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THE SYNTHESIS OF OLIGORIBONUCLEOTIDES—III*

MONOACYLATION OF RIBONUCLEOSIDES AND DERIVATIVES *VIA* ORTHOESTER EXCHANGE

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Abstract—Ribonucleosides and their 5'-derivs undergo acid-catalysed exchange with trimethyl orthoacetate and orthobenzoate to give the corresponding 2',3'-O-methoxy-ethylidene and -benzylidene derivs II (R' = Me and Ph) respectively. Treatment of the latter with aqueous acids, under very mild conditions, gives mixtures of the respective 2' (and 3')-acetates and benzoates, from which it is often possible to obtain a pure crystalline isomer (usually the 3'-ester), in good yield. The orthoester exchange reaction has been applied to the preparation of 3',5'-di-O-acyl-ribonucleoside derivs, which are required as intermediates in oligoribonucleotide synthesis.

ONE of the fundamental problems of oligoribonucleotide synthesis, and indeed of ribonucleoside chemistry, is the development of methods whereby the secondary OH groups of a ribonucleoside *cis*-2',3'-diol system may be differentiated. This problem consists essentially of two parts: the first requires the development of reactions which occur specifically at the 2'- or 3'-OH group or, alternatively, efficient methods of separating mixtures of 2'- and 3'-isomers; the second requires a technique which permits a distinction to be made between a pair of 2'- and 3'-isomers. In connection with the latter part of the problem, we have recently proposed[2] a general method of orientation, based on NMR spectroscopy, and have also developed a chemical procedure[3] which is suitable for the orientation of uridine derivatives. In the present discussion we wish to consider the first part of the problem and describe a convenient general method for the preparation of pure 3' (and, in some cases, 2')-O-acyl-ribonucleoside derivatives, which are the basic starting materials for our oligoribonucleotide synthesis.

The reactivities of the 2'- and 3'-OH functions towards electrophilic reagents appear to be of the same order, and pure derivatives may normally only be obtained by the fractionation of mixtures of isomers. Thus when uridine is allowed to react with an excess of triphenylmethyl chloride, a mixture of 2',5'- and 3',5'-di-O-trityl-uridines is obtained.[3,4] Similarly, the reaction between 5'-O-acetyl-uridine[5] or -adenosine[6] and toluene-*p*-sulphonyl chloride leads to a mixture of 2'- and 3'-tosylates. On the other hand, the diacetate fractions isolated from the products of partial

* For part II of this series, see Ref. 1.

[1] B. E. Griffin, M. Jarman, C. B. Reese and J. E. Sulston, *Tetrahedron* **23**, 2301 (1967).
[2] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston and D. R. Trentham, *Tetrahedron* **22**, 705 (1966).
[3] C. B. Reese and D. R. Trentham, *Tetrahedron Letters* 2459, 2467 (1965).
[4] N. C. Yung and J. J. Fox, *J. Amer. Chem. Soc.* **83**, 3060 (1961).
[5] D. M. Brown, Sir Alexander Todd and S. Varadarajan, *J. Chem. Soc.* 2388 (1956).
[6] A. H. Neilson, Ph.D. Thesis, Cambridge (1957).

acetylation of both 5′-O-acetyl-uridine[6] and -adenosine[7] crystallize from ethanol to give high recoveries of the pure crystalline 3′,5′-diacetates. However, this last observation may not be taken as evidence for the greater reactivity of the 3′-OH group, as the work-up procedure would have converted either pure isomer into an equilibrium mixture.[8]

The reaction between either of the above 5′-acetates and a stoicheiometric quantity of acetic anhydride leads to a mixture of triacetate, diacetate and unchanged starting material in approximately equimolecular amounts.[6,7] Although this mixture can be fractionated by counter-current distribution[6,7,8] or by chromatography on silicic acid,[9] the yield of diacetate is only ca. 35%, and thus the procedure is not very satisfactory. However, the observation[1,10] that 2′,3′-O-methoxymethylidene derivatives (II; R′ = H) are virtually quantitatively converted into mixtures of the corresponding 2′- and 3′-formates (respectively III and IV; R′ = H) by mild acidic hydrolysis suggested a much more efficient and, in theory, general method of monoacylation of a *cis*-diol system.

The application of this method to ribonucleoside systems is illustrated in Fig. 1:

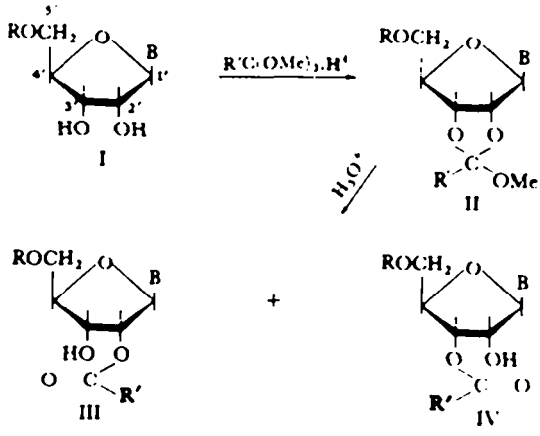


FIG. 1

the nucleoside or 5′-derivative (I) is allowed to undergo acid-catalysed orthoester exchange with the appropriate trimethyl orthoester, and the product II is then treated with aqueous acid to give the isomeric acyl derivatives III and IV. As it seemed at the outset that the extremely labile formate esters would be less suitable than the corresponding acetates and benzoates as intermediates in oligoribonucleotide synthesis, the acid-catalysed exchange between ribonucleosides (and 5′-derivatives) and trimethyl orthoacetate and orthobenzoate has been examined.*

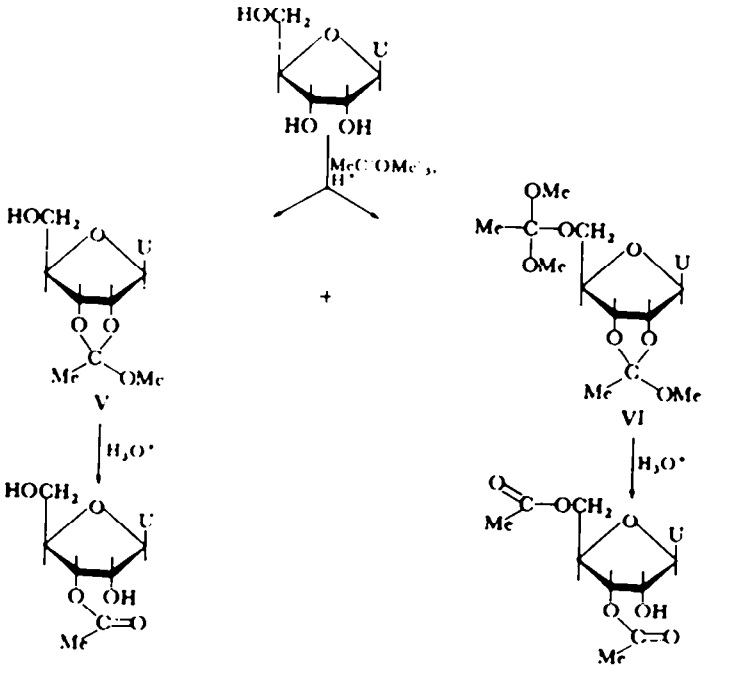
When uridine was allowed to react with an excess of trimethyl orthoacetate in the presence of toluene-*p*-sulphonic acid at 20°, it was converted into a mixture of two products. The major component, which could be isolated as a colourless glass, in

* A preliminary account of this work has already been published.[11]

[7] D. M. Brown, G. D. Fasman, D. I. Magrath and A. R. Todd, *J. Chem. Soc.* 1448 (1954).
[8] A. M. Michelson, L. Szabo and Sir Alexander Todd, *J. Chem. Soc.* 1546 (1956).
[9] B. E. Griffin, unpublished results.
[10] M. Jarman and C. B. Reese, *Chem. & Ind.* 1493 (1964).
[11] C. B. Reese and J. E. Sulston, *Proc. Chem. Soc.* 214 (1964).

73% yield, after chromatography of the mixture on neutral alumina, was characterized as 2',3'-O-methoxyethylideneuridine* (V); the minor component was tentatively assigned the bis-orthoester structure VI (see below). When V, which was shown by NMR spectroscopy to be a mixture of both possible diastereoisomers, was treated with acid under very mild conditions, it was quantitatively converted into a mixture of 2'- and 3'-O-acetyluridines.

A modified procedure was adopted to obtain 3'-O-acetyluridine (VII) on a preparative scale. Uridine and one-tenth of its weight of toluene-*p*-sulphonic acid were stirred with an excess of trimethyl orthoacetate. After 2 hr, examination of the



U = uracil-1

neutral† reaction solution by TLC showed that no uridine remained and that the products V and VI were present in the approximate respective proportions of 2:1. Brief treatment of these products with 5% acetic acid, followed by chromatography on silicic acid led to two main fractions: concentration of the first fraction and recrystallization from ethanol gave 3',5'-di-O-acetyluridine (VIII) in 34% yield; similar treatment of the second fraction gave 3'-O-acetyluridine (VII) in 46% yield. As it is extremely likely that 3',5'-di-O-acetyluridine (VIII) resulted from the hydrolysis of

* Strictly, it should be specified that this compound is a 1-methoxyethylidene deriv. However, this leads to a cumbersome system of nomenclature. In the same way, compounds of the type II (R' = Ph) will be referred to as 2',3'-O-methoxybenzylidene derivs.

† It is noteworthy that trimethyl orthoacetate shows more tendency to react with (and presumably esterify) toluene-*p*-sulphonic acid than does trimethyl orthoformate.

the minor product of the orthoester exchange reaction, the latter may be formulated as the bis-orthoester derivative (VI).

It was not proposed to discuss acyl migration and orientation in detail in the present paper as both these subjects have been considered elsewhere[2,3]. However, for the sake of clarity, they will be dealt with briefly. In the above experiment, although the diacetate and monoacetate fractions contained mixtures of 2'- and 3'-isomers, they both crystallized from ethanol to give the pure 3'-O-acetyl derivatives, in high yield. In each case, crystallization was slow and the material in solution was thus able to reequilibrate.[3] The acetyl group appears to be a particularly useful acyl protecting group: it migrates at a sufficiently great rate[3] to ensure the high recovery of a pure isomer and, at the same time, is not very susceptible to solvolysis.*

As will become apparent, when a pure compound crystallizes from a mixture of 2'- and 3'-isomers, it is usually found to be the thermodynamically more stable 3'-isomer. This is fortunate from the point of view of oligoribonucleotide synthesis,[12] but cannot easily be rationalized. The orientations of all the 2'- and 3'-O-acylribonucleoside derivatives described in this paper have been established by the NMR spectroscopic method.[2] In addition, the orientations of most of the uridine derivatives have been confirmed by the previously reported chemical method[3] (Experimental).

Adenosine and cytidine have also been converted into their 2',3'-O-methoxyethylidene derivatives, which were isolated in 67 and 74% yields, respectively. However, as in the preparation of their corresponding methoxymethylidene derivatives, these comparatively basic nucleosides required rather more than one molecular equivalent of acid to effect orthoester exchange, and it was then necessary to neutralize the products before submitting them to alumina chromatography. Although the adenosine derivative was obtained crystalline, it was clear from its NMR spectrum that it was a mixture of diastereoisomers. After several recrystallizations of this material, one of the diastereoisomers of 2',3'-O-methoxyethylideneadenosine was obtained pure. Crystallization of the acid hydrolysate of the mixed diastereoisomers from ethanol led to an 88% yield of pure 3'-O-acetyladenosine.

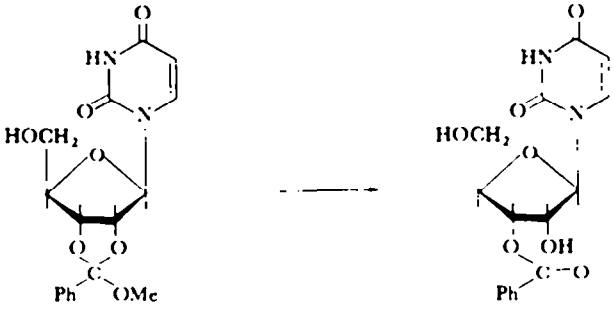
It was clearly also of interest to consider whether the methoxyethylidene group *per se* might be used as a protecting group for a *cis*-diol system, and to find the mildest conditions required for its hydrolysis. As 2',3'-O-methoxyethylidene-uridine and -adenosine were hydrolysed at immeasurably fast rates at and below pH 3, kinetic studies were carried out in sodium citrate buffer solution, at pH 5·6. The conversion of both the uridine and adenosine derivatives to their 2'(3')-acetates followed first order kinetics, and the half-times of hydrolysis were found to be 39 and 83 min, respectively. If it is assumed that the rate of hydrolysis is proportional to the concentration of hydrogen ions, then it follows that the methoxyethylidene derivatives are ca. 1000 times more labile than the corresponding methoxymethylidene derivatives.[1] Thus the methoxyethylidene protecting group is unlikely to find application in oligoribonucleotide synthesis; it is perhaps the most labile protecting group of its type so far reported.

2',3'-O-Methoxybenzylideneuridine (IX) was isolated in 79% yield by alumina

* The formyl group is more suitable from the standpoint of mobility but is very readily solvolysed, whereas the benzoyl group is insufficiently mobile to ensure a good yield of a pure isomer.

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

chromatography of the products of the reaction between uridine and an excess of trimethyl orthobenzoate in the presence of acid. The analytically pure glass, so obtained, was shown by NMR spectroscopy to be a 1:1 mixture of the two diastereoisomers. In another experiment, the initial products of orthoester exchange

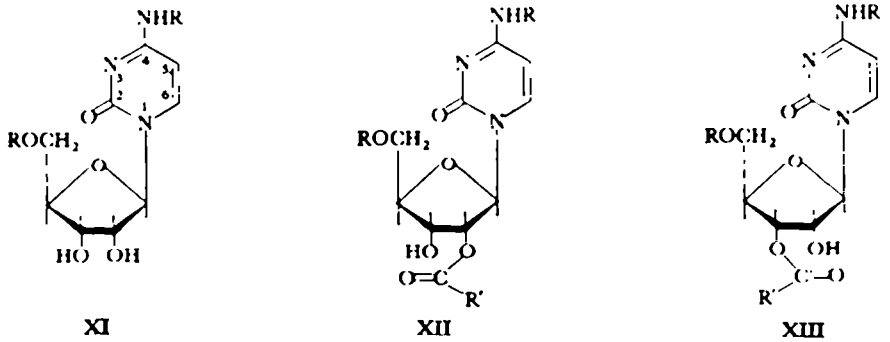


IX X

were not isolated but treated directly with acid and the resulting mixture fractionated on silicic acid: 3′-O-benzoyluridine (X) crystallized from the main fraction in 50% yield.

In addition to the above studies with unprotected nucleosides, the acylation of a number of 5′-O-acylribonucleoside derivatives has been undertaken in order to obtain the intermediates required for our oligoribonucleotide synthesis.[12] As the blocking of the 5′-OH group prevents the formation of bis-orthoester products such as VI, the reaction often proceeds quantitatively and, in some cases, pure compounds may be isolated without recourse to alumina or silicic acid chromatography. The results of some of these experiments are listed in Table 1.

It can be seen that 5′-O-acetyluridine, 5′-O-pivaloyluridine and 5′-O-acetyladenosine (Experiment Nos. 1, 2, 4) were all converted into their pure 3′-O-acetyl derivatives in good yields, whereas 5′-O-formyluridine (Experiment No. 3) gave a mixture of 2′- and 3′-O-acetates from which it was not possible to crystallize a pure isomer. It was also impossible to obtain crystalline 2′- or 3′-O-acetyl-5′-O-pivaloylguanosine from the mixture of isomers (Experiment No. 5), which readily formed a gel. Preliminary studies[13] indicate that more success is likely to be achieved with N-acylated derivatives of guanosine, which appear to have better crystallization properties.



XI XII XIII

[13] C. B. Reese and J. E. Sulston, unpublished results.

TABLE 1. MONOACYLATION OF 5′-O-ACYLRIBONUCLEOSIDE DERIVATIVES *via* ORTHOESTER EXCHANGE

Expt. No.	Nucleoside derivative (I)*	R	B	Orthoester	Product(s)†	% Yield‡
1.	5′-O-Acetyluridine	Ac	uracil	$MeC(OMe)_3$	3′,5′-Di-O-acetyluridine (IV; R′ = Me)	70
2.	5′-O-Pivaloyluridine	t-BuCO	uracil	$MeC(OMe)_3$	3′-O-Acetyl-5′-O-pivaloyluridine (IV; R′ = Me)	60
3.	5′-O-Formyluridine	HCO	uracil	$MeC(OMe)_3$	2′(3′)-O-Acetyl-5′-O-formyluridines (III and IV; R′ = Me)	56
4.	5′-O-Acetyladenosine	Ac	adenine	$MeC(OMe)_3$	3′,5′-Di-O-acetyladenosine (IV; R′ = Me)	60
5.	5′-O-Pivaloylguanosine	t-BuCO	guanine	$MeC(OMe)_3$	2′(3′)-O-Acetyl-5′-O-pivaloylguanosines (III and IV; R′ = Me)	36
6.	N^4,$O^{3'}$-Diacetylcytidine	Ac	N^4-acetylcytosine	$MeC(OMe)_3$	N^4,$O^{3'}$,$O^{5'}$-Triacetylcytidine (III; R′ = Me)	62
7.	N^4,$O^{3'}$-Dibenzoylcytidine	Bz	N^4-benzoylcytosine	$PhC(OMe)_3$	N^4,$O^{3'}$,$O^{5'}$-Tribenzoylcytidine (IV; R′ = Ph)	29
8.	N^4,$O^{3'}$-Dipivaloylcytidine	t-BuCO	N^4-pivaloylcytosine	$MeC(OMe)_3$	2′-O-Acetyl-N^4,$O^{3'}$-dipivaloylcytidine (III; R′ = Me)	29
9.	N^4,$O^{3'}$-Dipivaloylcytidine	t-BuCO	N^4-pivaloylcytosine	$PhC(OMe)_3$	2′-O-Benzoyl-N^4,$O^{3'}$-dipivaloylcytidine (III; R′ = Ph)	48

* See Fig. 1.

† If only one product is listed, it is crystalline and isomerically pure.

‡ In some cases, higher yields could be obtained by allowing the material in the liquors to equilibrate.

The chemistry of the cytidine derivatives listed in Table 1 differs sufficiently from that of the other compounds to merit a more detailed discussion. Following the procedure of Parihar,[14] the N^4, $O^{5'}$-diacylcytidine derivatives (XI; R = Ac, Bz, or t-BuCO), required as starting materials, were readily prepared by the action of 98% formic acid on N^4,$O^{5'}$-diacyl-2',3'-O-isopropylidenecytidines.* The most noteworthy aspect of the chemistry of these cytidine derivatives is that three of the four acylation experiments (Nos. 6, 8, 9) led to pure 2'-O-acyl compounds (XII). It should be mentioned that one of these compounds, N^4,$O^{2'}$,$O^{5'}$-triacetylcytidine (XII; R = Ac, R' = Me) was first obtained by Marinier[15] from the products of the reaction between acetic anhydride and the diacetate (XI; R = Ac); it was orientated by characterizing the derived 3'-O-tosylcytidine. We have confirmed this orientation by NMR spectroscopy,[2] and by converting the triacetate into 3'-O-mesylcytidine hydrochloride, and thence into the known 3'-O-mesyluridine.[4] In the light of present knowledge[3] about the state of the equilibrium between 2'- and 3'-O-acyl ribonucleosides, it is not surprising that the marginally less abundant isomer should sometimes crystallize from a solution containing both. Fortunately, in connection with our oligoribonucleotide synthesis,[12] one pure crystalline 3'-O-acyl derivative of cytidine, namely N^4,$O^{3'}$,$O^{5'}$-tribenzoylcytidine (XIII; R = Bz; R' = Ph) can be obtained. Thus it seems probable that a derivative of the required orientation† can be prepared if enough possibilities are examined. However, from the limited data so far available, it would appear that pure 3'-O-acyl derivatives are more accessible than their 2'-isomers.

Some of the compounds listed in Table 1 may be conveniently prepared by the route suggested in Fig. 2. The nucleoside is first converted into a 2',3'-O-methoxy-

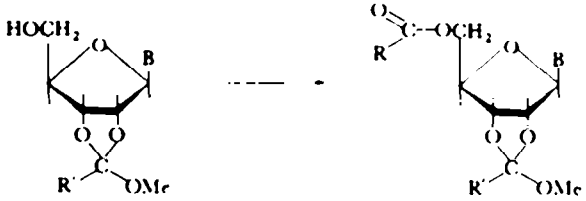


FIG. 2

alkylidene derivative XIV which, in some cases, may be isolated in a sufficiently pure state without recourse to alumina chromatography, and then acylated with the appropriate anhydride or acid chloride to give XV. The latter is then subjected to mild

* If the more usual aqueous acidic conditions were used to remove the isopropylidene group, concomitant N^4-deacylation occurred to an appreciable extent.

† In no instance have we succeeded in obtaining both the 2'- and 3'-isomers in a pure crystalline state. Rammler and Khorana[16] claim to have isolated both N^4, $O^{2'}$, $O^{5'}$- and N^4,$O^{3'}$,$O^{5'}$-tribenzoylcytidines as pure crystalline solids. The purity of the N^4,$O^{3'}$,$O^{5'}$-isomer, which was based on its melting-point, was not supported by phosphorylation studies.[16] We have found that these tribenzoylcytidines tend to cocrystallize (Experimental), and great care has to be exercised to obtain the pure crystalline N^4,$O^{3'}$,$O^{5'}$-isomer. It should be noted that benzoyl migration does not occur readily under the conditions of crystallization.

[14] D. B. Parihar, Ph.D. Thesis, Cambridge (1958).

[15] B. Marinier, unpublished results.

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

acidic hydrolysis and the products purified, if necessary, by fractionation on silicic acid. This route involves only two main steps, starting from a nucleoside. $N^4,O^{2'},O^{5'}$-Triacetylcytidine (XII: R = Ac, R' = Me) was readily prepared from cytidine in this way. A second example, the preparation of 3'-O-benzoyl-5'-O-formyluridine *via* the formylation of 2',3'-O-methoxybenzylideneuridine (XIV; B = uracil, R' = Ph) is analogous to the reported preparation[1] of 3',5'-di-O-formyluridine from 2',3'-O-methoxymethylideneuridine (XIV; B = uracil, R' = H). In the synthesis of such formyl derivatives it is advantageous to introduce the 5'-O-formyl group at as late a stage as possible in the reaction sequence, and thus minimize the number of stages involving comparatively labile intermediates.

It seems reasonable to conclude that the orthoester exchange reaction is likely to provide a general approach to the monoacylation of ribonucleoside-*cis*-2',3'-diol systems. Furthermore, this technique should be equally applicable to the monoacylation of simple glycols and related systems.*

Finally, the four principal ribonucleosides have been found to undergo ketal exchange with 2,2-dimethoxypropane, in the presence of toluene-*p*-sulphonic acid at room temperature, to give the corresponding 2',3'-O-isopropylidene derivatives in high yield. Exchange usually occurs rapidly, and the method may be adapted to any scale. Except in the case of uridine, more than one molecular equivalent of acid is required and it is sometimes convenient to add acetone† (or some other solvent) to keep the products in solution.

EXPERIMENTAL

UV absorption spectra were measured on a Cary recording spectrophotometer, model 14M-50. NMR spectra were measured with a Perkin–Elmer spectrometer, operating at 60 Mc/s. Chemical shifts are given in ppm on a τ scale; coupling constants are given in c/s. TMS and t-butanol were used as internal standards. D_2O (10–15%) was normally added to NMR solvents to promote exchange of active protons. In appropriate cases, the D_2O was acidified (M-with respect to AcOH) to suppress acyl migration.

Paper electrophoresis on Whatman No. 4 paper was conducted in a CCl_4-cooled apparatus (ca. 30 v/cm). The following solvents were used for paper chromatography: A, EtOH–water (7:3); B, propan-2-ol–ammonia (*d* 0·88)–water (7:1:2); C, butan-1-ol–AcOH–water (4:1:5); D, butan-1-ol–AcOH–water (5:2:3); E, butan-1-ol–water (86:14); F, isobutyric acid–ammonia (*d* 0·88)–water (66:1:33). Ascending chromatograms were run on Whatman No. 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 (1–20%) in $CHCl_3$. Mallinckrodt analytical grade silicic acid (100 mesh) and Woelm neutral alumina were used for adsorption chromatography.

Pyridine, trimethyl orthoacetate and 2,2-dimethoxypropane were dried by heating with CaH_2 under reflux, and then distilled before use. Trimethyl orthobenzoate was fractionated by distillation under reduced press (0·1 mm), but still contained methyl benzoate (ca. 10–20%).

*E.g., this technique has been used in the monoacetylation of a hexopyranoside deriv.[17] It has recently been brought to our attention that Gardi *et al.*[18] have monoacylated steroidal diols by a similar procedure.

† It seems improbable that acetone participates in this reaction, which involves acid-catalysed exchange between the nucleoside-*cis*-2'-3'-diol system and 2,2-dimethoxypropane to give a stable cyclic ketal. Thus the 2,2-dimethoxypropane is not simply acting as a dehydrating agent,[19] but is indeed the primary reagent. This conclusion was independently reached by Chládek and Smrt.[20]

[17] J. S. Brimacombe and D. Portsmouth, *Carbohyd. Res.* **1**, 128 (1965).
[18] R. Gardi, R. Vitali, and A. Ercoli, *Tetrahedron Letters* 448 (1961); *Gazz. Chim. Ital.* **93**, 431 (1963).
[19] A. Hampton, *J. Amer. Chem. Soc.* **83**, 3640 (1961).
[20] S. Chládek and J. Smrt, *Coll. Czech. Chem. Commun.* **28**, 1301 (1963).

2',3'-O-Methoxyethylideneuridine

Uridine (5 g), toluene-*p*-sulphonic acid, monohydrate (1 g, 0·25 mol) and trimethyl orthoacetate (15 ml) were stirred together at 20° for 16 hr. TLC revealed two products in the proportions of ca. 4:1, and no uridine. The reaction mixture was made slightly basic with methanolic MeONa, concentrated to an oil, dissolved in CH_2Cl_2 and chromatographed on a column (30 cm × 9 cm²) of alumina (grade III). The minor product was eluted with $CHCl_3$–1–4%* MeOH, and the required major product with $CHCl_3$–4% MeOH. Concentration of the latter fractions gave 2',3'-*O-methoxyethylideneuridine* as a colourless glass (4·47 g, 73%). (Found: C, 47·7; H, 5·6; N, 9·2. Calc. for $C_{12}H_{16}N_2O_7$: C, 48·0; H, 5·3; N, 9·3%.) R_F: 0·75 (system B), 0·63 (system E). UV (95% EtOH): λ_{max} 259 (log ε 3·96), λ_{min} 229 mμ (log ε 3·26). NMR spectrum in dimethylcyanamide/D_2O: τ 2·27 ($J \sim 8$), d, wt 1, assigned to H(6); τ 4·01 ($J = 2·3$) and τ 4·17 ($J = 2·2$), two ds, combined wt 1 (wt of low field to wt of high field d, 1:1·7), assigned to H(1') protons of two diastereoisomers; τ 4·30 ($J = 8·1$), d, wt 1, assigned to H(5); τ 5·05, m, wt 2, assigned to H(2') and H(3').

3'-O-Acetyluridine and 3',5'-di-O-acetyluridine from uridine

Uridine (10 g), toluene-*p*-sulphonic acid, monohydrate (1·0 g, 0·13 mol) and trimethyl orthoacetate (35 ml) were stirred together at 20°. After 2 hr, the reaction soln was no longer acidic and contained no uridine (TLC revealed 2',3'-O-methoxyethylideneuridine (2 parts) and a higher R_F component, 1 part). The products were concentrated under reduced press at 20° to yield a gum which was shaken with 5% AcOH (20 ml) until it dissolved. The resulting aqueous soln was extracted with ether (5 ml), and concentrated under reduced press to a gum which was dried by repeated evaporations with EtOH and benzene. This material was dissolved in $CHCl_3$ and chromatographed on a column (20 cm × 9 cm²) of silicic acid. Fraction 1, eluted with $CHCl_3$–2% MeOH contained 2'(3'),5'-di-O-acetyluridines. Recrystallization from EtOH gave 3',5'-di-O-acetyluridine (4·6 g, 34%) as colourless needles, m.p. 152–154° (lit.[8] 138–140°), undepressed by authentic material. Fraction 2, eluted with $CHCl_3$–4% MeOH contained 2'(and 3')-O-acetyluridines. Recrystallization from EtOH gave 3'-O-acetyluridine† as colourless needles, m.p. 172–174°. (Found: C, 46·5; H, 5·1; N, 9·8. Calc. for $C_{11}H_{14}N_2O_7$: C, 46·15; H, 4·9; N, 9·8%), yield 5·4 g (46%). R_F: 0·66 (system D), 0·43 (system E). Both 3'-O-acetyl- and 3',5'-di-O-acetyluridines were shown to be free from their respective 2'-isomers by NMR spectroscopy and by methanesulphonylation (see below).

2',3'-O-Isopropylideneuridine

Uridine (10 g) and toluene-*p*-sulphonic acid, monohydrate (1 g, 0·13 mol) were stirred with 2,2-dimethoxypropane (27·5 g) and acetone (150 ml) at 20° for 16 hr. After the acid had been neutralized with methanolic MeONa, the products were filtered through Hyflo-Supercel and concentrated, under reduced press, to a glass. This material was evaporated twice with water, and then recrystallized (charcoal) from water; yield of 2',3'-O-isopropylideneuridine 10·8 g (93%), m.p. 165–166° (lit.[10], 163–164°). R_F: 0·65 (system B), 0·79 (system D), 0·64 (system E).

5'-O-Acetyluridine‡

Acetic anhydride (7·5 ml, 2·5 mol) was added to a soln of 2',3'-O-isopropylideneuridine (9·2 g, 1 mol) in pyridine (70 ml), and the reaction allowed to proceed at 20° for 16 hr before MeOH (25 ml) was added. After a further period of 1 hr, the products were concentrated under reduced press and then re-evaporated with EtOH–water. The final residue, which was crystalline, was dissolved in 60% formic acid (100 ml) and allowed to stand at 20° for 4½ hr. Concentration of this soln gave a crystalline residue, which was left *in vacuo* (over KOH) for 1 hr before recrystallization from EtOH; yield

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

† Previously obtained (with m.p. 169–171°) by Dr. D. R. Trentham[21] from 2',5'-di-O-trityluridine,[5,6] in 74% overall yield.

‡ Preparation developed by Dr. M. Jarman. 5'-O-Acetyluridine may also be conveniently prepared from 2'-3'-O-methoxymethylideneuridine.[1]

[21] D. R. Trentham, Ph.D. Thesis, Cambridge (1965).

of 5'-O-acetyluridine, 7·7 g (83%), m.p. 163–165° (lit.[6], 163–164°). TLC indicated that the product was contaminated with a trace of uridine. R_F: 0·58 (system D), 0·30 (system E).

3',5'-Di-O-acetyluridine from 5'-O-acetyluridine

5'-O-Acetyluridine (5·0 g) and toluene-*p*-sulphonic acid, monohydrate (0·5 g, 0·13 mol) were stirred with trimethyl orthoacetate (15 ml) at 20°. After 1½ hr, the reaction soln, which had become neutral, was evaporated under reduced press and dissolved in 5% AcOH (50 ml). The aqueous soln was extracted with ether (5 ml), concentrated under reduced press and then re-evaporated with abs EtOH. The residue was allowed to stand *in vacuo* (over KOH) for 16 hr, and then crystallized from EtOH to give 3',5'-di-O-acetyluridine (2·3 g). The mother liquor was evaporated to dryness and the residue purified by silicic acid chromatography (see preparation of 3'-O-acetyluridine, above). When the appropriate fraction was evaporated and dissolved in EtOH, it slowly deposited pure 3',5'-di-O-acetyluridine (1·8 g); total yield, 4·1 g (70%), m.p. 152–155°. (Found: C, 47·6; H, 4·9; N, 8·4. Calc. for $C_{13}H_{16}N_2O_8$: C, 47·55; H, 4·9; N, 8·5%), R_F: 0·70 (system D), 0·55 (system E). The product was shown to be free from its 2',5'-isomer by NMR spectroscopy and by methanesulphonylation (see below).

3'-O-Acetyl-5'-O-pivaloyluridine

5'-O-Pivaloyluridine[22] (8·25 g) and toluene-*p*-sulphonic acid, monohydrate (0·48 g, 0·1 mol) were stirred with trimethyl orthoacetate (18 ml) at 20°. After 1 hr, the reaction soln was neutral and contained no unchanged starting material (as indicated by TLC). The products were concentrated under reduced press and then dissolved in 60% formic acid (25 ml). After ca. 5 min, the soln was evaporated under reduced press, and the residue re-evaporated with EtOH and benzene. The product was chromatographed on a column (20 cm × 9 cm²) of silicic acid. The required fraction was eluted with $CHCl_3$–1% MeOH. Recrystallization from AcOEt gave 3'-O-*acetyl*-5'-O-*pivaloyluridine* as colourless crystals, m.p. 165–168°. (Found: C, 52·1; H, 5·8; N, 7·7. $C_{16}H_{22}N_2O_8$ requires: C, 51·9; H, 5·95; N, 7·6%), yield 6·6 g (60%). UV (95% EtOH): λ_{max} 259 (log ε 4·00), λ_{min} 228 mμ (log ε 3·36). R_F: 0·85 (system D), 0·77 (system E). The product was shown to be free from its 2',5'-isomer by NMR spectroscopy and by methanesulphonylation (see below).

5'-O-Formyluridine

3',5'-Di-O-formyluridine[1] (4·3 g) was dissolved in a soln of morpholine (0·1 ml) in MeOH (80 ml). After the reaction soln had stood at 20° for 15 min, 98% formic acid (0·1 ml) was added, and the products evaporated under reduced press. The residue was slurried with silicic acid (50 g) and $CHCl_3$ and the slurry added to a column (6 cm × 9 cm², 50 g) of silicic acid. The required material was eluted with $CHCl_3$–6% MeOH. Evaporation of the solvents gave 5'-O-formyluridine as colourless crystals, m.p. 148–150° (lit.[23] 125–140°) (Found: C, 44·0; H, 4·6; N, 10·45. Calc. for $C_{10}H_{12}N_2O_7$: C, 44·1; H, 4·4; N, 10·3%); yield 2·1 g (54%). UV (95% EtOH containing 0·1% formic acid): λ_{max} 261 (log ε 3·98). λ_{min} 229 mμ (log ε 3·32). R_F: 0·50 (system D). This compound shows a positive periodate-Schiff spray test.

2'(3')-O-Acetyl-5'-O-formyluridines

5'-O-Formyluridine (2·0 g) and toluene-*p*-sulphonic acid, monohydrate (0·2 g, 0·14 mol) were stirred with trimethyl orthoacetate (6 ml) at 20°. After 2 hr, the reaction soln had become neutral and was free from starting material (as indicated by TLC). The products were cautiously concentrated under reduced press to yield a viscous oil, which was dissolved in $CHCl_3$ and the resulting soln applied to a column (10 cm × 3 cm²) of silicic acid. The column was then allowed to stand at 20° for 36 hr before it was eluted. The required fraction was subsequently eluted with $CHCl_3$–2% MeOH: this fraction was concentrated under reduced press to yield a glass which was then dissolved in EtOH (containing 0·1% formic acid). This soln deposited mixed crystals of 2'(and 3')-O-acetyl-5'-O-formyluridines. (Found: C, 46·0; H, 4·6; N, 8·9. Calc. for $C_{12}H_{14}N_2O_8$: C, 45·9; H, 4·5; N, 8·9%.); yield 1·3 g (56%). UV (95% EtOH): λ_{max} 258 (log ε 4·00), λ_{min} 228 mμ (log ε 3·41); R_F: 0·67 (system D), 0·45 (system E).

[22] M. Jarman, Ph.D. Thesis, Cambridge (1965).

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

The crystalline material was found to contain 2 parts of 2'- and 3 parts of 3'-O-acetate, by methanesulphonylation (see below).

2',3'-O-Methoxybenzylideneuridine

Uridine (3·0 g) and toluene-*p*-sulphonic acid, monohydrate (1·5 g, 0·64 mol) were stirred with trimethyl orthobenzoate (15 ml) at 20°. After 6 hr, a small amount of methanolic MeONa was added to the neutral products which were then concentrated under reduced press. The residue was dissolved in $CHCl_3$ and applied to a column (17 cm × 9 cm², 150 g) of alumina (grade II). The required 2',3'-O-*methoxybenzylideneuridine* was eluted with $CHCl_3$–4% MeOH and isolated as a colourless glass. (Found: C, 56·4; H, 5·0; N, 7·6. Calc. for $C_{17}H_{18}N_2O_7$: C, 56·3; H, 5·0; N, 7·7%); yield 3·52 g (79%); R_F: 0·80 (system B), 0·74 (system E). UV (95% EtOH): λ_{max} 260 (log ε 4·00), λ_{min} 229 mμ (log ε 3·52). NMR spectrum in dimethylcyanamide/D_2O: τ 2·23 (J ~ 8), d, wt 1, assigned to H(6); τ 2·51, m, wt 5, assigned to phenyl protons; τ 3·85 (J = 2·3) and τ 4·15 (J = 2·2), two ds. (of equal intensity), combined wt 1, assigned to H(1') protons of two diastereoisomers; τ 4·28 (J = 7·9), d, wt 1, assigned to H(5); τ 4·81, m, wt 1, assigned to H(2'); τ 5·00, m, wt 1, assigned to H(3').

3'-O-Benzoyluridine

Uridine (3·0 g), toluene-*p*-sulphonic acid, monohydrate (1·5 g) and trimethyl orthobenzoate (15 ml) were allowed to react together at 20°, as above. After 6 hr, 50% AcOH (20 ml) was added and after a further 30 min, the products were concentrated under reduced press and then re-evaporated several times with EtOH. The residue was dissolved in $CHCl_3$ and applied to a column (8 cm × 9 cm²) of silicic acid. Elution with $CHCl_3$–1% MeOH gave a higher R_F product (probably 2'(3'),5'-di-O-benzoyluridines), and elution with $CHCl_3$–4% MeOH gave the required product. The latter fraction was evaporated and the residue recrystallized from EtOH to give 3'-O-*benzoyluridine* as colourless crystals, m.p. 212–214°. (Found: C, 54·85; H, 4·8; N, 7·85. $C_{16}H_{16}N_2O_7$ requires: C, 55·2; H, 4·6; N, 8·05%); yield 2·2 g (50%); R_F: 0·85 (system D), 0·70 (system E). UV (95% EtOH): λ_{max} 231, 260 (log ε 4·19, 4·05), λ_{min} 247 mμ (log ε 3·98). NMR spectrum in dimethylcyanamide/D_2O (M with respect to AcOH): τ 1·77–2·45, m, wt 6, assigned to H(6) and phenyl protons; τ 3·94 (J = 6·4), d, wt 1, assigned to H(1'); τ 4·22, d, wt 1, assigned to H(5); τ 4·53, quartet, wt 1, assigned to H(3'). The product was free from its 2'-isomer.

3'-O-Benzoyl-5'-O-formyluridine

Formic acetic anhydride[24] (8 ml) was added to a soln of 2',3'-O-methoxybenzylideneuridine (4·0 g) in anhyd pyridine (40 ml) at −40°, and the reaction mixture allowed to stand at −15°. After 16 hr, EtOH (2 ml) was added and the products concentrated under reduced press at 20°. The resulting oil was dissolved in 90% formic acid (40 ml) and the soln similarly concentrated to give a residue which was then dissolved in abs EtOH. The latter soln deposited 3'-O-*benzoyl-5'-O-formyluridine* (1·1 g) as colourless crystals, m.p. 180–183°. (Found: C, 54·5; H, 4·3; N, 7·5. $C_{17}H_{16}N_2O_8$ requires: C, 54·25; H, 4·25; N, 7·45%.) The mother liquors were concentrated and then chromatographed on a column (19 cm × 9 cm²) of silicic acid. The required material was eluted with $CHCl_3$–2% MeOH; it was crystallized from EtOH to give a further crop (0·7 g) of 3'-O-benzoyl-5'-O-formyluridine; total yield, 1·8 g (45%). UV (95% EtOH): λ_{max} 258, 231 (log ε 4·06, 4·20), λ_{min} 248 mμ (log ε 4·01); R_F: 0·86 (system D), 0·73 (system E). NMR spectrum in dimethylcyanamide/D_2O (M with respect to AcOH): τ 1·71–τ 2·35, m, wt 7, assigned to H(6), formyl proton and phenyl protons; τ 3·99 (J = 5·8), d, wt 1, assigned to H(1'); τ 4·19 (J = 8·0), d, wt 1, assigned to H(5); τ 4·57, m, wt 1, assigned to H(3'). The product was isomerically pure (i.e., free from the 2'-O-benzoate).

*Identification of isomeric 2'- and 3'-O-acyluridines by methanesulphonylation**

A soln of 2'(3')-O-acyluridines (0·01 g) in anhyd pyridine (1 ml) at 0° is treated with methanesulphonyl chloride (0·02 ml, from a Microcap pipette). The reaction soln is allowed to stand at 0°. After 16 hr, water (0·1 ml) is added and the products are concentrated to very small volume below 30°

* The procedure described here is a qualitative adaption of a technique originally developed in connection with acyl migration studies. Certain modifications of the original procedure[9] have been introduced by Mr. R. Saffhill.

[24] A. Béhal, *Chem. Zentr.* 2, 750 (1900).

and then partitioned between CH_2Cl_2 (1 ml) and water (1 ml). The organic layer is washed with water (3 × 1 ml), evaporated to dryness, dissolved in methanolic ammonia (half-saturated at 0°; 1 ml), and then allowed to stand at 20° for 16 hr. The products (ca. 0·03 ml) are submitted to paper electrophoresis in 0·05M-sodium phosphate buffer (pH 7·5). Pure 2'- and 3'-O-acyluridine derivs lead to uncharged and cationic products, respectively. Mixtures of isomers lead to mixtures of uncharged and cationic products. A very approximate, qualitative estimate of the proportions of isomers in a mixture may be obtained by inspection of the electrophoretogram in UV light.

2',3'-O-Methoxyethylideneadenosine

Adenosine (1·07 g) and toluene-*p*-sulphonic acid, monohydrate (1·14 g, 1·5 mol) were stirred with trimethyl orthoacetate (6 ml) and dimethylformamide (3 ml) at 20°. After 3 hr, the colourless soln of products was found (TLC) to be free from adenosine and to contain one main product. Methanolic MeONa was added to the soln until it was just basic, and then the products were concentrated under reduced press to a viscous oil which was triturated with $CHCl_3$ (3 × 15 ml). The combined $CHCl_3$ extracts were filtered and concentrated under reduced press to give a pale yellow glass, which was dissolved in CH_2Cl_2 and chromatographed on a column (10·5 cm × 5 cm², 50 g) of alumina (grade III). A by-product R_F 0·89 (system B) was eluted with CH_2Cl_2 and the required 2',3'-O-*methoxyethylideneadenosine* was eluted with $CHCl_3$. Evaporation of the $CHCl_3$ gave the product as a glass which crystallized from EtOH (containing a trace of ammonia), m.p. 177°; yield 0·87 g (67%), R_F: 0·77 (system B). This material was unchanged after it had been allowed to stand in methanolic ammonia soln at 20° for 16 hr; it was shown by NMR spectroscopy to contain a 1:1 mixture of diastereoisomers (see below).

After several recrystallizations from EtOH (containing a trace of ammonia), a crystalline product with m.p. 219–222° was obtained. (Found: in material dried over P_2O_5 at 110°: C, 48·5; H, 5·4; N, 21·7. $C_{13}H_{17}N_5O_5$ requires: C, 48·2; H, 5·3; N, 21·6%.) UV (95% EtOH): λ_{max} 260 (log ε 4·11), λ_{min} 228 mμ (log ε 3·28). NMR spectrum (of material with m.p. 219–222°) in dimethylcyanamide/D_2O: τ 1·72, s, wt 1, assigned to H(2); τ 1·80, s, wt 1, assigned to H(8); τ 3·64 (J = 2·4), d, wt 1, assigned to H(1'). This spectrum indicated that the material was diastereoisomerically pure. By comparison of the latter signals with those of the spectrum of the mixture (m.p. 177°), some of the proton resonances of the other diastereoisomer can be obtained: τ 1·72, s, assigned to H(2); τ 1·80, s, assigned to H(8), τ 3·80 (J = 3·0), d, assigned to H(1').

3'-O-Acetyladenosine

2',3'-O-Methoxyethylideneadenosine (0·5 g) was dissolved in 60% AcOH, the soln lyophilized and the residue allowed to stand *in vacuo* over KOH. The semi-solid material, so obtained, was dissolved in hot abs EtOH (25 ml) and allowed to crystallize slowly. 3'-O-*Acetyladenosine* was obtained as colourless crystals, m.p. 180–181°. (Found: in material dried *in vacuo* over P_2O_5: C, 47·0; H, 4·8; N, 22·2. $C_{12}H_{15}N_5O_5$ requires: C, 46·6; H, 4·85; N, 22·6%); yield, 0·42 g (88%). UV (95% EtOH): λ_{max} 260 (log ε 4·15), λ_{min} 227 mμ (log ε 3·66), R_F: 0·63 (system C). The product was shown to be free from its 2'-isomer by NMR spectroscopy.

2',3'-O-Isopropylideneadenosine

Adenosine (2·67 g) and toluene-*p*-sulphonic acid, monohydrate (2·30 g, 1·2 mol) were stirred with 2,2-dimethoxypropane (25 ml) at 20° for 12 hr. After the reaction soln had been neutralized with N-methanolic MeONa, it was concentrated under reduced press and the resulting oil triturated with hot $CHCl_3$ (5 × 10 ml). The combined $CHCl_3$ extracts were filtered through Hyflo-Supercel and then evaporated under reduced press to give a product which was then crystallized from EtOH; yield 2·45 g (80%), m.p. 220° (lit.,[18] 216–217°). R_F: 0·66 (system A), 0·80 (system C).

5'-O-Acetyladenosine

To a soln of 2',3',5'-tri-O-acetyladenosine (9·0 g) in hot abs EtOH (150 ml) was added a soln of morpholine (4 g, 2 mol) in EtOH (40 ml). After the reactants had been heated under reflux for 20 hr, 5'-O-acetyladenosine was shown (TLC) to be the main product.* The latter, contaminated with a

* TLC also revealed trace quantities of adenosine, 2'(3'),5'-di-O-acetyladenosines, and starting material.

trace of adenosine, crystallized from the cooled products; yield of dry material, 5·1 g (72%); m.p. 143° (lit.,[24] 143°, after softening at 134°), R_P: 0·56 (system C). The product migrated as a single anionic species on a paper electrophoretogram in 0·2M-sodium borate buffer (pH 9).

5'-O-Pivaloyladenosine

Pivaloyl chloride (0·47 g, 1·2 mol) was added to a soln of 2',3'-O-isopropylideneadenosine (1·0 g, 1 mol) in pyridine (10 ml) and the reactants allowed to stand, with the exclusion of moisture, at 20° for 16 hr. EtOH (5 ml) was added and, after a further period of 1 hr, the products were concentrated under reduced press and re-evaporated with aqueous EtOH (1:1). The residue was dissolved in 90% formic acid (10 ml) and allowed to stand at 20°. After 18 hr it was concentrated under reduced press (bath temp <30°) and re-evaporated with water.

The product, which was apparently a formate salt, was dissolved in water (5 ml), and N KOH was added carefully until the pH of the soln had increased to 7–8. The aqueous soln was extracted with $CHCl_3$, the combined $CHCl_3$ extracts dried (Na_2SO_4) and evaporated to give a glass. Colourless crystals of *5'-O-pivaloyladenosine*, m.p. 162–163°, were obtained when the latter material was dissolved in EtOH and the soln allowed to cool. (Found; in material dried *in vacuo* over P_2O_5: C, 51·4; H, 6·4; N, 19·9. $C_{15}H_{21}N_5O_5$ requires: C, 51·4; H, 6·0; N, 19·9%); yield 0·78 g (68%). UV (95% EtOH): λ_{max} 260 (log ϵ 4·12), λ_{min} 228, mμ (log ϵ 3·62). R_P: 0·78 (system B), 0·87 (system C).

3',5'-Di-O-Acetyladenosine

5'-O-Acetyladenosine (8·8 g) and toluene-*p*-sulphonic acid, monohydrate (6·5 g, 1·2 mol) were stirred with trimethyl orthoacetate (30 ml) at 20°. After 4 hr, the reaction mixture was neutralized with N-methanolic MeONa and then concentrated under reduced press. The pale coloured gum, so obtained, was triturated with $CHCl_3$ (4 × 20 ml), and the combined $CHCl_3$ extracts filtered through Hyflo-Supercel and then evaporated to give a viscous oil. The latter material was dissolved in 5% AcOH (20 ml), concentrated under reduced press (bath temp <30°), then evaporated several times with abs EtOH and finally dissolved in $CHCl_3$ and applied to a column (35 cm × 3·5 cm^2, 100 g) of silicic acid. The required fraction was eluted with $CHCl_3$–2% MeOH. Evaporation of the solvents gave a glass (6·0 g, 60%) which crystallized slowly from abs EtOH (10 ml). The first crop of 3',5'-di-O-acetyladenosine weighed 5·0 g. (50%), m.p. 175–176·5° (lit.[8], 172–173°); R_P: 0·61 (system B), 0·72 (system C). The product was shown to be free from its 2',5'-isomer by NMR spectroscopy.

2',3'-O-Isopropylideneguanosine

Guanosine (10·0 g) and toluene-*p*-sulphonic acid, monohydrate (8·0 g, 1·2 mol) were stirred with 2,2-dimethoxypropane (36 ml) and dry acetone (1000 ml) at 20°. After 16 hr, the products were neutralized with M-methanolic MeONa, then concentrated under reduced press and dissolved in aqueous ammonia (*d* 0·88, 100 ml). The filtered soln slowly deposited crystals of 2',3'-O-isopropylideneguanosine, m.p. 290° dec (lit.[19], 292° dec); yield 8·24 g (70%). R_P: 0·62 (system B), 0·45 (system E).

2',3'-O-Isopropylidene-5'-O-pivaloylguanosine

Pivaloyl chloride (0·6 ml, 1·6 mol) was added to a soln of 2',3'-O-isopropylideneguanosine (1·0 g, 1 mol) in pyridine (10 ml). The resulting soln was allowed to stand at 20° with the exclusion of moisture. After 16 hr, MeOH (2 ml) was added and after a further 30 min the resulting soln was concentrated and re-evaporated with EtOH–water. The residue was partitioned between $CHCl_3$ and water and the dried (Na_2SO_4) $CHCl_3$ layer evaporated. Recrystallization of the product from EtOH gave *2',3'-O-isopropylidene-5'-O-pivaloylguanosine*, m.p. ~260° dec. (Found: C, 53·3; H, 6·5; N, 17·15. $C_{18}H_{25}N_5O_6$ requires: C, 53·1; H, 6·2; N, 17·2%); yield 0·64 g (50%). UV (95% EtOH): λ_{max} 255 (log ϵ 4·21), λ_{min} 222 mμ (log ϵ 3·49); R_P: 0·86 (system B), 0·90 (system D), 0·82 (system E).

5'-O-Pivaloylguanosine

A soln of 2',3'-O-isopropylidene-5'-O-pivaloylguanosine (1·14 g) in 60% formic acid (40 ml) was allowed to stand at 20°. After 16 hr, the soln was concentrated under reduced press, re-evaporated with abs EtOH, and finally crystallized from EtOH. The *5'-O-pivaloylguanosine*, so obtained

[24] D. M. Brown, L. J. Haynes and A. R. Todd, *J. Chem. Soc.* 3299 (1950).

had m.p. ~215°. (Found: C, 48·9; H, 5·8; N, 19·0. $C_{18}H_{21}N_5O_6$ requires: C, 49·0; H, 5·8; N, 19·05%); yield 0·84 g (82%). UV (95% EtOH) λ_{max} 255 (log ε 4·16), λ_{min} 222 mμ (log ε 3·43), R_P: 0·68 (system B), 0·76 (system D), 0·43 (system E).

2′(and 3′)-O-Acetyl-5′-O-pivaloylguanosines

5′-O-Pivaloylguanosine (0·5 g), toluene-*p*-sulphonic acid, monohydrate (0·025 g, 0·1 mol) and trimethyl orthoacetate (3 ml) were stirred together at 20°. After 4 hr, the neutral gel, thus obtained, was dissolved in EtOH (5 ml), concentrated and re-dissolved in 40% AcOH (10 ml). The soln was extracted with ether (2 ml), evaporated and re-dissolved in EtOH. A gelatinous ppt (0·08 g) was obtained. The mother liquors were evaporated and chromatographed on a column (12 cm × 3 cm²) of silicic acid. The required material was eluted with $CHCl_3$–4% MeOH. Evaporation of the solvents and crystallization from water gave 2′(and 3′)-O-acetyl-5′-O-pivaloylguanosines as mixed crystals m.p. 157–158°. (Found: C, 49·7; H, 5·9; N, 16·9. Calc. for $C_{17}H_{23}N_5O_7$: C, 49·9; H, 5·7; N, 17·1%); total yield, 0·20 g (36%). UV (95% EtOH): λ_{max} 255 (log ε 4·16), λ_{min} 220 mμ (log ε 3·40), R_P: 0·82 (system D), 0·68 (system E). The crystalline material was shown by NMR spectroscopy to be a mixture of isomers.

2′,3′-O-Methoxyethylidenecytidine•

Cytidine (0·976 g) and toluene-*p*-sulphonic acid, monohydrate (0·95 g, 1·25 mol) were stirred with trimethyl orthoacetate (2·5 ml) at 20°. The reaction mixture set solid within 45 min. After 2 hr, the products were neutralized with 2M-methanolic MeONa and $CHCl_3$ (10 ml) was added. The resulting mixture was thoroughly shaken and then filtered through Hyflo-Supercel. The filtrate and washings were concentrated under reduced press to give a colourless glass which was further purified by chromatography on a column (7 cm × 3 cm²) of alumina (grade III). The required fraction was eluted with $CHCl_3$–4% MeOH. Evaporation of the solvents gave 2′,3′-O-methoxyethylidenecytidine as a colourless glass. (Found: C, 48·0; H, 6·4; N, 13·75. Calc. for $C_{13}H_{17}N_3O_6$: C, 48·15; H, 5·7; N, 14·0%); yield, 0·89 g (74%); R_P: 0·66 (system B).

2′,3′-O-Isopropylidenecytidine

Cytidine (9·74 g) and toluene-*p*-sulphonic acid, monohydrate (8·27 g, 1·2 mol) were vigorously shaken with 2,2-dimethoxypropane (100 ml) at 20°. After 2 hr, the reaction mixture was neutralized with 2M-methanolic MeONa. MeOH (40 ml) and water (20 ml) were added and the clear soln concentrated under reduced press. The residue was dissolved in water (25 ml) and the soln continuously extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated under reduced press and then dried *in vacuo* (over P_2O_5) to give 2′,3′-O-isopropylidenecytidine as a colourless, chromatographically homogeneous hygroscopic glass; yield, 9·2 g (81%), R_P: 0·68 (system D).

N⁴,O⁵′-Diacetylcytidine

Acetic anhydride (15·0 ml, 3·5 mol) was added to a soln of 2′,3′-O-isopropylidenecytidine (10·5 g, 1 mol) in pyridine (40 ml) and the reactants stirred at 20°, with the exclusion of moisture. After 16 hr, EtOH (30 ml) was added and the products concentrated under reduced press, re-evaporated with EtOH (2 × 40 ml) and the residue partitioned between $CHCl_3$ (40 ml) and water (40 ml). The dried ($MgSO_4$) $CHCl_3$ layer was evaporated under reduced press to give an oil, which was then dissolved in 98% formic acid (700 ml). After the soln had stood at 20° for 24 hr, it was concentrated under reduced press to an oil which was shaken vigorously with anhyd ether (125 ml). The colourless amorphous powder, so obtained, was washed thoroughly with ether, kept *in vacuo* over KOH for 16 hr, and then crystallized from MeOH–$CHCl_3$ to give N⁴,O⁵′-diacetylcytidine as fine colourless needles, m.p. 172–176° (lit.[14], 185°). (Found: C, 47·6; H, 5·1; N, 12·8. Calc. for $C_{13}H_{17}N_3O_7$: C, 47·7; H, 5·2; N, 12·8%). Yield 5·65 g (47%). UV (MeOH): λ_{max} 247, 297 (log ε 4·18, 3·87), λ_{min} 226, 273 mμ (log ε 3·74, 3·61), R_P: 0·73 (system D). This compound gives a positive periodate-Schiff spray test.

N⁴,O⁵′-Dipivaloylcytidine

Pivaloyl chloride (12·7 g, 3·5 mol) was added to a soln of 2′,3′-O-isopropylidenecytidine (8·54 g, 1 mol) in pyridine (20 ml), and the reaction carried out as above. The oil obtained, after a similar

• This is a slight modification of a preparation first developed by Dr. M. Jarman.

work-up, was dissolved in 98% formic acid (1000 ml) at 20°. After 24 hr, the formic acid was removed by evaporation and the residue shaken with ether (250 ml). The powder obtained (8·5 g) was treated as above and then crystallized from MeOH–AcOEt to give *N⁴,O²′-dipivaloylcytidine* as fine prisms, m.p. 183–186°. (Found: C, 55·8; H, 6·8; N, 10·2. $C_{19}H_{29}N_3O_7$ requires: C, 55·5; H, 7·1; N, 10·2%). Yield: 5·12 g (42%). UV(MeOH): λ_{max} 248, 298 (log ε 4·20, 3·89), λ_{min} 226, 273 mμ (log ε 3·72, 3·62), R_F: 0·85 (system E). This compound gives a positive periodate-Schiff spray test.

N⁴,O²′-Dibenzoylcytidine

Benzoyl chloride (3·57 g, 2·2 mol) was added to a soln of 2′,3′-O-isopropylidenecytidine (3·29 g, 1 mol) in pyridine (10 ml) and the reactants stirred at 20°, with the exclusion of moisture. After 16 hr, water (2 ml) was added and, after a further period of 2 hr, the products were concentrated under reduced press. The oil, so obtained, was partitioned between CH_2Cl_2 (25 ml) and water (25 ml). The dried ($MgSO_4$) CH_2Cl_2 layer was evaporated and the residue dissolved in 98% formic acid (200 ml) and allowed to stand at 20° for 24 hr. The crude product, which was isolated as a powder (see preparation of N⁴,O²′-diacetylcytidine), was recrystallized from EtOH to give N⁴,O²′-dibenzoylcytidine as fibrous needles, m.p. 186–190° (lit.[16], 185–186°). (Found: C, 60·9; H, 4·9; N, 9·4. Calc. for $C_{23}H_{21}N_3O_7$: C, 61·2; H, 4·7; N, 9·3%); yield, 3·0 g (67%). UV (95% EtOH): λ_{max} 229, 261, 305 mμ (log ε 4·34, 4·38, 4·02), λ_{min} 245, 289 mμ (log ε 4·23, 3·92).

N⁴,O³′,O⁵′-Triacetylcytidine

(a) 2′,3′-O-Methoxyethylidenecytidine was prepared, as above, from cytidine (5·83 g), toluene-*p*-sulphonic acid, monohydrate (5·7 g, 1·25 mol) and trimethyl orthoacetate (15 ml). The product, which was not further purified by chromatography on alumina, was dissolved in pyridine (20 ml), and the soln treated with Ac_2O (5·65 ml, ca. 2·5 mol) at 20°. After 18 hr, water (6 ml) was added and after a further period of 1 hr, the products were concentrated under reduced press to give an oil which was dissolved in water (60 ml) and acidified (to pH 3·5) with AcOH. The products* were concentrated under reduced press, evaporated with EtOH (3 × 30 ml), and then crystallized from EtOH (50 ml) to give N⁴,O³′,O⁵′-Triacetylcytidine as colourless prisms, m.p. 182–185° (lit.,[18] 174°). (Found: C, 48·6; H, 5·5; N, 11·5. Calc. for $C_{15}H_{19}N_3O_8$: C, 48·8; H, 5·2; N, 11·4%); yield 2·3 g (26%, based on cytidine). UV (MeOH): λ_{max} 248, 297 (log ε 4·22, 3·86), λ_{min} 226, 273 mμ (log ε 3·74, 3·62). R_F: 0·83 (system D). This material was shown to be free from its N⁴,O³′,O⁵′-isomer by NMR spectroscopy.

(b) N⁴,O⁵′-Diacetylcytidine (2·1 g) and toluene-*p*-sulphonic acid, monohydrate (0·188 g, 0·17 mol) were stirred with trimethyl orthoacetate (14·5 ml) at 20°. After 7 hr, the reaction soln was neutralized with M-methanolic MeONa, the products concentrated and extracted with $CHCl_3$ (20 ml). The $CHCl_3$ soln was slurried with silicic acid (24 g) and the slurry added to the top of a column (8·5 cm × 12·6 cm²) of silicic acid. The column was allowed to stand at 20° for 36 hr before elution. The fraction containing N⁴,O³′(O²′),O⁵′-triacetylcytidines was eluted with $CHCl_3$–2·5% MeOH. Evaporation of the solvents and crystallization of the colourless glass, so obtained, gave N⁴,O³′,O⁵′-triacetylcytidine (1·44 g, 62%).

N⁴,O³′,O⁵′-Tribenzoylcytidine

N⁴,O⁵′-Dibenzoylcytidine (8·18 g) and mesitylenesulphonic acid (4·4 g, 1·2 mol) were stirred with trimethyl orthobenzoate (36 g) at 20°. After ½ hr, complete soln had occurred and after a further 2½ hr, the reaction soln was neutralized with 2M-methanol MeONa. $CHCl_3$ (100 ml) was added and the mixture filtered through Hyflo-Supercel. The filtrate was concentrated and the residue dissolved in 96% AcOH (100 ml). The latter soln was immediately evaporated under reduced press, the last traces of solvents being removed under high vacuum. The residue was dissolved in CH_2Cl_2 and the soln slurried with silicic acid (30 g). The slurry was added to a column (14 cm × 10 cm², 70 g) of silicic acid which was first eluted with CH_2Cl_2. The required fraction was eluted with AR $CHCl_3$ (which contains 2% EtOH) and the solvents evaporated. The residue (wt 8·7 g representing a 86% yield of mixed N⁴,O³′(O²′),O⁵′-tribenzoylcytidines) was recrystallized from boiling EtOH (500 ml) to give N⁴,O³′,O⁵′-*tribenzoylcytidine* as fibrous needles, m.p. 198–202° (lit.[16], 193–194°). (Found: C, 64·9;

* TLC examination of the crude products indicated that the overall yield of N⁴,O³′(O²′),O⁵′-triacetylcytidines was ca. 80%, based on cytidine.

H, 4·7; N, 7·2. $C_{26}H_{34}N_3O_6$ requires: C, 64·9; H, 4·5; N, 7·6%); yield, 2·9 g. UV (95% EtOH): λ_{max} 232, 262, 304 (log ε 4·54, 4·43, 4·00), λ_{min} 213, 247, 290 mμ (log ε 4·38, 4·32, 3·95). This material was shown by NMR spectroscopy to be free from its $N^4,O^{3'},O^{5'}$-isomer.

Two more crops of combined wt 3·37 g were obtained but this material was shown by NMR spectroscopy to contain the $N^4,O^{3'},O^{5'}$- and $N^4,O^{2'},O^{5'}$-isomers in the proportions of ca. 3:2. This material (3·37 g) was dissolved in dimethylformamide (60 ml) and water (10 ml) and the soln heated at 100° for 40 min. The solvents were removed under reduced press and finally under high vacuum to give the equilibrium mixture of $N^4,O^{3'},O^{5'}$- and $N^4,O^{2'},O^{5'}$-isomers (i.e. ca. 3 parts and 4 parts, respectively). No de-acylation could be detected by TLC. Recrystallization of the equilibrium mixture from EtOH gave more pure $N^4,O^{3'},O^{5'}$-isomer.

2'-O-Acetyl-$N^4,O^{5'}$-dipivaloylcytidine

$N^4,O^{5'}$-Dipivaloylcytidine (2·57 g) and toluene-*p*-sulphonic acid, monohydrate (0·183 g, 0·17 mol) were stirred with trimethyl orthoacetate (25 ml) at 20°. After 7 hr, the reaction soln was neutralized with M-methanolic MeONa, the products evaporated and the residue extracted with $CHCl_3$ (100 ml). The $CHCl_3$ extracts were slurried with silicic acid (18 g) and the slurry added to a column (3 cm × 3 cm^3) of silicic acid, which was allowed to stand at 20°. After 36 hr, the required fraction was eluted with $CHCl_3$–2–3% MeOH. Evaporation and crystallization from AcOEt gave *2'-O-acetyl-$N^4,O^{5'}$-dipivaloylcytidine* as colourless needles, m.p. 157–160°. (Found: C, 55·9; H, 7·1; N, 9·5. $C_{31}H_{51}N_3O_8$ requires: C, 55·6; H, 6·9; N, 9·3%); yield, 0·815 g (29%). UV (95% EtOH): λ_{max} 249, 299 (log ε 4·20, 3·86), λ_{min} 226, 276 mμ (log ε 3·62, 3·61). This material was shown by NMR spectroscopy to be free from the isomeric 3'-acetate.

2'-O-Benzoyl-$N^4,O^{5'}$-dipivaloylcytidine

$N^4,O^{5'}$-Dipivaloylcytidine (0·654 g) and mesitylenesulphonic acid (0·385 g, 1·2 mol) were stirred with trimethyl orthobenzoate (3·3 ml) at 20°. After 3 hr, the reaction soln was neutralized with 2M-methanolic MeONa and $CHCl_3$ (10 ml) was added. The products were filtered through Hyflo-Supercel, the filtrate concentrated under reduced press and treated with 98% AcOH (10 ml). The latter soln was evaporated under reduced press and finally under high vacuum. The residue was chromatographed on a column (8 cm × 2 cm^3) of silicic acid, and the required fraction was eluted with $CHCl_3$–2% MeOH. Evaporation and crystallization from AcOEt gave *2'-O-benzoyl-$N^4,O^{5'}$-dipivaloylcytidine* as colourless needles, m.p. 152–155°. (Found: C, 60·7; H, 6·9; N, 8·3. $C_{36}H_{53}N_3O_8$ requires: C, 60·6; H, 6·45; N, 8·15%); yield, 0·395 g (48%). UV (95% EtOH): λ_{max} 239, 300 (log ε 4·30, 3·85), λ_{min} 224, 277 mμ (log ε 4·20, 3·69). This material was shown by NMR spectroscopy to be free from the isomeric 3'-O-benzoate.

3'-O-Methanesulphonylcytidine hydrochloride

$N^4,O^{2'},O^{5'}$-Triacetylcytidine (0·503 g) was added to a freshly prepared soln of methanesulphonyl chloride (0·62 ml, 6 mol) in pyridine (50 ml) at 0°. After 22 hr, the products were allowed to warm up to 20° and stand for 2 hr. Water (3 ml) was added and after 2 hr, the products were concentrated under reduced press and the residue partitioned between CH_2Cl_2 (25 ml) and water (25 ml). The organic layer was washed with water (3 × 25 ml), dried ($MgSO_4$), evaporated, and the residue chromatographed on a column (5 cm × 2 cm^3) of silicic acid. The required fraction was eluted with $CHCl_3$–1% MeOH, the solvents evaporated, and the residue dissolved in methanolic ammonia (half-saturated at 0°, 75 ml) at 20°. After 16 hr, the soln was evaporated under reduced press and the residue dissolved in water (5 ml). The chromatographically homogeneous [R_F: 0·59 (system D), 0·25 (system E)] aqueous soln was treated with 0·2N HCl (14 ml), and then concentrated to an oil which crystallized from EtOH to give *3'-O-methanesulphonylcytidine hydrochloride* as colourless needles, m.p. 170–176°. (Found: C, 33·5; H, 4·6; Cl, 10·0; N, 11·95. $C_{10}H_{16}ClN_3O_7S$ requires: C, 33·6; H, 4·5; Cl, 9·9; N, 11·7%). Yield 0·324 g (67%). UV (0·01N HCl) : λ_{max} 277 (log ε 4·13), λ_{min} 240 mμ (log ε 3·37); R_F: 0·47 (system D), 0·73 (system F).

De-amination of 3'-O-methanesulphonylcytidine

A soln of 3'-O-methanesulphonylcytidine hydrochloride (0·015 g) in water (0·5 ml) was treated with AcOH (0·025 ml) and $NaNO_2$ (0·025 g) at 20°. After 2, 3, and 4 hr, further quantities (each of

0·025 g) of $NaNO_2$ were added. After a total reaction time of 4 hr, paper chromatography indicated a sole product [R_p: 0·61 (system F)], and no starting material. The product had λ_{max} 261 mμ and the same R_p as authentic 3′-O-methanesulphonyluridine[4]; it was unaffected by treatment with methanolic ammonia for 2 hr at 20°. When this ammonia-treated product was submitted to paper electrophoresis in 0·1M sodium borate buffer (pH 9), it behaved as an uncharged species.

Hydrolysis of 2′,3′-O-Methoxyethylidene-adenosine and -uridine at pH 5·6

The 2′,3′-O-Methoxyethylidene deriv (0·04 g) was dissolved in 0·1M sodium citrate buffer (pH 5·6, 8 ml), and the soln allowed to stand at 20°. After suitable time intervals, aliquots (0·5 ml) of the soln were removed and added to tubes containing M-aqueous ammonia (0·1 ml). A measured volume (0·025 ml) of the contents of each tube was applied to a Whatman No. 42 paper chromatogram which was subsequently developed in system B. Three bands corresponding to (a) unchanged starting material, (b) free nucleoside, and (c) an equal blank area were cut out, cut into strips and each allowed to soak in 0·1N HCl (6 ml), contained in a sealed tube, at 20° for 24 hr. The optical densities of the eluates of bands (a) and (b) were measured at λ_{max}; the eluate of band (c) was used as a blank in both cases. In this way, the half-times of hydrolysis of 2′,3′-O-methoxyethylideneadenosine and uridine were found to be 83 and 39 min, respectively.* In each case, first order kinetics was observed, and the pH of the solution remained constant.

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* Approximately 1:1 mixtures of diastereoisomers were used. The half-time of hydrolysis of diastereoisomerically pure 2′,3′-O-methoxyethylideneadenosine (m.p. 219–222°) was found to be 73 min. However, the latter compound dissolved slowly in the buffer and thus the deviation from the 83 min half-time observed for the mixture may be within the limits of experimental error.