*Deoxyribonucleosides and Related Compounds. Part V.** cyclo*Thymidines and other Thymidine Derivatives. The Configuration at the Glycosidic Centre in Thymidine.*

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A number of halogenodeoxythymidines have been prepared *via* the corresponding methanesulphonyl- or toluene-*p*-sulphonyl-thymidines, and have been converted by reduction into the corresponding deoxythymidines. When heated with silver acetate in methyl cyanide containing traces of base 3′-deoxy-3′- and 5′-deoxy-5′-iodothymidine gave halogen-free compounds formulated as O^2 : 3′-*cyclo*thymidine and O^2 : 5′-*cyclo*thymidine respectively. Similarly, treatment of 3′ : 5′-dimethanesulphonylthymidine with alcoholic ammonia yields 5′-methanesulphonyl O^2 : 3′-*cyclo*thymidine. The behaviour of these *cyclo*thymidines towards acid and alkali has been studied and their formation is in accord with the formulation of thymidine as 3-β-2′-deoxy-D-ribofuranosylthymine; the β-configuration has also been confirmed by *X*-ray crystallographic examination of 5′-bromo-5′-deoxythymidine. Silver dibenzyl phosphate reacts with 3′-acetyl-5′-deoxy-5′-iodothymidine, to give a product from which by removal of protecting groups thymidine-5′ phosphate is obtained.

Although the chemistry of the ribonucleosides and their derivatives has been extensively studied, the deoxyribonucleosides have received scant attention largely because of their relative inaccessibility. Levene and Tipson (*J. Biol. Chem.*, 1935, **109**, 623) treated thymidine (I; R = R′ = OH) with toluene-*p*-sulphonyl chloride and obtained a mixture of a ditoluene-*p*-sulphonylthymidine (presumably 3′ : 5′-) and a chloro-toluene-*p*-sulphonylthymidine (5′-chloro-5′-deoxy-3′-toluene-*p*-sulphonylthymidine ?) but their products were not characterised. They also observed (*loc. cit.*) that 5′-tritylthymidine gave a toluene-*p*-sulphonyl derivative which appeared to be very reactive in that when heated with sodium iodide for 2 hr. at 100°, *i.e.*, the standard conditions of Oldham and Rutherford (*J. Amer. Chem. Soc.*, 1932, **54**, 366), some 30% replacement of the toluene-*p*-sulphonyl group occurred. These preliminary studies formed the starting point for the work described in this paper, since both 3′-deoxy-3′-iodothymidine and 5′-deoxy-5′-iodothymidine were of interest to us as possible intermediates in polynucleotide synthesis using methods of the type described by Elmore and Todd (*J.*, 1952, 3681).

In view of Levene and Tipson's observations (*loc. cit.*) it was decided to use methanesulphonyl derivatives in the first instance, since the methanesulphonyl group is rather less readily replaced than the toluene-*p*-sulphonyl group. Thymidine (I; R = R′ = OH) reacted readily with methanesulphonyl chloride, giving a good yield of 3′ : 5′-dimethanesulphonylthymidine (I; R = R′ = $O\cdot SO_2\cdot Me$); when heated with sodium iodide the latter gave a rather complex mixture of products containing mainly 5′-deoxy-5′-iodo-3′-methanesulphonylthymidine (I; R = $O\cdot SO_2\cdot Me$, R′ = I). When heated with lithium bromide at 100° for 2 hours, however, the diester (I; R = R′ = $O\cdot SO_2\cdot Me$) gave 3′ : 5′-dibromo-3′ : 5′-dideoxythymidine (I; R = R′ = Br). Hydrogenation of this dibromo-compound under atmospheric pressure with a palladised barium sulphate catalyst gave a monobromo-compound. *X*-Ray crystallographic examination of this material by Mr. M. M. Woolfson of the Cavendish Laboratory showed that it was clearly 5′-bromo-3′ : 5′-dideoxythymidine (I; R = H, R′ = Br) (see Figure). Prolonged hydrogenation gave a small yield of 3′ : 5′-dideoxythymidine (I; R = R′ = H), although under the conditions employed some breakdown to thymine occurred. It should be noted that in these experiments replacement of a secondary sulphonyloxy-group in a nucleoside derivative by halogen occurred readily. This ease of replacement is probably to be ascribed to the absence of a hydroxyl group at position 2′ in the deoxyribonucleosides (cf. Matheson and Angyal, *J.*, 1952, 1133).

Methanesulphonylation of 5′-tritylthymidine gave 3′-methanesulphonyl-5′-tritylthymidine (I; R = $O\cdot SO_2\cdot Me$, R′ = $O\cdot CPh_3$) from which, by heating with 80% acetic acid, 3′-

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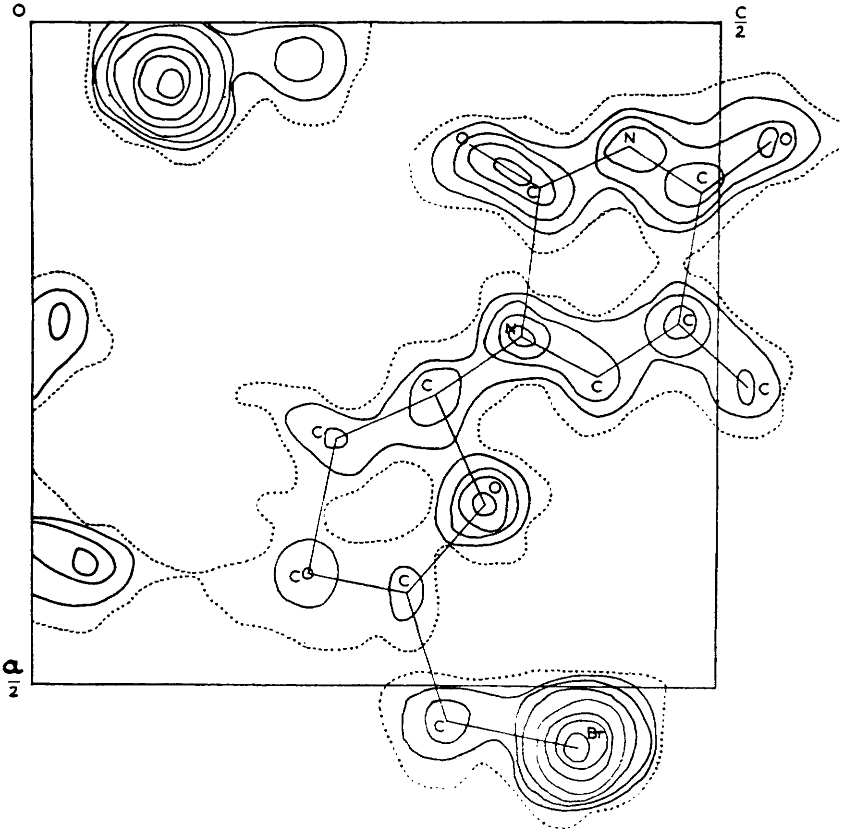
* Part IV, *J.*, 1954, 1882.

methanesulphonylthymidine (I; R = O·SO$_2$·Me, R′ = OH) was obtained. Treatment of the ether ester (I; R = O·SO$_2$·Me, R′ = O·CPh$_3$) with lithium bromide under the usual conditions gave 3′-bromo-3′-deoxy-5′-tritylthymidine (I; R = Br, R′ = O·CPh$_3$), and with sodium iodide 3′-deoxy-3′-iodo-5′-tritylthymidine (I; R = I, R′ = O·CPh$_3$) was similarly obtained; the latter gave 3′-deoxy-3′-iodothymidine (I; R = I, R′ = OH) which was smoothly reduced to 3′-deoxythymidine (I; R = H, R′ = OH).

5′-Bromo-3′ : 5′-dideoxythymidine.

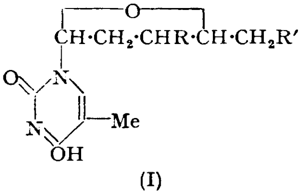


A comparable series of reactions leading to 5′-deoxythymidine was also carried out. 5′-Toluene-*p*-sulphonylthymidine (I; R = OH, R′ = O·SO$_2$·C$_7$H$_7$) was prepared by deacetylating 3′-acetyl-5′-toluene-*p*-sulphonylthymidine (Michelson and Todd, *J.*, 1953, 951); when heated with sodium iodide it gave 5′-deoxy-5′-iodothymidine (I; R = OH, R′ = I) which underwent ready reduction to 5′-deoxythymidine (I; R = OH, R′ = H). When lithium bromide was used instead of sodium iodide the ester (I; R = OH, R′ = O·SO$_2$·C$_7$H$_7$) gave 5′-bromo-5′-deoxythymidine (I; R = OH, R′ = Br) which, like the other deoxyhalogenothymidines, was dimorphic. *X*-Ray crystallographic examination of the compound (I; R = OH, R′ = Br) by Dr. W. Cochran and Mr. M. Huber of the Cavendish Laboratory confirmed the correctness of the structure assigned and also showed conclusively the β-configuration at the glycosidic centre; these results, incidentally, establish conclusively the correctness of the structures assigned to the intermediates employed in the synthesis of the thymidine mononucleotides (Michelson and Todd, *loc. cit.*).

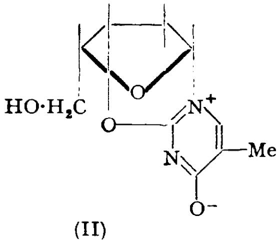
As expected, 3′-acetyl-5′-deoxy-5′-iodothymidine with silver dibenzyl phosphate yielded a product from which, by removal of protecting groups, thymidine-5′ phosphate was obtained; this was identified by comparison with an authentic specimen (Michelson and Todd, *loc. cit.*). Phosphorylation of 5′-deoxy-5′-iodothymidine with dibenzyl phosphorochloridate gave a good yield of 5′-deoxy-5′-iodothymidine-3′ dibenzyl phosphate.

As a preliminary to the above experiments on the replacement of iodine atoms in deoxyiodothymidine derivatives by dibenzyl phosphate residues their reaction with silver acetate in methyl cyanide was examined. To our surprise 3'-deoxy-3'-iodothymidine (I; R = I, R' = OH), although it reacted readily enough with silver acetate on being heated in this medium did not yield the expected 3'-acetylthymidine. Instead, a crystalline high-melting substance $C_{10}H_{12}O_4N_2$ was obtained. A similar experiment with 5'-deoxy-5'-iodothymidine gave a crystalline isomeric compound $C_{10}H_{12}O_4N_2$ as main product, although a certain amount of 5'-acetylthymidine was also produced. 3'-Acetyl-5'-deoxy-5'-iodothymidine gave with silver acetate in the same way a substance $C_{12}H_{14}O_5N_2$ accompanied by a small amount of a second product which, from its paper chromatographic behaviour, was almost certainly 3' : 5'-diacetylthymidine. In these initial experiments the solvent employed was a laboratory sample of methyl cyanide which had not been specially purified. With highly purified, dry methyl cyanide, no reaction occurred. It was ultimately found that the presence of traces of aliphatic amines in the methyl cyanide originally used was essential and that when purified methyl cyanide was used it was necessary to add a small amount of, *e.g.*, diethylamine.

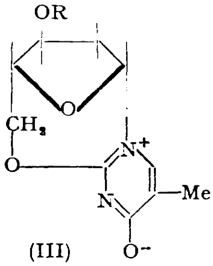
In previous papers we described the formation of *cyclo*nucleoside salts by heating the 5'-toluene-*p*-sulphonates of adenosine and cytidine (Clark, Todd, and Zussman, *J.*, 1951, 2952) or of deoxyadenosine and deoxycytidine (Andersen, Hayes, Michelson, and Todd, *J.*, 1954, 1882) in inert solvents. The easy formation of these salts by intramolecular alkylation was doubtless due in part to the markedly basic nature of the purine and pyrimidine derivatives involved. Nevertheless, if thymidine also has the β-configuration at the glycosidic linkage, it might well be that treatment of suitable deoxyiodothymidine derivatives with silver acetate could also yield analogous *cyclo*nucleoside derivatives. The properties of the products described above, and in particular their ultra-violet absorption spectra, lend strong support to this view. All three compounds show an absorption maximum at 250 mμ in place of the characteristic thymidine maximum at 268 mμ, the shift being very similar to that observed in passing from cytidine (max. 270 mμ) to *O*² : 5'-*cyclo*-cytidine toluene-*p*-sulphonate (max. 252—253 mμ). The infra-red spectra of these three compounds are in accord with their formulation as *O*² : 3'-*cyclo*thymidine (II), *O*² : 5'-*cyclo*-thymidine (III; R = H), and 3'-acetyl-*O*² : 5'-*cyclo*thymidine (III; R = Ac).



(I)



(II)



(III)

The cyclisation between position 3' and the oxygen attached to $C_{(2)}$ in the pyrimidine ring which is postulated in (II) presents no steric difficulty; on the evidence of molecular models it seems to be even easier than the closure postulated in (III) and, indeed, this accords with the observed ease of formation of the compounds and their relative stabilities. A change of configuration at position 3' appears to be involved (and may have occurred during the preparation of the 3'-iodo-derivative), so that (II) is a derivative of 2-deoxy-D-xylose rather than of 2-deoxy-D-ribose. In any case it should be noted that the acceptance of structures (II) and (III) requires the formulation of thymidine as a β-2-deoxy-D-ribofuranoside since cyclisation could not take place if it were an α-glycoside.

In view of the ready cyclisation of 3'-deoxy-3'-iodothymidine, the effect of alkali on the sulphonyl derivatives of thymidine was examined. While 5'-toluene-*p*-sulphonylthymidine is quite stable, treatment of 3' : 5'-dimethanesulphonylthymidine with ethanolic ammonia under mild conditions gave a good yield of the highly crystalline 5'-methanesulphonyl-O^2 : 3′-*cyclo*thymidine, inversion at position 3′ occurring with elimination of one molecule of methanesulphonic acid, to give a derivative of 2-deoxy-D-xylose.

The behaviour of the cylic compounds (II) and (III; R = H) when subjected to alkaline and acid hydrolysis is of interest. Both compounds are hydrolysed by 0·1N-aqueous sodium hydroxide or ammonia at room temperature, reaction being much the faster with (III; R = H). The product from (III; R = H) was identified as thymidine. That from (II) was very similar to, but apparently not identical with, thymidine; although not rigidly identified it is believed to be the isomeric thymine 2-deoxy-D-xylofuranoside. Towards acid the compounds were more stable; (II) was unaffected and (III; R = H) was only slowly attacked by 0·1N-hydrochloric acid at room temperature. At 100° there was in each case rapid hydrolysis, first to a substance similar in its chromatographic behaviour to thymidine and apparently containing the pyrimidine system (ultra-violet absorption) and the deoxy-sugar residue (cysteine spray), and then by further action of the acid to thymine and a free deoxy-sugar. The sugar obtained in this way from (III; R = H) appeared to be 2-deoxy-D-ribose but that from (II) differed in its R_F value on paper chromatograms. Although lack of material has prevented actual isolation of the sugar from (II) comparison of its paper chromatographic behaviour with that of authentic 2-deoxy-D xylose leaves little room for doubt as to its identity with this substance. It should be noted that thymidine itself is unaffected by 0·1N-sodium hydroxide and only very slightly by 0·1N-hydrochloric acid under the conditions used in these hydrolyses.

The results of the above hydrolyses can be satisfactorily explained on the basis of structures (II) and (III; R = H) if it be assumed that alkaline hydrolysis splits the linkage between the oxygen attached to $C_{(2)}$ and the pyrimidine residue, yielding thymidine from (III; R = H) and the isomeric 2-deoxy-D-xyloside from (II), and that acid effects cleavage at $N_{(3)}$ in the pyrimidine system in the first instance, giving deoxy-sugars attached to the thymine residue at $C_{(2)}$ by an ether linkage which can be hydrolysed further to yield thymine and free deoxy-sugars.

EXPERIMENTAL

3′ : 5′-Dimethanesulphonylthymidine.—Methanesulphonyl chloride (6 c.c.) was added to a solution of thymidine (5 g.) in dry pyridine (60 c.c.) at 0°, and the mixture kept at 0° overnight. Ice-water (4 c.c.) was added, the mixture kept at 0° for 1 hr., then poured into ice-water (800 c.c.) with vigorous stirring, and kept at 0° for several hours. The crystalline precipitate of *3′ : 5′-dimethanesulphonyl thymidine* (7·55 g.) was collected and dried. Recrystallised from 90% ethanol it formed colourless needles, m. p. 168—169° (decomp.) (Found, in material dried for 15 hr. at 60°/10^{-3} mm.: C, 36·4; H, 4·6; N, 7·0. $C_{12}H_{18}O_9N_2S_2$ requires C, 36·2; H, 4·5; N, 7·0%).

5′-Deoxy-5′-iodo-3′-methanesulphonylthymidine.—A solution of 3′ : 5′-dimethanesulphonyl thymidine (3·5 g.) and sodium iodide (3·5 g.) in dry acetone (40 c.c.) was heated at 100° for 2 hr. Sodium methanesulphonate was removed and the filtrate evaporated to dryness. Water (50 c.c.) was added, the mixture shaken well, and the pale yellow amorphous solid collected. Several recrystallisations, first from ethanol and finally from water, gave needles of *5′-deoxy-5′-iodo-3′-methanesulphonylthymidine* (1·84 g.), m. p. 161—162° (decomp.) (Found, in material dried at 80°/10^{-3} mm. for 6 hr. : C, 30·6; H, 3·6; N, 6·4. $C_{11}H_{15}O_6SN_2I$ requires C, 30·7; H, 3·5; N, 6·5%).

3′ : 5′-Dibromo-3′ : 5′-dideoxythymidine.—A solution of 3′ : 5′-dimethanesulphonylthymidine (1 g.) and lithium bromide (1 g.) in anhydrous acetone (10 c.c.) was heated at 100° for 2 hr. Lithium methanesulphonate (0·47 g.; theor., 0·51 g.) was collected and the filtrate taken to dryness under reduced pressure. The residue was well washed with water and recrystallised twice from ethanol, giving the product (0·17 g.) as needles, m. p. 159° (decomp.) (Found, in material dried for 12 hr. at 60°/10^{-3} mm. : C, 33·2; H, 3·4; N, 7·4. $C_{10}H_{12}O_3N_2Br_2$ requires C, 32·6; H, 3·3; N, 7·6%).

5′-Bromo-3′ : 5′-dideoxythymidine.—A solution of 3′ : 5′-dibromo-3′ : 5′-dideoxythymidine (0·6 g.) in aqueous ethanol was hydrogenated in the usual way with palladised barium sulphate at pH 9. Catalyst was removed and the filtrate taken to small volume under reduced pressure. The *5′-bromo-3′ : 5′-dideoxythymidine* which separated was collected (0·4 g.) and recrystallised twice from ethanol, forming needles, m. p. 155—156° (Found, in material dried at 100°/10^{-3} mm. for 12 hr. : C, 41·4; H, 4·4; N, 9·9. $C_{10}H_{13}O_3N_2Br$ requires C, 41·5; H, 4·5; N, 9·7%). A solution of 5′-bromo-3′ : 5′-dideoxythymidine (100 mg.) in aqueous ethanol (10 c.c. of 50%)

at pH 9 with ammonium hydroxide was hydrogenated at atmospheric temperature and pressure during 24 hr. with palladised barium sulphate. Catalyst was removed and the filtrate taken to dryness under reduced pressure. The residue was extracted twice with hot ethyl acetate, leaving a residue of thymine and ammonium bromide. The ethyl acetate extracts were taken to dryness, the residue was triturated with 1 : 1 ether–light petroleum (b. p. 40—60°), and the mixture kept at 0° for several hours. The crystalline deposit was recrystallised from ether–light petroleum (b. p. 60—80°) and finally sublimed at 120°/10^{-1} mm., giving crude 3′ : 5′-dideoxythymidine (40 mg.) as needles, m. p. 105°, still contaminated with a trace of 5′-bromo-3′ : 5′-dideoxythymidine (Found : C, 55·0; H, 6·1. $C_{10}H_{14}O_3N_2$ requires C, 57·1; H, 6·7%).

3′-Methanesulphonyl-5′-tritylthymidine.—Methanesulphonyl chloride (2·5 c.c.) was added to a solution of 5′-tritylthymidine (5 g.) in pyridine (50 c.c.), and the mixture kept at 0° overnight, with exclusion of moisture. Ice-water (1 c.c.) was then added and after 1 hr. at 0° the mixture was poured into ice-water (500 c.c.) with vigorous stirring. The precipitate of *3′-methanesulphonyl-5′-tritylthymidine* was filtered off, well washed with water, and dried (5·4 g.) (Found, in material dried for 15 hr. at 60°/10^{-3} mm. : C, 63·9; H, 5·6; N, 5·0. $C_{30}H_{30}O_7N_2S$ requires C, 64·1; H, 5·3; N, 5·0%).

3′-Methanesulphonylthymidine.—A solution of 3′-methanesulphonyl-5′-tritylthymidine (3·9 g.) in 80% acetic acid (40 c.c.) was heated under reflux for 6 min. Acetic acid was then removed under reduced pressure and the residue shaken repeatedly with ether to remove triphenylmethanol. The ether-insoluble residue was recrystallised from hot water; *3′-methanesulphonylthymidine* crystallised in needles (1·1 g.), m. p. 116° (Found, in material dried at 80°/10^{-3} mm. for 12 hr. : C, 41·2; H, 5·4; N, 9·0. $C_{11}H_{16}O_7N_2S$ requires C, 41·3; H, 5·0; N, 8·8%).

3′-Bromo-3′-deoxy-5′-tritylthymidine.—A solution of 3′-methanesulphonyl-5′-tritylthymidine (1 g.) and lithium bromide (1 g.) in anhydrous acetone (10 c.c.) was heated at 100° for 2 hr. Lithium methanesulphonate (0·15 g.; theory, 0·18 g.) was removed and the filtrate taken to dryness under reduced pressure. Water (40 c.c.) was added to the residue, the mixture shaken vigorously, and the amorphous solid collected, washed well with water, and dried (0·9 g.). Recrystallised first from methanol and then from acetone–methanol, *3′-bromo-3′-deoxy-5′-tritylthymidine* formed cubes, m. p. 144° (Found, in material dried for 12 hr. at 60°/10^{-3} mm. : C, 63·5; H, 5·1; N, 5·3. $C_{29}H_{27}O_4N_2Br$ requires C, 63·6; H, 4·9; N, 5·1%).

3′-Deoxy-3′-iodo-5′-tritylthymidine.—A solution of 3′-methanesulphonyl-5′-tritylthymidine (6 g.) and sodium iodide (3·5 g.) in anhydrous acetone (50 c.c.) was heated at 100° for 2 hr. Sodium methanesulphonate (1·11 g.; theory, 1·26 g.) was removed and the filtrate taken to dryness under reduced pressure, water (100 c.c.) added to the residue, and the mixture shaken vigorously. The yellow amorphous solid was collected, well washed with water, and dried (6·0 g.). Recrystallised from methanol and then from acetone–methanol, *3′-deoxy-3′-iodo-5′-tritylthymidine* formed colourless cubes, m. p. 147° (Found, in material dried for 12 hr. at 60°/10^{-3} mm. : C, 58·8; H, 5·0; N, 4·8. $C_{29}H_{27}O_4N_2I$ requires C, 58·6; H, 4·5; N, 4·7%).

3′-Deoxy-3′-iodothymidine.—A solution of 3′-deoxy-3′-iodo-5′-tritylthymidine (5·0 g.) in 80% acetic acid (35 c.c.) was heated under reflux for 7 min. Acetic acid was removed under reduced pressure and the residue shaken several times with ether to remove triphenylmethanol. The ether-insoluble residue was recrystallised twice from water, giving 3′-deoxy-3′-iodothymidine as needles (1·0 g.), m. p. 166—167° (decomp.) (Found, in material dried for 6 hr. at 90°/10^{-3} mm. : C, 34·8; H, 3·7; N, 7·7. $C_{10}H_{13}O_4N_2I$ requires C, 34·1; H, 3·7; N, 7·9%).

3′-Deoxythymidine.—A solution of 3′-deoxy-3′-iodothymidine (0·5 g.), in aqueous ethanol brought to pH 10 with ammonia, was hydrogenated at atmospheric temperature and pressure with palladised barium sulphate. When hydrogenation was complete (*ca.* 3 hr.) catalyst was removed and the filtrate taken to dryness under reduced pressure below 30°. The residue was extracted several times with hot ethyl acetate, and the combined ethyl acetate extracts were taken to small volume and cooled. *3′-Deoxythymidine* separated as needles, m. p. 145° (0·2 g.), and was recrystallised twice from acetone–light petroleum (b. p. 40—60°) without change in m. p. (Found, in material dried at 90°/10^{-3} mm. for 6 hr. : C, 53·3; H, 6·5; N, 12·2. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4%).

*5′-Toluene-*p*-sulphonylthymidine.*—A solution of 3′-acetyl-5′-toluene-*p*-sulphonylthymidine (Michelson and Todd, *J.*, 1953, 951) (9·7 g.) in methanol half saturated with ammonia (75 c.c.) was kept at 0° overnight. Methanol was removed under reduced pressure and the crystalline residue of *5′-toluene-*p*-sulphonylthymidine* recrystallised from methanol as needles (6·5 g.), m. p. 172° (decomp.) (Found, in material dried for 15 hr. at 80°/10^{-3} mm. : C, 51·6; H, 5·3; N, 6·9. $C_{17}H_{20}O_7N_2S$ requires C, 51·5; H, 5·1; N, 7·1%).

5′-Deoxy-5′-iodothymidine.—A solution of 5′-toluene-*p*-sulphonylthymidine (5·326 g.) and sodium iodide (5 g.) in acetone (20 c.c.) was heated at 100° for 2 hr. Sodium toluene-*p*-sulphonate (2·59 g.; theory, 2·60 g.) was removed and the filtrate taken to dryness under reduced pressure. Water (40 c.c.) was added to the residue, and the resultant crystalline mass collected, washed with water, and dried [4·6 g.; m. p. 172° (decomp.)]. Recrystallised from water, 5'-*deoxy-5'-iodothymidine* formed needles, m. p. 168° (decomp.) (Found, in material dried for 12 hr. at 60°/10^{-3} mm.: C, 34·2; H, 3·6; N, 7·9. $C_{10}H_{13}O_4N_2I$ requires C, 34·1; H, 3·7; N, 7·9%).

5'-*Deoxythymidine*.—A solution of 5'-deoxy-5'-iodothymidine (0·6 g.) in aqueous ethanol at pH 10 (ammonia) was hydrogenated with palladised barium sulphate. When hydrogenation was complete, catalyst was removed and washed with hot ethanol, and the combined filtrate and washings were taken to small volume; 5'-*deoxythymidine* crystallised (0·250 g.). Recrystallised from ethanol it had m. p. 188° (Found, in material dried at 90°/10^{-3} mm. for 6 hr.: C, 53·3; H, 6·1; N, 12·3. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4%).

5'-*Bromo-5'-deoxythymidine*.—A solution of 5'-toluene-*p*-sulphonylthymidine (0·50 g.) and lithium bromide (0·50 g.) in anhydrous acetone (5 c.c.) was heated at 100° for 2 hr. Acetone was removed under reduced pressure, water (20 c.c.) added to the residue, and the resultant crystalline mass collected, washed with water, and dried [0·335 g.; m. p. 154° (decomp.)]. Recrystallised twice from water, 5'-*bromo-5'-deoxythymidine* formed needles, m. p. 129° (decomp.) (Found, in material dried for 12 hr. at 60°/10^{-3} mm.; C, 38·8; H, 4·3; N, 9·2. $C_{10}H_{13}O_4N_2Br$ requires C, 39·3; H, 4·3; N, 9·2%).

Action of Silver Dibenzyl Phosphate on 3'-Acetyl-5'-deoxy-5'-iodothymidine.—A mixture of silver dibenzyl phosphate (100 mg.) and 3'-acetyl-5'-deoxy-5'-iodothymidine (100 mg.) in dry benzene (15 c.c.) was refluxed with stirring for 2 hr. Silver iodide was removed by filtration, the benzene solution washed with sodium hydrogen carbonate solution, then water, and dried (Na_2SO_4), and solvent removed under reduced pressure to give a colourless gum. This was dissolved in aqueous ethanol, hydrogenated with a palladium catalyst, the acetyl group removed by treatment with barium hydroxide solution, and the nucleotide worked up as previously described (Michelson and Todd, *J.*, 1953, 951), giving barium thymidine-5' phosphate (55 mg.) identical with authentic material.

5'-*Deoxy-5'-iodothymidine-3' Dibenzyl Phosphate*.—A solution of anhydrous 5'-deoxy-5'-iodothymidine (1 g.) in dry pyridine (20 c.c.) at −30° was treated with dibenzyl phosphorochloridate (from 3 g. of dibenzyl phosphite), and the mixture kept just above its f. p. for 6 hr. and then at 0° overnight. Sodium carbonate (1·5 g.) and water (10 c.c.) were added, and the mixture was taken to dryness under reduced pressure. To the residue water and chloroform were added and the chloroform extract was washed with sodium hydrogen carbonate solution, then water, and dried (Na_2SO_4). Solvent was removed under reduced pressure and the residual gum taken to dryness twice with ethanol, then dissolved in acetone (5 c.c.), a 1 : 1 mixture (100 c.c.) of ether and light petroleum (b. p. 40—60°) was added, and the solution kept at 0° overnight. The supernatant liquid was decanted from the gelatinous mass which had separated and the precipitation repeated. The final gel was dissolved in acetone and taken to a glass under reduced pressure (yield, 1·1 g.). For analysis the gum was dissolved in acetone, and the solution filtered and poured into water, giving 5'-*deoxy-5-iodothymidine-3' dibenzyl phosphate* as a white amorphous solid (Found, in material dried at room temp./1 mm. for 2 weeks: C, 47·6: H, 4·6; N, 4·2. $C_{24}H_{26}O_7N_2PI$ requires C, 47·1; H, 4·3; N, 4·6%).

Paper Chromatography of Deoxythymidine Derivatives.—Whatman No. 1 paper. Solvent system, *n*-butanol–water (86 : 14) in ascending chromatograms. Cysteine spray (Buchanan, *Nature*, 1951, **168**, 1091). The results are tabulated.

	R_F	Cysteine spray colour
3'-Methanesulphonylthymidine	0·46	Pink
3'-Deoxy-3'-iodothymidine	0·71	Pink
3'-Deoxythymidine	0·53	Faint yellow
5'-Bromo-5'-deoxythymidine	0·66	Pink
5'-Deoxy-5'-iodothymidine	0·67	Pink
5'-Deoxythymidine	0·56	Brown
5'-Toluene-*p*-sulphonylthymidine	0·70	Pink
3' : 5'-Dibromo-3' : 5'-dideoxythymidine	0·79	Pink
5'-Bromo-3' : 5'-dideoxythymidine	0·71	Faint yellow
Thymidine	0·40	Pink
2-Deoxyribose	0·27	Pink
2-Deoxyxylose	0·30	Pink

5'-*Methanesulphonyl*-O^2 : 3'-cyclo*thymidine*.—Saturated methanolic ammonia (5 c.c.) was added to a solution of 3' : 5'-dimethanesulphonylthymidine (0·13 g.) in 90% ethanol (5 c.c.), and the mixture kept at room temperature overnight. The crystalline deposit was collected and recrystallised from 90% ethanol, giving *5'-methanesulphonyl* O^2 : 3'-cyclo*thymidine* as needles, m. p. 176° (decomp.) (0·08 g.) (Found, in material dried for 12 hr. at 100°/1 mm. : C, 43·7; H, 4·5; N, 9·3. $C_{11}H_{14}O_6N_2S$ requires C, 43·7; H, 4·6; N, 9·3%). Light absorption in H_2O : max. 250—252, min. 218 mμ.

O^2 : 3'-cyclo*Thymidine*.—A solution of 3'-deoxy-3'-iodothymidine (200 mg.) in dry laboratory-grade methyl cyanide (25 c.c.) was heated and stirred under reflux with powdered silver acetate (400 mg.) for 1¼ hr. The solution was filtered hot, hydrogen sulphide was passed through the filtrate to remove silver ions, and, after further filtration, the solution was evaporated under reduced pressure. The residue was extracted with hot ethanol and the filtered extract concentrated to small bulk and cooled. O^2 : 3'-cyclo*Thymidine* separated as colourless needles (110 mg.); recrystallised from 90% ethanol it had m. p. 230° (Found, in material dried for 12 hr. at 100°/10^{-3} mm. : C, 53·8; H, 5·7; N, 12·3. $C_{10}H_{12}O_4N_2$ requires C, 53·6; H, 5·4; N, 12·5%). Light absorption : in H_2O, max. 252 (ε 7900), min. 220 (ε 4900); in N/100-HCl, max. 254 (ε 7800), min. 220 (ε 4900); in N/100-NaOH, max. 254 (ε 7800), min. 219 (ε 4900). Thymidine itself had the following light absorption : in H_2O, max. 269 (ε 9300), min. 235 mμ (ε 1900); in N/100-HCl, max. 267—268 (ε 9200), min. 235 (ε 1900); in N/100-NaOH, max. 266 (ε 7200), min. 243 mμ (ε 4200).

O^2 : 5'-cyclo*Thymidine*.—5'-Deoxy-5'-iodothymidine (500 mg.) in methyl cyanide (45 c.c.) was refluxed and stirred for 1¼ hr. with silver acetate (750 mg.). Working up as described above gave an ethanolic solution which deposited O^2 : 5'-cyclo*thymidine*, which recrystallised from 90% ethanol as colourless needles (100 mg.), m. p. 230° (on one occasion m. p. 218°) (Found, in material dried 12 hr. at 100°/10^{-3} mm. : C, 53·3; H, 5·8; N, 12·1. $C_{10}H_{12}O_4N_2$ requires C, 53·6; H, 5·4; N, 12·5%). Light absorption : in H_2O, max. 250—253 (ε 10,400), min. 218 mμ (ε 3500); in N/100-HCl, max. 251—252 (ε 9000), min. 219 (ε, 4000); in N/100-NaOH, max. 252 (ε 10,400), min. 219 mμ (ε 5600).

5'-Acetylthymidine.—Paper-chromatographic examination of the mother-liquor from the first crystallisation of *O*^{2} : *5'-cyclo*thymidine showed the presence of a material with a considerably higher R_F value. The mother-liquor was therefore taken to dryness, the residue dissolved in acetone, and ether was added to slight turbidity. The mixture was set aside at 0° overnight, the clear solution decanted from deposited gum, and light petroleum (b. p. 40—60°) added to slight turbidity. During several days at 0° colourless needles of *5'-acetylthymidine* separated, which recrystallised from acetone–light petroleum (b. p. 40—60°) as needles (50 mg.), m. p. 146° (Found, in material dried for 8 hr. at 90°/10^{-3} mm. : C, 50·5; H, 5·8; N, 10·1. $C_{12}H_{16}O_6N_2$ requires C, 50·7; H, 5·6; N, 9·9%).

3' : *5'-Diacetylthymidine*.—A solution of thymidine (1 g.) in dry pyridine (15 c.c.) and acetic anhydride (5 c.c.) was kept at room temperature for 15 hr., then poured into ice-water (150 c.c.). The aqueous solution was extracted thrice with chloroform, and the combined extracts were washed successively with ice-cold dilute sulphuric acid, sodium hydrogen carbonate, and water, then dried (Na_2SO_4) and evaporated. The residue, which crystallised on trituration with dry ether, was recrystallised from ethanol, giving 3' : *5'-diacetylthymidine* (1·1 g.) as colourless prisms, m. p. 125° (Found, in material dried for 8 hr. at 80°/10^{-3} mm. : C, 51·7; H, 5·7; N, 8·7. $C_{14}H_{18}O_7N_2$ requires C, 51·5; H, 5·5; N, 8·6%).

3'-*Acetyl*-O^2 : 5'-cyclo*thymidine* —A solution of 3'-acetyl-5'-deoxy-5'-iodothymidine (500 mg.) in laboratory-grade methyl cyanide (25 c.c.) was stirred and refluxed with silver acetate for 1¼ hr., then worked up as described above for analogous experiments. 3'-*Acetyl*-O^2 : 5'-cyclo*thymidine* (125 mg.), crystallised from 90% ethanol, had m. p. 252° (Found, in material dried for 12 hr. at 100°/10^{-3} mm. : C, 54·1; H, 5·0; N, 11·0. $C_{12}H_{14}O_5N_2$ requires C, 54·1; H, 5·3; N, 10·5%). Light absorption : in 50% EtOH, max. 249—250 (ε 11,000), min. 220 (ε 3600); in N/100-HCl, max. 248—249 (ε 10,700), min. 218 (ε 3700); in N/100-NaOH, max. 248—249 (ε 10,500), min. 218 mμ (ε 3500).

Paper chromatography showed the presence of a faster-running material [R_F 0·63 in *n*-butanol–water (86 : 14)] in the mother-liquor from the first crystallisation of 3'-acetyl-*O*^{2} : 5'-*cyclo*thymidine; although it was not isolated this material is believed to be 3' : 5'-diacetylthymidine since this showed an identical R_F (0·63) when run alongside it.

Paper Chromatography of cyclo*Thymidines*.—Ascending chromatograms, Whatman No. 1 paper. Solvent systems : A, *n*-butanol–water (86 : 14); B, *iso*propanol–water–concentrated aqueous ammonia (70 : 20 : 10); C, *n*-propanol–2N-HCl (3 : 1). R_F values found were :

	A	B	C
Thymidine	0·39	0·57	0·74
O^2 : 3'-*cyclo*Thymidine	0·22	0·61	0·46
O^2 : 5'-*cyclo*Thymidine	0·23	0·59	0·96
3'-Acetyl-*O*^{2} : 5'-*cyclo*thymidine	0·39	0·62	0·89

Paper Electrophoresis of Some Thymidine Derivatives.—Whatman No. 1 paper at 220 v. Movements (cm.), towards negative pole in all cases, were :

	N/10-AcOH, 6 hr.	N/10-NH_3, 7 hr.
Thymidine	0·8	0·0
5′-Deoxythymidine	0·5	0·3
O^2 : 3′-*cyclo*Thymidine	0·7	2·5
O^2 : 5′-*cyclo*Thymidine	0·8	2·3
3′-Acetyl-O^2 : 5′-*cyclo*thymidine	0·5	2·3

Hydrolysis of O^2 : 3′-cyclo*Thymidine and* O^2 : 5′-cyclo*Thymidine.*—(a) *With* 0·1N-*sodium hydroxide.* After 2 hr. at room temperature partial hydrolysis of the 3′-compound had occurred, giving a compound travelling exactly like thymidine on paper chromatograms ; the 5′-compound had undergone complete hydrolysis to a similar product. Hydrolysis of the 3′-compound was nearly complete after 24 hr.

The hydrolysis product from both compounds ran almost exactly like thymidine on paper chromatograms in *n*-butanol–water, *iso*propanol–ammonia, or *n*-propanol–hydrochloric acid and gave a strong cysteine spray colour only on the area showing ultra-violet absorption. The ultra-violet absorption of the product was identical with that of natural thymidine (comparison of $\lambda_{max.}$, $\lambda_{min.}$, and optical density ratios 250/260 and 280/260 mμ both in N/20-acid and N/20-alkali).

Closer examination of the alkaline hydrolysis, with dilute ammonia as reagent, showed that the product from O^2 : 5′-*cyclo*thymidine was in fact thymidine (infra-red spectrum, m. p., and mixed m. p.). O^2 : 3′-*cyclo*Thymidine, however, gave a product moving slightly faster (R_F 0·41) than thymidine (R_F 0·40) in paper chromatograms (*n*-butanol–water). The infra-red spectrum of this material was similar to, but not identical with, that of thymidine ; scarcity of material prevented thorough study of the product but it is almost certainly the expected thymine 2-deoxy-D-xylofuranoside.

(b) *With* 0·1N-*hydrochloric acid.* After 48 hr. at room temperature the 3′-compound was unchanged and only slight hydrolysis had occurred with the 5′-compound.

After 10 minutes at 100° the 3′-compound was almost completely converted into a faster-moving substance with the same R_F as thymidine in *n*-butanol–water, showing ultra-violet absorption and a strong cysteine spray reaction, together with a smaller amount of a substance revealed on paper chromatograms as a spot with a weak cysteine spray reaction and no ultra-violet absorption (R_F/R_F of thymidine = 0·75). On similar treatment the 5′-compound was completely destroyed, yielding a substance with the same R_F as thymidine (or thymine) in *n*-butanol–water, giving a weak cysteine spray reaction, and another travelling on paper chromatograms as a spot devoid of ultra-violet absorption but with a strong cysteine spray reaction (R_F/R_F of thymidine = 0·67).

After 30 minutes the 3′-compound was completely converted into one ultra-violet-absorbing substance with greatly decreased cysteine spray reaction, the non-absorbing cysteine spray-positive substance having correspondingly increased in amount. With the 5′-compound the cysteine spray reaction of the material showing ultra-violet absorption was very weak indeed.

After 1 hr. at 100° both compounds appeared to have been hydrolysed completely, giving a product with ultra-violet absorption and another with a cysteine spray reaction. The material showing ultra-violet absorption was identified in each case as thymine by paper chromatography in 3 solvent systems, by measurement of ultra-violet absorption ($\lambda_{max.}$, $\lambda_{min.}$, and optical density ratios at 250/260 and 280/260 mμ in N/20-acid and -alkali), and by isolation in crystalline form. The cysteine spray-positive product from the 5′-compound agreed in paper-chromatographic behaviour with 2-deoxy-D-ribose (R_F/R_F thymidine = 0·67) and that from the 3′-compound with 2-deoxy-D-xylose (R_F/R_F thymidine = 0·75) (both sugars treated with N/10-hydrochloric acid for 1 hr. at 100°).

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