Synthetic Analogues of Polynucleotides. Part XII.[1] Synthesis of Thymidine Derivatives containing an Oxyacetamido- or an Oxyformamido-linkage instead of a Phosphodiester Group

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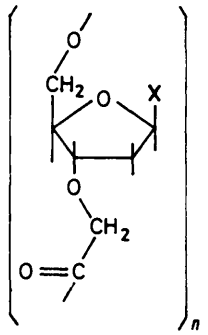
The dinucleotide analogue thymidinylacetamido-[3'(*O*) ⟶ 5'(*C*)]-5'-deoxythymidine was synthesised. The compound showed a hypochromic effect of about 10% at 268 nm. At pH 6·0—7·5 and 20° the compound was stable; in M-sodium hydroxide at 37° it was 50% hydrolysed after 3·4 h. 5'-Azido-5'-deoxythymidine was converted into its 3'-*O*-carboxymethyl derivative and this was reduced to give 5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine. Attempts to polymerise this compound were unsuccessful because of the ready formation of a lactam. Thymidinylformamido-[3'(*O*) ⟶ 5'(*C*)]-5'-deoxythymidine was also synthesised. It showed a hypochromic effect of about 8% at 267 nm and was more stable to alkali than the acetamido-compound.

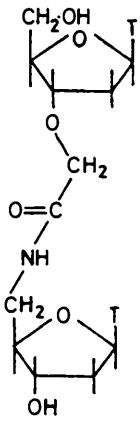
ANALOGUES of oligo- and poly-nucleotides of structure (I) in which the nucleosides are joined by acetate ester linkages have been synthesised and shown to interact with natural polynucleotides [2] and in some cases to inhibit the binding of tRNA to ribosomes in the presence of messenger RNA [2e,3] and to interfere with protein biosynthesis in a cell-free system.[2e] Analogues containing this linkage suffer from two disadvantages, namely the relative instability of the linkage and the low solubility of the compounds under physiological conditions; both factors made it difficult to test the compounds for biological activity. The synthesis of analogues in which the ribose carbon atoms of nucleosides were joined by either an oxyacetamido- or an oxyformamido-linkage was attempted because it appeared that such compounds would not suffer from these disadvantages. Molecular models showed that the oxyacetamido-linkage is as favourable with regard to the internucleoside distance as is the acetate ester linkage, both giving distances close to that found in polynucleotides, whereas the oxyformamido-linkage gives a distance which is about 0·5 Å shorter.

The dinucleotide analogue thymidinylacetamido-[3'(*O*) ⟶ 5'(*C*)]-5'-deoxythymidine (II) was synthesised by condensation of 3'-*O*-carboxymethyl-5'-*O*-triphenylmethylthymidine [2a] with 5'-amino-5'-deoxythymidine [4] in the presence of dicyclohexylcarbodi-imide and removal of the triphenylmethyl group under acidic conditions. Its structure was established by elemental analysis, n.m.r. spectroscopy, and the fact that the compound gave 3'-*O*-carboxymethylthymidine and 5'-amino-5'-deoxythymidine upon alkaline hydrolysis. As expected, it was stable under physiological conditions, no hydrolysis being detected after 7 days at pH 6—7·5 and 20°. At pH 14 and 37° the half life was 3·4 h. The compound was readily soluble in water, in contrast to similar compounds containing the acetate ester linkage. A hypochromic effect at 268 nm of about 10% indicated the occurrence of base stacking.

These properties indicated the desirability of synthesising a polymer of structure (III). For this purpose,

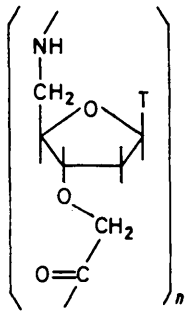


(I) X = purine or pyrimidine residue

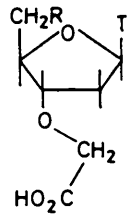


(II) T = thymine residue

5'-azido-5'-deoxythymidine was treated with sodium chloroacetate in dimethyl sulphoxide in the presence of 2·4 molar proportions of sodium hydride. Under



(III)



(IV)

similar conditions 5'-*O*-triphenylmethylthymidine gives almost exclusively the 3'-*O*-carboxymethylated product with yields of about 95% and less than 1% of bis-

[1] Part XI, A. Hodgson and A. S. Jones, *Tetrahedron Letters*, 1972, 5349.

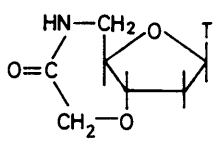
[2] (*a*) M. H. Halford and A. S. Jones, *J. Chem. Soc.* (*C*), 1968, 2667; (*b*) M. D. Edge and A. S. Jones, *ibid.*, 1971, 1933; (*c*) A. Hodgson, A. S. Jones, and R. T. Walker, *J.C.S. Perkin I*, 1972, 1991; (*d*) M. D. Edge, A. Hodgson, A. S. Jones, M. MacCoss, and R. T. Walker, *ibid.*, 1973, 290; (*e*) A. S. Jones, M. MacCoss and R. T. Walker, *Biochim. Biophys. Acta*, 1973, 294, 365.

[3] G. J. Cowling, A. S. Jones, and R. T. Walker, *Biochim. Biophys. Acta*, 1971, 254, 452.

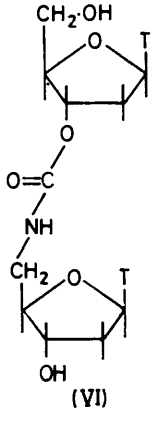
[4] J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, 1962, **27**, 3045.

carboxymethylated material. In the present case the reaction was far less selective; although the major product (60—70%) was a monocarboxymethylated compound, as determined by paper electrophoresis, 20—25% of starting material remained and about 10% of a biscarboxymethylated compound was produced. The major product, isolated with difficulty, was identified as 5′-azido-3′-*O*-carboxymethyl-5′-deoxythymidine (IV; R = N_3) from the i.r. spectrum, which showed the presence of an azide group, and from the fact that upon strong acidic hydrolysis, thymine was produced, not 3-carboxymethylthymine. The reason for the lack of selectivity in this carboxymethylation reaction as compared with that of 5′-*O*-triphenylmethylthymidine may be that in the latter the bulkier substituent at C-5′ prevents reaction at the nitrogen at position 3.

Catalytic hydrogenation readily converted this azido-compound into the 5′-amino-compound (IV; R = NH_2). Attempts to polymerise this amine to give (III) under similar conditions to those used to obtain the analogues of structure (I) failed, however; the product was mainly the lactam (V) and no polymer was formed. The structure (V) was assigned on the basis of the high R_F value of the compound upon t.l.c., its elemental analysis, and its mass spectrum. Attempts to polymerise compound (V) under similar conditions to those used for the polymerisation of caprolactam were also unsuccessful; the compound appeared to be remarkably stable.



(V)



(VI)

As an example of a dinucleotide analogue containing the oxyformamido-linkage, thymidinylformamido-[3′(*O*) ⟶ 5′(*C*)]-5′-deoxythymidine (VI) was synthesised by treating 2,2,2-trichloroethyl 5′-*O*-triphenylmethylthymidine 3′-carbonate with 5′-amino-5′-deoxythymidine and removing the triphenylmethyl group by acidic hydrolysis. This compound is analogous to the dinucleoside carbonates synthesised by Tittensor.[5] The structure (VI) was established by n.m.r. spectroscopy and by hydrolysis to the constituent nucleoside units. The compound was slightly less soluble in water and more stable to alkaline hydrolysis than (II), and showed a hypochromicity of about 8% at 267 nm which indicated the occurrence of base stacking.

A number of attempts were made to synthesise polymers containing the oxyformamido-linkage, but without success. It appeared that a six-membered lactam was produced instead, but this was not characterised.

These results show that although analogues containing an oxyacetamido- or an oxyformamido-linkage have the required stability and solubility, alternative methods for their synthesis are required.

EXPERIMENTAL

N.m.r. Spectra.—Except where stated these were obtained with a Perkin-Elmer R14 spectrometer (100 MHz) for solutions in $(CD_3)_2SO$. In the assignment of signals for compounds containing two nucleoside units no distinction between these units is made.

5′-O-*Triphenylmethylthymidinylacetamido*-[3(O) ⟶ 5′-(C)]-5′-*deoxythymidine*.—To a dry solution of 3′-*O*-carboxymethyl-5′-*O*-triphenylmethylthymidine pyridinium salt (0·80 mmol) [2a] and 5′-amino-5′-deoxythymidine (0·84 mmol) [4] in pyridine (5 ml) was added dicyclohexylcarbodiimide (8·2 mmol), and the mixture was kept at 20° for 4 days. The white solid was filtered off and washed with pyridine, and the combined filtrate and washings were evaporated to dryness. Final traces of pyridine were removed by repeated co-evaporation with acetone. To the resulting solid were added chloroform (80 ml) and water (80 ml). The chloroform layer and chloroform extracts of the aqueous layer were combined, dried, and evaporated to a small volume. Addition of light petroleum (300 ml) gave a white solid which was filtered off and chromatographed on silica gel (80 g) with ethanol–chloroform (1 : 9) as eluant to give the *product* (334 mg, 53%), m.p. 146—149°; $\lambda_{max.}$ (EtOH) 267 nm (ε 18,400), $\lambda_{min.}$ 242 nm (Found: C, 63·9; H, 5·7; N, 8·8. $C_{41}H_{43}N_5O_{10}$ requires C, 64·3; H, 5·7; N, 9·1%); homogeneous upon t.l.c. (R_F 0·6 in ethanol–chloroform, 2 : 23); δ 7·50—7·45 (17H, m, Ph_3C and H-6), 6·2—6·1 (2H, m, H-1′), and 1·80 and 1·45 (6H, s, Me).

Thymidinylacetamido-[3′(O) ⟶ 5′(C)]-5′-*deoxythymidine* (II).—The foregoing compound (325 mg, 0·42 mmol) was dissolved in 98% formic acid (10 ml) and kept at 20° for 5 min. The solution was then evaporated to dryness and residual formic acid removed by co-evaporation with ethanol. To the residue were added chloroform (40 ml) and water (40 ml). The aqueous layer was separated, washed with chloroform (3 × 40 ml), and freeze-dried to give the *product* (166 mg, 76%), m.p. 137—142°; λ_{max} (H_2O) 268 nm (ε 18,000), $\lambda_{min.}$ 237 nm (Found: C, 50·7; H, 5·7; N, 13·1. $C_{22}H_{29}N_5O_{10}$ requires C, 50·5; H, 5·6; N, 13·4%); homogeneous by t.l.c. (R_F 0·55 in acetonitrile–water, 17 : 3); δ 11·25br (2H, H-3), 7·95br (1H, 5′-NH), 7·70 and 7·50 (2H, s, H-6), 6·2—6·1 (2H, m, H-1′), 5·4—5·0 (2H, m, 3′- and 5′-OH), 4·3—4·1 (2H, m, H-3′), 4·05—2·1 (10H, m, H-2′, H-4′, H-5′, O·CH_2·CO), and 1·78 (6H, s, Me).

Stability of the internucleoside linkage. Samples of the compound were subjected to the following reagents: M-hydrochloric acid; 0·05M-sodium phosphate, pH 6·0; 0·05M-sodium cacodylate, pH 7·0; 0·05M-Tris buffer, pH 7·5; M-sodium hydroxide, all at 20°. The extent of hydrolysis was determined by t.l.c. followed by u.v. spectrophotometry. Except for the last case there was no hydrolysis even after 7 days. In the last case, 75% hydrolysis had occurred after 24 h. Treatment of the compound with

[5] J. R. Tittensor, *J. Chem. Soc.* (*C*), 1971, 2656.

M-sodium hydroxide at 37° resulted in 50% hydrolysis in 3·4 h.

Hypochromicity. The hypochromicity value obtained by direct measurement of the extinction coefficient at 268 nm was 7 ± 3%, whereas that obtained by hydrolysing the compound into its two ' nucleoside units ' and determining the increase in u.v. absorption at 268 nm was 13 ± 4%.

5'-Azido-3'-O-carboxymethyl-5'-deoxythymidine.—To a solution of sodium hydride (57 mg, 2·4 mmol) in dry dimethyl sulphoxide was added 5'-azidothymidine (267 mg, 1·0 mmol),[4] and the mixture was stirred for 15 min. Sodium chloroacetate (127 mg, 1·1 mmol) was then added and the mixture was stirred at 20° for 18 h. Ethanol–water (1 : 1) was added and the pH was adjusted to 7 with dilute hydrochloric acid. The solution was evaporated to dryness and the residue chromatographed on silica gel (40 g). Elution with acetonitrile–water (19 : 1) gave starting material; subsequent elution with acetonitrile–water (17 : 3) gave the *product* as the *sodium salt* (R_F 0·3—0·4 in this solvent). This was isolated as two fractions, one (234 mg) was only 88% pure, the other (70 mg) was pure; m.p. 135—145 (decomp.) (total yield 79%), $\lambda_{max.}$ (H_2O) 268 nm (ε 9800), $\lambda_{min.}$ 236 nm (Found: C, 41·8; H, 4·3; N, 19·9. $C_{12}H_{14}N_5NaO_6$ requires C, 41·5; H, 4·1; N, 20·2%); $\nu_{max.}$ (KBr) 2100 cm^{-1} (azide); behaves as a monocarboxylic acid upon paper electrophoresis; δ 7·50 (1H, s, H-6), 6·18 (1H, m, H-1'), 4·10 (3H, m, H-3' and $O{\cdot}CH_2{\cdot}CO$), 3·65—2·20 (4H, m, H-2', H-4', H-5'), and 1·80 (3H, s, Me).

The impure material was satisfactory for the following reaction.

5'-Amino-3'-O-carboxymethyl-5'-deoxythymidine Hydrochloride.—The foregoing azido-compound (3·25 mmol) was dissolved in dry methanol (200 ml) and hydrogenated at 1 atm for 4 h at 20° over platinum oxide (350 mg). The product obtained by filtration and evaporation was dissolved in water (10 ml) and applied to a column (20 cm × 2·5 cm diam.) of Amberlite 120 (H^+) resin. The column was eluted with water (100 ml) and then with ammonia solution (pH 10·9). The ammoniacal eluate was evaporated to dryness, the residue was dissolved in water (50 ml), and the solution was acidified with hydrochloric acid and freeze-dried to give the *product* (696 mg, 64%), m.p. (under N_2) 178° (Found: C, 42·8; H, 5·5; Cl, 10·4; N, 12·6. $C_{12}H_{18}ClN_3O_6$ requires C, 42·9; H, 5·4; Cl, 10·6; N, 12·5%), $\lambda_{max.}$ (H_2O) 267 nm (ε 9700), $\lambda_{max.}$ 236 nm; homogeneous by t.l.c. in butan-2-ol–water (7 : 5; organic phase) (R_F 0·15) and in acetonitrile–water (17 : 3) (R_F 0·06); δ (D_2O) 7·45 (1H, s, H-6), 6·18 (1H, m, H-1'), 5·00 (1H, m, H-3'), 4·15 (2H, s, $O{\cdot}CH_2{\cdot}CO$), 4·28—2·50 (4H, m, H-2', H-4', H-5'), and 1·88 (3H, s, Me).

Attempted Polymerisation of 5'-Amino-3'-O-carboxymethyl-5'-deoxythymidine.—A number of systems were tried: dicyclohexylcarbodi-imide in pyridine and in dimethylformamide; a water-soluble carbodi-imide in water; the mixed anhydride method using ethyl chloroformate and triethylamine; tetraethyl pyrophosphate and triethylamine in diethyl phosphite. In no case was any polymer detected but in each case a lactam was produced. The following procedure gave the best yield of this.

To a solution of 5'-amino-3'-*O*-carboxymethyl-5'-deoxythymidine hydrochloride (125 mg), in dry diethyl phosphite (1 ml) were added triethylamine (30 μl) and tetraethyl pyrophosphate (0·2 ml), and the mixture was heated at 100° for 30 min. T.l.c. showed the presence of a major product with an R_F 0·8 in acetonitrile–water (17 : 3) and R_F 0·5 in chloroform–ethanol (9 : 1). The mixture was reduced in volume as much as possible by evaporation *in vacuo*. To the residue were added chloroform (20 ml) and water (20 ml). The major product was extracted into the chloroform layer, which was dried and evaporated to dryness. The oily residue was chromatographed on a silica column (20 g). Elution with ethanol–chloroform (3 : 97) removed traces of diethyl phosphite; subsequent elution with ethanol–chloroform (3 : 22) gave, after concentration and precipitation with light petroleum, *5'-amino-3'-O-carboxymethyl-5'-deoxythymidine lactam* (V) (27 mg, 26%), m.p. 245°, $\lambda_{max.}$ 263 nm (ε 9250), $\lambda_{min.}$ 236 nm (Found: C, 51·0; H, 5·4; N, 14·6. $C_{12}H_{15}N_3O_5$ requires C, 51·2; H, 5·4; N, 14·9%), *m/e* 281, 156 (base + H), and 126 (sugar).

The compound was heated to 210° for 2·5 h under nitrogen in the presence of a trace of 5'-amino-5'-deoxythymidine. T.l.c. showed little change.

5'-O-Triphenylmethylthymidinylformamido-[3'(O) ⟶ 5'(C)]-5'-deoxythymidine.—2,2,2-Trichloroethyl 5'-*O*-triphenylmethylthymidine 3'-carbonate (165 mg) [5] was dissolved in dry dimethylformamide (0·5 ml), 5'-amino-5'-deoxythymidine (73 mg) was added, and the mixture was stirred at 20° for 2 days. It was then evaporated to dryness to give a solid which was crystallised from ethanol–chloroform to give the *product* (82 mg, 44%), m.p. 218—222°, $\lambda_{max.}$ (EtOH) 266 nm (ε 18·500), $\lambda_{min.}$ 242 nm (Found: C, 63·1; H, 5·7; N, 9·2. $C_{40}H_{41}N_5O_{10}$ requires C, 63·9; H, 5·5; N, 9·3%); homogeneous by t.l.c. in chloroform–ethanol (9 : 1) (R_F 0·5); δ 11·30br (2H, H-3), 7·50 (1H, s, H-6), 7·45 (16H, m, Ph_3C and H-6), 6·2 (2H, m, H-1'), 5·3—5·2 (2H, m, H-3', 3'-OH), 4·10 (1H, m, H-3'), 4·85—2·1 (8H, m, H-2', H-4', H-5'), and 1·79 and 1·45 (6H, s, Me).

Thymidinylformamido-[3'(O) ⟶ 5'(C)]-5'-deoxythymidine (VI).—The foregoing compound (211 mg) was dissolved in 98% formic acid (10 ml) and the solution was kept at 20° for 10 min. The formic acid was then removed by evaporation and co-evaporation with ethanol and to the residue were added chloroform (30 ml) and water (30 ml). The aqueous layer was washed with chloroform and then freeze-dried to give the *product* (127 mg, 87%), m.p. 130°, $\lambda_{max.}$ (H_2O) 267 nm (ε 17,300) (Found: C, 48·4; H, 5·3; N, 13·7. $C_{21}H_{27}N_5O_{10},0{\cdot}5H_2O$ requires C, 48·6; H, 5·3; N, 13·5%); homogeneous by t.l.c. in butan-2-ol–water (7 : 5; organic phase) (R_F 0·7) and in acetonitrile–water (17 : 3) (R_F 0·65); δ 11·3br (2H, H-3), 8·25br (1H, 5'-NH), 7·72 and 7·48 (2H, s, H-6), 6·1 (2H, m, H-1'), 5·1 (2H, m, H-3', 3'-OH), 4·1 (1H, m, H-3'), 7·9—6·1 (9H, m, H-2', H-4', H-5', and H_2O), and 1·80 (6H, s, Me).

A hypochromicity at 267 nm of 9 ± 3% was estimated by direct measurement of the extinction coefficient and a value of 7 ± 4% was obtained by the alkaline hydrolysis procedure. No detectable hydrolysis of the compound had occurred at 20° after 20 h in M-hydrochloric acid or at pH values of 6·0, 7·0, and 7·5. In M-sodium hydroxide at 37°, 50% hydrolysis had occurred after 8 h. The products were identified as thymidine and 5'-amino-5'-deoxythymidine.

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