309. *Nucleotides. Part XXXVI.* Adenosine-2′ Uridine-5′ Phosphate.*

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Adenosine-2′ uridine-5′ phosphate has been synthesised by condensing 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphorochloridate with 2′ : 3′-di-*O*-acetyluridine and subsequently removing protecting groups. The synthetic product showed the expected properties in both chemical and enzymic reactions. A convenient method for preparing 3′ : 5′-di-*O*-acetyladenosine by transacetylation is described.

Michelson and Todd [1] recently reported the first synthesis of a dinucleotide containing the 3′ : 5′-internucleotidic linkage characteristic of the natural nucleic acids. The synthetic compound (dithymidine dinucleotide) showed the same behaviour towards enzymes as the dinucleotides found in hydrolysates of natural deoxyribonucleic acids, thus providing valuable confirmation of the structural pattern of the latter. Clearly corresponding syntheses of diribonucleotides would be of at least equal value in the chemistry of ribonucleic acids, and, indeed, because of the ready availability of ribonucleosides as starting materials, preliminary although successful experiments on such syntheses were begun in these laboratories more than two years ago by Dr. R. H. Hall. As in the deoxyribonucleotide series, the first objective was the synthesis of a dinucleoside phosphate which would serve as a model for di- and higher poly-nucleotide syntheses. Diribonucleoside-5′ phosphates had already been prepared [2] but these were of little interest since there is good evidence that 5′ : 5′-internucleotidic linkages do not occur in ribonucleic acids; [2,3] only those containing 3′ : 5′- and 2′ : 5′-linkages were likely to give information of structural value.

The presence of the *cis*-α-glycol group in the ribonucleosides, *e.g.*, in adenosine (I) or uridine (II), complicates the synthesis of the diribonucleoside-2′ : 5′- and -3′ : 5′ phosphates in several ways. The only obvious method of unambiguous synthesis is the condensation of a suitably protected nucleoside, *e.g.*, (IV; U = uracil residue), with a protected nucleoside phosphorochloridate, *e.g.*, (III; A = adenine residue), or with a mixed anhydride of a protected nucleotide with some stronger acid. Phosphorochloridates in the ribonucleoside series are normally prepared by halogenation of the corresponding nucleoside benzyl phosphites, *i.e.*, they are nucleoside benzyl phosphorochloridates, and hence when they react with a nucleoside derivative the first product (*e.g.*, V) must be a triester of phosphoric acid. Such a compound can have reasonable stability only if there is no free hydroxyl group in either nucleoside residue adjacent to the point of attachment of the phosphate group.[4] In other words, with this type of synthesis, complete removal of the benzyl residue before there is any loss of other protecting groups is essential; moreover, the protecting groups on the nucleosides must be such that they can subsequently be removed under conditions which avoid hydrolytic breakdown of the labile dinucleotide structure (VI).

3′ : 5′-Di-*O*-acetyladenosine has been prepared by Brown, Fasman, Magrath, and Todd [5] by partial acetylation of 5′-*O*-acetyladenosine. This method was not very convenient for large-scale preparation but a satisfactory alternative was found employing the principle of transacetylation. 2′ : 3′ : 5′-Tri-*O*-acetyladenosine is readily accessible and from it by partial hydrolysis 5′-*O*-acetyladenosine. When these two compounds, carefully dried, are mixed in equimolecular proportion and heated just above the melting point of the mixture transacetylation occurs. Working up the product by countercurrent distribution gives 3′ : 5′-di-*O*-acetyladenosine in good yield in addition to some unchanged starting

* Part XXXV, *J.*, 1956, 1371.

[1] Michelson and Todd, *J.*, 1955, 2632. In this paper, *J.*, 1955, 2637, line 27; *for* water (9 c.c.), and 0·4N-sulphuric acid (4·5 c.c.), then *read* water (90 c.c.), and 0·4N-sulphuric acid (4·5 c.c.), for 1 hr. then.

[2] Elmore and Todd, *J.*, 1952, 3681.

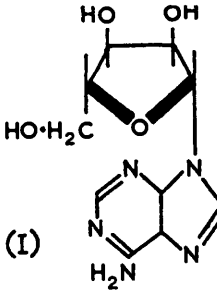
[3] Brown and Todd, *J.*, 1952, 52.

[4] Brown, Magrath, and Todd, *J.*, 1954, 1442.

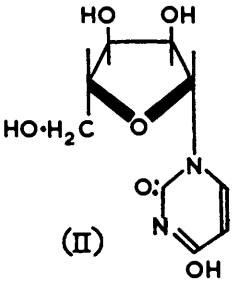
[5] Brown, Fasman, Magrath, and Todd, *J.*, 1954, 1448.

materials which can be re-processed. It is of interest that neither by this method nor by partial acetylation have we ever isolated any 2′ : 5′-di-*O*-acetyladenosine. Whether this is due to selective acetylation at $C_{(3')}$ or to migration of the acetyl group from $C_{(2')}$ to $C_{(3')}$ during the prolonged countercurrent distribution process in aqueous media is still an open question; only one spot corresponding to a diacetyladenosine is observed on paper chromatography of the crude product obtained by either method, but in the absence of a pure reference sample of 2′ : 5′-di-*O*-acetyladenosine it is difficult to assess the value of this observation.

The fusion method of preparation having made 3′ : 5′-di-*O*-acetyladenosine an accessible starting material it was decided to use it in attempts to prepare a dinucleoside-2′ : 5′-



(I)



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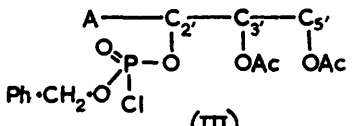

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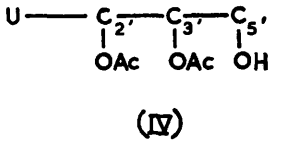

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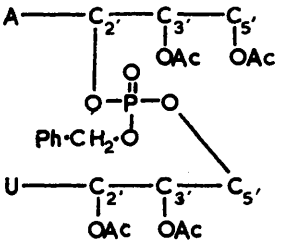

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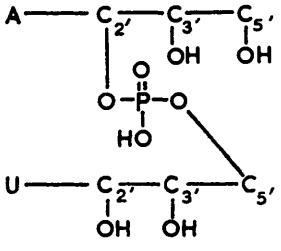

(VI)

phosphate. 3′ : 5′-Di-*O*-acetyladenosine-2′ benzyl phosphate [5] was chosen as an appropriate model for an acetylated dinucleoside phosphate in hydrolysis experiments; it was found that removal of acetyl groups without affecting the benzyl group was best effected by cold aqueous barium hydroxide at pH 9·6.

3′ : 5′-Di-*O*-acetyladenosine was converted *via* 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphite into 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphorochloridate [5] (III) which was not isolated but was prepared in solution as required and used immediately. With anhydrous 2′ : 3′-di-*O*-acetyluridine [6] (IV) at room temperature in presence of dry 2 : 6-lutidine, this gave a complex mixture, which was hydrogenated in presence of acetic acid to remove the benzyl group, then deacetylated and subjected to anion-exchange chromatography on Dowex 2 resin (formate form). Adenosine-2′ uridine-5′ phosphate (VI) was eluted with 0·05N-formic acid and crystallised from aqueous ethanol in colourless needles. The dinucleoside phosphate showed all the properties predicted on the basis of the known behaviour of simple nucleotide monoesters and ribonucleic acids. With 0·1N-aqueous sodium hydroxide at 37° it yielded uridine and a mixture of adenosine-2′ and adenosine-3′

[6] Kenner, Todd, Webb, and Weymouth, *J.*, 1954, 2288.

phosphate. Purified snake-venom diesterase hydrolysed it to uridine-5′ phosphate and adenosine [7] (Calculated molar ratio, 1 : 1; found, 1·2 : 1). Pancreatic ribonuclease had no action on the substance (absence of $C_{(3')}$ linkage on the pyrimidine nucleoside residue),[8] nor was it affected by spleen 3′-diesterase.[9] Periodate oxidation followed by incubation at pH 10 in a glycine buffer for 18 hours gave uracil and adenosine-2′ phosphate.[10,11] We are indebted to Dr. L. A. Heppel for his kindness in carrying out the enzyme experiments on the synthetic material.

The yield of adenosine-2′ uridine-5′ phosphate obtained, even with vigorous drying of all starting materials and solvents, was low (*ca.* 8%). After its removal from the ion-exchange column further elution gave mainly a mixture of adenosine-2′ and -3′ phosphate, followed by a more firmly held but rather unstable material which, by analogy with the observations made in the synthesis of dithymidine-3′ : 5′ phosphate,[1] may have been P^1P^2-diadenosine-2′ pyrophosphate. Whether the low yield of dinucleoside phosphate is due to lack of reactivity in either one of the starting materials or to preferential formation of large amounts of unstable pyrophosphate is uncertain; in either case the major end products would be adenosine-2′ and adenosine-3′ phosphate. It is, however, clear that synthesis of a 2′ : 5′-dinucleotide by this type of method should be feasible and there is every reason to expect that the 3′ : 5′-compound should be accessible by an analogous procedure.

Experimental

5′-O-*Acetyladenosine by Partial Hydrolysis of* 2′ : 3′ : 5′-*Tri*-O-*acetyladenosine*.—Methanolic ammonia (120 c.c., saturated at 0°) was added to a solution of 2′ : 3′ : 5′-tri-*O*-acetyladenosine (12 g.) in ethanol (1300 c.c.) and the mixture kept at room temperature for 45 min. Solvent was removed under reduced pressure, water (250 c.c.) was added to the residue, and the aqueous solution extracted once with chloroform (the chloroform extract gave on evaporation 3 g. of unchanged triacetyladenosine) and taken to half volume under reduced pressure. The solution was kept at 0° overnight, whereupon 5′-*O*-acetyladenosine (4 g.) separated as needles, m. p. and mixed m. p. 135°.

Paper-chromatographic examination of the crude product from the above experiment showed no formation of diacetyl- or of other monacetyl-adenosine; the only other product was free adenosine.

3′ : 5′-*Di*-O-*acetyladenosine*.—(a) *By partial acetylation*. Partial acetylation[5] of 5′-*O*-acetyladenosine (24 g.) and separation of the products by countercurrent distribution (100 transfers; ethyl acetate–water) gave 5′-*O*-acetyladenosine (7 g.; m. p. 135°), 3′ : 5′-di-*O*-acetyladenosine (8·5 g.; m. p. 169—170°), and 2′ : 3′ : 5′-tri-*O*-acetyladenosine (7·4 g.; m. p. 168°). Recrystallised first from acetone–light petroleum (b. p. 60—80°) and then from ethanol–light petroleum (b. p. 60—80°) 3′ : 5′-di-*O*-acetyladenosine formed needles, m. p. 170° (Found, in material dried at 105°/10^{-3} mm.: C, 48·1; H, 5·0; N, 19·6. Calc. for $C_{14}H_{17}O_6N_5$: C, 47·9; N, 19·9%).

(b) *By trans-acetylation*. A finely ground mixture of anhydrous 5′-*O*-acetyladenosine (6·9 g.) and 2′ : 3′ : 5′-tri-*O*-acetyladenosine (10 g.) was heated under reduced pressure to 160° for 5 min. to ensure complete melting, then kept *in vacuo* in a closed system at 130° for 12 hr. The cooled melt was shown to contain mono-, di-, and tri-*O*-acetyladenosine by paper chromatography, and it was subjected to countercurrent distribution in an automatically operated machine (20 c.c. phases; 100 transfers; ethyl acetate–water). This yielded 5′-*O*-acetyladenosine (tubes 3—25; 3·4 g.; m. p. 135°), 3′ : 5′-di-*O*-acetyladenosine (tubes 33—63; 7·5 g.; a glass), and 2′ : 3′ : 5′-tri-*O*-acetyladenosine (tubes 75—93; 4·5 g.; m. p. 168°). The 3′ : 5′-di-*O*-acetyladenosine was recrystallised as described under (a) above and had m. p. 170°; further recrystallisation from water raised the m. p. to 172—173° (yield 6 g.) (Found, in material dried at 105°/10^{-3} mm.: C, 47·7; H, 5·1; N, 19·7%).

Adenosine-2′ Uridine-5′ Phosphate.—*N*-Chlorosuccinimide (0·682 g.) was added to a solution of 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphite[5] (2·13 g.) in dry benzene (15 c.c.) and the mixture kept at room temperature for 4 hr. Anhydrous 2′ : 3′-di-*O*-acetyluridine[6] (1·26 g.) was then added, followed by 2 : 6-lutidine (2·5 c.c.) and acetonitrile (50 c.c.), and the mixture was

[7] Cohn and Volkin, *J. Biol. Chem.*, 1953, **203**, 319.
[8] Brown and Todd, *J.*, 1953, 2040.
[9] Brown, Heppel, and Hilmoe, *J.*, 1954, 40.
[10] Brown, Fried, and Todd, *J.*, 1955, 2206.
[11] Whitfield and Markham, *Nature*, 1953, **171**, 1151.

shaken at room temperature till it formed a clear solution (*ca.* 2 hr.), then set aside for 36 hr. at 25°. Solvent was removed under reduced pressure at room temperature and the residue freed from traces of lutidine *in vacuo* over phosphoric oxide. The product was next dissolved in aqueous ethanol (30 c.c. of 65%), glacial acetic acid (1 c.c.) was added, and the solution hydrogenated at room temperature and atmospheric pressure with a palladium oxide catalyst. Catalyst was filtered off and aqueous barium hydroxide added to pH 9·6. The solution was maintained at this pH by cautious addition of barium hydroxide during 2 days at room temperature and then put through a column of Dowex 2 resin (formate form; 9 × 3·5 cm.), the column being well washed with water. *Adenosine-2′ uridine-5′ phosphate* was eluted with 0·05N-formic acid, an automatic fraction-collector being used. Appropriate fractions were pooled, concentrated to small bulk under reduced pressure, and freeze-dried. The residue recrystallised from aqueous ethanol, giving the dinucleoside phosphate as colourless needles, (250 mg.), m. p. 175—180° (decomp.) (Found, in air-dried material: C, 36·5; H, 4·7; N, 15·5; loss in wt. at 70°/1 mm. during 10 hr., 9·2. $C_{19}H_{24}O_{12}N_7P,3H_2O$ requires C, 36·4; H, 4·8; N, 15·6; loss on drying, 8·6. Found, in anhyd. material: P, 5·4. $C_{19}H_{24}O_{12}N_7P$ requires P, 5·4%). R_F values (Whatman No. 1 paper) were (*a*) 0·3 in *n*-butanol–acetic acid–water (5 : 2 : 3), (*b*) 0·39 in 95% ethanol–M-ammonium acetate (75 : 30), (*c*) 0·62 in propan-1-ol–ammonia–water (6 : 3 : 1), (*d*) 0·18 in propan-2-ol–ammonia–water (7 : 1 : 2), and (*e*) 0·57 in propan-2-ol–1%-ammonium sulphate (3 : 2).

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