Synthetic Analogues of Polynucleotides. Part IX.[1] Synthesis of 3′-*O*-Carboxymethyl-2′-deoxyribonucleosides and their Use in the Synthesis of an Analogue of 2′-Deoxyadenylyl-(3′ → 5′-)-thymidine 3′-Phosphate

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3′-*O*-Carboxymethyl-5′-O-triphenylmethyl-2′-deoxycytidine, 3′-*O*-carboxymethyl-2′-deoxyguanosine, 3′-*O*-carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine and 3′-*O*-carboxymethyl-2′-deoxyadenosine have been synthesised by the action of sodium chloroacetate on suitably protected deoxyribonucleosides. The 2,2,2-trichloroethyl group has been shown to be suitable for the protection of the carboxy-group during the synthesis of polynucleotide analogues containing acetate ester linkages, and superior to the 2-cyanoethyl group used hitherto. The dinucleotide analogue. 2′-deoxyadenosinylacetyl-(3′ → 5′)-thymidin-3′-ylacetic acid has been synthesised; it shows a hyperchromic effect of 5% at 248 nm and 6·7% at 260 nm upon hydrolysis of the internucleoside linkage.

THE synthesis of 3′-*O*-carboxymethylthymidine and its 5′-*O*-triphenylmethyl derivative and their use in the synthesis of analogues of oligonucleotides have already been described.[1-3] This paper reports work on the corresponding derivatives of other naturally occurring deoxyribonucleosides. The 3′-*O*-carboxymethyl derivatives are obtained by alkylating a suitably protected deoxyribonucleoside with sodium chloroacetate. To synthesise the deoxycytidine derivative, *N*(4)-acetyl-5′-*O*-triphenylmethyl-2′-deoxycytidine was obtained by acetylating 2′-deoxycytidine selectively on the 4-amino-group [4] and then introducing the triphenylmethyl group on the 5′-*O*-position in the usual way. The reaction of

[1] Part VIII, M. D. Edge, A. Hodgson, A. S. Jones, and R. T. Walker, *J.C.S. Perkin I*, 1972, 1991.

[2] M. H. Halford and A. S. Jones, *J. Chem. Soc.* (*C*), 1968, 2667.

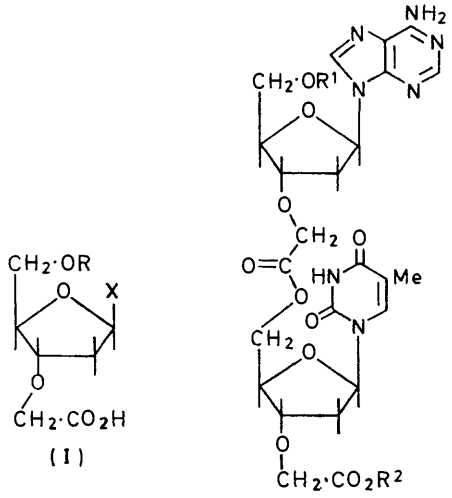
[3] M. D. Edge and A. S. Jones, *J. Chem. Soc.* (*C*), 1971, 1933.

[4] K. A. Watanabe and J. J. Fox, *Angew. Chem.*, 1966, **78**, 589.

this compound with sodium chloroacetate in dimethyl sulphoxide in the presence of three molar proportions of sodium hydride gave a single product in over 50% yield, which was identified as 3′-*O*-carboxymethyl-5′-*O*-triphenylmethyl-2′-deoxycytidine (I; R = Ph_3C, X = cytosine residue) by the fact that it migrated as a monobasic acid upon paper electrophoresis at pH 6·8, had the same u.v. spectrum as 2′-deoxycytidine, and gave only cytosine and no substituted cytosines upon strong acid hydrolysis. It appeared that the *N*-acetyl group was removed during the isolation of the product.

In the case of 2′-deoxyguanosine the use of a number of blocking groups was investigated. The most satisfactory reaction was given with *N*(2)-dimethylaminomethylene-5′-*O*-bis-(4-methoxyphenyl)phenylmethyl-2′-deoxyguanosine. This compound was alkylated with sodium chloroacetate under similar conditions to those already described. The reaction did not go to completion and at the same time compounds containing two or more carboxymethyl groups were easily formed. The highest yield of the required monocarboxylic acid was obtained by the use of 2·5 molar proportions of sodium hydride. During the isolation procedure the blocking groups were partly removed; they were then completely removed by successive treatment with dilute ammonia and dilute acid to give 3′-*O*-carboxymethyl-2′-deoxyguanosine (I; R = H, X = guanine residue) in 30% yield. The compound ran as a monobasic acid on paper electrophoresis at pH 6·8, its u.v. spectrum was similar to that of 2′-deoxyguanosine, and upon acid hydrolysis it gave guanine, but no carboxymethylguanines. Thus the compound contained a carboxymethyl group but not on the guanine ring. It must therefore have been in the 3′-*O*-position, because of the structure of the starting material.

For the synthesis of 3′-*O*-carboxymethyl-2′-deoxyadenosine, the adenine residue of 2′-deoxyadenosine was protected with the dimethylaminomethylene group and the 5′-hydroxy-group with the 4-methoxyphenyldiphenylmethyl group. The dimethylaminomethylene group was removed during the carboxymethylation and so the product which was isolated was 3′-*O*-carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine (I; R = 4MeO·C_6H_4·CPh_2, X = adenine residue) (65% yield), the remainder of the material being non-carboxymethylated and more highly carboxymethylated compounds. The product was identified as in the other two cases. Hydrolysis with dilute acid gave 3′-*O*-carboxymethyl-2′-deoxyadenosine (I; R = H, X = adenine residue), which upon treatment with N-sodium hydroxide at 20° for 15 min gave a small amount (2·9%) of adenine; 2′-deoxyadenosine was completely stable under these conditions. A possible explanation of this may be the participation of the carboxylate group in the displacement of the adenine residue. Molecular models confirmed the stereochemical feasibility of the mechanism and it is known that the adenine residue in nucleosides is susceptible to displacement by alkali although the conditions are much more drastic than those used here.[5]



The condensation of 3′-*O*-carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine to form an analogue of a dinucleotide has been studied. In previous similar work,[1,3] the 2-cyanoethyl group was used to block the carboxy-group. This was selectively removed by the use of potassium t-butoxide in dry dimethylformamide. This procedure caused about 5% hydrolysis of the internucleoside linkages and so there was room for improvement. For this purpose the use of the 2,2,2-trichloroethyl group has been investigated. The model system 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine was converted into its 2,2,2-trichloroethyl ester by condensation with 2,2,2-trichloroethanol in the presence of dicyclohexylcarbodi-imide. This compound was then heated with zinc–copper couple in dimethylformamide;[6] this gave complete conversion into the free carboxylic acid. The protected dinucleotide analogue, 2′,5′-*O*-bis(triphenylmethyl)uridinylacetyl-(3′ ⟶ 5′)-(2′,3′-*O*-isopropylidene)uridine,[7] was unchanged when treated with zinc–copper couple in a similar way.

3′-*O*-Carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine was condensed with 3′-*O*-(2,2,2-trichloroethoxy)carbonylmethylthymidine, obtained by removal of the triphenylmethyl group from the corresponding 5′-*O*-triphenylmethyl derivative, in the presence of dicyclohexylcarbodi-imide to give the fully protected dinucleotide analogue (II; R^1 = 4-MeO·C_6H_4·CPh_2, R^2 = CCl_3·CH_2). Upon alkaline hydrolysis the product gave equimolar amounts of 3′-*O*-carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine and 3′-*O*-carboxymethylthymidine, and the u.v. spectrum showed that the adenine residue was not acylated. This compound was then treated with zinc–copper couple in dimethylformamide to give a compound, which, although it was not completely

[5] A. S. Jones, A. M. Mian, and R. T. Walker, *J. Chem. Soc.* (*C*), **1966**, 692.
[6] E. LeGoff, *J. Org. Chem.*, 1964, **29**, 2048.
[7] A. Hodgson and A. S. Jones, *Tetrahedron Letters*, in the press.

characterised because of the presence of zinc salts, was concluded to be the partly protected dinucleotide analogue (II; R^1 = 4-MeO·C_6H_4·CPh_2, R^2 = H). This conclusion was based upon the fact that the compound was a carboxylic acid, contained a 4-methoxyphenyldiphenylmethyl group, had the expected u.v. spectrum, and differed chromatographically from starting material and the products which would arise from scission of the internucleoside linkage. As no measurable amounts of these scission products were produced during the removal of the 2,2,2-trichloroethyl group we conclude that the use of this group in syntheses of this type is highly specific and superior to use of the 2-cyanoethyl group.

The 4-methoxyphenyldiphenylmethyl group was removed from the partly protected dinucleotide analogue by mild acidic hydrolysis to give 2′-deoxyadenosinyl-(3′ → 5′)-thymidin-3′-ylacetic acid (II; R^1 = R^2 = H). This compound had the expected u.v. spectrum, reacted as a carboxylic acid, and gave equimolar amounts of 3′-*O*-carboxymethyl-2′-deoxyadenosine and 3′-*O*-carboxymethylthymidine upon alkaline hydrolysis. It showed a hyperchromic effect, upon hydrolysis into its two components, of 5·0% at 248 nm and 6·7% at 260 nm, thus indicating that there was base stacking as in natural dinucleotides. The hyperchromic effect for thymidylyl-(3′ → 5′)-2′-deoxyadenosine 3′-phosphate is about 10% [8] and for adenylyl-(3′ → 5′)-uridine about 4%.[9]

EXPERIMENTAL

Chromatography.—For t.l.c., silica gel powder, MN Silica Gel UV/254 (Machery, Nagel, and Co.) and for column chromatography, Keiselgel, 0·05—0·2 mm (70—325 mesh ASTM), type 7734 (Merck) were used. The following solvents were used for chromatography on Whatman No. 1 paper or silica gel (t.l.c.): (1) butan-1-ol–ethanol–water (4 : 1 : 5; organic phase); (2) propan-2-ol–ammonia(*d* 0·88)–water (35 : 3 : 15); (3) propan-2-ol–10N-HCl–water (136 : 33 : 31); (4) butan-2-ol saturated with water; (5) butan-2-ol saturated with water to which 5% formic acid has been added; (6) butan-2-ol–ammonia(*d* 0·88)–water (7 : 1 : 4).

N(4)-*Acetyl*-5′-O-*triphenylmethyl*-2′-*deoxycytidine*.—2′-Deoxycytidine (4 g) was dissolved in dry methanol (400 ml), acetic anhydride (4 ml) was added, and the mixture was boiled under reflux for 5 h with additions of more acetic anhydride (4 ml portions) at hourly intervals. The methanolic solution was evaporated to dryness and the residue was dried *in vacuo* (NaOH) and dissolved in dry pyridine (40 ml). Triphenylmethyl chloride (5·4 g) was added and the mixture kept at room temperature for 1 week. The resulting solution was poured into ice–water (2·5 l) with stirring, and after 2 h the white precipitate was filtered off, washed with water, and air-dried. This crude solid, which contained a small amount of 5′-*O*-triphenylmethyl-2′-deoxycytidine,[10] was dissolved in the minimum volume of chloroform and the mixture was fractionated on silica gel (450 g). The column was eluted with chloroform (1 column volume) and then with ethanol–chloroform (2 : 23) to give the *product* (3·5 g) (Found: C, 70·1; H, 5·5; N, 8·7. $C_{30}H_{29}N_3O_5$ requires C, 70·5; H, 5·7; N, 8·2%), $\lambda_{max.}$ 248 (ε 13,800) and 300 nm, $\lambda_{min.}$ 264 nm (in EtOH).

3′-O-*Carboxymethyl*-5′-O-*triphenylmethyl*-2′-*deoxycytidine*.—The foregoing compound (1·02 g, 2 mmol) was dissolved in dry dimethyl sulphoxide (20 ml), sodium hydride (144 mg, 6 mmol) was added, and the solution was stirred for 1 h. Sodium chloroacetate (350 mg, 3 mmol) was then added and the mixture stirred until all the solid had dissolved (*ca.* 2 h). The solution was then kept at room temperature for 18 h, after which time a white precipitate had formed. [The product at this stage showed $\lambda_{max.}$ 298, $\lambda_{min.}$ 274 nm (EtOH) and a mobility upon paper electrophoresis at pH 6·8 of 4·8 cm kV^{-1} h^{-1}, showing that it was probably *N*(4)-acetyl-3′-*O*-carboxymethyl-5′-*O*-triphenylmethyl-2′-deoxycytidine.] To the mixture were added water (20 ml) and ethanol (10 ml) and the solution was kept at room temperature for 2 h. It was then evaporated to dryness *in vacuo* and the residue dissolved in solvent (2) and applied to a column of silica gel (70 g). Elution with the same solvent gave the *product* as a hydrate (0·52 g) (Found: C, 65·0; H, 5·9; N, 8·0. $C_{30}H_{29}N_3O_6,1{\cdot}5H_2O$ requires C, 65·0; H, 5·8; N, 7·6%), $\lambda_{max.}$ (EtOH) 271 nm (ε 8650). It migrated as a monobasic acid upon electrophoresis at pH 6·8.

5′-O-*Bis*-(4-*methoxyphenyl*)*phenylmethyl*-N(2)-*dimethylaminomethylene*-2′-*deoxyguanosine*.— *N*(2)-Dimethylaminomethylene-2′-deoxyguanosine [11] (452 mg) was stirred with a solution of bis-(4-methoxyphenyl)phenylmethyl chloride (511 mg) in pyridine (20 ml) for 24 h. Methanol (15 ml) was then added and the mixture kept at room temperature for 18 h. Water was then added and the mixture extracted with chloroform (2 × 150 ml). The chloroform layer was washed with water, dried, and concentrated to a small volume. Ether was added until precipitation was complete and the precipitate was filtered off and dried (687 mg). Crystallisation of the white solid from acetone–ethyl acetate–light petroleum (b.p. 60—80°) gave the pure *product* (Found: C, 63·4; H, 6·0; N, 12·8. $C_{34}H_{36}N_6O_6,H_2O$ requires C, 63·5; H, 6·0; N, 13·1%), $\lambda_{max.}$ 276, 282, and 302, $\lambda_{min.}$ 256 and 278 nm (EtOH).

3′-O-*Carboxymethyl*-2′-*deoxyguanosine*.— 5′-*O*-Bis-(4-methoxyphenyl)phenylmethyl-*N*(2)-dimethylaminomethylene-2′-deoxyguanosine (197 mg, 0·35 mmol) was dissolved in dry dimethyl sulphoxide (5 ml) and sodium hydride (44 mg, 0·9 mmol) was added. After 90 min, sodium chloroacetate (46 mg, 0·4 mmol) was added and the mixture kept at room temperature for 5 days. The dimethyl sulphoxide was distilled off *in vacuo* and the resulting solid dissolved in 4N-ammonia. The solution was kept at room temperature for 18 h, then extracted with butan-1-ol (3 × 50 ml); the aqueous layer was evaporated to dryness and the residue extracted with methanol. The combined butan-1-ol and methanol extracts upon evaporation to dryness gave a solid (145 mg) which appeared to be a mixture of mono- and di-carboxymethylated products in a ratio of 9 : 1, and other products. This was dissolved in acetic acid–water (4 : 1) and kept at room temperature for 25 min. The solution was then evaporated to dryness *in vacuo*. To the residue were added ether (40 ml) and water (40 ml); the aqueous layer was separated and extracted with ether, and the aqueous solution was evaporated to dryness. The residue was dissolved in M-ammonia and applied to a column of DEAE

[8] R. L. Sinsheimer, *J. Biol. Chem.*, 1954, **208**, 445.
[9] A. M. Michelson, 'The Chemistry of Nucleosides and Nucleotides,' Academic Press, New York, 1963, p. 446.
[10] A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 1954, 34.
[11] J. Zemlicka and A. Holy, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3159.

cellulose (10 g) which was then eluted with M-ammonia. Deoxyguanosine was eluted first, followed by the required product. This was converted into the pyridinium salt, which was freeze-dried to give 3′-O-*carboxymethyl-2′-deoxyguanosine* as the dihydrate (31 mg) (Found: C, 39·9; H, 5·3; N, 19·6. $C_{12}H_{15}N_5O_6,2H_2O$ requires C, 39·9; H, 5·3; N, 19·4%), $\lambda_{max.}$ 254 and 270sh, $\lambda_{min.}$ 226 (pH 1), $\lambda_{max.}$ 255—265 nm (pH 13). Paper electrophoresis at pH 6·8 showed a single component with a mobility characteristic of a monobasic acid. The compound was heated with 98% formic acid at 175° for 1 h and the product separated by paper chromatography in solvent (3). Only guanine (no carboxymethylguanines) was detected.

N(6)-*Dimethylaminomethylene-5′*-O-(*4-methoxyphenyldiphenylmethyl*)-*2′-deoxyadenosine*. *N*(6)-Dimethylaminomethylene-2′-deoxyadenosine (5·2 g, 17 mmol)[11] and 4-methoxyphenyldiphenylmethyl chloride (5·76 g, 18·7 mmol) were dried together *in vacuo* and then dissolved in dry pyridine (120 ml); the mixture was stirred at room temperature for 2 days. An excess of methanol was then added; the mixture was kept at room temperature for 4 h, added to ice–water (500 ml), and extracted with chloroform (3 × 200 ml). The combined extracts were evaporated to dryness. The residue, dissolved in benzene (100 ml), was added dropwise to cyclohexane (500 ml) to give a precipitate which was filtered off. T.l.c. showed the presence of a considerable amount of 5′-*O*-(4-methoxytriphenylmethyl)-2′-deoxyadenosine in addition to the desired product, so the solid was dissolved in dimethylformamide (100 ml), dimethylformamide dimethyl acetal (12 ml) was added, and the mixture was stirred at room temperature for 18 h. The solution was then evaporated to dryness *in vacuo* and the residue, dissolved in the minimum of chloroform was added dropwise to an excess of light petroleum (b.p. 60—80°). The precipitate was filtered off and dried to give the required *product* (6·3 g) (Found: C, 65·5; H, 6·1; N, 14·1. $C_{33}H_{34}N_6O_3,1·5H_2O$ requires C, 65·5; H, 6·1; N, 13·9%), m.p. 110—114°, $\lambda_{max.}$ 232 and 312 (ε 34,800), $\lambda_{min.}$ 260 nm (EtOH).

3′-O-*Carboxymethyl-5′*-O-(*4-methoxyphenyldiphenylmethyl*)-*2′-deoxyadenosine*.—The foregoing compound (2·4 g, 4·1 mmol) was dried *in vacuo* and dissolved in dry dimethylsulphoxide (24 ml), and sodium hydride (0·20 g, 8·3 mmol) was added. After evolution of hydrogen was complete (90 min), sodium chloroacetate (0·55 g, 4·7 mmol) was added and the solution was kept at room temperature for 3 days. The dimethyl sulphoxide was evaporated off *in vacuo* at 40° and to the residue was added aqueous ammonia (*d* 0·88; *ca.* 20 ml). The mixture was left at room temperature for 18 h in order to remove the dimethylaminomethylene group from the adenine residue. Neutralisation and counter-current distribution in ethyl acetate–water then gave the product, which was isolated as the *triethylammonium salt* (1·5 g) (Found: C, 63·5; H, 6·6; N, 11·8. $C_{38}H_{46}N_6O_6,2H_2O$ requires C, 63·5; H, 6·95; N, 11·7%), m.p. 118—121° (decomp.) $\lambda_{max.}$ 234 and 260 (ε 14,500), $\lambda_{min.}$ 246 nm (EtOH). The compound was shown to be homogeneous by t.l.c. in solvent (1) (R_F 0·31) and by electrophoresis at pH 6·8 (mobility, 2·4 cm kV^{-1} h^{-1}). Hydrolysis with 98% formic acid at 100° for 4 h gave adenine as the only heterocyclic base; no carboxymethyladenines were detected.

3′-O-*Carboxymethyl-2′-deoxyadenosine*.— 3′-*O*-Carboxymethyl-5′-*O*-(4-methoxytriphenylmethyl)2′-deoxyadenosine (166 mg) dissolved in acetic acid–water (4 : 1; 10 ml) was kept at room temperature for 3 h. The solution was then evaporated to dryness and water (100 ml) and chloroform (100 ml) were added to the residue. After shaking, complete dissolution was achieved and the two layers were separated. The aqueous layer was washed once with chloroform (100 ml) and then freeze-dried to give 3′-O-*carboxymethyl-2′-deoxyadenosine* (75 mg) (Found: C, 40·3; H, 5·7. $C_{12}H_{15}N_5O_5,2·7H_2O$ requires C, 40·3; H, 5·7%), $\lambda_{max.}$ 261, $\lambda_{min.}$ 230 nm [ε_{260} 14·9 × 10^3 (pH 5·5)].

Alkaline Hydrolysis of 3′-O-*Carboxymethyl-2′-deoxyadenosine*.—3′-*O*-Carboxymethyl-2′-deoxyadenosine (1 mg) and 2′-deoxyadenosine (1 mg) were separately dissolved in N-sodium hydroxide (1 ml) and the solutions kept at room temperature for 15 min. The hydrolysates were then examined by paper chromatography in solvent (1). The 2′-deoxyadenosine reaction showed only starting material. The carboxylic acid reaction showed starting material and a component which had R_F value and u.v. spectrum (at pH 5 and 13) identical with those of adenine (2·9% of the total product).

3′-O-(2,2,2-*Trichloroethoxycarbonyl*)*methyl-5′*-O-*triphenylmethylthymidine*.— 3′-*O*-Carboxymethyl-5′-*O*-triphenylmethylthymidine (sodium salt) [2] (0·57 g, 1 mmol) was converted into the pyridinium salt by use of the pyridinium form of Zeo-Karb 225 resin. The salt was dried and 2,2,2-trichloroethanol (0·11 ml, 1·1 mmol), dicyclohexylcarbodiimide (2·1 g), and dry pyridine (0·2 ml) were added. The mixture was kept at room temperature for 18 h, pyridine (2 ml) was added, and the precipitate of dicyclohexylurea was filtered off. The solvents were evaporated from the filtrate to give a brown oil to which acetone was added. A small amount of white solid was filtered off, the filtrate was evaporated to dryness, and the residue was dissolved in benzene. This solution was added dropwise to an excess of n-pentane and the precipitate was filtered off to give a yellow solid (0·99 g). [Silica gel t.l.c. in acetone–benzene (1 : 4) showed one u.v. absorbing component (R_F 0·49).] This was dissolved in benzene and applied to a column of silica gel (70 g), which was then eluted with acetone–benzene (1 : 4) to give the *product* (0·35 g) (Found: C, 58·8; H, 4·5; Cl, 15·8; N, 4·1. $C_{33}H_{31}Cl_3N_2O_7$ requires C, 58·8; H, 4·6; Cl, 15·8; N, 4·2%), $\lambda_{max.}$ 232 and 266 (ε 9500), $\lambda_{min.}$ 242 nm (EtOH).

3′-O-(2,2,2-*Trichloroethoxycarbonyl*)*methylthymidine*.— The foregoing compound (0·51 g) dissolved in acetic acid–water (4 : 1; 10 ml) was heated at 100° for 15 min. The solution was then evaporated to dryness to give a white solid. [T.l.c. in acetone–benzene (1 : 4) showed that a new component (R_F 0·1) had been formed.] The solid was dissolved in benzene and applied to a column of silica gel (40 g). The required *compound* was eluted with acetone–benzene (1 : 4) and then acetone–benzene (7 : 3) to give a white solid (0·24 g) (Found: C, 38·7; H, 3·9; Cl, 24·5; N, 6·4. $C_{14}H_{17}Cl_3N_2O_7$ requires C, 38·9; H, 3·9; Cl, 24·7; N, 6·5%), $\lambda_{max.}$ 267, $\lambda_{min.}$ 235 (pH 5·0), $\lambda_{max.}$ 267, $\lambda_{min.}$ 247 nm (pH 13) [ε_{267} (pH 13)/ε_{267} (pH 5·0) = 0·7].

Selective Removal of the 2,2,2-*Trichloroethyl Group*.— (*a*) 3′-*O*-(2,2,2-Trichloroethoxycarbonyl)methyl-5′-*O*-triphenylmethylthymidine (5 mg) was dissolved in dry dimethylformamide (5 ml) and freshly prepared zinc–copper couple [6] (20 mg) added. The mixture was vigorously shaken at 50° for 1 h, then centrifuged, and the supernatant liquid was examined by t.l.c. in acetone–ethyl acetate (1 : 49) and by paper chromatography in solvent (1). In both systems one component, identical with 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine, was detected.

(*b*) 2′,5′-*O*-Bis(triphenylmethyl)uridinylacetyl-(3′ → 5′)-(2′,3′-*O*-isopropylidene)uridine [7] was treated with the

zinc–copper couple and the product examined by chromatography as in (*a*). The only detectable u.v.-absorbing component was unchanged starting material.

2,2,2-*Trichloroethyl* 5′-O-(4-*Methoxyphenyldiphenylmethyl*)-2′-*deoxyadenosinylacetyl*-(3′ ⟶ 5′)-*thymidin*-3′-*ylacetate* (II).—The triethylammonium salt of 3′-*O*-carboxymethyl-5′-*O*-(4-methoxytriphenylmethyl)-2′-deoxyadenosine (16 mg, 0·024 mmol) was converted into the pyridinium salt in the usual way and dried; 3′-*O*-(2,2,2-trichloroethoxycarbonyl)methylthymidine (130 mg, 0·3 mmol) was added. The mixture was dissolved in dry pyridine; the solvent was then evaporated off and the residue rigorously dried *in vacuo*. Dicyclohexylcarbodi-imide (48 mg, 0·23 mmol) and dry pyridine (0·5 ml) were then added and the mixture was kept for 18 h at room temperature. It was then evaporated to dryness, acetone was added to the residue, and the precipitate of dicyclohexylurea was filtered off. The filtrate was evaporated to a small volume and added to a large volume of cyclohexane. The solid so formed (38 mg) was filtered off, dissolved in a minimum volume of ethyl acetate, and applied to a column of silica gel (5 g). Elution with acetone–ethyl acetate (1 : 49) removed all unchanged 3′-*O*-(2,2,2-trichloroethoxycarbonyl)methylthymidine; elution with acetone–ethyl acetate (2 : 3) then gave the *product* (8·5 mg) [Found (after correction for 2% water): C, 55·8; H, 5·1; N, 9·3. $C_{46}H_{46}Cl_3N_7O_{12}$ requires C, 55·5; H, 4·7; N, 9·8%], $\lambda_{max.}$ 234 and 262, $\lambda_{min.}$ 226 and 243—244 nm (EtOH). The compound was chromatographically homogeneous in solvent (1) (R_F 0·80) and in acetone–ethyl acetate (7 : 13) (R_F 0·53).

Hydrolysis of this compound with 0·1N-NaOH at 20° gave an equimolar mixture of 3′-*O*-carboxymethylthymidine and 3′-*O*-carboxymethyl-5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosine. Acidic hydrolysis with 98% formic acid at 175° for 60 min gave adenine and thymine in the molar ratio 1 : 1·03.

2′-*Deoxyadenosinylacetyl*-(3′ ⟶ 5′)-*thymidin*-3′-*ylacetic Acid*.—The foregoing compound (47 mg) was dissolved in dry dimethylformamide (20 ml); zinc–copper couple (400 mg) was added and the suspension vigorously shaken at 50° for 1 h. T.l.c. in solvent (1) showed that no starting material (R_F 0·8) remained and that only one u.v.-absorbing component (R_F 0·27) had been produced. The bulk of the suspension was filtered and to the filtrate an equal volume of water was added. The precipitate was centrifuged off, washed with water, and dried. The compound gave with perchloric acid and acetic acid the yellow colour characteristic of a 4-methoxyphenyldiphenylmethyl derivative. It ran as a single u.v.-absorbing component on t.l.c. in solvent (4) (R_F 0·34) and contained a free carboxy-group as shown by the use of a Bromocresol Green spray. It was concluded, therefore, that the compound was 5′-*O*-(4-methoxyphenyldiphenylmethyl)-2′-deoxyadenosinylacetyl-(3′ ⟶ 5′)-thymidin-3′-ylacetic acid. Elemental analysis showed, however, that a considerable amount of a zinc-containing impurity was present. Because of the difficulty of removing this impurity without decomposing the compound, the next stage was carried out on the impure product. A solution in acetic acid–water (4 : 1; 4 ml) was kept at room temperature for 18 h. It was then evaporated to dryness at 30° and to the residue were added water (2 ml), chloroform (2 ml), and 8-hydroxyquinoline (100 mg). The mixture was shaken and centrifuged, and the aqueous layer was separated from the chloroform layer and the yellow solid. The aqueous layer was washed (2×) with chloroform and evaporated to dryness. The residue was crystallised from aqueous methanol at −40° to give the *product* (7 mg) [Found: (after correction for 7·6% ash): C, 46·3; H, 5·0. $C_{23}H_{27}N_7O_{11},H_2O$ requires C, 46·4; H, 4·9%], $\lambda_{max.}$ 261 nm (pH 5·0) (ε 19,800). The compound was shown to be homogeneous by t.l.c. in solvents (4) (R_F 0·18), (5) (R_F 0·37), and (6) (R_F 0·40), and reacted as a carboxylic acid to the Bromocresol Green spray. It was hydrolysed with aqueous triethylamine at room temperature for 6 h; the products were separated by t.l.c. in solvent (5) and identified and estimated by u.v. spectroscopy. 3′-*O*-Carboxymethylthymidine (R_F 0·71) and 3′-*O*-carboxymethyl-2′-deoxyadenosine (R_F 0·46) were present in the molar ratio 0·97 : 1.

The hyperchromicity of the compound was determined by measuring the change in optical density on hydrolysis of the internucleoside linkage with 0·1N-sodium hydroxide. There was a rise of 5·0% at 248 nm and of 6·7% at 260 nm (after making allowance for the difference in extinction coefficient of the products at pH 5 and 13).

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