615. *Deoxynucleosides and Related Compounds. Part VI.* The Synthesis of 2-Thiouridine and of 3′-Deoxyuridine.*

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2′ : 3′-*O*-*iso*Propylidene-O^2 : 5′-*cyclo*uridine and 2′ : 3′-*O*-*iso*propylidene-5′-*O*-toluene-*p*-sulphonyluridine both react with sodium ethyl sulphide to give 5′-deoxy-5′-ethylthio-2′ : 3′-*O*-*iso*propylideneuridine. The same reaction applied to O^2 : 2′-*cyclo*uridine affords 3′-deoxy-3′-ethylthiouridine from which 3′-deoxyuridine is obtained by treatment with Raney nickel. 2′ : 3′-*O*-*iso*Propylidene-O^2 : 5′-*cyclo*uridine with hydrogen sulphide and triethylamine gives 2′ : 3′-*O*-*iso*propylidene-2-thiouridine from which 2-thiouridine can be obtained. The thiolation reaction also affords polysulphides of the probable structure R-S_{2n}-R where $n = 1$, 2, or 3, and R is an *iso*propylidene-2-deoxy-2-uridinyl residue.

The structures of the naturally occurring deoxyribonucleosides have been completely elucidated;[1] they are all β-2′-deoxy-D-ribofuranosides. Several unsuccessful attempts have been made in the past to effect their synthesis. Thus, following the work of Levene and Cortese,[2] Davoll and Lythgoe[3] condensed silver theophylline with, *inter alia*, 3 : 4-di-*O*-acetyl-2-deoxyribosyl chloride and obtained both α- and β-2′-deoxyribopyranosyltheophylline. The lability of halides derived from furanose sugars would almost certainly complicate any extension of this method to deoxyribofuranose derivatives. In addition, recent views[4] on the mechanism of such condensations suggest that the formation of the β-anomer in ribonucleoside syntheses depends on participation by the neighbouring 2-*O*-acyl group; in deoxynucleoside syntheses this control is lacking, as was indeed shown by Davoll and Lythgoe[3] when they isolated both anomeric forms. As another approach, methods involving ring-opening of 2′ : 3′-anhydropentofuranosides were studied[5] but it was found

* Part V, *J.*, 1955, 816.

[1] Lythgoe, *Ann. Reports*, 1944, **41**, 200; Brown and Lythgoe, *J.*, 1950, 1990; Andersen, Hayes, Michelson, and Todd, *J.*, 1954, 1882; Michelson and Todd, *J.*, 1955, 816.

[2] Levene and Cortese, *J. Biol. Chem.*, 1931, **92**, 53.

[3] Davoll and Lythgoe, *J.*, 1949, 2526.

[4] Ness, Fletcher, and Hudson, *J. Amer. Chem. Soc.*, 1951, **73**, 296; Baker, in the Ciba Foundation Symposium on "The Chemistry and Biology of Purines," J. and A. Churchill, London, 1957, p. 120.

[5] Davoll, Lythgoe, and Trippett, *J.*, 1951, 2230.

that, in agreement with the experience of others,[6,7] attack was predominantly at the 3′-position and that only traces of the desired 2′-deoxy-compounds were formed.

Since 5-protected 2-deoxy-D-ribose derivatives are now available [8] it is probable that a deoxynucleoside synthesis based on, for example, the type used successfully for adenosine [9] could be elaborated. But it seemed that syntheses from the available ribonucleosides would be more attractive in that the required lactol ring-size and glycosidic configuration would be already established in the starting material. We describe here experiments on these lines which, however, led to 3′-deoxyuridine, and in the succeeding paper [10] a route is developed for the synthesis of 2′-deoxyuridine and of thymidine.

Our earlier studies of the *cyclo*nucleosides (II and VII) derived from uridine showed that in reactions involving ring-opening, nucleophilic attack occurred at $C_{(2)}$ of the pyrimidine ring. Thus, O^2 : 2′-*cyclo*uridine (VII) was converted into the arabinoside, spongouridine, by acid.[11] The O^2 : 5′-*cyclo*uridine derivative (II) gave uridine with acid, and with methanolic ammonia gave O^2-methyl-2′ : 3′-*iso*propylideneuridine, which by longer treatment with the same reagent yielded *iso*propylidene*iso*cytidine.[12] We have now found that O^2 : 2′-*cyclo*uridine (VII) also reacts with methanolic ammonia to give 3-β-D-arabofuranosyl*iso*cytosine (VI), the structure of which follows from its elementary analysis and the identity of its ultraviolet spectral characteristics with those of *iso*propylidene-*iso*cytidine.*

It was of interest to find if nucleophilic displacement on the *cyclo*nucleosides could be effected with alkyl–oxygen fission, *i.e.*, on a sugar ring-carbon atom, since this might give a method for deoxynucleoside synthesis. The *cyclo*uridine (II) reacted readily with hydrogen sulphide and triethylamine [13] in dimethylformamide but the major product was 2′ : 3′-*O*-*iso*propylidene-2-thiouridine (I). The constitution of this product followed from its composition and the close similarity of its ultraviolet absorption spectrum to that of 3-methyl-2-thiouracil.[14] On hydrolysis with dilute acetic acid 2-thiouridine was obtained.† It is probably identical with the thiouracil riboside formed enzymically from thiouracil and ribofuranose 1-phosphate.[16] Syntheses of 2-thiouridine [17] and the related 3-glucopyranosyl-2-thiouracil [18] by other routes have also been described. In the reaction of the *cyclo*uridine (II) with hydrogen sulphide, three other products, A, B, and C, were also obtained, A being formed when the least amount of triethylamine was used and C when a large excess of base was present. Their empirical formulæ indicated that they were polysulphides. Their ultraviolet spectra were different from those of uridine and 2-thiouridine. They had almost identical infrared spectra. Thus derivatives of 5′-thiouridine were excluded on spectral grounds. We formulate A, B, and C tentatively as di-, tetra-, and hexa-sulphides with the general structure (IV; $n = 1, 2, 3$). This would account

* We thank Dr. D. Shugar, Academy of Sciences, Warsaw, for drawing our attention to the presence of an impurity in a sample of O^2 : 2′-*cyclo*uridine which we had provided. This impurity has been identified as compound (VI) and is formed during the preparation of the *cyclo*uridine from 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine by the action of methanolic ammonia (see p. 3032).

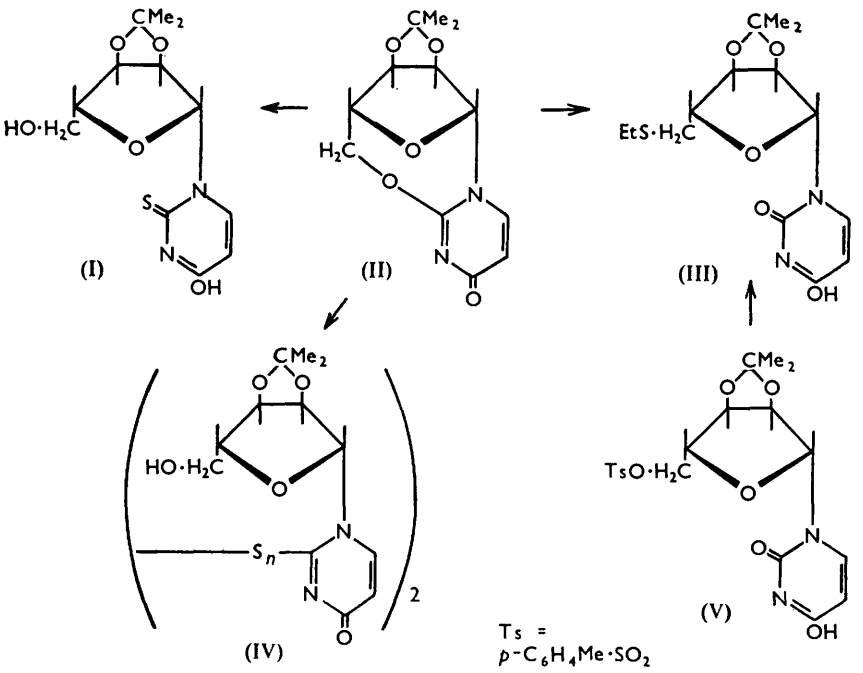
† A preliminary announcement of this result and some other observations on *cyclo*nucleoside chemistry have already been made.[15]

[6] Mukherjee and Todd, *J.*, 1947, 969.
[7] Allerton and Overend, *J.*, 1951, 1480.
[8] Kenner and Richards, *J.*, 1954, 3277.
[9] Kenner, Taylor, and Todd, *J.*, 1949, 1621.
[10] Brown, Parihar, Reese, and Todd, following paper.
[11] Brown, Todd, and Varadarajan, *J.*, 1956, 2388.
[12] *Idem*, *J.*, 1957, 868.
[13] Fairfull, Lowe, and Peak, *J.*, 1952, 742.
[14] Shugar and Fox, *Bull. Soc. chim. belges*, 1952, **61**, 293.
[15] Brown, Todd, and Varadarajan in the Ciba Foundation Symposium on "The Chemistry and Biology of Purines," J. and A. Churchill, Ltd., London, 1957, p. 108.
[16] Strominger and Friedkin, *J. Biol. Chem.*, 1954, **208**, 663; see also Jeener and Roseeles, *Biochim. Biophys. Acta*, 1953, **11**, 438; Jeener, *ibid.*, 1954, **13**, 148; Matthews, *ibid.*, 1956, **19**, 559.
[17] Shaw and Warrener, *Proc. Chem. Soc.*, 1957, 351.
[18] Fox, Chang, and Davoll, *Fed. Proc.*, 1954, **13**, 211.

for their insolubility in alkali and the fact that B and C, when heated to 180°, liberate sulphur and are converted into A.

When the action of the more powerful nucleophilic reagent sodium ethyl sulphide was studied it was found that *O*^{2} : 5′-*cyclo*uridine (II) was converted smoothly into 5′-deoxy-5′-ethylthio-2′ : 3′-*O-iso*propylideneuridine (III). This compound had a uridine-like ultraviolet spectrum and, moreover, was formed directly with the same reagent from 2′ : 3′-*O-iso*propylidene-5′-*O*-toluene-*p*-sulphonyluridine (V).[19] Thus ring-opening in the *cyclo*nucleoside could be effected in either direction depending on the reagent and conditions employed.

With this knowledge we decided to study the action of sodium ethyl sulphide on

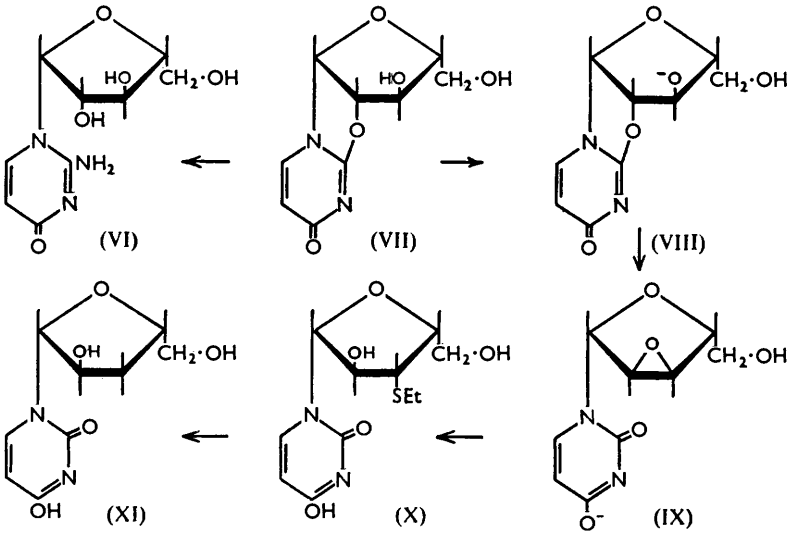


O^2 : 2′-*cyclo*uridine (VII) as a possible route to 2′-deoxyuridine. Reaction was slow in dimethylformamide but at 100° with a large excess of reagent a product was obtained in good yield. From its composition it was clearly a deoxyethylthiouridine and since its ultraviolet spectrum was very similar to that of uridine substitution had occurred on the sugar residue. Surprisingly, too, it gave a reaction, on paper chromatograms, with the cysteine–sulphuric acid reagent,[20] hitherto considered [21,22] specific for 2-deoxy-sugars and their glycosides.

The material was acetylated, and heated in aqueous ethanol with Raney nickel. Reaction was slow and even after 30 hours starting material appeared to be present, but a product very similar to 2′-deoxyuridine had been formed. Reduction of the pyrimidine ring also seemed to occur in part and model experiments showed that conversion of 3-methyluracil into 4 : 5-dihydro-3-methyluracil was essentially complete in five hours. A reaction time of two hours was finally chosen and counter-current distribution of the deacetylated mixture separated unchanged starting material from the product which was formed in small yield. It was a deoxynucleoside which could not be distinguished from

[19] Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113.
[20] Buchanan, *Nature*, 1951, **168**, 1091
[21] Stumpf, *J. Biol. Chem.*, 1947, **169**, 367.
[22] Dische, *Proc. Soc. Exp. Biol. Med.*, 1944, **55**, 217.

natural 2′-deoxyuridine [23] on paper chromatograms and, like it, gave a positive cysteine–sulphuric acid reaction. But the crystalline material had a higher melting point than 2′-deoxyuridine and the infrared spectra of the two compounds, although very similar, were distinguishable. The synthetic substance was degraded by reduction followed by hydrolysis [24] and the sugar produced compared with all the 2- and 3-deoxypentoses. Paper electrophoresis in borate buffer [25] and paper chromatography established its identity as 3-deoxyribose. Thus the original product is 3′-deoxyuridine (XI), so the ethylthio-compound must have been 3′-deoxy-3′-ethylthiouridine. A probable explanation for the production of the latter from O^2 : 2′-*cyclo*uridine is that the primary action of sodium



ethyl sulphide is to remove a proton from the 3′-hydroxyl group. The anion (VIII) then undergoes conversion into the 2′ : 3′-anhydrouridine anion (IX) and indeed these may be in mobile equilibrium. The latter is then attacked by the reagent, to give the 3′-deoxy-3′-ethylthio-compound, which must on this basis have the *xylo*-configuration (X). It is well known that the epoxy-ring in 2 : 3-anhydropentosides is opened predominantly by attack at the 3-position, only 1—2% of reaction at the 2-position being observed. The crude deoxynucleoside recovered from mother-liquors, when degraded to the sugar, gave, in addition to 3-deoxyribose, a small amount of 2-deoxyribose, further evidence of the formation of a 2 : 3-anhydro-intermediate.

When 5′-*O*-acetyluridine is toluene-*p*-sulphonylated, substitution occurs mainly in the 2′-position.[11] Some 3′-*O*-toluene-*p*-sulphonyluridine is also produced, however, and has now been isolated from the mother-liquors from the 2′-compound by treatment with methanolic ammonia. The 3′-isomer, the structure of which was established by degradation, is unaffected by the latter reagent, in contrast to the rapid formation of O^2 : 2′-*cyclo*uridine from the 2′-*O*-toluene-*p*-sulphonyl derivative.

Experimental

Evaporations were done under reduced pressure. Paper chromatography was carried out with Whatman No. 1 paper and the solvent systems (A) butan-1-ol saturated with water, (B) butan-1-ol–acetic acid–water (5 : 2 : 3), and (C) propan-2-ol–aqueous 1%$(NH_4)_2SO_4$ (2 : 1). Counter-current distributions were effected in an automatically operated apparatus (20·5 c.c. phase) with the ethyl acetate–water system.

[23] Dekker and Todd, *Nature*, 1950, **166**, 157.
[24] Burke, *J. Org. Chem.*, 1955, **20**, 643.
[25] Foster, *Chem. and Ind.*, 1952, 828.

3′-O-*Toluene*-p-*sulphonyluridine*.—To a solution of 5′-*O*-acetyluridine (13 g.) in dry pyridine (70 c.c.) was added toluene-*p*-sulphonyl chloride (9·51 g.), and the solution set aside overnight. Ethanol (100 c.c.) was added and solvents were removed. Water (200 c.c.) was added to the red syrup and after 10 hr. the solid was collected, triturated with ethanol, and crystallised from methanol, giving needles (9·8 g.) of 5′-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyluridine, m. p. 176—177° as described earlier.[11] The aqueous filtrate and alcoholic washings were combined and evaporated. The residual syrup was treated with half-saturated methanolic ammonia (300 c.c.) and left overnight, then methanol and ammonia were removed and the residue submitted to counter-current distribution (47 transfers). O^2 : 2′-*cyclo*Uridine was present in tubes 1–4 and the product in tubes 32—45. The contents of the latter tubes were pooled and evaporated. The residue crystallised from ethanol (20 c.c.). 3′-O-*Toluene*-p-*sulphonyluridine* formed needles (3·4 g.), m. p. 205—206° (Found: C, 48·1; H, 4·3; N, 6·9. $C_{16}H_{18}O_8N_2S$ requires C, 48·2; H, 4·5; N, 7·0%). The substance had R_F 0·79 in solvent B and gave a negative reaction with the periodate–benzidine spray reagent.[26] It had $\lambda_{max.}$ 261—262 and 225 mμ, $\lambda_{min.}$ 241 mμ in 95% EtOH.

The compound was recovered unchanged when kept in methanolic ammonia for 7 days at room temperature. No isolable product was obtained with ammonia at 100° in a sealed tube, or with sodium methoxide (1 mol.) in methanol. No sodium toluene-*p*-sulphonate separated when a solution of the compound in acetonylacetone containing sodium iodide was heated at 100° for 24 hr.

Action of Methanolic Ammonia on 5′-O-*Acetyl*-2′-O-*toluene*-p-*sulphonyluridine*: *Isolation of* 3-β-D-*Arabofuranosyl*iso*cytosine and* O^2 : 2′-cyclo*Uridine*.—The 2′-*O*-toluene-*p*-sulphonyl derivative [11] (10·0 g.) was dissolved in methanol (400 c.c.), saturated methanolic ammonia (400 c.c.) added, and the solution kept at room temperature for 7 hr. The solvent was removed and the crystalline residue (5·22 g.) washed with a little ethanol, then recrystallised, slowly, from 80% ethanol (40 c.c.). 3-β-D-*Arabofuranosyl*iso*cytosine* formed prisms which on one further recrystallisation had m. p. 235—236° (yield 1·91 g.), R_F (in C) 0·60 (Found: C, 45·1; H, 5·6; N, 17·1. $C_9H_{13}O_5N_3$ requires C, 44·4; H, 5·3; N, 17·3%). Light absorption: in water, $\lambda_{max.}$ 257 mμ (ε 6370), $\lambda_{min.}$ 237 mμ (ε 5950); in 0·1N-NaOH, $\lambda_{max.}$ 260, 228 mμ (ε 6150, 11,130), $\lambda_{min.}$ 250 mμ (ε 5800); in 0·1N-HCl, $\lambda_{max.}$ 258, 219 mμ (ε 8500, 10,340), $\lambda_{min.}$ 239 mμ (ε 5390). Bands at 3330, 3260, 1589 cm.$^{-1}$ due probably to the NH_2 group were present in the infrared spectrum. The substance gave a negative reaction with the periodate reagent [27] on paper chromatograms.

To the mother-liquors, after isolation of the *iso*cytosine derivative, water was added to give a 50% aqueous ethanol solution, which was kept at 0° for several days. O^2 : 2′-*cyclo*-Uridine separated slowly and formed stout rods (2·7 g.), m. p. 234—236°, from 95% ethanol. It had R_F 0·68 in solvent C, and was identical in all respects with an authentic specimen.[11] Paper chromatography showed that after treatment for 5 days with methanolic ammonia it was converted into the above *iso*cytosine derivative.

5′-*Deoxy*-5′-*ethylthio*-2′ : 3′-iso*propylideneuridine*.—(*a*) Anhydrous sodium ethyl sulphide (200 mg.) was added to a solution of 2′ : 3′-*O*-*iso*propylidene-O^2 : 5′-*cyclo*uridine [12] (100 mg.) in anhydrous dimethylformamide (30 c.c.); after 15 hr. at room temperature water and dilute acetic acid were added and the solution was extracted with chloroform. The extract was evaporated and the residue crystallised from ethanol, giving the *product* (65 mg.) as colourless rods, m. p. 140° (Found: C, 51·2; H, 6·2; N, 8·4. $C_{14}H_{20}O_5N_2S$ requires C, 51·2; H, 6·1; N, 8·5%), $\lambda_{max.}$ 259 mμ (ε 9850), $\lambda_{min.}$ 229 mμ (ε 2460) in 95% EtOH, infrared absorption bands in the carbonyl region were present at 1622, 1687, 1711, and 1773 cm.$^{-1}$.

(*b*) 2′ : 3′-*iso*Propylidene-5′-*O*-toluene-*p*-sulphonyluridine [19] (1·5 g.) was dissolved in dry dimethylformamide (30 c.c.), and sodium ethyl sulphide (3·0 g.) added. The solution was heated at 100° for 2 hr. with exclusion of moisture, then cooled in ice, diluted with ice-water (60 c.c.), and acidified with dilute sulphuric acid. The solution was extracted with chloroform (2 × 100 c.c.), and the extract washed with water (50 c.c.) and evaporated to dryness. The residue was evaporated twice with ethanol *in vacuo* and finally crystallised from hot ethanol. The product (0·41 g.), R_F 0·91 in solvent B (Found: C, 51·2; H, 6·3; N, 8·4%), had m. p. 142° undepressed by the compound prepared by method (*a*); the infrared spectra of the two materials were identical.

[26] Sykes and Todd, " The Chemistry of Penicillin," Oxford Univ. Press, 1949, p. 185.
[27] Buchanan, Dekker, and Long, *J.*, 1950, 3162.

5′-Deoxy-5′-ethylthiouridine.—The mother-liquors from the crystallisation of the *iso*propylidene derivative in preparation (*b*) above were kept for some time in the cold. *5′-Deoxy-5′-ethylthiouridine* separated and on recrystallisation from ethanol formed small prisms (0·58 g.), m. p. 177—178°, R_F 0·74 in B (Found: C, 45·8; H, 5·7; N, 9·75. $C_{11}H_{16}O_5N_2S$ requires C, 45·8; H, 5·6; N, 9·7%). It gave a positive periodate reaction [27] on paper chromatograms.

2′ : 3′-O-isoPropylidene-2-thiouridine and Di-(2-deoxy-2′ : 3′-O-isopropylidene-2-uridinyl) Disulphide (IV; $n = 1$).—Dry hydrogen sulphide was passed through a solution of *2′ : 3′-O-iso*propylidene-O^2 : *5′-cyclo*uridine (1·5 g.) in anhydrous dimethylformamide (20 c.c.) containing triethylamine (1 c.c.) for 10 hr. The green solution was set aside overnight, then evaporated to dryness. The residue was dissolved in ethanol (50 c.c.) and on concentration to 25 c.c. deposited crystals (350 mg.). Recrystallisation of these from ethanol afforded the *disulphide* in thin plates, m. p. 205—206° (Found: C, 47·9; H, 5·4; N, 9·0; S, 10·7. $C_{24}H_{30}O_{10}N_4S_2$ requires C, 48·2; H, 5·1; N, 9·4; S, 10·7%), $\lambda_{infl.}$ 210—212 mμ (ε 12,700) in 95% EtOH. Infrared absorption bands in the carbonyl region were at 1675, 1714, 1735 cm.$^{-1}$.

The mother-liquors on evaporation gave a yellow oil which, after several days, deposited crystals (710 mg.). These were washed with cold ethanol and recrystallised, affording *2′ : 3′-O-isopropylidene-2-thiouridine* in prisms, m. p. 192°, R_F 0·85 in A (Found: C, 48·5; H, 5·2; N, 9·3; S, 10·6. $C_{12}H_{16}O_5N_2S$ requires C, 48·0; H, 5·4; N, 9·3; S, 10·7%).

Di-(2-deoxy-2′ : 3′-O-isopropylidene-2-uridinyl) Tetrasulphide (V; $n = 2$).—*iso*Propylidene-O^2 : *5′-cyclo*uridine (0·5 g.) was dissolved in a mixture of dimethylformamide (6 c.c.) and triethylamine (1 c.c.). Passing in hydrogen sulphide changed the colour of the solution to green, blue, orange, and finally red. The solution was worked up as above and yielded the *tetrasulphide* (90 mg.) which formed colourless plates or needles, shrinking at 180° and melting at 232° (decomp.) (Found: C, 43·3; H, 4·7; N, 8·1; S, 20·3. $C_{24}H_{30}O_{10}N_4S_4$ requires C, 43·5; H, 4·6; N, 8·5; S, 19·4%).

The mother-liquors on concentration yielded *iso*propylidene-2-thiouridine.

The tetrasulphide was converted into the above disulphide with loss of sulphur when it was heated in an open tube at 180—185° in a paraffin bath. Identity was established by m. p., mixed m. p., and analysis.

Di-(2-deoxy-2′ : 3′-O-isopropylidene-2-uridinyl) Hexasulphide (V; $n = 3$).—The reaction was carried out as above but with *cyclo*nucleoside (0·5 g.) in dimethylformamide (6 c.c.) and triethylamine (5 c.c.). The *hexasulphide* crystallised from ethanol in colourless needles (0·23 g.) which became yellow at 175° and decomposed at 215° (Found: C, 39·1; H, 3·9; N, 7·8; S, 26·5. $C_{24}H_{30}O_{10}N_4S_6$ requires C, 39·7; H, 4·2; N, 7·7; S, 26·5%), $\lambda_{max.}$ 262 mμ (ε 2565), $\lambda_{min.}$ 253 mμ (ε 3040) in 95% EtOH.

The mother-liquors from the reaction afforded *2′ : 3′-O-iso*propylidene-2-thiouridine.

The hexasulphide when heated at 180° gave the disulphide and sulphur.

2-Thiouridine.—A solution of *2′ : 3′-O-iso*propylidene-2-thiouridine (0·3 g.) in 40% acetic acid (120 c.c.) was heated under reflux for 2 hr. The residue after removal of solvent was crystallised twice from ethanol, affording the *product* (155 mg.) in thick pale yellow prisms, m. p. 205—207°, with sintering at 195° (Found: C, 41·6; H, 4·9; N, 10·6. $C_9H_{12}O_5N_2S$ requires C, 41·5; H, 4·6; N, 10·8%), $\lambda_{max.}$ 272—273, 218 mμ (ε 11,300, 13,100), $\lambda_{min.}$ 243 mμ (ε 4230) in H_2O, $\lambda_{max.}$ 270, 240 mμ (ε 12,700, 19,300), $\lambda_{min.}$ 260·5 mμ (ε 12,200) in 0·1N-NaOH.

3′-Deoxy-3′-ethylthiouridine.—*O^2 : 2′-cyclo*Uridine (5·0 g.) and sodium ethyl sulphide (25 g.) were heated at 100° in anhydrous dimethylformamide (650 c.c.) with stirring for 10 hr.; sodium formate separated during this period. A further 25 g.) of sodium ethyl sulphide was added and heating continued for 12 hr. After cooling, solid carbon dioxide was added in excess, solvent was removed, and the solid residue dissolved in water (200 c.c.). Sodium ions were removed by passage through a column (2·6 × 25 cm.) of Dowex-50 resin (H^+ form), and the column washed with water (5 l.). Eluate and washings were taken to dryness and the pale yellow residue was subjected to counter-current distribution (166 transfers). The contents of tubes 31—72 (which appeared to contain a single substance of R_F 0·66 in solvent A) were pooled and evaporated. The residue did not crystallise, so its ethanolic solution was filtered and taken to dryness, to give the *product* as a colourless glass (3·5 g.) (Found, in material dried at 50°/0·1 mm. for 12 hr. over P_2O_5: C, 45·9; H, 6·0; N, 9·9. $C_{11}H_{16}O_5N_2S$ requires C, 45·8; H, 5·6; N, 9·7%).

It had $\lambda_{max.}$ 260 mμ, $\lambda_{min.}$ 230 mμ. On oxidation with metaperiodate an uptake of 0·71 mole/mole in 18 hr. was observed. Acetylation of the oxidation solution and extraction gave

a crude product whose infrared spectrum showed a band at 1040 cm.$^{-1}$, possibly indicative of a sulphoxide group.[26]

3′-*Deoxyuridine.*—3′-Deoxy-3′-ethylthiouridine (3·4 g.) was acetylated in pyridine (20 c.c.) with acetic anhydride (35 c.c.) at room temperature overnight and the solution worked up by addition of ethanol and evaporation. The acetylated material (4·5 g.) (single spot on chromatograms, R_F 0·87 in A) was dissolved in 1 : 3 water–ethanol (150 c.c.), six drops of aqueous ammonia and Raney nickel (22 c.c. of sludge) were added, and the mixture was boiled for 2 hr. under reflux. Catalyst was filtered off and washed with warm ethanol (500 c.c.), and filtrate and washings were evaporated, giving a syrup (3·4 g.). This was deacetylated with half-saturated methanolic ammonia (60 c.c.) during 7 hr., and solvent again removed. The product when chromatographed in solvent A showed two spots: starting material of R_F 0·66, and the product of R_F 0·29, both positive to the cysteine spray reagent. The mixture was submitted to counter-current distribution (149 transfers). The starting material (2·1 g.) was recovered from tubes 28—70. The required deoxyuridine was present in tubes 1—14. Except for those of tube 1, which contained some uncharacterised impurities, the contents of the tubes 2—14 were pooled and evaporated. The residue (482 mg.) was dissolved in dry ethanol (4 c.c.). After 24 hr. crystalline uracil (12 mg.) was deposited and removed. The mother-liquor was concentrated (3 c.c.), dry ether (3 c.c.) added, and the product allowed to crystallise. Recrystallised from the same solvent 3′-*deoxyuridine* formed needles (88 mg.), R_F 0·29 (in A), m. p. 178° depressed to 155° in admixture with 2′-deoxyuridine (Found: C, 47·8; H, 5·3; N, 12·4. $C_9H_{12}O_5N_2$ requires C, 47·4; H, 5·3; N, 12·3%), $\lambda_{max.}$ 262 mμ (ε 9660), $\lambda_{min.}$ 230 mμ (ε 1710) in 95% EtOH.

The substance had R_F values identical with those of 2′-deoxyuridine in all of the four solvent systems but the pink colour produced with the cysteine–sulphuric acid spray reagent required 1·5 times as long to develop at 85°. Paper electrophoresis did not distinguish the two materials.

The deoxynucleoside (15 mg.) was reduced and hydrolysed by Burke's method,[24] and the deoxy-sugar obtained was compared with all the 2- and 3-deoxypentoses on chromatograms in the butan-1-ol–ethanol–water (4 : 1 : 5) system (40 hr. irrigation) and on electrophoretograms in borate buffer.[25] The results are collected in the Table.

Deoxypentose	R_F	Spray 1	Spray 2	Spray 3	Electrophoretic migration (cm.)
2-Deoxyribose	0·39	Yellow-brown	Pink (5 min.)	Purple (5 min.)	+1·1
2-Deoxyxylose	0·44	,,	,,	,,	−0·4
3-Deoxyribose	0·45	Pink	Pink (10—15 min.)	—	+6·8
Hydrolysis product ...	0·45	,,	,,	—	+6·8
3-Deoxyarabinose	0·41	,,	,,	—	+5·6
D-Glucose	—	Brown	—	—	+9·0

Spray reagents: (1) aniline phthalate (Partridge, *Nature*, 1949, **164**, 443); (2) cysteine–sulphuric acid; [20] (3) diphenylamine.[27]

The impure 3′-deoxyuridine isolated from mother-liquors when reduced and hydrolysed as above contained, in addition to 3-deoxyribose, small amounts of 2-deoxyribose.

3-*Deoxyribose.*—This was prepared in solution by reduction of methyl 2 : 3-anhydro-α-riboside [6] (40 mg.) with lithium aluminium hydride by the method of Allerton and Overend,[7] followed by acid hydrolysis. Its chromatographic characteristics are recorded in the Table. With the diphenylamine spray reagent a trace of 2-deoxyribose was detected as a contaminant.

3-*Deoxyarabinose.*—This was prepared as above by lithium aluminium hydride reduction of 2′ : 3′-anhydrolyxofuranosyltheophylline [5] (100 mg.) followed by hydrolysis. The substance was not isolated but was used directly for the chromatographic study.

Isolation of Ribose from 3′-O-*Toluene*-p-*sulphonyluridine.*—The toluene-*p*-sulphonyl derivative (400 mg.) was reduced with sodium amalgam, then hydrolysed by the procedure applied earlier [28] to methylated methyl 2-*O*-toluene-*p*-sulphonylribofuranoside. The sugar was obtained as a syrup. Paper chromatography in three solvent systems showed it to be identical with D-ribose and distinguishable from lyxose, xylose, and arabinose. The syrup in methanol (10 c.c.) was treated with toluene-*p*-sulphonylhydrazine (200 mg.), boiled for 0·5 hr., then set aside in the cold for some days. Ribose toluene-*p*-sulphonylhydrazone separated in plates

[28] Brown, Fasman, Magrath, and Todd, *J.*, 1954, 1448.

and on recrystallisation from 95% ethanol had m. p. 159—160° undepressed by an authentic specimen.[29] The infrared spectra of the samples were identical.

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[29] Easterby, Hough, and Jones, *J.*, 1951, 3416.