212. *Nucleotides. Part XLVII.* The Catalytic Oxidation of Nucleosides and Nucleotides: A Projected Stepwise Degradation of Polynucleotides.*

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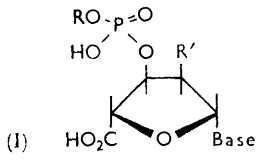
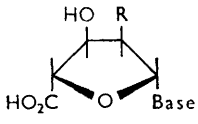
The nucleosides uridine, adenosine, guanosine, and thymidine, and the nucleotides, thymidine-3′ phosphate and dithymidine-3′,5′ phosphate, have been oxidised by oxygen in the presence of reduced Adams catalyst to their corresponding 5′-carboxylic acids. The properties of the uridine acid have been investigated in detail. When heated in N-sodium hydroxide the two nucleotide carboxylic acids underwent decarboxylative elimination reactions but much less readily than a specially designed model compound. A projected stepwise degradation of polynucleotides is discussed in the light of these results.

The development of chemical methods for the stepwise degradation of polynucleotides could be of major importance in furthering our knowledge of nucleic acid structure. One such method depending upon the periodate oxidation of the *cis*-1,2-glycol system present in a terminal residue of a polyribonucleotide, followed by subsequent removal of the oxidised residue by base-catalysed elimination, has already been described.[1,2] According to the original procedure elimination of the oxidised residue was effected at pH 9—10 and 37°, but subsequent modifications permit its operation under even milder conditions.[3]

As this method cannot, of course, be applied to polydeoxyribonucleotides which lack a *cis*-glycol system, we have sought to develop a somewhat analogous procedure applicable to both types of nucleic acid. In this projected scheme, the primary alcoholic group (either pre-existing or generated by enzymic action) in a terminal residue would be oxidised to carboxyl and the resulting acid (I; R = remainder of polynucleotide chain, R′ = H or OH) subjected to simple or decarboxylative elimination, thus removing the terminal residue. This process could in theory be repeated on the remaining polynucleotide after enzymic removal of the terminal 5′-phosphate group. A preliminary report on our initial oxidation experiments on nucleosides has already been published.[4] The present communication presents the results of these experiments in detail, together with our further work on nucleotide oxidation and on the projected degradation procedure.



(I)



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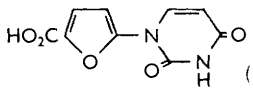

(III)

For the projected degradation to be of value it was necessary to be able to oxidise nucleosides and nucleotides to the corresponding 5′-carboxylic acids selectively, under mild conditions, and in high yield. With these considerations in mind catalytic oxidation with oxygen in the presence of a noble-metal catalyst seemed attractive, since Heyns and Paulsen [5] showed this method to be fairly specific for the oxidation of primary hydroxyl groups in polyols. In practice we found the method satisfactory for the selective oxidation of nucleosides and nucleotides and we have used it throughout our work. Since our original

* Part XLVI, *J.*, 1961, 2316.

[1] Brown, Fried, and Todd, *Chem. and Ind.*, 1953, 352; *J.*, 1955, 2206.
[2] Whitfield and Markham, *Nature*, 1953, **171**, 1151; Whitfield, *Biochem. J.*, 1954, **58**, 390.
[3] Ogur and Small, *J. Biol. Chem.*, 1960, **235**, PC60; Yu and Zamecnik, *Biochim. Biophys. Acta*, 1960, **45**, 148; Khym and Cohn, *J. Biol. Chem.*, 1961, **236**, PC9.
[4] Reese, Schofield, Shapiro, and Todd, *Proc. Chem. Soc.*, 1960, 290.
[5] Heyns and Paulsen, *Angew. Chem.*, 1957, **69**, 600.

observations, analogous oxidations have been reported in which chromium trioxide in pyridine [6] and hydrogen peroxide and reduced platinum oxide [7] were used. Before our work the only nucleoside carboxylic acid described [8] was " 5-nitrouridine-5'-carboxylic acid " (II; Base = 5-nitrouracil-3, R = OH) obtained during the nitration of uridine.

Uridine in aqueous solution buffered at pH 8·8 was smoothly oxidised by oxygen in presence of reduced platinum oxide (Adams catalyst), giving the crystalline carboxylic acid (II; Base = uracil-3, R = OH), which yielded an isopropylidene derivative with acetone. Treatment of the acid (II; Base = uracil-3, R = OH) with diazomethane gave its crystalline methyl ester, or, if a large excess of diazomethane was used, methyl 1-methyluridine-5'-carboxylate. When heated with acetic anhydride uridine-5'-carboxylic acid did not give the expected diacetate. Instead, an acid $C_9H_6N_2O_5$ was obtained whose properties correspond to (III), a structure first suggested in discussion by Professor R. B. Woodward; presumably it owes its formation to acetylation followed by a double elimination.

Under similar conditions adenosine was oxidised to the zwitterionic " adenosine-5'-carboxylic acid " (II; Base = adenine-9, R = OH), although the oxidation was slower than with uridine. Guanosine was oxidised even more slowly and the acidic product could not be obtained crystalline. The reason for the slower oxidation of the purine nucleosides is obscure. Thymidine, like uridine, was very readily oxidised to " thymidine-5'-carboxylic acid " (II; Base = thymine-3, R = H). Nucleotides appear to be much more difficult to oxidise catalytically than nucleosides. Thymidine-3' phosphate could only be oxidised to the corresponding carboxylic acid (I; Base = thymine-3, R = R' = H) in the presence of a large excess of platinum at 100° and pH 2. In the same conditions the dinucleoside phosphate, dithymidine-3',5' phosphate yielded the carboxylic acid (I; Base = thymine-3, R = thymidine-5', R' = H) in comparable yield. Both these nucleotide carboxylic acids were isolated as their lithium salts.

As a preliminary to studies on base-catalysed elimination of phosphate or phosphate ester groups from nucleotide-5'-carboxylic acids (I), it was decided to examine the elimination of bromide ion from 3'-bromo-3'-deoxythymidine-5'-carboxylic acid. However, 3'-bromo-3'-deoxythymidine from which it might have been prepared cyclised with great ease to O^2,3'-cyclothymidine [9] (a fact which, incidentally, supports the assigned *ribo*-configuration of the bromine atom); it was accordingly methylated with diazomethane, giving 3'-bromo-3'-deoxy-1-methylthymidine. When the latter, in neutral solution, was treated with oxygen in presence of platinum at 100° very little acidic material was formed, the main product being 1-methylthymine, probably formed as a result of breakdown of the initial product of decarboxylative elimination. It thus seemed that the projected elimination procedure applied to a nucleotide-5'-carboxylic acid might lead directly to production of free base which would be an added advantage in a stepwise degradation method for polynucleotides. Unfortunately elimination of the phosphate groups in such compounds proved difficult to achieve. Thus, even when heated with aqueous N-sodium hydroxide at 100°, thymidine-3'-phosphate-5'-carboxylic acid was only half converted into thymine in 48 hours, while the carboxylic acid (I; Base = thymine-3, R = thymidine-5', R' = H) although more susceptible still required 6 hours in these conditions for 50% conversion into thymine and thymidine-5' phosphate.

Although these experiments establish that oxidation followed by base-catalysed elimination is feasible for oligodeoxyribonucleotide degradation, it is clear that in its present form it is not likely to have much practical significance. Viszolyi and Tener [7] have meanwhile reported that the *N*-propylamide derived from the carboxylic acid (I; Base =

[6] Jones and Williamson, *Chem. and Ind.*, 1960, 1624.
[7] Vizsolyi and Tener, *Chem. and Ind.*, 1962, 263.
[8] Levene and La Forge, *Ber.*, 1912, **45**, 608; Wempen, Doerr, Kaplan, and Fox, *J. Amer. Chem. Soc.*, 1960, **82**, 1624.
[9] Michelson and Todd, *J.*, 1955, 816.

thymine-3, R = thymidine-5′, R′ = H) yields thymidine-5′ phosphate in 97% yield after 40 minutes' heating at 100° with 0·25N-aqueous sodium hydroxide. This represents a substantial improvement although the conditions are still relatively severe and the preliminary two-step conversion of the acid into its *N*-propylamide is an added complication which limits the practical use of the method.

Experimental

Paper Chromatography and Electrophoresis.—Ascending chromatograms on Whatman No. 1 paper (unless otherwise stated) were run in the following solvent systems: A, propan-2-ol–ammonia–water, 7 : 1 : 2; B, butan-1-ol–acetic acid–water, 5 : 2 : 3; C, butan-1-ol–acetic acid–water, 5 : 1 : 3; D, isobutyric acid–N-ammonia–0·1M-ethylenediaminetetra-acetic acid, 100 : 60 : 1·6; E, butan-1-ol–water, 86 : 14; F, ethyl acetate–water–formic acid, 60 : 35 : 5 (upper layer).

Electrophoretograms were run at 2000 v (40 v/cm.) on Whatman No. 1 and No. 4 paper in the following buffers: 1, 0·1M-phosphate, pH 6·8; 2, 0·025M-borate, pH 9; 3, 0·05M-acetate, pH 4·5; 4, 0·05M-carbonate, pH 10·5.

" *Uridine-5′-carboxylic Acid* " (II; Base = uracil-3, R = OH).—Recrystallised uridine (0·794 g., 3·25 mmoles) was dissolved in a solution (pH 8·8) of sodium hydrogen carbonate (0·275 g., 3·25 mmoles) and sodium carbonate decahydrate (0·12 g.) in water (110 c.c.). Reduced platinum oxide [10] (0·57 g.) was added, and oxygen was bubbled into the rapidly stirred suspension, maintained at 80°. Paper chromatography (system A) and paper electrophoresis (buffer 1) indicated that two-thirds of the uridine was oxidised after 3 hr. and that the reaction was virtually complete after 22 hr. The catalyst was collected by filtration, and the filtrate concentrated to 25 c.c. under reduced pressure, treated with Amberlite IR-120 (H^+ form; 10 c.c. + 2 c.c.) and further evaporated to a pale yellow oil which crystallised. Crude *uridine-5′-carboxylic acid* (0·624 g., 74%), obtained by triturating the product with ethanol, recrystallised from ethanol and had m. p. (corr.) 220·5—222° (decomp.) (Found, in material dried at 75°/5 × 10^{-4} mm. for 6 hr.: C, 41·9; H, 4·3; N, 10·8. $C_9H_{10}N_2O_7$ requires C, 41·9; H, 3·9; N, 10·9%). It migrated as an anion at pH 6·8 (buffer 1) and consumed periodate. It had a potentiometrically determined pK_a, 3·07, $[\alpha]_D^{20}$ +18° (*c* 2·5 in H_2O), $\nu_{max.}$ (in Nujol) 1710 cm.$^{-1}$, R_F 0·65 (system A), 0·46 (system B), $\lambda_{max.}$ (in H_2O) 260 (log ε 4·00), $\lambda_{min.}$ 229 mμ.

The alcoholic mother-liquors deposited a neutral crystalline compound (0·043 g.) which, recrystallised from methanol–acetone, had m. p. 237—239°, consumed periodate, and had $\nu_{max.}$ (in Nujol) 1734 cm.$^{-1}$, R_F 0·69 (system B), $\lambda_{max.}$ (in H_2O) 260.(log ε 3·96), $\lambda_{min.}$ 229 mμ. It was assumed to be *ethyl uridine-5′-carboxylate* (Found, in material dried at 80°/10^{-3} mm. for 6 hr.: C, 45·5; H, 4·7; N, 10·1. $C_{11}H_{14}N_2O_7$ requires C, 46·1; H, 4·9; N, 9·8%).

Methylation of Uridine-5′-carboxylic Acid with Diazomethane.—(*a*) Ethereal diazomethane (15 mol.) was added to a solution of uridine-5′-carboxylic acid (0·05 g., 1 mol.) in *warm* methanol (10 c.c.). After 5 min., the solution was concentrated to a white crystalline mass, which recrystallised from aqueous methanol. *Methyl uridine-5′-carboxylate* (0·028 g.), m. p. 236—238°, was obtained after further crystallisation from aqueous ethanol (Found, in material dried at 80°/10^{-3} mm. for 6 hr.: C, 44·1; H, 4·4; N, 10·2. $C_{10}H_{12}N_2O_7$ requires C, 44·1; H, 4·4; N, 10·3%). It was neutral, consumed periodate, and had R_F 0·63 (system B, Whatman No. 4), $\nu_{max.}$ (in Nujol) 1755 cm.$^{-1}$, $\lambda_{max.}$ (in water), 260 (log ε 3·99), $\lambda_{min.}$ 230 mμ.

(*b*) Ethereal diazomethane (10 mol.) was added to a solution of uridine-5′-carboxylic acid (0·20 g., 1 mol.) in methanol (20 c.c.) at room temperature. The yellow colour was not discharged. When the solution was concentrated and chilled it deposited colourless crystals (0·107 g., 49%) of *methyl 1-methyluridine-5′-carboxylate*. Recrystallised from aqueous methanol this formed needles, m. p. 168—170° (Found, in material dried at 80°/10^{-3} mm. for 6 hr.: C, 46·1; H, 5·1; N, 9·8. $C_{11}H_{14}N_2O_7$ requires C, 46·2; H, 4·9; N, 9·8%). This material consumed periodate, and had R_F 0·78 (system B, Whatman No. 4), $\nu_{max.}$ (in Nujol) 1752 cm.$^{-1}$, $\lambda_{max.}$ (in water) 260 (log ε 3·96), $\lambda_{min.}$ 231 mμ. The same compound was obtained by further methylating methyl uridine-5′-carboxylate with diazomethane.

[10] Heyns and Beck, *Chem. Ber.*, 1957, **90**, 2443.

2′,3′-O-*Isopropylideneuridine-5′-carboxylic Acid*.—A suspension of uridine-5′-carboxylic acid (0·05 g.) and an excess of powdered, dried (by azeotropic distillation with benzene) Amberlite IR-120 (H^+ form; 0·5 g.) in dry acetone (15 c.c.) was heated under reflux for 24 hr. The resin was collected and the filtrate evaporated to dryness and then extracted with boiling chloroform. The chloroform extract was evaporated to a colourless gum of 2′,3′-O-*isopropylideneuridine-5′-carboxylic acid* which, crystallised from acetone–ether, had m. p. 187—188° (0·025 g., 45%) (Found, in material dried at 80°/10^{-3} mm. for 6 hr.: C, 48·4; H, 4·6; N, 9·1. $C_{12}H_{14}N_2O_7$ requires C, 48·3; H, 4·7; N, 9·4%). This acidic compound did not consume periodate; it had R_F 0·67 (system A), $\nu_{max.}$ (in Nujol) 1724 cm.$^{-1}$, $\lambda_{max.}$ (in H_2O) 259 (log ε 3·98), $\lambda_{min.}$ 229 mμ.

Treatment of Uridine-5′-carboxylic Acid with Acetic Anhydride.—Uridine-5′-carboxylic acid gradually dissolved when treated with boiling acetic anhydride (3 c.c.) for 15 min. Water was added to the cooled product which then deposited colourless crystals (0·095 g., 77%) assigned the constitution 5-*uracil-3′-yl-2-furoic acid* (III) [Found, in material recrystallised from glacial acetic acid and dried at 100°/10^{-3} mm. for 6 hr.: C, 48·8; H, 2·7; N, 12·2%; equiv., 106. $C_9H_6N_2O_5$ requires C, 48·7; H, 2·7; N, 12·6%; equiv. (for a dibasic acid), 111], R_F 0·70 (system B), $\nu_{max.}$ (in Nujol) 1724 cm.$^{-1}$. The material fluoresced in ultraviolet light and had $\lambda_{max.}$ 258 (log ε 4·15), $\lambda_{min.}$ 227 mμ.

When this acid (III) (0·011 g.) was heated above 250°, it did not melt but sublimed with decomposition to yield needles (0·005 g.) of a neutral compound (as indicated by paper electrophoresis in buffer 1), R_F 0·81 (system B), $\lambda_{max.}$ 257, $\lambda_{min.}$ 233 mμ.

"*Adenosine-5′-carboxylic Acid*" (II; Base = adenine-9, R = OH).—Recrystallised adenosine (0·885 g., 3 mmoles) was dissolved in a solution (pH 9) of sodium hydrogen carbonate (0·252 g., 3 mmoles) and sodium carbonate decahydrate (0·12 g.) in water (110 c.c.). Platinum catalyst (0·88 g.) was added and oxygen was bubbled through the stirred suspension, maintained at 90°, for 28 hr. Paper chromatography (system A) indicated that over half the adenosine had reacted. Catalyst was removed and the filtrate concentrated under reduced pressure to 5 c.c. The pH (measured by a meter) was adjusted to 4 by adding dilute sulphuric acid. *Adenosine-5′-carboxylic acid* (0·457 g., 52%) separated and was reprecipitated as needles by dissolving it in aqueous sodium hydroxide (charcoal) and then adding dilute sulphuric acid (Found, in material dried at 105°/6 × 10^{-4} mm. for 6 hr.: C, 42·8; H, 3·9; N, 24·4. $C_{10}H_{11}N_5O_5$ requires C, 42·7; H, 4·0; N, 24·9%). The material did not melt below 320°; it migrated as an anion at pH 6·8 (buffer 1), and had R_F 0·31 (system A), $[\alpha]_D^{20}$ −14° (*c* 2 in 0·1N-NaOH), $\lambda_{max.}$ (in water) 258 (log ε 4·18), $\lambda_{min.}$ 228 mμ. The mother-liquors contained an unidentified material, R_F 0·39 (system A).

Oxidation of Guanosine.—Recrystallised guanosine (0·958 g., 3 mmoles), dissolved in the buffer used for adenosine, was oxidised in the presence of platinum catalyst (0·90 g.) for 96 hr. at 90°. Paper electrophoresis (buffer 1) indicated that about two-thirds of the guanosine had reacted. Catalyst was removed and the filtrate concentrated to 50 c.c. Dilute sulphuric acid was added to give pH 4. A gelatinous precipitate, which was shown by electrophoresis to be a mixture of an acidic and a neutral component, was obtained; it was not purified.

Thymidine-5′-carboxylic Acid (II; Base = thymine-3, R = H).—Recrystallised thymidine (0·785 g., 3·25 mmoles) was dissolved in a solution (pH 9) of sodium hydrogen carbonate (0·275 g., 3·25 mmoles) and sodium carbonate decahydrate (0·12 g.) in water (120 c.c.). Platinum catalyst (0·52 g.) was added and the oxidation was conducted at 77° for 8 hr. Paper chromatography (system A) indicated that virtually no thymidine remained after 6 hr. Catalyst was removed and the filtrate concentrated to 50 c.c., treated with Amberlite IR-120 (H^+ form; 12 c.c.), and evaporated to dryness under reduced pressure. *Thymidine-5′-carboxylic acid* (0·565 g., 68%) melted with decomp. at 263—265° (corr.) after recrystallisation first from methanol–chloroform and then from water (Found, in material dried at 110°/10^{-3} mm. for 8 hr.: C, 46·9; H, 4·5; N, 10·6. $C_{10}H_{12}N_2O_6$ requires C, 46·9; H, 4·7; N, 10·9%). It had a potentiometrically determined pK_a 3·18, $[\alpha]_D^{20}$ +27° (*c* 2 in 0·1N-NaOH), R_F 0·51 (system A), $\lambda_{max.}$ 267 (in water) (log ε 3·97), $\lambda_{min.}$ 234 mμ. Three other unidentified products with R_F's 0·14, 0·39, 0·67 (system A) were revealed by paper chromatography of the mother-liquors.

Thymidine-3′-phosphate-5′-carboxylic Acid (I; Base = thymine-3, R = R′ = H).—A solution of dilithium thymidine-3′ phosphate [11] (0·25 g.) in 5% acetic acid (30 c.c.) was oxidised

[11] Turner and Khorana, *J. Amer. Chem. Soc.*, 1959, **81**, 4651.

in the presence of platinum catalyst (2·86 g.) for 7 hr. at 100°. The filtrate, after separation of the catalyst, was evaporated under reduced pressure to remove acetic acid. The residue was treated with aqueous barium hydroxide to give pH 7·5, filtered, and lyophilised, dissolved in a small volume of water, and absorbed on a Dowex 2 column (Cl^- form; 12 cm. × 1·5 cm.²). The column was washed with water which eluted thymidine (9% of ultraviolet-absorbing material), and then subjected to gradient-elution from 0—0·05N-hydrochloric acid over a volume of 2 l., 10 c.c. fractions being collected. Fractions 40—59 contained thymidine-5′-carboxylic acid (6%); fractions 60—109, thymidine-3′ phosphate (7%). Fractions 110—195 which contained the required product (78%) were combined and treated with aqueous lithium hydroxide to pH 7·6, concentrated to small volume, and centrifuged. The product, obtained by lyophilisation of the supernatant liquid, was dissolved in a small volume of methanol and precipitated with acetone, then dissolved in water, passed through an Amberlite IR-120 column (H^+ form), treated with aqueous lithium hydroxide to give pH 7·5, and concentrated to small volume. *Trilithium thymidine-3′-phosphate-5′-carboxylate* was precipitated as a white amorphous solid (0·132 g., 50%) on addition of acetone (Found, in material dried at 20°/1 mm. for 48 hr.: C, 26·4; H, 4·6; N, 6·2; P, 6·8. $C_{10}H_{10}Li_3N_2O_9P,5\frac{1}{2}H_2O$ requires C, 26·5; H, 4·6; N, 6·2; P, 6·8%). It had R_F 0·06 (system A), 0·44 (system C), 0·26 (system D), $[\alpha]_D^{20}$ +31·2° (*c* 0·48 in water), $\nu_{max.}$ (in Nujol) 1690 cm.$^{-1}$, potentiometric pK_a (of carboxylate) 2·9, $\lambda_{max.}$ 267 (log ε 3·95), $\lambda_{min.}$ 234 mμ, and electrophoretic mobility (relative to thymidine-5′ phosphate) 1·26 (buffer 2).

Thymidine-5′-phosphate-3′-thymidine-5′-carboxylic Acid (I; Base = thymine-3, R = thymidine-5′, R′ = H).—A solution of lithium dithymidine-3′,5′ phosphate [12] (0·246 g.) in 5% acetic acid (30 c.c.) was oxidised in the presence of platinum catalyst (2·68 g.) for 10 hr. at 100°. The filtrate, after removal of the catalyst, was brought to pH 7·6 with triethylamine, lyophilised, dissolved in a small volume of water, and absorbed on a Dowex 2 column (Cl^- form; 6 cm. × 5·25 cm.²). The column was washed with water which eluted thymidine (15% of ultraviolet-absorbing material) and then gradient-eluted from 0—0·1N-hydrochloric acid over 2 l., 10 c.c. fractions being collected. Fractions 65—81 contained dinucleoside phosphate and mononucleotide (7%). Fractions 82—192, which contained the required product (78%), were treated with aqueous lithium hydroxide to give pH 7·5, concentrated to small volume and finally lyophilised. When acetone was added to a solution of this material in methanol, a white amorphous precipitate (0·148 g., 58%) of *dilithium thymidine-5′-phosphate-3′-thymidine-5′-carboxylate* was obtained (Found, in material dried at 20°/1 mm. for 48 hr.: C, 37·3; H, 4·7; N, 7·9; P, 4·6. $C_{20}H_{23}Li_2N_4O_{14}P,4H_2O$ requires C, 37·2; H, 4·8; N, 8·7; P, 4·8%), having $\nu_{max.}$ (KBr disc.) 1700 cm.$^{-1}$, R_F 0·20 (system A), 0·41 (system C), 0·41 (system D), and electrophoretic mobility (relative to thymidine-5′ phosphate) 0·88 (buffer 2).

3′-*Bromo*-3′-*deoxythymidine*.—A solution of 3′-bromo-3′-deoxy-5′-*O*-tritylthymidine [9] (1·35 g.) in 80% acetic acid (10 c.c.) was heated at 60° for 1 hr. The products were poured into water (180 c.c.), the mixture was filtered, and the filtrate evaporated to dryness. The residue recrystallised from water, to yield colourless needles (0·351 g., 47%) of 3′-*bromo*-3′-*deoxythymidine*, m. p. 150° (deomp.) (Found, in material dried at 70°/1 mm. for 12 hr.: C, 39·9; H, 4·6; N, 9·4. $C_{10}H_{13}BrN_2O_4$ requires C, 39·4; H, 4·3; N, 9·2%), R_F 0·77 (system A), 0·78 (system E).

3′-*Bromo*-3′-*deoxy*-1-*methylthymidine*.—Ethereal diazomethane (20 mols.) was added to a solution of 3′-bromo-3′-deoxythymidine (0·187 g., 1 mol.) in methanol (10 c.c.). After 1 hr., the unchanged diazomethane and solvents were removed under reduced pressure and the residue recrystallised to give colourless needles (0·124 g., 64%) of 3′-*bromo*-3′-*deoxy*-1-*methylthymidine*, m. p. 140° (Found, in material dried at 70°/1 mm. for 12 hr.: C, 41·6; H, 4·9; N, 8·7. $C_{11}H_{15}BrN_2O_4$ requires C, 41·4; H, 4·7; N, 8·8%), R_F 0·88 (system E).

1-*Methylthymine*.—A solution of 1-methylthymidine (0·099 g.) (provided by Mr. J. A. Haines) in 10% sulphuric acid (3 c.c.) was heated under reflux for 10 hr. An excess of barium carbonate was added and the filtrate passed through a column of mixed cation-exchanger (H^+ form) and anion-exchanger (OH^- form). The eluate was concentrated to dryness and recrystallised from ethyl acetate as needles (0·028 g., 52%) of 1-methylthymine,[13] m. p. 208° (Found, in material dried at 55°/1 mm. for 12 hr.: C, 51·6; H, 6·2; N, 19·8. Calc. for $C_6H_8N_2O_2$: C, 51·4; H, 5·8; N, 20·0%). This had R_F 0·75 (system F) and, at pH 1, $\lambda_{max.}$ 264 (log ε 3·87), $\lambda_{min.}$ 235 mμ, and, at pH 13, $\lambda_{max.}$ 289 (log ε 4·01), $\lambda_{min.}$ 249 mμ.

[12] Gilham and Khorana, *J. Amer. Chem. Soc.*, 1958, **80**, 6212.
[13] Johnson and Clapp, *J. Biol. Chem.*, 1908, **5**, 49.

1-*Methylthymidine-5′-carboxylic Acid.*—A solution of 1-methylthymidine (0·02 g.) in phosphate buffer (pH 7; 20 c.c.) was oxidised in the presence of platinum catalyst (0·10 g.) for 4 hr. at 100°. Catalyst was collected, and the filtrate was treated with Amberlite IR-120 (H^+ form), then with an excess of barium hydroxide, followed by carbon dioxide, and finally with Amberlite IR-120 (H^+ form) again; on concentration it deposited colourless needles (0·008 g., 36%) of 1-methylthymidine-5′-carboxylic acid, m. p. 188°, R_F 0·76 (system A), 0·56 (system D).

Oxidation of 3′-Bromo-3′-deoxy-1-methylthymidine.—A solution of 3′-bromo-3′-deoxy-1-methylthymidine (0·136 g.) in phosphate buffer (pH 7; 25 c.c.) was oxidised in the presence of platinum catalyst (0·136 g.) for 12 hr. at 100°. The filtrate, after removal of catalyst, was passed through a column of Amberlite IR-120 (H^+ form), then treated with aqueous barium hydroxide to give pH 7·6, filtered, and passed through a column of Dowex 1 (Cl^- form). This column was first well washed with water, and then gradient-eluted from 0—0·03N-hydrochloric acid over 1 l., 10 c.c. fractions being collected. The initial aqueous washings (54% of ultraviolet absorption) were concentrated and then continuously extracted with ethyl acetate. The concentrated organic phase deposited colourless prisms (0·027 g.) of 1-methylthymine, m. p. 211°. The combined fractions 14—52 of the acid eluate (33% of ultraviolet absorption) were concentrated to small volume, lyophilised, and crystallised from acetone as a colourless material (0·003 g.), m. p. 236°. Mixed m. p. and infrared spectrum (KBr disc) confirmed that this was not 1-methylthymidine-5′-carboxylic acid, although both compounds had virtually identical electrophoretic properties in buffers 2, 3, and 4. It had R_F 0·67 (system A), 0·65 (system D).

Action of Alkali on Thymidine-5′-carboxylic Acid and Nucleotide Derivatives.—(*a*) A solution of thymidine-5′-carboxylic acid (1·3 mg.) in N-sodium hydroxide (1 c.c.) was unchanged after being heated at 100° for 5 days.

(*b*) A solution of trilithium thymidine-3′-phosphate-5′-carboxylate (2·1 mg.) in N-sodium hydroxide (1 c.c.) decomposed at 100° with a half-life of *ca.* 48 hr., to give thymine (R_F 0·36, system F). Thymidine-3′ phosphate was stable under these conditions. The reaction was followed by electrophoresis (buffer 2).

(*c*) A solution of dilithium thymidine-5′-phosphate-3′-thymidine-5′-carboxylate (2·1 mg.) in N-sodium hydroxide (1 c.c.) decomposed at 100° with a half-life of *ca.* 6 hr. to give mainly thymine and mononucleotide (detected by electrophoresis, buffer 2). A trace of thymidine-3′-phosphate-5′-carboxylic acid was observed. Dithymidine-3′,5′ phosphate decomposed very slowly under the same conditions to thymidine and mononucleotide.

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