165. *Nucleotides. Part XL.** O^2 : 5′-cyclo*Uridine and a Synthesis of* iso*Cytidine.*

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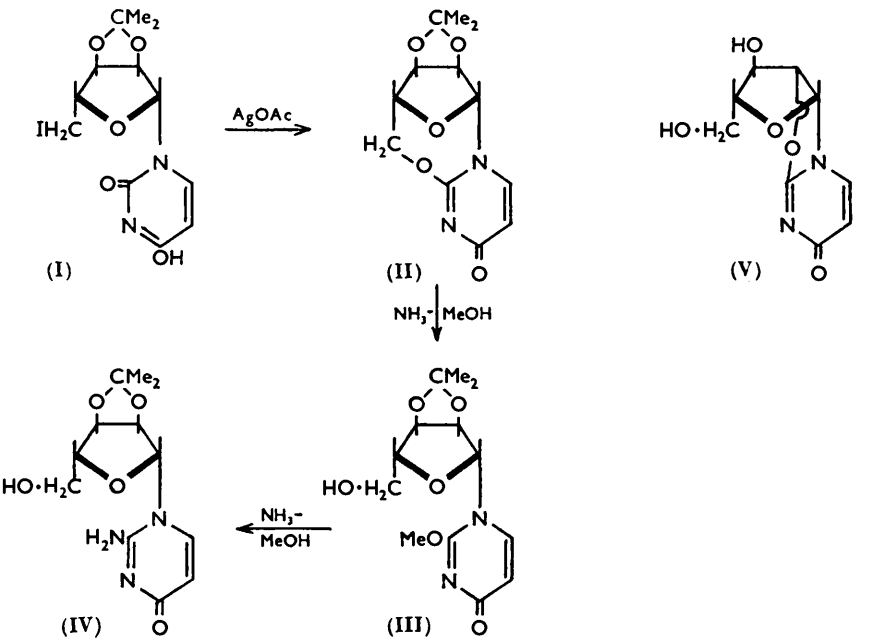
5′-Deoxy-5′-iodo-2′ : 3′-*O*-*iso*propylideneuridine (I) when treated with silver acetate in methanol gives 2′ : 3′-*O*-*iso*propylidene-O^2 : 5′-*cyclo*uridine (II). Hydrolysis of the latter by acid or alkali yields *iso*propylideneuridine, and base-catalysed methanolysis leads to 2′ : 3′-*O*-*iso*propylidene-O^2-methyluridine which is converted by methanolic ammonia into 2′ : 3′-*iso*propylidene-*iso*cytidine (IV). Acid-hydrolysis of (IV) yields *iso*cytidine. Analogous transformations are described with 2′ : 3′-di-*O*-acetyluridine derivatives.

During synthetic studies in the nucleotide field an attempt was made to prepare 5′-*O*-acetyluridine by the action of silver acetate on 5′-deoxy-5′-iodouridine in methanol. A product was formed which was evidently not the required acetyl derivative but which was too insoluble to permit complete purification. In order to avoid solubility difficulties we carried out similar experiments with 5′-deoxy-5′-iodo-2′ : 3′-*O*-*iso*propylideneuridine (I) and the 2′ : 3′-di-*O*-acetyl analogue. The iodo-compound (I) when treated with silver



acetate in methanol rapidly formed a product giving analytical values corresponding to an anhydro*iso*propylideneuridine and an ultraviolet absorption spectrum with $\lambda_{max.}$ 237 mμ (uridine has $\lambda_{max.}$ 260 mμ). The product was evidently analogous to that obtained by

* Part XXXIX, *J.*, 1956, 4873.

Michelson and Todd [1] when they treated 5′-deoxy-5′-iodothymidine with silver acetate in methyl cyanide containing traces of aliphatic amines and formulated as O^2 : 5′-*cyclo*thymidime. Although we find that the reaction with the compound (I) does not proceed in methyl cyanide either with or without the addition of base, nevertheless the reactions of the anhydro-compound justify its formulation as 2′ : 3′-*O*-*iso*propylidene-O^2 : 5′-*cyclo*uridine (II).

It is significant that, while 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine is rapidly converted into O^2 : 2′-*cyclo*uridine (V) by methanolic ammonia,[2] neither 5′-*O*-toluene-*p*-sulphonyluridine nor 5′-deoxy-5′-iodouridine is affected by this reagent or by a variety of other bases. For this reason the reaction of the 5′-iodo-derivatives promoted by silver ion is probably to be considered as one involving a carbonium-ion intermediate as distinct

FIG. 1. FIG. 2.

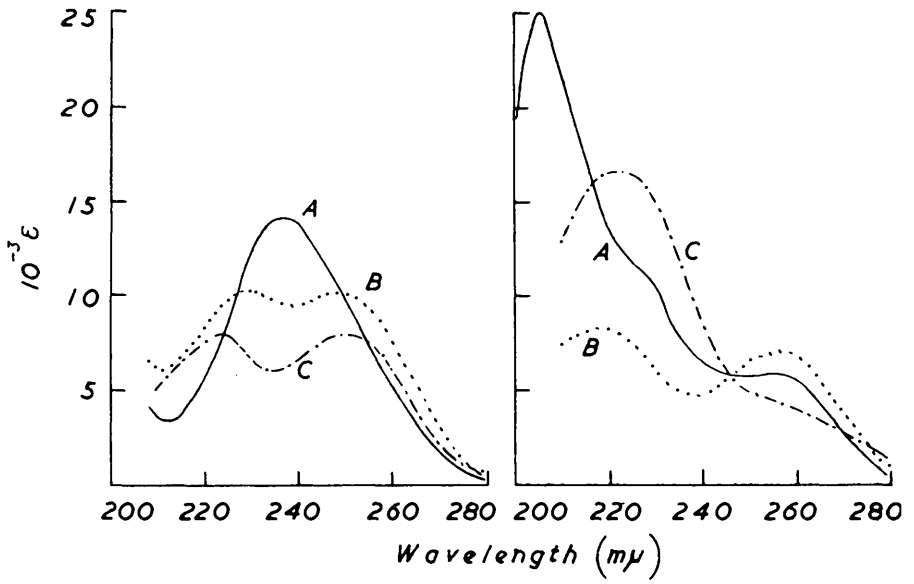


FIG. 1. *Ultraviolet spectra of* (*A*) 2′ : 3′-O-iso*propylidene*-O² : 5′-cyclo*uridine in* 95% EtOH, (*B*) O²-*methyl*-2′ : 3′-O-iso*propylideneuridine in* 95% EtOH, *and* (*C*) O² : 2′-cyclo*uridine in* H_2O.

FIG. 2. *Ultraviolet spectra of* 2′ : 3′-O-iso*propylidene*isocytidine, (*A*) *in* H_2O, (*B*) *in* 0·1N-HCl, *and* (*C*) *in* 0·1N-NaOH.

from that leading to O^2 : 2′-*cyclo*uridine (V) where a stereochemically controlled attack by the pyrimidine ring-carbonyl group with expulsion of toluene-*p*-sulphonate ion must occur.[2]

2′ : 3′-*O*-*iso*Propylidene-O^2 : 5′-*cyclo*uridine (II) is very readily hydrolysed. It is quickly converted into *iso*propylideneuridine by 0·3N-sodium hydroxide or by 25% aqueous acetic acid at room temperature and is therefore much less stable than O^2 : 2′-*cyclo*uridine which requires for hydrolysis 0·1N-sulphuric acid at 100°.[2] Acid hydrolysis in these cases probably involves attack at the cationoid centre, $C_{(2)}$, and apparently differs from the acid hydrolysis of O^2 : 5′-*cyclo*thymidine where an initial cleavage of the glycosidic linkage occurs.[1]

The *cyclo*nucleoside (II) reacts readily with methanolic ammonia, yielding a compound which, from its composition, appears to have been formed by the addition of elements of methanol. The reaction is base-catalysed since the same substance is produced when methanolic triethylamine is used. Ethanolic triethylamine yields a homologous product with closely similar properties. Two possible structures for the methanol addition product

[1] Michelson and Todd, *J.*, 1955, 816.
[2] Brown, Todd, and Varadarajan, *J.*, 1956, 2388.

from (II) were visualised, *viz.*, O^2-methyl-2′ : 3′-*O-iso*propylideneuridine (III) and another derived by addition of methanol to the 4 : 5-double bond in (II). Additions of the latter type to uracil and uridine have been described but are photochemically activated.[3] The methanol addition product is hydrolysed by acid to uridine, but more slowly than the *cyclo*nucleoside (II), requiring conditions similar to those used for the conversion of O^2 : 2′-*cyclo*uridine (V) into spongouridine.[2] The ultraviolet absorption spectrum of the adduct is closely similar to that of (V), differing only in intensity (Fig. 1), so that the chromophores must be closely related. For these reasons structure (III) is preferred. The spectrum of the O^2 : 5′-*cyclo*uridine (II) (Fig. 1) is markedly different from those of (III) and (V). We tentatively attribute this to strain in the O^2 : 5′-*cyclo*-compound (II), a factor which would also account for its easy hydrolysis. It is to be noted, however, that some slight strain exists even in O^2 : 2′-cyclouridine (V), since an *X*-ray crystallographic structural analysis[4] of the 5′-deoxy-5′-iodo-derivative shows the the $C_{(1')}$-N^3 glycosidic linkage is slightly deformed from the direction N^3-$C_{(6)}$; this may account for the lower intensity of absorption noted for (V), as compared with (III). The infrared spectra, too, show related effects; derivatives of O^2 : 5′-*cyclo*uridine have one band in the carbonyl region at about 1650 cm.$^{-1}$, while this appears as a doublet in derivatives of O^2-methyluridine and in O^2 : 2′-*cyclo*uridine (see Table).

	Infrared bands in the carbonyl region (cm.$^{-1}$)
2′ : 3′-*O-iso*Propylidene-O^2 : 5′-*cyclo*uridine	1637
O^2-Methyl-2′ : 3′-*O-iso*propylideneuridine	1642, 1663
2′ : 3′-Di-*O*-acetyl-O^2 : 5′-*cyclo*uridine	1658, 1745
2′ : 3′-Di-*O*-acetyl-O^2-methyluridine	1630, 1641, 1745
O^2 : 2′-*cyclo*Uridine	1626, 1650

When 2′ : 3′-*O-iso*propylidene-O^2 : 5′-*cyclo*uridine (II) is dissolved in methanolic ammonia, O^2-methyl-2′ : 3′-*O-iso*propylideneuridine (III) is first formed but prolonged reaction leads to its disappearance and the formation of a new crystalline substance, which is also formed by the action of ammonia in ethanol, *via* the intermediate ethanol addition compound. Analysis indicates that the new compound is formed by displacement of an alkoxyl by an amino-group and we therefore formulate it as 2′ : 3′-*O-iso*propylidene*iso*cytidine (IV). Although no direct analogy is available, the changes in ultraviolet spectrum with pH (Fig. 2) are reminiscent of those observed with other aminohydroxypyrimidines.[5] The *iso*propylidene derivative (IV) is converted by cold formic acid into the parent nucleoside, *iso*cytidine, which has so far resisted attempts at crystallisation. Confirmation of its structure comes from the conversion of the compound into uridine by the action of nitrous acid.

An analogous series of compounds is obtained by similar methods from 2′ : 3′-di-*O*-acetyl-5′-deoxy-5′-iodouridine. Thus with silver acetate in methanol the latter substance readily affords 2′ : 3′-di-*O*-acetyl-O^2 : 5′-*cyclo*uridine. If the reaction mixture is evaporated to dryness, the product isolated by extraction is 2′ : 3′-di-*O*-acetyl-O^2-methyluridine and a similar process using silver acetate in ethanol affords the corresponding O^2-ethyluridine diacetate. O^2-Methyluridine is obtained from the diacetyl-O^2 : 5′-*cyclo*uridine by short treatment with triethylamine in methanol. With methanolic ammonia over a more extended period, *iso*cytidine is obtained.

Experimental

Paper chromatography was carried out using Whatman No. 1 paper and the solvent system butan-1-ol–water (86 : 14). The R_F values recorded are for this system; wherever possible authentic specimens were run concurrently for comparison.

2′ : 3′-O-iso*Propylidene*-O² : 5′-cyclo*uridine*.—A solution of 5′-deoxy-5′-iodo-2′ : 3′-*O-iso*propylideneuridine (2·43 g.) in anhydrous methanol (600 c.c.) was boiled under reflux with silver acetate (4·5 g.) for 15 min., and the mixture filtered through Hyflo Supercel. Silver ions

[3] Moore and Thompson, *Science*, 1955, **122**, 594.
[4] Brown, Cochran, Medlin, and Varadarajan, *J.*, 1956, 4873.
[5] Shugar and Fox, *Biochim. Biophys. Acta*, 1952, **9**, 199, 369.

were removed from the filtrate by hydrogen sulphide and, after concentration to 20 c.c., benzene was added. The crystalline *product* (1·3 g.) which separated was recrystallised from ethanol, forming colourless plates which sintered at 190° and then darkened but did not melt (Found: C, 54·2; H, 5·1; N, 11·0. $C_{12}H_{14}O_5N_2$ requires C, 54·1; H, 5·3; N, 10·5%). It had R_F 0·62. Light absorption in 95% EtOH: $\lambda_{max.}$ 237 mμ (ε 14,100); $\lambda_{min.}$ 212 mμ (ε 3290).

The substance was converted into 2′ : 3′-*O-iso*propylideneuridine [6] (R_F 0·78) by 25% acetic acid or 0·3N-sodium hydroxide at room temperature during 4 hr.

O^2-*Methyl*-2′ : 3′-O-iso*propylideneuridine*.—(i) The above *cyclo*uridine derivative (0·3 g.) in methanol (100 c.c.) was mixed with saturated methanolic ammonia (1 c.c.) and after 4 hr. at room temperature the solvent was removed *in vacuo*. The *product* formed stout colourless rods (from aqueous methanol), m. p. 155—156° (Found: C, 51·9; H, 5·9; N, 9·2. $C_{13}H_{18}O_6N_2$ requires C, 52·3; H, 6·1; N, 9·4%). It had R_F 0·78 and was easily soluble in methanol and ethanol. Light absorption in 95% EtOH: $\lambda_{max.}$ 248—249, 229 mμ (ε 10,000, 10,200); $\lambda_{min.}$ 238, 212 mμ (ε 9400, 5970).

(ii) 2′ : 3′-*O-iso*Propylidene-O^2 : 5′-*cyclo*uridine (0·1 g.) was dissolved in methanol (50 c.c.) and triethylamine (1 c.c.). Evaporation of the solution after 8 hr. and crystallisation of the residue gave O^2-methyl-2′ : 3′-*O-iso*propylideneuridine (55 mg.), m. p. and mixed m. p. 155—156°.

The substance was stable to cold 25% acetic acid but was converted into uridine (R_F 0·19) by 0·1N-sulphuric acid at 100° in 1 hr. *iso*Propylideneuridine was formed in a few minutes by treatment with 0·3N-sodium hydroxide at room temperature.

O^2-*Ethyl*-2′ : 3′-O-iso*propylideneuridine*.—To a solution of 2′ : 3′-*O-iso*propylidene-O^2 : 5′-*cyclo*uridine (0·3 g.) in ethanol (100 c.c.), triethylamine (5 c.c.) was added. Evaporation of the solution at the end of 10 days yielded O^2-*ethyl*-2′ : 3′-O-iso*propylideneuridine*, which crystallised from ethanol as prisms, m. p. 171—172° (Found: C, 54·0; H, 6·5; N, 9·0. $C_{14}H_{20}O_6N_2$ requires C, 53·8; H, 6·5; N, 9·0%). Light absorption in 95% EtOH: $\lambda_{max.}$ 248, 229 mμ (ε 10,400, 11,300); $\lambda_{min.}$ 241, 213 mμ (ε 10,100, 5990). It had R_F 0·86.

2′ : 3′-O-iso*Propylidene*isocytidine.—2′ : 3′-*O-iso*Propylidene-O^2 : 5′-*cyclo*uridine (0·3 g.) was dissolved in anhydrous methanol (10 c.c.), and saturated methanolic ammonia (35 c.c.) added. After 5 days, formation of the product (R_F 0·58) was complete. The solution was evaporated to dryness and the residue crystallised from ethanol. 2′ : 3′-O-iso*Propylidene*iso*cytidine* formed thick colourless prisms, m. p. 206—207° (Found: C, 50·8; H, 6·2; N, 14·6. $C_{12}H_{17}O_5N_3$ requires C, 50·9; H, 6·1; N, 14·8%). Light absorption in water: $\lambda_{max.}$ 254—255, 205 mμ (ε 5820, 25,400), $\lambda_{min.}$ 248 mμ (ε 5690); in 0·1N-HCl: $\lambda_{max.}$ 256, 220 mμ (ε 7110, 8390), $\lambda_{min.}$ 239 mμ (ε 4790); in 0·1N-NaOH: $\lambda_{max.}$ 223—224 mμ (ε 16,500). The infrared spectrum of the substance showed a band in the carbonyl region at 1645 $cm.^{-1}$.

The same substance was obtained by treating an ethanol solution of the *cyclo*uridine with ethanolic ammonia. The initially formed O^2-ethyl*iso*propylideneuridine (R_F 0·86; $\lambda_{max.}$ 248, 229 mμ) changed into *iso*propylidene*iso*cytidine (R_F 0·58) during 5 days. When isolated it had m. p. 206—207° alone or when mixed with the material prepared as described above.

iso*Cytidine* (3-β-D-*Ribofuranosyl*iso*cytosine*).—The above *iso*propylidene derivative (0·2 g.) was dissolved in formic acid (20 c.c. of 98%). After 4 hr. at room temperature, formic acid was removed by evaporation *in vacuo* with repeated additions of ethanol. The *product* was a hygroscopic glass, very soluble in water but insoluble in ethanol. It was precipitated by concentration of a 98% ethanolic solution under reduced pressure (Found, in a sample dried for 12 hr. at 110°/0·1 mm. over phosphoric oxide: C, 44·6; H, 5·4; N, 17·1. $C_9H_{13}O_5N_3$ requires C, 44·4; H, 5·3; N, 17·3%) and had R_F 0·12.

Sodium nitrite (75 mg.) was added to the synthetic *iso*cytidine (50 mg.) in 2N-acetic acid. After 4 hr. at room temperature, the solution was de-ionised by passage through a column of a mixture of Amberlite IR-4B (OH form) and Amberlite IRC-50 (H form) (75 g. of each), and the eluate and washings were collected and concentrated to about 1 c.c. Paper chromatography showed a single spot (R_F 0·19). An aqueous eluate of the spot had the characteristic ultraviolet absorption spectrum of uridine ($\lambda_{max.}$ 260 mμ; $\lambda_{min.}$ 230 mμ).

5′-O-*Toluene*-p-*sulphonyluridine*.—2′ : 3′-*O-iso*Propylidene-5′-*O*-toluene-*p*-sulphonyluridine [6] (1·0 g.) was dissolved in 98% formic acid (20 c.c.), and the solution set aside for 3 hr. After removal of formic acid *in vacuo*, the glassy residue was crystallised from ethanol. The *toluene*-p-*sulphonyl derivative* formed thin plates (0·83 g.), m. p. 162—163°, R_F 0·66 (Found: C, 47·8; H, 4·8; N, 7·1. $C_{16}H_{18}O_8N_2S$ requires C, 48·2; H, 4·6; N, 7·0%).

[6] Levine and Tipson, *J. Biol. Chem.*, 1934, **106**, 113.

The substance was recovered unchanged when dissolved in saturated methanolic ammonia and left for 24 hr. at room temperature.

5′-*Deoxy*-5′-*iodouridine* (with Dr. D. T. ELMORE).—5′-Deoxy-5′-iodo-2′ : 3′-*O*-*iso*propylideneuridine [6] (2·5 g.) was treated as above with formic acid. After removal of formic acid, the residual glass was evaporated several times with ethanol. The *iodo-compound* crystallised from ethanol and after recrystallisation formed colourless needles (1·8 g.), m. p. 182—183°, R_F 0·57 (Found : C, 30·6; H, 3·1; N, 7·7. $C_9H_{11}O_5N_2I$ requires C, 30·5; H, 3·1; N, 7·9%).

The substance was unaffected by methanolic ammonia at 60°.

2′ : 3′-*Di*-O-*acetyl*-5′-*deoxy*-5′-*iodouridine*.—The above iodo-compound (1·3 g.) was treated with acetic anhydride (10 c.c.) and anhydrous pyridine (1 c.c.) and set aside for 15 hr. Ethanol was added and then solvents were removed *in vacuo*. The *diacetate* crystallised from ethanol in stout prisms (1·15 g.), m. p. 162—163°, R_F 0·90 (Found : C, 35·5; H, 4·0; N, 6·2. $C_{13}H_{15}O_7N_2I$ requires C, 35·6; H, 3·5; N, 6·4%).

2′ : 3′-*Di*-O-*acetyl*-O^2 : 5′-cyclo*uridine*.—The above diacetate (0·85 g.) in anhydrous methanol (150 c.c.) was refluxed with silver acetate (2·0 g.) for 30 min. Silver iodide began to separate as soon as the solution was brought to the boil. The hot solution was filtered through Hyflo Supercel, and silver ions were removed by hydrogen sulphide. Concentration of the solution gave the cyclo*uridine* (0·43 g.) which crystallised from methanol in clusters of fine needles, sintering at 240° with decomposition at 245—265°. It had R_F 0·37 (Found : C, 50·7; H, 5·1; N, 9·5. $C_{13}H_{14}O_7N_2$ requires C, 50·3; H, 4·6; N, 9·0%). It was sparingly soluble in methanol and ethanol. Light absorption in 95% EtOH : $\lambda_{max.}$ 238 mμ (ε 13,900); $\lambda_{min.}$ 212 mμ (ε 2900).

(*a*) The substance was treated in the cold with 25% acetic acid. Conversion into uridine diacetate (R_F 0·69; $\lambda_{max.}$ 260, $\lambda_{min.}$ 230 mμ) was complete in 4 hr.

(*b*) With 0·3N-sodium hydroxide conversion into uridine was complete in 5 min. The product was identified by its R_F (0·19), ultraviolet absorption, and a positive reaction to the periodate–Schiff spray reagent.[7]

(*c*) After reaction of the *cyclo*uridine diacetate with aqueous ammonia paper chromatography showed the presence of uridine (R_F 0·19; $\lambda_{max.}$ 260, $\lambda_{min.}$ 230 mμ) and *iso*cytidine (R_F 0·12; $\lambda_{max.}$ 255, 205 mμ).

(*d*) Use of methanolic ammonia for 6 hr. gave two products, separable by paper chromatography and identified as *iso*cytidine (R_F 0·12; $\lambda_{max.}$ 255, 205 mμ) and O^2-methyluridine (see below) (R_F 0·22; $\lambda_{max.}$ 249, 229 mμ). Prolonged treatment yielded only the former compound.

O^2-*Methyluridine*.—To a solution of 2′ : 3′-di-*O*-acetyl-O^2 : 5′-*cyclo*uridine (0·18 g.) in hot methanol (70 c.c.) was added triethylamine (1 c.c.), and the solution was kept overnight at room temperature. After evaporation to dryness the residue was crystallised from ethanol, a small amount of a sparingly soluble product (40 mg.), m. p. 210° (decomp.), separating. Analytical values were poor but light-absorption data ($\lambda_{max.}$ 235 mμ) suggested that the substance was O^2 : 5′-*cyclo*uridine. Evaporation of the mother-liquors to small bulk (4 c.c.) yielded O^2-*methyluridine* which crystallised from ethanol in prisms, m. p. 173° (Found : C, 45·6; H, 5·5; N, 10·3. $C_{10}H_{10}O_6N_2$ requires C, 46·5; H, 5·5; N, 10·8%), R_F 0·22. Light absorption in H_2O : $\lambda_{max.}$ 249, 229 mμ (ε 9890, 9360); $\lambda_{min.}$ 237·5, 213 mμ (ε 8710, 5240).

2′ : 3′-*Di*-O-*acetyl*-O^2-*methyluridine*.—2′ : 3′-Di-*O*-acetyl-5′-deoxy-5′-iodouridine (1·0 g.) and silver acetate (3·0 g.) were boiled under reflux in anhydrous methanol for 30 min. After filtration from silver salts the solution was taken to dryness under reduced pressure, the grey residue was dissolved in methanol, and silver ions were removed by hydrogen sulphide. Removal of solvent gave a colourless product easily soluble in methanol. Recrystallisation from this solvent gave the *product* (0·49 g.) in long rectangular rods, m. p. 198—200°, R_F 0·69 (Found : C, 49·2; H, 5·1; N, 8·2. $C_{14}H_{18}O_8N_2$ requires C, 49·1; H, 5·3; N, 8·2%). Light absorption in 95% EtOH : $\lambda_{max.}$ 245, 231 mμ (ε 10,200, 10,100); $\lambda_{min.}$ 236—240 mμ (ε 9900).

2′ : 3′-*Di*-O-*acetyl*-O^2-*ethyluridine*.—This *compound* was prepared as above but with ethanol instead of methanol as solvent. It formed thin colourless plates, m. p. 183—185°, R_F 0·85 (Found : C, 50·3; H, 5·7; N, 8·3. $C_{15}H_{20}O_8N_2$ requires C, 50·6; H, 5·7; N, 7·9%). Light absorption in 95% EtOH : $\lambda_{max.}$ 246—248, 229—230 mμ (ε 10,900 11,100); $\lambda_{min.}$ 239—240, 212—213 mμ (ε 10,500, 6300).

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[7] Buchanan, Dekker, and Long, *J.*, 1950, 3165.