616. *Deoxynucleosides and Related Compounds. Part VII.* The Synthesis of 2′-Deoxyuridine and of Thymidine.*

By D. M. Brown, D. B. Parihar, C. B. Reese, and Sir Alexander Todd.

5′-*O*-Acetyl-2′-*O*-toluene-*p*-sulphonyluridine reacts with sodium iodide in hot acetonylacetone, to give 5′-*O*-acetyl-2′-deoxy-2′-iodouridine from which by hydrogenation and deacetylation 2′-deoxyuridine is obtained. The same route starting from 5′-*O*-acetyl-3-β-D-ribofuranosylthymine leads to thymidine. The synthetic deoxyribonucleosides are identical with the naturally occurring compounds.

In the preceding paper * synthetic approaches to the naturally occurring 2′-deoxyribonucleosides were discussed and it was suggested that methods involving displacement reactions at $C_{(2)}$ of the sugar residue in derivatives of the ribonucleosides might lead to compounds readily convertible into the deoxynucleosides. Preliminary experiments along these lines using uridine derivatives led in fact to 3′-deoxyuridine. 2′-*O*-Toluene-*p*-sulphonyladenosine and 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine were readily available from earlier work [1,2] and we therefore sought to bring about a direct exchange of the toluene-*p*-sulphonyloxy-group in these compounds by iodide, although it has been claimed that the Oldham–Rutherford reaction is not usually applicable to other than toluene-*p*-sulphonates of primary alcohols.[3]

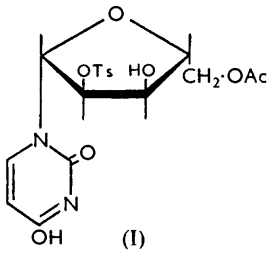
It was found that 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine [2] (I) reacted readily with sodium iodide in hot acetonylacetone and from the reaction mixture a crystalline iodonucleoside was obtained. This was a 2′-deoxy-2′-iodo-compound since hydrogenation over a palladium catalyst gave 5′-*O*-acetyl-2′-deoxyuridine, from which 2′-deoxyuridine (II; R = H) was obtained in high yield. This was identical in all respects with the naturally occurring nucleoside.[4,5]

Whether the intermediate iodo-compound is the *arabo*- (III) or the *ribo*-isomer (IV) has not been established. Its formation might be expected to involve an inversion at position 2′, leading to compound (III). However, the substance, like 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine,[2] is readily converted by methanolic ammonia into *O*[2] : 2′-*cyclo*uridine (V). This suggests either that the compound has the *ribo*-configuration (IV), or that it is (III) and that very easy formation of the intermediate 2′ : 3′-anhydrouridine (VI) from it can occur under these mildly basic conditions.[6] The *cyclo*-compound (V) would then be formed by a second inversion.[7] In contrast to the ready reaction between the toluenesulphonate (I) and the iodide ion, Dr. A. M. Michelson found earlier in unpublished experiments that 2′-*O*-methanesulphonyladenosine reacts only very sluggishly with

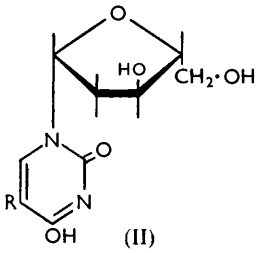
* Part VI, preceding paper.

[1] Brown, Fasman, Magrath, and Todd, *J.*, 1954, 1448.
[2] Brown, Varadarajan, and Todd, *J.*, 1956, 2388.
[3] Tipson, *Adv. Carbohydrate Chem.*, 1953, **8**, 192; cf., however, Michelson and Todd, *J.*, 1955, 816.
[4] Dekker and Todd, *Nature*, 1950, **166**, 157.
[5] See Brown, Parihar, Reese, and Todd, *Proc. Chem. Soc.*, 1957, 321, for preliminary account.
[6] See Davoll and Lythgoe, *J.*, 1949, 2526, for a possible analogy.
[7] See preceding paper.

various anions. In addition it was noted [7] that 3′-*O*-toluene-*p*-sulphonyluridine was unaffected by sodium iodide under the usual conditions. If evidence based on ring-opening in 2 : 3-anhydropentofuranosides is relevant to the present discussion, then, other things being equal, nucleophilic displacement at the 3′-position occurs more readily than



(I)



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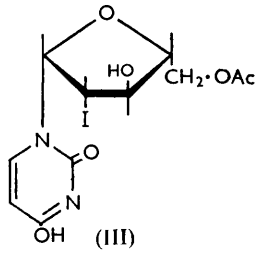

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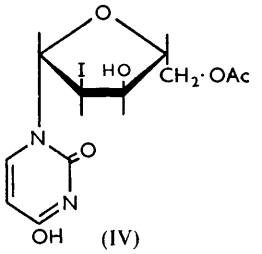

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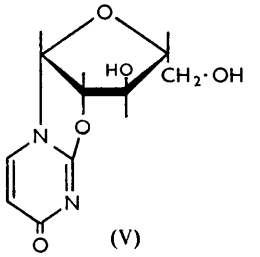
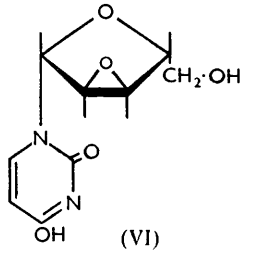


(V)



(VI)

at the 2′-position.[8,9] It does appear then that in the present case the uracil 2-carbonyl group may play some part in the displacement reaction. This may become clear when the precise structure of the iodo-compound has been established.

The route evolved for the synthesis of 2′-deoxyuridine was applied to the synthesis of thymidine (II; R = Me). 3-β-D-Ribofuranosylthymine [10] was converted *via* its 2′ : 3′-*O*-*iso*propylidene derivative [11] into the 5′-acetate. It was found that toluene-*p*-sulphonylation proceeded with more difficulty than in the uridine series and that only about 25% conversion could be achieved. Methanesulphonylation was even less satisfactory. Counter-current distribution allowed the complete separation of starting material from 5′-*O*-acetyltoluene-*p*-sulphonylribosylthymine. This could not be crystallised, so it was treated directly with sodium iodide and then the crude material was hydrogenated and deacetylated. The product was then separated by counter-current distribution from minor contaminants which were, on paper chromatographic evidence, unchanged toluene-*p*-sulphonyl derivatives, probably mainly the 3′-isomer. The product was found to be identical with natural thymidine, and it gave on reduction and hydrolysis 2-deoxyribose,[12] identified by paper chromatography.

Recent biochemical work [13] has shown with some certainty that the direct conversion of ribo- into deoxyribo-nucleosides occurs in living organisms, but the mechanism is obscure. Our syntheses can have no direct relevance to possible biosynthetic pathways except in so far as they demonstrate that displacement reactions can be effected at the 2′-position in ribonucleosides. It is possible that, in Nature, displacement of phosphate by hydride ion (or its biochemical equivalent) could occur. Conceivably, too, a 2′-*cyclo*-nucleoside might be an intermediate in the process although on stereochemical grounds we would expect that this would only be possible with the pyrimidine nucleosides. It is of

[8] Mukherjee and Todd, *J.*, 1947, 969; Allerton and Overend, *J.*, 1951, 1480.
[9] Davoll, Lythgoe, and Trippett, *J.*, 1951, 2230.
[10] Fox, Yung, Davoll, and Brown, *J. Amer. Chem. Soc.*, 1956, **78**, 2117.
[11] Griffin and Todd, unpublished work.
[12] Burke, *J. Org. Chem.*, 1955, **20**, 643.
[13] Grossman and Hawkins, *Biochim. Biophys. Acta*, 1954, **26**, 657; Amos and Magasanik, *J. Biol. Chem.*, 1957, **229**, 653.

interest that since the completion of the work described in this paper Shaw and Warrener [14] have reported a synthesis of thymidine from S^2 : 2′-*cyclo*-5-methyl-2-thiouridine in small yield.

Experimental

Evaporations were carried out under reduced pressure. Counter-current distributions were made in the ethyl acetate–water system in an automatic machine (20·5 c.c. phase). Paper chromatograms were run on Whatman No. 1 paper, the R_F values recorded below being for the butan-1-ol–acetic acid–water (5 : 2 : 3 v/v) system.

5′-O-*Acetyl*-2′-*deoxy*-2′-*iodouridine*.—5′-*O*-Acetyl-2′-*O*-toluene-*p*-sulphonyluridine [2] (5·36 g.) and dry sodium iodide (5·36 g.) were dissolved in freshly distilled acetonylacetone (53 c.c.) and the solution was heated for 2·75 hr. on the water-bath with exclusion of moisture, then cooled. Sodium toluene-*p*-sulphonate (2·2 g.; 96%) was removed by filtration, and washed with acetone and ether. Filtrate and washings were evaporated, finally at 100° *in vacuo*, yielding a thick red syrup which was dissolved in water (20 c.c.). A few crystals of sodium thiosulphate were added, then the solution was submitted to counter-current distribution (80 transfers). The contents of tubes 50—70 which contained a single substance R_F 0·78 were evaporated to a glass (3·74 g.), which crystallised from ethanol (5 c.c.)–ether (2 c.c.) as colourless needles. Recrystallised from dry ethanol it gave needles (2·22 g.), m. p. 167° (Found, in material dried at 85°/0·1 mm. for 4 hr. over P_2O_5: C, 33·35; H, 3·3; N, 6·8. $C_{11}H_{13}O_6N_2I$ requires C, 33·33; H, 3·3; N, 7·1%), $\lambda_{max.}$ 259 mμ (ε 10,770), $\lambda_{min.}$ 229 mμ (ε 2690) in 95% EtOH.

The substance (25 mg.) was set aside with half-saturated methanolic ammonia. After 7 hr. at room temperature, paper chromatography (propan-2-ol–1% ammonium sulphate system) showed the presence of O^2 : 2′-*cyclo*uridine (R_F 0·68) and 3-β-D-arabofuranosyl*iso*cytosine (R_F 0·60), the starting material having disappeared. The former was isolated after 3 days as needles, m. p. 234—235° undepressed on admixture with an authentic specimen of O^2 : 2′-*cyclo*uridine; infrared spectra were identical.

5′-O-*Acetyl*-2′-*deoxyuridine*.—The above iodo-compound (0·325 g.) was dissolved in 50% ethanol (30 c.c.), sodium acetate (0·41 g.) added, and the solution hydrogenated over 5% palladium–barium sulphate (0·29 g.) at room temperature and pressure. Reaction was complete in 4 hr.; after removal of catalyst and solvent the residue was dissolved in water (10 c.c.), and the solution extracted three times with ethyl acetate. The extract was taken to dryness and the syrup dissolved in dry ethanol (5 c.c.). After several weeks in the cold 5′-O-*acetyl*-2′-*deoxyuridine* separated in thick rods which shrank at 90° and melted at 96°; it had R_F 0·68 (Found, in material dried at 40°/0·1 mm.: C, 49·1; H, 5·4; N, 10·7. $C_{11}H_{14}O_6N_2$ requires C, 48·9; H, 5·2; N, 10·4%), and $\lambda_{max.}$ 261 mμ (ε 9550), $\lambda_{min.}$ 230 mμ (ε 1960) in 95% EtOH.

2′-*Deoxyuridine*.—5′-*O*-Acetyl-2′-deoxy-2′-iodouridine (1·6 g.) was dissolved in 50% ethanol (50 c.c.), ammonia added to pH 9, and the solution hydrogenated over palladium–barium sulphate (1·60 g.). Uptake of hydrogen ceased before the theoretical volume had been taken up, so the solution was again brought to pH 9 and a further quantity of catalyst (1·0 g.) was added. Hydrogenation was complete in 24 hr. and after removal of catalyst and solvent the residual gum, which contained 2′-deoxyuridine (R_F 0·54) and its acetate (R_F 0·68), was dissolved in water (10 c.c.), and the solution extracted with ethyl acetate continuously for 4 hr. Evaporation of the ethyl acetate extract gave a glass which was treated with half-saturated methanolic ammonia (100 c.c.) for 8 hr. at room temperature. Removal of solvent and crystallisation of the residue from absolute ethanol (5 c.c.) gave 2′-deoxyuridine. Recrystallised twice from the same solvent this formed stout needles (0·76 g.), m. p. 167° undepressed on admixture with the natural substance (Found: C, 47·2; H, 5·3; N, 12·6. Calc. for $C_9H_{12}O_5N_2$: C, 47·4; H, 5·3; N, 12·3%). Its infrared and ultraviolet spectra and characteristics on paper chromatography and electrophoresis were identical with those of natural 2′-deoxyuridine.[4]

5′-O-*Acetyl*-3-β-D-*ribofuranosylthymine*.—2′ : 3′-*iso*Propylidene-3-β-D-ribofuranosylthymine [11] (14·3 g.) was dissolved in dry pyridine (100 c.c.), acetic anhydride (20 c.c.) was added, and the mixture left for 4 hr. at room temperature. Ethanol (200 c.c.) was added with cooling and after 30 min. solvents were removed. The product, a pale yellow glass (16·2 g.) which gave one spot on chromatograms (R_F 0·90), was presumably the 5′-*O*-acetyl-2′ : 3′-*iso*propylidene derivative. It (15 g.) was dissolved in 20% acetic acid (300 c.c.), and the solution

[14] Shaw and Warrener, *Proc. Chem. Soc.*, 1958, 81.

boiled under reflux for 1 hr. The solvent was removed and the residual gum was subjected to counter-current distribution (94 transfers), to remove traces of uncharged starting material and ribosylthymine. The contents of tubes 8—23 were pooled and evaporated. The residual 5′-*acetate* crystallised from ethanol as needles (11·1 g.), m. p. 95°, R_F 0·67 (Found: C, 48·0; H, 5·5; N, 9·3. $C_{12}H_{16}O_7N_2$ requires C, 48·0; H, 5·3; N, 9·3%), $\lambda_{max.}$ 265 mμ (ε 8675), $\lambda_{min.}$ 234 mμ (ε 1870) in 95% EtOH.

Thymidine (2′-*Deoxy*-3-β-D-*ribofuranosylthymine*).—The above 5′-*O*-acetyl compound (5·0 g.) was dissolved in dry pyridine (30 c.c.), and toluene-*p*-sulphonyl chloride (3·49 g., 1·1 mol.) added. The clear solution was set aside overnight at room temperature. Ethanol (100 c.c.) was added and solvents were then removed under reduced pressure. Water (20 c.c.) was added to the residual syrup, and after 6 hr. in the cold the solution was adjusted to pH 7 with sodium hydroxide and then extracted with ethyl acetate (4 × 200 c.c.). The aqueous phase contained an ultraviolet-absorbing substance (R_F 0·34), probably a *cyclo*nucleoside. The ethyl acetate extract, on being set aside, deposited some 5′-*O*-acetylribofuranosylthymine, from which it was decanted. After removal of solvent the glass (2·4 g.) was subjected to counter-current distribution (85 transfers). The contents of tubes 3—14 yielded pure 5′-*O*-acetylribofuranosylthymine (total recovered, 2·4 g.). Tubes 72—85 gave a gum (1·3 g.) showing two spots on chromatograms which were periodate-negative, probably corresponding to 2′(and 3′)-toluene-*p*-sulphonyl derivatives.

The gum from tubes 72—85 was dried thoroughly, then heated at 100° with sodium iodide (1·28 g.) in acetonylacetone (12·8 c.c.) for 3 hr. The red solution was cooled, filtered from sodium toluene-*p*-sulphonate (0·233 g.; theor. 0·50 g.), and evaporated. The residual syrup was dissolved in water (10 c.c.) and treated with a little sodium thiosulphate, and the solution continuously extracted with ethyl acetate for 3 hr. The ethyl acetate (100 c.c.) extract was washed with water (2 × 25 c.c.), then evaporated to a syrup (1·25 g.). This material showed an intense spot on paper chromatograms (R_F 0·79), giving a pink colour with the cysteine–sulphuric acid spray reagent, probably due to 5′-*O*-acetyl-2′-iodothymidine together with minor impurities (probably 3′-*O*-toluene-*p*-sulphonyl derivatives) at R_F 0·85 and 0·71.

The syrup and sodium acetate (1·5 g.) dissolved in 50% ethanol (50 c.c.) were hydrogenated over palladium–barium sulphate (1·3 g.). Reaction ceased after uptake of 10 c.c. of hydrogen. Fresh catalyst (1·0 g.) was added and hydrogenation was completed in 8 hr. The residue after removal of catalyst and solvent was dissolved in water (10 c.c.) and continuously extracted with ethyl acetate for 4 hr. The extract was taken to dryness and the yellow syrup dissolved in half-saturated methanolic ammonia (50 c.c.). After 24 hr. the solvent was removed and the residue, which contained thymidine as a major component (R_F 0·62; cysteine-positive) together with two minor components (R_F 0·82, 0·70), was subjected to counter-current distribution (89 transfers). Tubes 1—15 contained thymidine (crude yield 263 mg.), a trace of ultraviolet-absorbing impurity (R_F 0·70) being present in tubes 1—4. The residual gummy crystals from tubes 5—15 were recrystallised thrice from absolute ethanol (4 c.c.) and formed needles, m. p. 182—185° undepressed on admixture with natural thymidine (Found: C, 49·3; H, 5·6. Calc. for $C_{10}H_{14}O_5N_2$: C, 49·6; H, 5·8%). The infrared spectrum (in Nujol and in hexachlorobutadiene) of the product was identical with that of thymidine. Paper-chromatographic (R_F 0·62) and electrophoretic behaviour were also the same; the product, like thymidine, gave a negative periodate reaction, a purple spot with Dische's diphenylamine reagent, and a persistent deep pink colour with the cysteine–sulphuric acid spray.[15]

A portion (10 mg.) of the syrup from tubes 1—4 was reduced and then hydrolysed,[12] and the sugar obtained compared with all the other 2- and 3-deoxypentoses [7] by chromatography. When chromatograms were sprayed with the aniline phthalate reagent the material showed a major component corresponding to 2-deoxyribose in colour (yellow-brown) and R_F (0·39) and a minor one (pink, R_F 0·45) of 3-deoxyribose [butan-1-ol–ethanol–water (4 : 1 : 5) system]. Paper electrophoresis led to the same conclusion.

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[15] Wyatt, in "The Nucleic Acids," Ed. Chargaff and Davidson, 1955, Vol. I, 243.