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SYNTHETIC ANALOGUES OF POLYNUCLEOTIDES, PART XI. THE SYNTHESIS AND PROPERTIES OF URIDINYLACETYL-(3'→5')-URIDINE, AN ANALOGUE OF URIDYLYL-(3'→5')-URIDINE

A. Hodgson and A. S. Jones

Chemistry Department, The University of Birmingham, Birmingham. B15 2TT

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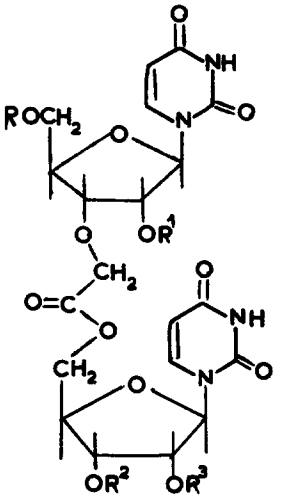
Analogues of oligodeoxyribonucleotides in which the phosphodiester linkages of the natural compounds have been replaced by acetate ester linkages (.CH_2CO_2.) and in which the base residues were either thymine or adenine, have been synthesised and shown to interact with their complementary polynucleotides in solution[1,2,3]. The adenine-containing analogue has been shown to inhibit the binding of phe-$tRNA^{Phe}$ to ribosomes in the presence of polyuridylic acid and to inhibit the biosynthesis of polyphenylalanine in an Escherichia coli cell-free system containing polyuridylic acid as messenger[3,4]. Because of the more direct involvement of polyribonucleotides in protein biosynthesis it was of interest to synthesise similar analogues of oligoribonucleotides.

In the case of the deoxyribonucleotide analogues, 3'-O-carboxymethyldeoxyribonucleosides were polymerised. For the ribonucleotide analogues it was decided to adopt a similar arrangement despite the possibility of an easier synthesis of 5'-O-carboxymethylribonucleosides[1,5]. This was because if the latter were polymerised migration of the internucleoside linkage from the 3'-O-position to the 2'-O-position would be possible. Initially the synthesis of a dinucleotide analogue was undertaken to determine whether the internucleoside linkage was stable enough for biological tests to be carried out on this type of compound.

2',5'-O-Bis(triphenylmethyl)uridine was treated with sodium chloroacetate in the presence of 2.5 molecular proportions of sodium hydride to give 3'-O-carboxymethyl-2',5'-O-bis(triphenylmethyl)uridine (I). The structure of this compound was established by elemental analysis, U.V. spectroscopy, behaviour upon chromatography and electrophoresis and by the fact that upon strong acid hydrolysis it gave uracil but no carboxymethyluracil. Compound I was condensed with 2',3'-O-isopropylideneuridine to give II (R,R^1 = triphenylmethyl; R^2,R^3 = isopropylidene).



II

The structure of this compound followed from the fact that it gave equimolar amounts of the two units upon alkaline hydrolysis and that the n.m.r. spectrum was consistent with II. The protecting groups were removed from this compound by mild acid hydrolysis to give uridinylacetyl-(3'→5')-uridine (II,R,R^1,R^2,R^3 = H) which was characterised by hydrolysis into its constituent units, U.V. absorption spectrum and n.m.r. spectrum. The compound was only about 90% pure, however, as shown by its elemental analysis and extinction coefficient. Its instability precluded further purification, however. It showed a hyperchromism of about 4% upon hydrolysis into its constituent units, a value similar to that shown by uridylyl(3'→5')uridine[6].

Uridinylacetyl-(3'→5')uridine was more easily hydrolysed than a simular deoxyribonucleoside derivative. Thus the half life of the former at 20° at pH 5.0 was only 4 hr. whereas that of thymidinylacetyl-(3'→5')-thymidinylacetyl-(3'→5')uridine[7] was about 127 hr. at pH 6.0 at 37°. This decreased stability could have been due to the participation of the 2' hydroxyl group in the scission of the internucleoside linkage. The instability of these compounds appears to render them unsuitable for biological testing.

Experimental

3'-O-Carboxymethyl-2',5'-O-bis(triphenylmethyl)uridine. 2',5'-O-Bis(triphenylmethyl)uridine[8] (7.0g) in dimethylsulphoxide (60ml) was stirred with sodium hydride (0.58g) for 2 hr. and then with sodium chloroacetate (1.1g) for 3 days at 20°. The products were fractionated by chromatography on silica gel using chloroform-ethanol mixtures as eluant to give finally the required product as the sodium salt (4.3g). An analytically pure sample was obtained as the free acid m.p. 213-214° (Found: C,74.5; H,5.5; N,3.4. $C_{49}H_{42}N_2O_8$ requires C,74.8; H,5.3; N,3.6%) λ_{max} 263.5nm; λ_{min} 245nm. $\varepsilon_{263.5}$ 9.1 x 10^3 (ethanol). Hydrolysis with 72% perchloric acid at 100° gave uracil but no 3-carboxymethyluracil.

2',5'-O-Bis(triphenylmethyl)uridinylacetyl-(3'→5') (2',3'-O-isopropylidene)uridine. The foregoing compound (508mg, as the pyridine salt) was condensed with 2',3'-O-isopropylidenuridine (900mg) in pyridine (6ml) and dimethylformamide (0.5ml) in the presence of dicydohexylcarbodiimide (1.5g) at 20° for 2 days and the product isolated by a procedure similar to those described previously for similar compounds[1,7]. The required compound was obtained as a white solid (555mg) which was homogeneous by t.l.c. A crystalline sample was obtained from ethanol. m.p. 157.5-159° (Found: C,68.4; H,5.0; N,5.3. $C_{61}H_{56}N_4O_{13}H_2O$ requires C,68.4; H,5.4; N,5.2%) λ_{max} 260.5nm; ε_{max} 17.6 x 10^3. The n.m.r. spectrum confirmed the presence of water and was consistent with the structure. In particular signals for the N-H protons and those at H-5, H-6, H-1' and $-CH_3$ were as expected. The absence of H-5' at the position obtained for uridine was also consistent with results on similar compounds[2]. Alkaline hydrolysis gave equimolar amounts of the constituent units.

Uridinylacetyl-(3'→5')uridine. The foregoing compound (125mg) was dissolved in formic acid -water (9:1) and kept at 20° for 2.5 hr. After removal of triphenylmethanol the product was purified by chromatography on silica gel, but owing to its instability the recovery was only 20%. It was finally obtained in a chromatographically pure form except for a trace of uridine. The compound was only 90% pure, however, (Found: C,45.4; H,4.9; N,9.3. $C_{20}H_{24}N_4O_{13}$ requires C,45.5; H,4.6; N,10.6%) λ_{max} 263nm. ε_{max} 18.0 x 10^3 (water). N.m.r. showed NH, H-6, H-5 and H-1' as expected. Two of the H-5' protons were in the same position as those in uridine; the other two were shifted as expected for the uridine residue substituted at the 5'-O-position[2]. Upon hydrolysis with alkali the extinction coefficient increased by 3.8%. Equimolar amounts of uridine and 3'-O-carboxymethyluridine were formed.

Stability of the Internucleoside linkage. The above compound was dissolved in water and kept at

20°. At intervals the amount of hydrolysis was determined by t.l.c. and spectrophotometry. The % hydrolysis was 33, 40 and 45 after 1, 2 and 3 hr. respectively.

Acknowledgements

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