861. *Deoxynucleosides and Related Compounds. Part VIII.*[1] *Some Further Transformations of* O^2 : 2′-cyclo*Uridine.*

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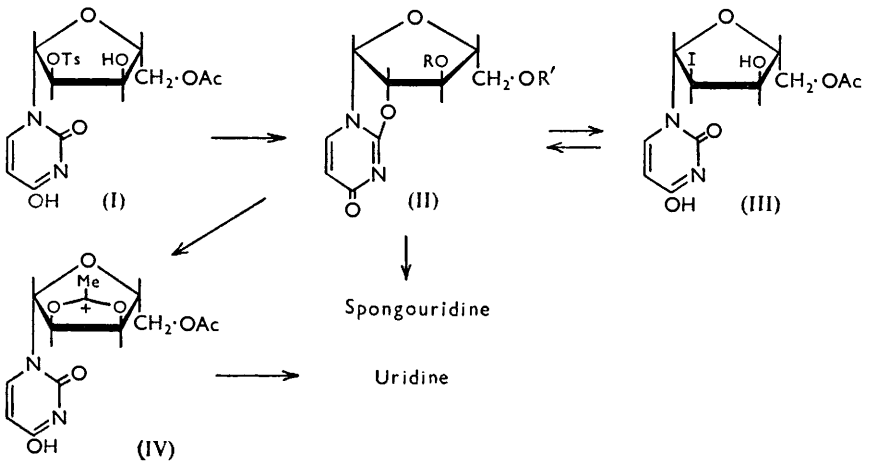
Heating 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine (I) with either sodium azide in methyl cyanide or sodium acetate in acetonylacetone affords 5′-*O*-acetyl-O^2 : 2′-*cyclo*uridine (II; R = H, R′ = Ac). The latter is converted into 5′-*O*-acetyl-2′-deoxy-2′-iodouridine (III) by sodium iodide in acetonylacetone containing glacial acetic acid; the iodide (III), also obtained directly from sulphonyl derivative (I) with sodium iodide, is now assigned the *ribo*-configuration.

3′ : 5′-Di-*O*-acetyl-O^2 : 2′-*cyclo*uridine on acid hydrolysis gives both uridine and spongouridine, in contrast to O^2 : 2′-*cyclo*uridine which gives only the latter. The chemistry of the oxazolidine ring in *cyclo*uridine derivatives is discussed and a possible pathway is suggested for the biochemical conversion of pyrimidine ribo- into deoxyribo-nucleosides.

In the synthesis of the naturally occurring 2′-deoxyuridine described earlier[1] a key step was the conversion of 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine (I) by sodium iodide in acetonylacetone into the 5′-*O*-acetyl-2′-iodo-derivative. The orientation of the iodo-group was not then established. Direct displacement of the toluene-*p*-sulphonyloxy-group would presumably have given an iodo-compound with the *arabo*-configuration, but the greater reactivity of compound (I) than of analogous adenosine derivatives[2] suggested that some other factor was involved in the reaction. Moreover the iodo-compound, like the sulphonate (I), with methanolic ammonia readily gave O^2 : 2′-*cyclo*uridine (II; R = R′ = H), which favoured its formulation as the *ribo*-isomer (III). Evidence which is considered to establish this orientation has now been obtained.



When the sulphonyl derivative (I) was heated with either sodium acetate in acetonylacetone or sodium azide in methyl cyanide, there was no evidence for the introduction of an acetoxy- or azido-group: instead, the sole product was an anhydrouridine acetate. That this was the *cyclo*uridine (II; R = H, R′ = Ac) was evident from its ultraviolet spectrum and the fact that acetylation gave the diacetate (II; R = R′ = Ac) identical with the product of acetylation of O^2 : 2′-*cyclo*uridine[3] itself. The salts had merely

[1] Part VII, Brown, Parihar, Reese, and Todd, *J.*, 1958, 3035.
[2] Unpublished work by Drs. A. M. Michelson and T. L. V. Ulbricht.
[3] Brown, Todd, and Varadarajan, *J.*, 1956, 2388.

buffered the toluene-*p*-sulphonic acid produced in the internal displacement reaction and the non-formation of acetoxy- and azido-compounds is probably to be ascribed, simply, to the low solubility of the salts compared with that of sodium iodide. The formation of the compound (II; R = H, R′ = Ac) in these reactions makes it very probable that the *cyclo*nucleoside derivative was an intermediate in the reaction with sodium iodide too, and that the deoxyiodo-compound was formed by a second displacement on $C_{(2')}$ with opening of the oxazolidine ring. In attempting to establish this it was found that, although the *cyclo*-compound (II; R = H, R′ = Ac) was but little affected by anhydrous hydrogen iodide in methyl cyanide, sodium iodide in acetonylacetone, containing a little glacial acetic acid as buffer, converted it into the deoxyiodo-compound, identical with that obtained by the action of sodium iodide on the toluene-*p*-sulphonate (I). Sodium azide in methyl cyanide re-converted the iodo-compound into the *cyclo*-compound (II; R = H, R′ = Ac). On current views of the mechanism of displacement reactions, these facts allow the assignment of structure (III) to the iodo-compound, and the earlier observations,[1] mentioned above, then find a ready interpretation.

In seeking a further example of a displacement at $C_{(2')}$ with fission of the oxazolidine ring the effect of a neighbouring acetoxy-group was studied. When *O*^{2} : 2′-*cyclo*uridine was hydrolysed with aqueous acid spongouridine was the only product.[3] Hydrolysis of the corresponding diacetate (II; R = R′ = Ac) gave spongouridine and uridine, the latter representing 35% of the total product. Presumably the reaction proceeded, in part, through the intermediate ion (IV) which was hydrolysed without further inversion, as would be expected.[4] The postulated intervention of the neighbouring hydroxyl group in the reaction of *cyclo*uridine with sodium thioethoxide to give the transient intermediate 2′ : 3′-anhydrouridine [5] is now rendered more certain.

Much interest attaches at present to the biosynthetic pathways leading to deoxyribonucleic acid. Evidence has been presented for the conversion of acetaldehyde and triose phosphate into deoxyribose 5-phosphate [6] but the incorporation of the latter into nucleotides has not yet been observed and, at least in one organism, another pathway must also exist.[7] A number of studies lead to the conclusion that the direct conversion of ribonucleosides into deoxyribonucleosides occurs although it is not clear whether this occurs at the nucleoside or the nucleotide level.[8] With regard to the latter possibility, displacement of a group on $C_{(2')}$ in a pyrimidine ribonucleoside to give a *cyclo*nucleoside followed by reductive fission would offer a simple route to the deoxynucleoside. Since Dekker [9] has shown that *cyclo*nucleoside formation can occur by displacement of phosphate on $C_{(2')}$, such a route to deoxynucleosides could have biochemical significance.

Experimental

Unless otherwise stated, butan-1-ol–acetic acid–water (5 : 2 : 3) and Whatman No. 1 paper were used for paper chromatography.

5′-O-*Acetyl*-O^2 : 2′-cyclo*uridine*.—(*a*) A solution of 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine [3] (0·5 g.) in pure methyl cyanide (15 c.c.) was refluxed with stirring for 4 hr. with powdered sodium azide (327 mg., 4·4 mol.). Sodium toluene-*p*-sulphonate and unchanged sodium azide were removed by filtration, and filtrate and washings were concentrated to a syrup which was evaporated with methanol. After dissolution in warm ethanol and cooling in the ice-box the *product* separated. Recrystallised from ethanol it formed plates (258 mg.),

[4] Winstein and Buckles, *J. Amer. Chem. Soc.*, 1942, **64**, 2787.
[5] Brown, Parihar, Todd, and Varadarajan, *J.*, 1958, 3028.
[6] Racker, *J. Biol. Chem.*, 1952, **196**, 347; McGeown and Malpress, *Nature*, 1952, **170**, 575.
[7] Lanning and Cohen, *J. Biol. Chem.*, 1955, **216**, 413.
[8] Rose and Schweigert, *ibid.*, 1953, **202**, 635; Roll, Weinfeld, and Carroll, *ibid.*, 1956, **220**, 455; Reichard, *Acta Chem. Scand.*, 1955, **9**, 1275; 1957, **11**, 11; Grossman and Hawkins, *Biochim. Biophys. Acta*, 1957, **26**, 657; Grossman, *Fed. Proc.*, 1958, **17**, 235; Amos and Magasanik, *J. Biol. Chem.*, 1957, **229**, 653; Bagatell, Wright, and Sable, *Biochim. Biophys. Acta*, 1958, **28**, 216.
[9] Dekker, personal communication; see also Abs. Papers, 133rd Amer. Chem. Soc. Meeting, San Francisco 1958, p. 4D.

m. p. 168—169°, R_F 0·65 (Found: C, 49·4; H, 4·4; N, 10·7. $C_{11}H_{12}O_6N_2$ requires C, 49·25; H, 4·5; N, 10·4%), $\lambda_{max.}$ 248, 224 mμ (ε 7450, 9010), $\lambda_{min.}$ 237 mμ (ε 6800) in 95% EtOH.

(*b*) The above 2′-*O*-toluene-*p*-sulphonyl derivative (0·104 g.) was treated in the same way with freshly fused sodium acetate (0·104 g.) in dry acetonylacetone (4 c.c.) at 100° for 3 hr. The product was isolated as above and formed plates (51 mg.), m. p. 168—169° alone or mixed with O^2 : 2′-*cyclo*uridine-5′-acetate; infrared spectra and R_F values were identical. The substance gave no reaction with the periodate spray reagent on chromatograms.

(*c*) 5′-*O*-Acetyl-2′-deoxy-2′-iodouridine [1] (215 mg.) was heated for 15 hr. with sodium azide (247 mg.) in dry methyl cyanide (10 c.c.), and the solution worked up as above, to give 5′-*O*-acetyl-O^2 : 2′-*cyclo*uridine (116 mg.), R_F 0·65, m. p. and mixed m. p. 169—170° (Found: C, 49·4; H, 4·25; N, 10·8%). The product had infrared and ultraviolet spectra identical with those of the *cyclo*uridine acetate isolated as above.

3′ : 5′-*Di*-O-*acetyl*-O² : 2′-cyclo*uridine*.—O^2 : 2′-*cyclo*Uridine (100 mg.) was shaken with dry pyridine (5 c.c.) and acetic anhydride (5 c.c.) for one day. Ethanol (20 c.c.) was added with cooling, solvents were removed *in vacuo*, and the residue was evaporated with ethanol until free from pyridine. The *product* crystallised from dry ethanol (4 c.c.) forming stout prisms (82 mg.), m. p. 186—187°, R_F 0·71 (Found: C, 50·3; H, 4·65; N, 9·1. $C_{13}H_{14}O_7N_2$ requires C, 50·3; H, 4·5; N, 9·0%), $\lambda_{max.}$ 248, 224 mμ (ε 8030, 9725), $\lambda_{min.}$ 238 mμ (ε 7450) in 95% EtOH.

The same substance was obtained by acetylation of 5′-*O*-acetyl-O^2 : 2′-*cyclo*uridine.

Action of Sodium Iodide on 5′-O-*Acetyl*-O² : 2′-cyclo*uridine*.—A solution of 5′-*O*-acetyl-O^2 : 2′-*cyclo*uridine (93 mg.), dry sodium iodide (224 mg.) and anhydrous (recrystallised) acetic acid (50 mg.) in acetonylacetone was heated at 100° for 12 hr. with exclusion of moisture. Paper chromatography showed the presence only of starting material and product (*ca.* 60%). The solvents were removed *in vacuo*, the residue was dissolved in water containing a little sodium thiosulphate, and the product isolated by extraction with ethyl acetate (2 × 10 c.c.). Removal of the solvent followed by crystallisation at 0° for 4 days from dry ethanol (2 c.c.) gave the product. On recrystallisation it formed needles (23 mg.), m. p. and mixed m. p. 167—168° with 5′-*O*-acetyl-2′-deoxy-2′-iodouridine.[1] The compounds had identical infrared spectra and R_F values (0·77) on paper chromatograms (pink colour with the cysteine–sulphuric acid spray reagent).

Acid Hydrolysis of 3′ : 5′-*Di*-O-*acetyl*-O² : 2′-cyclo*uridine*.—(*a*) The *cyclo*uridine diacetate (20 mg.) was heated in 0·1N-hydrochloric acid for 1 hr. at 100°. The solution was than taken to dryness under reduced pressure, giving a syrup. (*b*) The *cyclo*uridine diacetate (20 mg.) was heated at 100° for 1 hr. with 80% acetic acid, then the solvent was removed. The residual product was deacetylated by treatment with half-saturated methanolic ammonia at room temperature for 8 hr. and the solvent was then removed *in vacuo*, giving a syrup.

Paper chromatography of the products from (*a*) and (*b*) showed the presence in each of two ultraviolet absorbing substances, of R_F 0·44 (periodate positive) and 0·49 (periodate negative), corresponding to uridine and spongouridine. Electrophoresis on paper in borate buffer (pH 10; 260 v for 10 hr.) showed two products migrating 4·9 and 1·2 cm., again corresponding to uridine and spongouridine. The relative amounts, estimated by the optical density of extracts of excised spots, were, for reaction (*a*) 1 : 1·35 and for reaction (*b*) 1 : 2.

Degradation of the reaction products by reduction and hydrolysis [10] gave ribose and ribinose, characterised chromatographically as the only detectable sugars.

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[10] Burke, *J. Org. Chem.*, 1955, **20**, 643.