of 2′,3′-O-methoxymethylideneguanosine 5′-phosphate. UV (water at pH 7): λ_{max} 253, λ_{min} 222 mμ. Paper electrophoretic mobility (borate buffer, pH 8·5) was 0·71 that of guanosine 5′-phosphate.

The minor component (10%; R_f in system F, 0·96) was converted into guanosine 5′-phosphate (R_f 0·17) by treatment with 0·1N HCl for 4 hr at 20°, followed by neutralization.

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