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SYNTHETIC ANALOGUES OF POLYNUCLEOTIDES—XIII

THE RESOLUTION OF DL-β-(THYMIN-1-YL)ALANINE AND POLYMERISATION OF THE β-(THYMIN-1-YL)ALANINES

J. D. BUTTREY, A. S. JONES and R. T. WALKER
Chemistry Department, The University of Birmingham, Birmingham, B15 2TT

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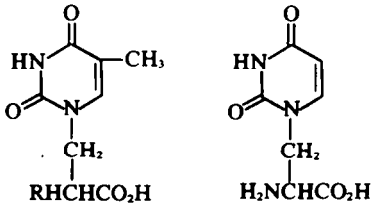
Abstract—DL-β-(Thymin-l-yl)alanine has been resolved into D(+) and L(−) forms. The pure D(+) form was obtained by fractional crystallisation of the (+)α-methylphenylethylamine salts of the α-N-formyl derivatives. The pure L(−) isomer was obtained on a small scale by chromatography of the same salts. The optically active amino acids and the DL-mixture were polymerised by the mixed anhydride procedure to give polymers which showed no evidence of base stacking or of interaction with polyadenylic acid. The molecular weights of the polymers were in the range 2–4 × 10^3. These were determined by end group assay which involved the synthesis of α-*N*-(2,4-dinitrophenyl)-DL-β-(thymin-l-yl)alanine as a standard.

Previous work has shown that a number of synthetic analogues of polynucleotides in which the sugar-phosphodiester backbone of the molecules has been replaced by backbones of considerably different structure, interact with polynucleotides. These synthetic analogues include copolymers of 5'-O-acryloyluridine with acrylamide,[1,2] polymers formed by the reaction of polyacrylic acid hydrazide with ribonucleoside dialdehydes[3] and a 1-carboxymethylthymine derivative of dextran.[4] In addition Pitha *et al.* have synthesised polyvinyl derivatives of purines and pyrimidines some of which interact with their complementary polynucleotides.[5–8] The hybrid of poly (1-vinylcytosine) with polyinosinic acid has been shown to have antiviral activity.[9] These results indicate that significant interaction with polynucleotides can be obtained with analogues which are structurally substantially different from polynucleotides. In view of this it appeared to us to be of interest to synthesise polypeptides in which the side chains of the amino acids are the purine and pyrimidine bases of the nucleic acids as another example of this type of compound. In this connection it should be noted that the spacing between alternate amino acids side chains in a fully extended polypeptide chain (7·27A) is close to that of the internucleoside spacing in polynucleotides (7·1A). Synthesis of the required amino acids, namely β-purin-9-yl- and β-pyrimidin-l-yl alanines have been described by us[10] and by others,[11–14] as have methods for the synthesis of small peptides of these amino acids.[15] Work has also been carried out on the synthesis of similar but not identical polypeptides.[16]

The amino acid used for this work was β-(thymin-l-yl)alanine (1, R = H). Hitherto this has been obtained only as the racemic mixture. Because of the importance of obtaining a polypeptide of known stereochemistry it was essential to resolve this compound. This was achieved by converting it into its α-N-formyl derivative (1, R = CHO) which was resolved by fractional crystallisation of its (+)α-methyl-phenylethylamine salts from ethanol. The less soluble diastereomer was recrystallised to constant optical rotation and then reconverted to the amino acid (1, R = H) to give a product of $[\alpha]_D + 15°$. The more soluble diastereomer could not be obtained pure by crystallisation; upon reconversion to the free amino acid it gave a product of $[\alpha]_D - 10°$. A pure sample was obtained on a small scale by chromatography and this on conversion to the free amino acid gave a product of $[\alpha]_D - 14{\cdot}3°$.



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The closely related compound β-(uracil-l-yl)alanine (2) occurs naturally as the L form (Willardiine).[17] The racemic compound has been synthesised and resolved by Dewar and Shaw[18] who showed that the L-isomer has an $[\alpha]_D$ of −20°. In view of the close structural similarity of 1 (R = H) and 2 and to the fact that the Me group of 1 is well removed from the assymmetric centre it can be concluded that the dextrorotatory isomer of 1 (R = H) has D configuration. This conclusion is supported by the fact that uridylic acid and thymidylic acid show similar ORD curves in the visible region.[19]

In order to polymerise the β-(thymin-l-yl)alanines, the mixed anhydride procedure, using 2-methylpropan-l-yl chloroformate and N-methylmorpholine, was employed. The conditions used were expected to give very little racemisation. Considerable difficulty was experienced

because of the very low solubility of 1 (R = H) in the solvents usually used for polypeptide synthesis and in many other solvents which might be considered suitable. Ethyleneglycol was used as the solvent although this was not entirely satisfactory because of its reactivity. The D(+) isomer, the impure L(−) isomer and the DL mixture of 1 (R = H) were separately polymerised to give in each case about 10% yield of a polymer. The molecular weights were determined by end group assay. This was carried out by forming the 2,4-dinitrophenyl derivative of the terminal amino acid unit and determining the amount present spectrophotometrically. For a standard compound, α-N-(2,4-dinitrophenyl)-DL-β-(thymin-1-yl)alanine was synthesised. The molecular weights of the polymers from the impure L(−) isomer and from the DL form were about 4×10^3 whereas that from the D(+) isomer was only $1{\cdot}9 \times 10^3$.

The UV absorption spectra of the polymers at 20° were identical with those of the monomers, the optical densities being the same as that expected from a summation of the optical densities of the monomers. There was no change in optical density on heating the polymers to 85°. There was, therefore, no evidence of secondary structure and base stacking in these polymers. The optical rotation of the polymers obtained from the optically active amino acids was somewhat lower than that of the monomers indicating that some racemisation had occurred. The polymers showed no hypochromic effect on mixing with polyadenylic acid. This is surprising in view of the effect obtained with polyvinylpyrimidines[5-8] but could be attributed to the low molecular weight of the poly-β-(thymin-1-yl)alanines.

EXPERIMENTAL

DL-β-(Thymin-1-yl)alanine and its α-N-formyl derivative were synthesised as previously described.[10,15]

Resolution of DL-β-*(thymin-1-yl)alanine.* α-N-Formyl-DL-β-(thymin-1-yl)alanine (0·6 g, 2·5 mmoles) was dissolved with stirring in warm EtOH (50 ml) containing (+)α-methylphenylethylamine (0·43 ml, 2·5 mmole). The soln was reduced in volume to about 20 ml, kept at 20° for 72 h and the resulting white crystals of the α-methylphenylethylamine salt filtered off. It was crystallised from EtOH until a constant value for the optical rotation was obtained (yield 0·4 g). This compound was then boiled under reflux with 6N HCl for 50 min, the soln evaporated to dryness and the HCl removed by co-evaporation with MeOH. The residue was crystallised from water to give D(+)-β-*(thymin-1-yl)alanine* (0·2 g), $[\alpha]_D^{22}$ + 15° (*c*, 1·2 NHCl) (Found: C, 43·2; H, 5·4; N, 18·8. $C_8H_{11}N_3O_4.O.5H_2O$ requires: C, 43·2; H, 5·4; N, 18·9%); λ_{max} 269 nm (ε, $9{\cdot}81 \times 10^3$), λ_{min} 238 nm (ε, $1{\cdot}54 \times 10^3$) at pH 1. λ_{max} 271 nm (ε, $7{\cdot}60 \times 10^3$), λ_{min} 247 nm (ε, $3{\cdot}2 \times 10^3$) at pH 12.

The mother liquors from the separation of the α-methylphenylethylamine salts was reduced in volume to about 10 ml and light petroleum (b.p. 40–60°) added until the soln became cloudy. After standing at 4° a white crystalline ppt was obtained. This was recrystallised from EtOH-light petroleum to give an α-methylphenylethylamine salt (0·5 g). This was hydrolysed with 6N HCl as described above to give optically impure L(−)-β-(thymin-1-yl)alanine (0·26 g), $[\alpha]_D^{22}$ − 10° (*c*, 1·15 in NHCl) (Found: C, 43·5; H, 5·4; N, 18·9. $C_8H_{11}N_3O_4.O.5H_2O$ requires: C, 43·2; H, 5·4; N, 18·9%); λ_{max} 269 nm (ε, $9{\cdot}76 \times 10^3$), λ_{min} 238 nm (ε, $1{\cdot}55 \times 10^3$) at pH 1; λ_{max} 271 nm (ε, $7{\cdot}6 \times 10^3$), λ_{min} 247 nm (ε, $3{\cdot}20 \times 10^3$) at pH 12.

Chromatographic separation of the diastereomeric salts. This was carried out by TLC on silica gel in butan-1-ol: EtOH: water (4:1:5, organic phase). Two components of R_f 0·11–0·24 and 0·30–0·49 were obtained. These were isolated and converted into the free amino acids as described above. The two components had identical chromatographic and UV spectral properties to DL-β-(thymin-1-yl)alanine and had $[\alpha]_D^{19}$ + 15° (*c*, 0·5 in NHCl) and −14·4° (*c*, 0·5 in NHCl) respectively.

Polymerisation of D-, L- *and* DL-β*(thymin-1-yl)alanine.* The β-(thymin-1-yl)alanine (0·1 g, 0·47 mmole) was suspended in ethylene glycol (2·5 ml) and the suspension stirred with warming for 12 h to achieve maximum soln. The soln was then cooled to −15°, N-methylmorpholine (47 mg, 0·47 mmole) and 2-methylpropan-1-yl chloroformate (68 mg, 0·5 mmole) were added with stirring and the resulting white suspension stirred at −15° for 2 min. It was then allowed to warm to room temp and then stirred for 180 h and then dialysed exhaustively against distilled water and freeze dried. In the three cases the yield was about 10 mg. The extinction coefficients were identical with that of the amino acid both in acid and alkali. There was no change in optical density of the soln upon heating to 85° at neutral pH. The optical rotation ($[\alpha]_D$) of the polymer obtained from the D-amino acid was +11·5° and that from the impure L-amino acid was −7·3°.

Molecular weight determination. This was carried out by determining the amount of end group present by reacting the polymer with 2,4-dinitrofluorobenzene and determining the 2,4-dinitrophenyl groups spectrophotometrically. For a standard the 2,4-dinitrophenyl derivative of DL-β-(thymin-1-yl)alanine was synthesised.

α-N-2,4-*Dinitrophenyl*-DL-β-*(thymin-1-yl)alanine.* DL-β-(thymin-1-yl)alanine (20 mg, 0·09 mmole) was dissolved in 0·025M sodium tetraborate (2 ml). 1-Fluoro-2,4-dinitrobenzene (90 mg, 0·48 mmole) was added and the mixture stirred in the dark at 40° for 80 min the soln being maintained at pH 9 by the dropwise addition of N NaOH. The mixture was then acidified with N HCl to give a yellow oil which solidified on standing. This was filtered off in the dark and washed with diethyl ether and then with dil HCl. It was crystallised from aqueous acetone to give the *required product* (28 mg, 80%), m.p. 220(d) (Found: C, 43·3; H, 3·6; N, 18·3. $C_{14}H_{13}N_5O_8.O.5H_2O$ requires: C, 43·2; H, 3·6; N, 18·0%); λ_{max} 272 nm (ε, $2{\cdot}5 \times 10^4$), λ_{min} 239 nm (ε, $6{\cdot}4 \times 10^3$), λ_{max} 360 nm (ε, $1{\cdot}76 \times 10^4$) at pH 1; λ_{max} 273 nm (ε, $2{\cdot}45 \times 10^4$), λ_{min} 248 nm (ε, $7{\cdot}5 \times 10^3$), λ_{max} 363 nm (ε, $1{\cdot}94 \times 10^4$) at pH 5. The IR and NMR spectra were consistent with the assigned structure.

End group assay. The polymer (1 mg) was dissolved in 0·025M sodium tetraborate (1 ml), 1-fluoro-2,4-dinitrobenzene (1 mg) added and the soln stirred in the dark at 40° for 80 min, the reaction being maintained at pH 9. The mixture was then dialysed against distilled water for 4 h and the material which remained inside the bag purified by paper chromatography in butan-1-ol: EtOH: water (4:1:5, organic phase). The fraction containing the polymer (R_f 0·00–0·24) was eluted with water and the proportion of 2,4-dinitrophenyl end groups determined from the optical densities at 268 nm and 363 nm. The polymer from the D amino acid was an average nine units long (mol wt $1{\cdot}9 \times 10^3$) and those from the DL and the impure L amino acid both about 20 units long (mol wt $4{\cdot}3 \times 10^3$).

Measurement of interaction with polyadenylic acid. The polymers obtained as described above were mixed in various proportions with polyadenylic acid in 0·3M NaCl, 0·03M sodium citrate. Measurement of the optical densities of the solns showed that there was no hypochromic effect even at 0°.

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REFERENCES

[1]F. Cassidy and A. S. Jones, *Eur. Polymer J.* **2**, 319 (1966)
[2]M. G. Boulton, A. S. Jones and R. T. Walker, *J. Chem. Soc.* (C), 1216 (1968)
[3]M. G. Boulton, A. S. Jones and R. T. Walker, *Biochim. Biophys. Acta* **246**, 197 (1971)
[4]A. S. Jones, P. Lewis and S. F. Withers, *Tetrahedron* **29**, 2293 (1973)
[5]P. M. Pitha and J. Pitha, *Biopolymers* **9**, 965 (1970)
[6]J. Pitha, P. M. Pitha and P. O. P. Ts'O, *Biochim. Biophys. Acta* **204**, 39 (1970)
[7]P. M. Pitha and A. M. Michelson, *Ibid.* **204**, 381 (1970)
[8]F. Reynolds, D. Grunberger, J. Pitha and P. M. Pitha, *Biochemistry* **11**, 3261 (1970)
[9]J. Pitha and P. M. Pitha, *Science* **172**, 1146 (1971)
[10]M. T. Doel, A. S. Jones and N. Taylor, *Tetrahedron Letters* 2285 (1969)
[11]S. A. Giller, M. Yu Lidak, Y. Y. Shluke and Y. P. Shvachkin, *Chem. Heterocyclic Compd.* (Acad. Sci. Latvian Rep.) **2**, 124 (1968)
[12]M. Yu Lidak, Y. Y. Shluke and Y. P. Shvachkin, *Ibid.* **2**, 955 (1968)
[13]M. Yu Lidak, R. A. Paegle, K. Y. Petz, M. G. Plate and Y. P. Shvachkin, *Ibid.* **2**, 193 (1968)
[14]S. A. Giller, M. Yu Lidak, R. A. Paegle, M. G. Plate and Y. P. Shvachkin, *First all Union Conference on the Chemotherapy of Malignant Tumours* (Chem. Sect.) 103 (1968)
[15]M. T. Doel, A. S. Jones and R. T. Walker, *Tetrahedron* **30**, 2755 (1974)
[16]H. de Koning and U. K. Pandit, *Rec. Trav. Chim.* **91**, 1069 (1971)
[17]R. Gmelin, *Z. Physiol. Chem.* **316**, 164 (1959)
[18]J. H. Dewar and G. Shaw, *J. Chem. Soc.* 583 (1962)
[19]J. S. Yang, T. Samejima and P. K. Sarkar, *Biopolymers* **4**, 623 (1966)