521. *Nucleotides. Part III. Mononucleotides derived from Adenosine, Guanosine, Cytidine, and Uridine.*

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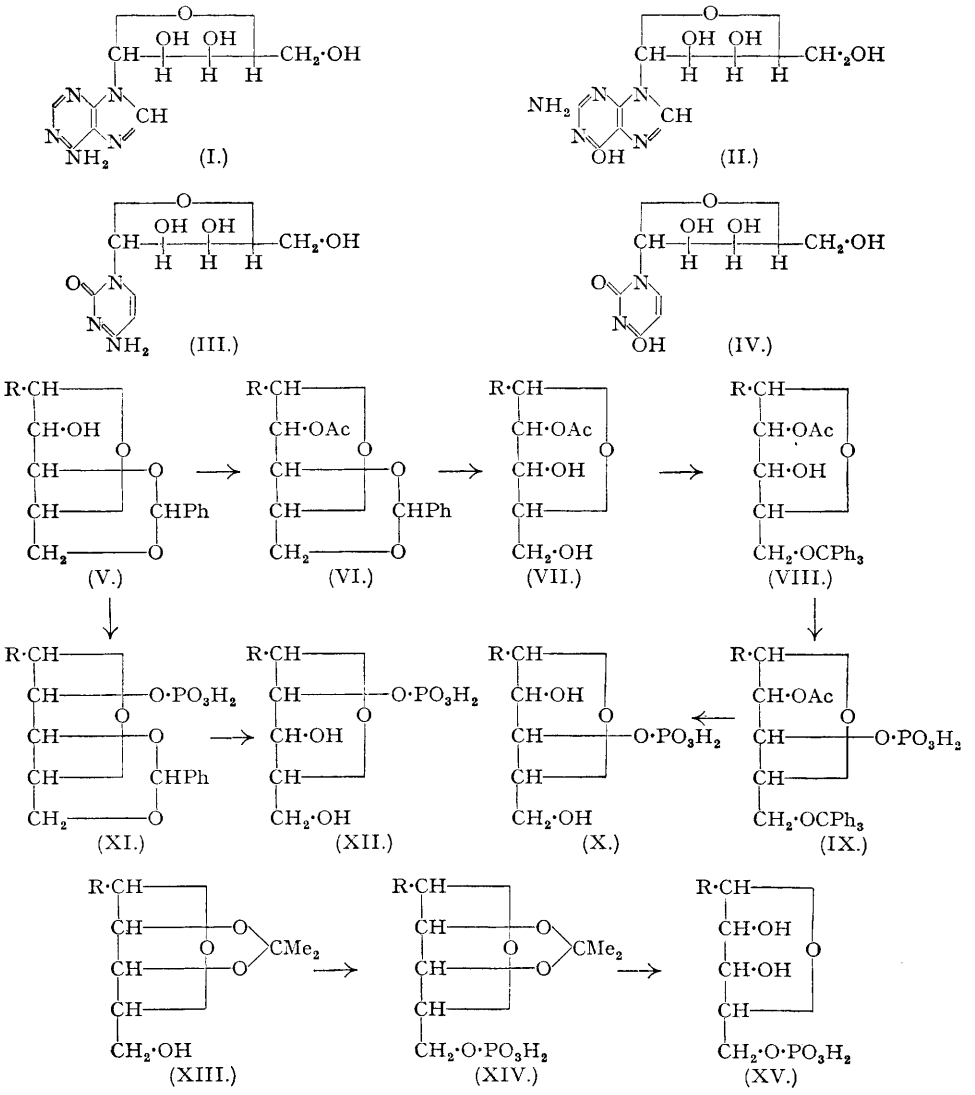
The following nucleotides have been synthesised by procedures clearly defining the position of the phosphoryl group: *adenosine-2′ phosphate*, adenosine-3′ phosphate (yeast adenylic acid), *guanosine-2′ phosphate*, *guanosine-5′ phosphate*, uridine-3′ phosphate (uridylic acid), uridine-5′ phosphate, cytidine-2′ phosphate, and *cytidine-5′ phosphate*. Unambiguous synthesis of the 3′-phosphates gives conclusive proof of the structures of the natural nucleotides of adenosine, uridine, and cytidine, derived from yeast ribonucleic acid; the structure of guanylic acid is deduced by elimination. The preferential phosphorylation of the 3′-hydroxyl group in 5′-trityl-nucleosides is noted and an improved method for the synthesis of D-ribofuranose-5 phosphate presented. Correlation of the hydrolytic stabilities of all twelve possible ribonucleotides shows that the ready alkaline fission of yeast ribonucleic acid cannot be ascribed to the marked lability of a single specific phospho-ester linkage.

Only in the case of muscle adenylic acid (adenosine-5′ phosphate) has the location of the phosphate residue in a simple nucleotide obtained from natural sources been confirmed by unambiguous synthesis (Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131; Bredereck, Berger, and Ehrenberg, *Ber.*, 1940, **73**, 269; Baddiley and Todd, *J.*, 1947, 648). Convincing evidence in favour of the 3′-phosphate structure for the four nucleotides derived from yeast ribonucleic acid (yeast adenylic, guanylic, cytidylic, and uridylic acids) has been presented, but such syntheses as have been reported are ambiguous and, as practical methods of preparation, very unsatisfactory. In addition to these five natural nucleotides, seven other nucleotides derived from adenosine (I), guanosine (II), cytidine (III), and uridine (IV) are theoretically possible; of these uridine-5′ phosphate (Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113), uridine-2′ phosphate (Gulland and Smith, *J.*, 1947, 338), and cytidine-2′ phosphate (Gulland and Smith, *J.*, 1948, 1527) have been synthesised by methods which leave no doubt as to their constitution. As a preliminary to studies on the nature of the internucleotidic linkage in the ribonucleic acids, it was clearly desirable that all twelve possible nucleotides should be compared as regards their

stability to hydrolytic agents and also made accessible as intermediates for synthetic work. Attention has therefore been directed to the synthesis of the missing members of the series and, at the same time, of nucleotides derived from yeast ribonucleic acid.

Hitherto the only reported synthesis of yeast adenylic acid has been that of Barker and Gulland (*J.*, 1942, 231), who obtained it in very small yield by direct treatment of adenosine with phosphoryl chloride in presence of barium hydroxide, a method which is ambiguous as



regards location of the phosphate group. Condensation of benzaldehyde with adenosine gave 3′ : 5′-*benzylidene adenosine* (V; R = adenine), and acetylation of this gave N^6 : 2′-*diacetyl* 3′ : 5′-*benzylidene adenosine* (VI; R = N^6-acetyladenine), in which one acetyl residue was believed (by analogy with other cases of acylated adenosine derivatives) to be located on the nitrogen atom of the 6-amino-group. Acid hydrolysis yielded 2′-*acetyl adenosine* (VII; R = adenine), which, on tritylation, gave a mixture of monotrityl (presumably 5′-) and ditrityl (presumably N^6 : 5′-) derivatives. As the presence of a trityl residue on the amino-nitrogen could not interfere with the synthesis envisaged, the mixture was directly phosphorylated with dibenzyl chlorophosphonate. Removal of protecting groups gave the crystalline *adenosine-3′ phosphate* (X; R = adenine), identical in all respects with adenylic acid obtained by hydrolysing

yeast ribonucleic acid. This synthesis provides final confirmation of the structure of yeast adenylic acid, which has hitherto rested on the production of an optically inactive ribitol phosphate on reduction of the ribose phosphate obtained from the deaminated nucleotide (Levene and Harris, *J. Biol. Chem.*, 1932, **98**, 9).

Dibenzyl chlorophosphonate reacted readily with 3′ : 5′-benzylidene adenosine to give, in good yield, 3′ : 5′-*benzylidene adenosine-2′ dibenzyl phosphate* from which 3′ : 5′-*benzylidene adenosine-2′ phosphate* (XI; R = adenine) was obtained by catalytic hydrogenation. Removal of the benzylidene residue by acid furnished the amorphous *adenosine-2′ phosphate* (XII; R = adenine), characterised as its crystalline *brucine* and *acridine* salts.

Previous attempts to phosphorylate guanosine and its derivatives have met with little success, perhaps owing to their low solubility in common organic solvents and the difficulty of purifying the products. Gulland and Hobday (*J.*, 1940, 746) claimed the synthesis of guanosine-3′ and guanosine-5′ phosphate (albeit in minute yield) by phosphorylating the unprotected nucleoside with phosphoryl chloride in aqueous barium hydroxide and pyridine respectively, but presented little clear evidence as to the location of the phosphate residues. Bredereck and Berger (*Ber*, 1940, **73**, 1124) prepared a series of derivatives leading to 2′-acetyl 5′-trityl guanosine, but were unable to phosphorylate either this compound or 5′-trityl guanosine with diphenyl chlorophosphonate. In our experiments guanosine was condensed with acetone in presence of zinc chloride, yielding 2′ : 3′-iso*propylidene guanosine* (XIII; R = guanine). Tritylation of the product was readily effected giving 5′-*trityl* 2′ : 3′-iso*propylidene guanosine*. Although reaction with triphenylmethyl chloride is not always confined to primary hydroxyl groups, the ease of reaction in this case, coupled with the known tendency of acetone to condense with adjacent *cis*-hydroxyl groups, justifies the orientation allotted to these derivatives. A number of attempts were made to phosphorylate 2′ : 3′-*iso*propylidene guanosine, both with dibenzyl and with diphenyl chlorophosphonates. In every case reaction occurred, but the products before and after removal of the protecting groups were amorphous and heterogeneous, and could not be purified; it is difficult to explain the lack of success in these experiments. Phosphorylation with phosphoryl chloride was next tried; since 2′ : 3′-*iso*propylidene guanosine is virtually insoluble in pyridine, it was dissolved in dry dimethylformamide, excess of pyridine added, the mixture cooled at once to −10°, and phosphoryl chloride added. This procedure proved effective and the product obtained in moderate yield was isolated as *barium* 2′ : 3′-iso*propylidene guanosine-5′ phosphate* (cf. XIV; R = guanine). Mild acid hydrolysis of this substance gave the crystalline *guanosine-5′ phosphate* (XV; R = guanine), characterised as its *barium* and *brucine* salts.

For the synthesis of *guanosine-2′ phosphate* (XII; R = guanine), guanosine was condensed with benzaldehyde to yield 3′ : 5′-benzylidene guanosine, a compound which has since been described by Gulland and Overend (*J.*, 1948, 1380), who prepared it in like manner. Phosphorylation of this substance with dibenzyl chlorophosphonate was not very satisfactory, removal of protecting groups from the product giving rather small yields of guanosine-2′ phosphate which was difficult to purify. Contrary to our experience with guanosine-5′ phosphate, phosphoryl chloride was even less satisfactory. In view of the difficulty encountered in synthesising this nucleotide and the unsuccessful attempts to prepare guanosine-3′ phosphate by Bredereck and Berger (*loc. cit.*), we did not pursue the synthesis of the latter. It is in any case clear from our synthesis of the 2′- and 5′-isomers that guanylic acid, which differs from both, must, of necessity, be guanosine-3′ phosphate.

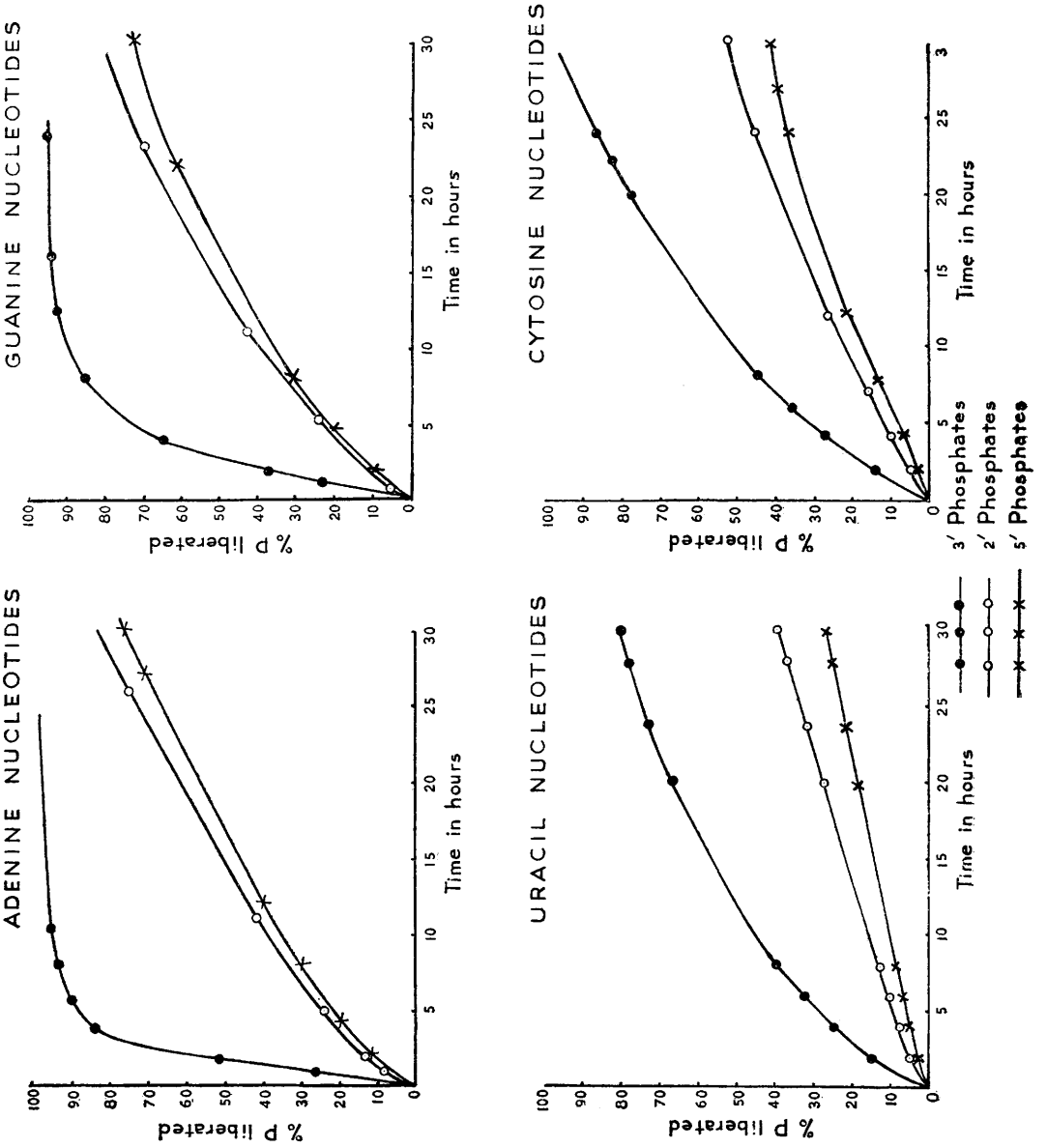
Convincing evidence that the phosphate residue in the natural pyrimidine nucleotides uridylic and cytidylic acid is located at $C_{(3')}$ has been provided by the unambiguous synthesis of uridine-5′ phosphate (Levene and Tipson, *loc. cit.*), uridine-2′ phosphate, and cytidine-2′ phosphate (Gulland and Smith, *loc. cit.*), all of which differ from the natural compounds, coupled with the conversion of cytidylic into uridylic acid by deamination (Bredereck, *Z. physiol. Chem.*, 1934, **224**, 79). The synthesis of uridylic acid by direct phosphorylation of uridine with phosphoryl chloride (Gulland and Hobday, *loc. cit.*) gives such low yields that it has little value as a preparative method. We have synthesised uridine-3′ phosphate (X; R = uracil) by an unambiguous route analogous to that employed for adenosine-3′ phosphate. 3′ : 5′-Benzylidene uridine (V; R = uracil) (Gulland and Smith, *J.*, 1947, 338) was acetylated, and the resulting 2′-*acetyl* 3′ : 5′-*benzylidene uridine* (VI; R = uracil) hydrolysed to 2′-*acetyl uridine* (VII; R = uracil). Tritylation of the last-named gave 2′-*acetyl* 5′-*trityl uridine* (VIII; R = uracil), which, on treatment with dibenzyl chlorophosphonate followed by removal of protecting groups, furnished uridine-3′ phosphate identical with uridylic acid from yeast ribonucleic acid. Since, as mentioned above, uridylic acid can be prepared by deamination of cytidylic acid (X; R = cytosine), the

synthesis of the former also provides valid confirmation of the structure of the latter. Uridine-5′ phosphate (XV; R = uracil) was readily prepared from 2′ : 3′-*iso*propylidene uridine (XIII; R = uracil) by use of dibenzyl chlorophosphonate as phosphorylating agent; contrary to the statements of Gulland and Hobday (*loc. cit.*) and of Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113), this nucleotide gives a beautifully crystalline barium salt. A similar phosphorylation of 2′ : 3′-iso*propylidene cytidine* (XIII; R = cytosine) with dibenzyl chlorophosphonate, followed by removal of protecting groups, yielded crystalline *cytidine-5′ phosphate* (XV; R = cytosine), characterised as its *brucine* salt. Treatment of 3′ : 5′-benzylidene cytidine with dibenzyl chlorophosphonate and removal of protecting groups from the product yielded, similarly, cytidine-2′ phosphate (XII; R = cytosine). Gulland and Smith (*J.*, 1948, 1527) claim to have prepared this compound from 3′ : 5′-benzylidene cytidine by reaction with phosphoryl chloride, followed by hydrolytic removal of the benzylidene residue; their product, however, had a considerably lower optical rotation than ours, and the rate of hydrolysis with acid reported by them was quite anomalous. As can be seen from the figure, cytidine-2′ phosphate as prepared by us shows a behaviour on acid hydrolysis exactly analogous to that of the other purine and pyrimidine nucleotides bearing the phosphoryl residue in the 2′-position of the carbohydrate residue, *i.e.*, the hydrolysis curve lies between those of the 3′- and 5′-phosphate. On the figures given by Gulland and Smith (*loc. cit.*), the curve for cytidine-2′ phosphate would lie not only below that of cytidine-5′ phosphate but actually below the curve for any of the other ten nucleotides; it is difficult to account for their results except by assuming that their product was not homogeneous. One further point in connexion with the cytidine phosphates should be mentioned. Gulland and Smith (*loc. cit.*) attempted to prepare cytidine-2′ phosphate from 3′ : 5′-benzylidene cytidine using diphenyl chlorophosphonate; they reported simultaneous phosphorylation of the sugar hydroxyl and the amino-group at position 6 in the pyrimidine nucleus, and further, found that attempts to remove the phenyl groups by hydrogenation brought about reduction and deamination of the pyrimidine ring with liberation of ammonia. Neither with 2′ : 3′-*iso*propylidene cytidine nor 3′ : 5′-benzylidene cytidine did we find any evidence of preferential *N*-phosphorylation when dibenzyl chlorophosphonate was used, and in our experiments no difficulty was experienced in removing the benzyl groups from the products by catalytic hydrogenation, the pyrimidine portion of the molecule being quite unaffected.

In the course of preliminary efforts to prepare adenosine-3′ : 5′ and adenosine-2′ : 3′ diphosphate, 2′-acetyl adenosine and N^6 : 5′-dityril adenosine were phosphorylated with dibenzyl chlorophosphonate. The product from 2′-acetyl adenosine was a rather intractable mixture which probably contained some of the desired diphosphate, but hydrogenation of the product from N^6 : 5′-dityril adenosine gave N^6 : 5′-*dityril adenosine*-3′ *phosphate* in an overall yield of 75%. The structure of the latter follows from its hydrolysis to adenosine-3′ phosphate. This preferential phosphorylation at $C_{(3')}$ was also observed when 5′-trityl uridine was treated with dibenzyl chlorophosphonate; working up in the normal fashion gave a 50% yield of uridine-3′ phosphate, and there was no evidence of phosphorylation at $C_{(2')}$. Bredereck and his co-workers (*Ber.*, 1940, **73**, 269, 1124) have already reported the producton of the natural nucleotides on phosphorylating 5-trityl uridine and 5-trityl cytidine and removing the trityl groups, but in these cases the yields were so small that it was uncertain whether, in fact, there had been a preferential phosphorylation at $C_{(3')}$. In the course of our work we also had occasion to prepare D-ribofuranose-5 phosphate. This compound we obtained readily in 86% yield by treatment of 2 : 3-*iso*propylidene methyl-D-ribofuranoside with dibenzyl chlorophosphonate, followed by removal of the protecting groups. This method of preparation is greatly superior to the phosphorylation with phosphoryl chloride described by Levene and Stiller (*J. Biol. Chem.*, 1934, **104**, 299), which gives poor yields and necessitates a tedious purification procedure. This synthesis and that of all the nucleotides described in this paper, with the exception of guanosine-5′ phosphate, serve to emphasise the value and convenience of dibenzyl chlorophosphonate as a phosphorylating agent.

Comparison of the stability, towards alkali, of all twelve mononucleotides derived from adenosine, guanosine, cytidine, and uridine shows clearly that the alkali-lability of yeast ribonucleic acid cannot be attributed to the specific lability of a known phospho-ester linkage. All the mononucleotides are only slightly affected when kept at room temperature for 3 days in 1% aqueous sodium hydroxide, although under the same conditions yeast ribonucleic acid is hydrolysed to a mixture of nucleotides (Levene, *J. Biol. Chem.*, 1923, **55**, 9). The observation of Gulland and Smith (*J.*, 1948, 1532) that diuridine-2′ : 2′ phosphate is similarly stable to cold alkali suggests that alkali-lability is not a characteristic feature of dinucleoside phosphates. Whether the instability of the natural polynucleotides is to be ascribed to the presence of two

Hydrolysis of nucleotides with N/10*-sulphuric acid at* 100°.



phosphate ester linkages on each sugar residue, or to some unusual type of phosphate linkage (*e.g.*, 1′ : 3′-) between nucleoside molecules, can be settled only by further investigation. For purposes of reference, acid hydrolysis curves for the twelve mononucleotides are recorded in the figure.

Experimental.

3′ : 5′-*Benzylidene Adenosine*.—Adenosine (12·5 g., dried for 24 hours at 120°/1 mm.), powdered anhydrous zinc chloride (35 g.), and pure dry benzaldehyde (175 c.c.) were shaken in a stoppered flask at room temperature for 24 hours. The clear viscous solution was then slowly poured into ether (2·5 l., dried over potassium carbonate) with shaking, and the precipitated zinc chloride double salt rapidly filtered off, washed with ether, dried, and dissolved in "Cellosolve" (200 c.c.). Aqueous sodium hydroxide (120 c.c. of 10%) was added, the mixture was set aside for 10 minutes, and then carbon dioxide was passed through it until the solution was neutral to phenolphthalein. Precipitated inorganic salts were filtered off, washed well with hot "Cellosolve," and the combined filtrate and washings evaporated to small volume under reduced pressure. Cold water was cautiously added to the semi-solid mass, and the precipitated crude products were collected, washed well with water to remove sodium salts, and recrystallised from aqueous ethanol, to give fibrous needles of 3′ : 5′-*benzylidene adenosine* (12·5 g., 75%), m. p. 224° (decomp.), $[\alpha]_D^{18}$ −150° (*c*, 1·41 in pyridine) (Found, in material dried for 3 hours at 116°/1 mm.: C, 57·5; H, 4·9; N, 19·9. $C_{17}H_{17}O_4N_5$ requires C, 57·5; H, 4·8; N, 19·7%). The *picrate* crystallised from ethanol had m. p. 203° (decomp.) (Found, in material dried for 3 hours at 80°/1 mm.: C, 47·3; H, 3·5; N, 19·2. $C_{17}H_{17}O_4N_5,C_6H_3O_7N_3$ requires C, 47·3; H, 3·4; N, 19·2%).

3′ : 5′-*Benzylidene Adenosine*-2′ *Dibenzyl Phosphate*.—Dibenzyl chlorophosphonate (from 8 g. of dibenzyl phosphite) was added to a solution of 3′ : 5′-benzylidene adenosine (4 g., dried at 110°/1 mm. for 12 hours) in anhydrous pyridine (50 c.c.) at −30°, and the mixture kept just above its freezing point for 6 hours and then kept at room temperature overnight. Water (20 c.c.) and sodium carbonate (4 g.) were added, and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform, washed with aqueous sodium hydrogen carbonate and then with water, and dried (Na_2SO_4); removal of the solvent under reduced pressure gave a gum which was evaporated twice with ethanol and finally dissolved in hot ethanol (80 c.c.); this was filtered and then cooled. The clear supernatant liquid was decanted from a brown gummy precipitate (*A*) and evaporated to small volume under reduced pressure. Ether was added to turbidity; when the mixture was kept, fine needles of 3′ : 5′-*benzylidene adenosine*-2′ *dibenzyl phosphate* separated. Recrystallised from ethanol–ether, the product (4·2 g., 60%) had m. p. 68° (Found, in material dried for 3 hours at 50°/1 mm.: C, 59·9; H, 5·0; N, 11·2; P, 5·0. $C_{31}H_{30}O_7N_5P$ requires C, 60·5; H, 4·9; N, 11·4; P, 5·0%). The precipitate *A* was dissolved in chloroform and reprecipitated by pouring the solution into light petroleum, to give a light-brown powder (2 g.), m. p. 80—90°, probably 3′ : 5′-*benzylidene adenosine*-N^6 *dibenzyl phosphate* (Found, in material dried for 12 hours at 50°/1 mm.: N, 11·3. $C_{31}H_{30}O_7N_5P$ requires N, 11·4%).

3′ : 5′-*Benzylidene Adenosine*-2′ *Phosphate*.—3′ : 5′-Benzylidene adenosine-2′ dibenzyl phosphate (3 g.) in 1 : 1 aqueous ethanol (200 c.c.) was hydrogenated at room temperature/1 atm. with a mixture of palladium and palladised charcoal catalysts. The theoretical amount of hydrogen for removal of two benzyl groups was absorbed in 2½ hours. The hot solution was filtered from catalyst, the latter extracted with hot aqueous ethanol, and the combined filtrate and extracts were evaporated to 75 c.c. under reduced pressure and set aside overnight at 0°. 3′ : 5′-*Benzylidene adenosine*-2′ *phosphate* separated as a white powder (1·3 g., 60%), m. p. 225° (decomp.) (Found, in material dried for 3 hours at 120°/1 mm.: C, 47·7; H, 4·4; N, 15·8; P, 6·9. $C_{17}H_{18}O_7N_5P$ requires C, 46·9; H, 4·2; N, 16·1; P, 7·1%).

Adenosine-2′ *Phosphate*.—A solution of 3′ : 5′-benzylidene adenosine-2′ phosphate (1 g.) in dilute sulphuric acid (water 74 c.c., dioxan 25 c.c., N-sulphuric acid 1 c.c.) was heated under reflux gently for 1 hour, neutralised with baryta, and filtered through Hyflo supercel, and neutral lead acetate solution was added to the filtrate. The lead salt was collected, washed with water, suspended in hot water (100 c.c.), and decomposed with hydrogen sulphide. The filtered aërated solution was concentrated to 3 c.c. under reduced pressure and again filtered, and acetone (25 c.c.) was slowly added. The fine white precipitate of *adenosine*-2′ *phosphate* was collected, washed, and dried (0·35 g., 44%). On heating, the acid shrank and began to turn brown at 170—180° and melted with decomposition at 205—215° It was very soluble in water and could not be obtained crystalline (Found, in material dried at room temperature: C, 31·0; H, 4·5; N, 18·1; P, 8·0, 8·2. $C_{10}H_{14}O_7N_5P,2H_2O$ requires C, 31·3; H, 4·7; N, 18·3; P, 8·1%).

Brucine (97 mg.) in methanol (1 c.c.) was added to a solution of the nucleotide (39 mg.) in water (2 c.c.) and the solution evaporated to dryness in a desiccator. The residue, recrystallised twice from water, gave the *dibrucine* salt as colourless needles with an indefinite m. p. (165—175°) (Found, in air-dried material: C, 54·8; H, 6·2; N, 10·0. $C_{10}H_{14}O_7N_5P,2C_{23}H_{26}O_4N_2,5H_2O$ requires C, 54·8; H, 6·2; N, 10·3%). The *acridine* salt crystallised from water, containing a little ethanol, as yellow needles, m. p. 215° (decomp.) (Found, in air-dried material: C, 52·2; H, 4·9; N, 14·9; P, 5·6. $C_{10}H_{14}O_7N_5P,C_{13}H_9N,C_2H_5{\cdot}OH$ requires C, 52·4; H, 4·9; N, 14·7; P, 5·4%. Found, in material dried for 4 hours at 110°/1 mm.: N, 15·9. $C_{10}H_{14}O_7N_5P,C_{13}H_9N$ requires N, 16·0%).

N^6 : 2′-*Diacetyl* 3′ : 5′-*Benzylidene Adenosine*.—A suspension of fused sodium acetate (0·1 g.) in redistilled acetic anhydride (30 c.c.) was heated to gentle ebullition in a round-bottomed flask fitted with an air-condenser and silica-gel drying-tube. The flame was removed and benzylidene adenosine (2·5 g.) slowly added, the mixture being shaken after each addition. Excess of acetic anhydride was removed under reduced pressure, the resulting thick gum dissolved in dry acetone (40 c.c.) and filtered through sintered glass to remove sodium acetate, and the filtrate evaporated to dryness, giving a colourless frothy glass which could not be obtained crystalline. Precipitation from chloroform by pouring into light petroleum (b. p. 60—80°) gave a white amorphous powder (3 g., 97%), m. p. 55—65°, which retained traces of light petroleum tenaciously (Found, in material dried for 6 hours at 40°/1 mm.: C, 58·5; H,

4·6; N, 15·4. $C_{21}H_{21}O_6N_5$ requires C, 57·5; H, 4·8; N, 15·9%. Found, after drying for a further 6 hours at 40°/1 mm.: N, 15·6%), $[\alpha]_D^{18}$ −47·5° (*c*, 3·4 in chloroform).

2′-Acetyl Adenosine.—N^6 : 2′-Diacetyl 3′ : 5′-benzylidene adenosine (8 g.) in ethanol (400 c.c.) was added to dilute sulphuric acid (1200 c.c.; N/75) (*i.e.*, total volume 1600 c.c. of 0·01 N-sulphuric acid), and the solution heated under reflux for 1 hour. Barium hydroxide and carbonate were added to neutrality, barium sulphate was remove by filtration through Hyflo supercel, and the filtrate evaporated to small bulk under reduced pressure. After being set aside at 0° for several hours, the *acetate* was filtered off (addition of alcohol to the filtrate gave a precipitate of barium acetate) and recrystallised from water, giving large colourless irregular plates (4·8 g., 85%), m. p. 67—70° (hydrated) [Found, in air-dried material: C, 41·6; H, 5·4; N, 20·1, 20·2. $C_{12}H_{15}O_5N_5,2H_2O$ requires C, 41·7; H, 5·5; N, 20·3%. Found, in material dried for one week at room temperature/1 mm. (m. p. 97—102°) and then for 6 hours at 80°/1 mm.: C, 46·7; H, 4·9; N, 22·5. $C_{12}H_{15}O_5N_5$ requires C, 46·6; H, 4·9; N, 22·6%], $[\alpha]_D^{17}$ −60° (*c*, 0·67 in pyridine).

Tritylation of 2′-Acetyl Adenosine.—A solution of 2′-acetyl adenosine (2 g., dried for 24 hours at 80°/1 mm.) and triphenylmethyl chloride (4·2 g., 2·2 mols.) in dry pyridine (60 c.c.) was heated at 100° for 3 hours and then set aside at room temperature overnight with exclusion of moisture. The mixture was poured into ice-water (400 c.c.) with vigorous stirring and kept at 0° overnight. The crude product was filtered off, dried, dissolved in chloroform, and filtered into light petroleum (400 c.c.; b. p. 60—80°) with stirring, and the pale yellow amorphous precipitate collected (3·3 g., 65%; m. p. *ca.* 110°) (Found, in material dried for 15 hours at 80°/1 mm.: N, 10·5. 2′-Acetyl 5′-trityl adenosine, $C_{31}H_{29}O_5N_5$, requires N, 12·7%; 2′-acetyl N^6 : 5′-ditrityl adenosine, $C_{50}H_{43}O_5N_5$, requires N, 8·8%). The mixture of mono- and di-tritylated compounds could not be separated. Tritylation with 1·1 mols. of triphenylmethyl chloride gave a similar mixture, composed mainly of the monotrityl compound (Found, in material dried for 12 hours at 50°/1 mm.: C, 70·0; H, 5·5; N, 12·1%. $C_{31}H_{29}O_5N_5$ requires C, 67·5; H, 5·3; N, 12·7%. $C_{50}H_{43}O_5N_5$ requires C, 75·7; H, 5·4; N, 8·8%).

Adenosine-3′ Phosphate.—Dibenzyl chlorophosphonate (from 5 g. of dibenzyl phosphite) was added to a solution of the above impure acetyl trityl adenosine (3 g.; dried for 12 hours at 80°/1 mm.) in dry pyridine (40 c.c.) at −40°, and the mixture kept just above its m. p. for 6 hours and then set aside at room temperature overnight. The product was worked up in the usual way to give a thick resin, which was dissolved in a small amount of chloroform and poured into ether (200 c.c.), yielding a viscous semi-solid gum. The gum was dissolved in hot 1 : 1 aqueous ethanol (150 c.c.), boiled with palladised charcoal to remove catalyst poisons, filtered, and kept at room temperature overnight. A small amount of deposited solid was filtered off and the solution hydrogenated with a mixture of palladised charcoal and palladium oxide as catalyst (hydrogen uptake, 470 c.c. in 10 hours). Catalyst was removed by filtration, and the filtrate neutralised with sodium hydroxide and treated with excess of 20% lead acetate solution. The lead salt was spun off, washed with water, suspended in hot water (250 c.c.), and decomposed with hydrogen sulphide. Lead sulphide and precipitated triphenylmethyl alcohol were removed by filtration through Hyflo supercel, and the solution was aërated till free from hydrogen sulphide. Barium hydroxide was added to pH 10, and the solution kept at 30° for 30 minutes to remove the 2′-acetyl group, the pH being maintained at 10 by addition of barium hydroxide from time to time. Carbon dioxide was then passed into the mixture until it was neutral, the whole filtered, and lead acetate solution added. The precipitated lead salt was spun off, washed with water, suspended in hot water (200 c.c.), and decomposed with hydrogen sulphide. The filtrate was aërated and concentrated to small volume under reduced pressure, the temperature being kept below 30°. Acetone was added to precipitate the nucleotide (0·45 g., *ca.* 30%) which was recrystallised from water. While impure, the product is fairly soluble in water; with increasing purity the solubility deacreases, and the pure adenosine-3′ phosphate is only sparingly soluble in hot water, from which it crystallises in long colourless transparent rods, m. p. 194° (decomp.), undepressed in admixture with a sample of authentic yeast adenylic acid (m. p. 193°) prepared by hydrolysis of yeast ribonucleic acid (Found, in air-dried material: P, 8·3. $C_{10}H_{14}O_7N_5P,H_2O$ requires P, 8·5%. Found, in material dried for 15 hours at 110°/1 mm.: C, 34·1; H, 4·1; N, 20·2; P, 8·6. $C_{10}H_{14}O_7N_5P$ requires C, 34·6; H, 4·0; N, 20·2; P, 8·9%). The dibrucine salt, prepared in the usual manner, crystallised from water in long thin rods and had m. p. 177°, followed by effervescence and darkening at 225° (decomp.), undepressed in admixture with the brucine salt prepared from authentic yeast adenylic acid. Levene (*J. Biol. Chem.*, 1919, **40**, 415) records a heptahydrate which on heating began to contract at 177°, effervesced at 195°, and showed a second effervescence, with darkening, at 225° (Found, in air-dried material: C, 53·3; H, 6·5; N, 9·9. Calc. for $C_{10}H_{14}O_7N_5P,2C_{23}H_{26}O_4N_2,7H_2O$: C, 53·3; H, 6·3; N, 10·0%). The monoacridine salt was obtained as small balls of fine yellow needles, m. p. 175° (decomp.), undepressed in admixture with the acridine salt (m. p. 175°) prepared from authentic yeast adenylic acid (Berlin and Westerberg, *Z. physiol. Chem.*, 1944, **281**, 98). Bredereck, quoted in a paper by Wagner-Jauregg and Griesshaber (*Ber.*, 1937, **70**, 1458) on the incomplete combustion of acridine salts, reports the acridine salt of yeast adenylic acid as an amorphous yellow solid. Tipson (*J. Biol. Chem.*, 1937, **120**, 621) describes a diacridine salt, m. p. 184°, but we were unable to confirm its existence.

N^6 : 5′*Ditrityl Adenosine-3′ Phosphate.*—Dibenzyl chlorophosphonate (from 2 g. of dibenzyl phosphite) was added to a solution of N^6 : 5′-ditrityl adenosine (0·9 g.) (Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131) in dry pyridine (10 c.c.) at −40° and the mixture kept just above its m. p. for 6 hours and then at room temperature overnight. Sodium carbonate and water were added and the product was worked up in the usual manner, to give a colourless gum which was dissolved in ethanol, kept at 0° for 12 hours, and filtered from a small amount of solid material [m. p. 200° (decomp.)]. The filtrate was concentrated to small volume under reduced pressure, and the phosphorylated ditrityl adenosine precipitated as a gum by addition of ether. This gum was dissolved in aqueous ethanol and hydrogenated (palladium and palladised charcoal), and the solution filtered from catalyst and reduced to small bulk *in vacuo*. The white solid which separated was collected, dried, dissolved in chloroform, and filtered into light petroleum (b. p. 40—60°) giving the *product* as a fine white powder, m. p. 150—160° (0·75 g., 75%) (Found, in material dried for 3 hours at 80°/1 mm.: N, 8·4; P, 3·6. $C_{48}H_{42}O_7N_5P$ requires N, 8·4; P, 3·7%).

Detritylation of N^6 : 5′-*Ditrityl Adenosine*-3′ *Phosphate*.—The phosphate (1 g.) was dissolved in aqueous ethanol, and neutral lead acetate solution added to precipitate the lead salt, which was centrifuged off, washed with water, suspended in hot water (200 c.c.), and decomposed with hydrogen sulphide. Filtration from the lead sulphide and triphenylmethyl alcohol, and concentration of the filtrate under reduced pressure, followed by addition of acetone, gave the free nucleotide (0·22 g., 53%). Direct determination of the rate of acid hydrolysis on this unpurified material gave a curve identical with that of " natural " yeast adenylic acid, showing the complete absence of adenosine-2′ phosphate. Recrystallisation from water gave adenosine-3′ phosphate as needles, m. p. 192—194° (decomp.), undepressed in admixture with authentic yeast adenylic acid or the synthetic adenosine-3′ phosphate described above (Found, in material dried for 15 hours at 110°/1 mm.: C, 34·3; H, 4·0; N, 19·5; P, 8·8. Calc. for $C_{10}H_{14}O_7N_5P$: C, 34·6; H, 4·0; N, 20·2; P, 8·9%). The acridine salt, recrystallised from water, had m. p. 174—175° (decomp.), undepressed in admixture with the acridine salt, m. p. 175° (decomp.), of authentic yeast adenylic acid.

2′ : 3′-iso*Propylidene Guanosine*.—Levene and Tipson (*J. Biol. Chem.*, 1937, **121**, 131, footnote) record that they have prepared this substance, but give no characteristics or analysis.

Guanosine (35 g.; dried for 24 hours at 110°/1 mm.) was added to a solution of anhydrous zinc chloride (85 g.) in dry acetone (600 c.c.) and heated under reflux for 5 hours under anhydrous conditions, a clear solution being obtained. Acetone was removed under reduced pressure, the resulting syrup dissolved in a small amount of " Cellosolve " (2-ethoxyethanol), and dry ether (1500 c.c.) added with shaking. The hygroscopic zinc chloride double salt was rapidly collected, washed with ether, and dried. The powdered salt was dissolved in warm " Cellosolve " (250 c.c.), a solution of barium hydroxide (200 g. of octahydrate) in water (700 c.c.) added, the mixture well shaken, and carbon dioxide passed through it until neutral to phenolphthalein. The mixture was then filtered and the voluminous precipitate washed well with hot water (*ca.* 2 l.) and a small amount of hot " Cellosolve." The combined filtrate and washings (*ca.* 3 l.), on cooling, deposited the crude product. Recrystallised twice from hot water, 2′ : 3′-iso*propylidene guanosine* formed colourless needles (22·5 g., 64%), m. p. 299° (decomp.) (Found, in material dried for 3 hours at 127°/1 mm.: C, 47·9; H, 5·1; N, 21·6. $C_{13}H_{17}O_5N_5$ requires C, 48·2; H, 5·2; N, 21·6%), $[\alpha]_D^{18}$ −36·3° (*c*, 4·98 in dimethylformamide); it was soluble in dimethylformamide, glacial acetic acid, and hot water, but insoluble in pyridine, ethanol, chloroform, or other common organic solvents.

2′ : 3′-iso*Propylidene* 5′-*Trityl Guanosine*.—2′ : 3′-*iso*Propylidene guanosine (2 g.; dried for 24 hours at 110°/1 mm.) was dissolved in anhydrous dimethylformamide (10 c.c.) and pyridine (10 c.c.). Triphenylmethyl chloride (2·5 g.) was added, and the mixture heated on the water-bath for 3 hours, with exclusion of moisture, and then cooled to 0° overnight. The clear solution was slowly poured into ice–water (400 c.c.) with vigorous stirring, and the crude trityl compound filtered off, washed well with cold water, and dried. Recrystallisation from pyridine–ethanol gave 2′ : 3′-iso*propylidene* 5′-*trityl guanosine* as long colourless needles (2·1 g., 60%), m. p. 278—279° (Found, in material dried for 2 hours at 110°/1 mm.: C, 67·4; H, 5·5; N, 12·3. $C_{32}H_{31}O_5N_5$ requires C, 68·0; H, 5·5; N, 12·4%), $[\alpha]_D^{18}$ +63·6° (*c*, 0·42 in pyridine). The mother-liquors were evaporated to dryness under reduced pressure and heated under reflux with light petroleum (b. p. 60—80°) to remove triphenylmethyl alcohol, and the residue was powdered and shaken with cold acetone (25 c.c.); after filtration from a small amount of monotrityl compound, the solvent was removed leaving a white flaky glass—presumably *N* : 5′-ditrityl 2′ : 3′-*iso*propylidene guanosine—which could not be crystallised.

2′ : 3′-iso*Propylidene Guanosine*-5′ *Phosphate*.—Phosphoryl chloride (1·32 g., 1 mol.) in anhydrous pyridine (12 c.c.) was added dropwise during 20 minutes to a vigorously stirred solution of 2′ : 3′-*iso*propylidene guanosine (2·75 g.; dried for 24 hours at 120°/1 mm.) in dimethylformamide (20 c.c.) and pyridine (30 c.c.) at −10°, and stirring was continued for a further 2 hours. Ice-cold aqueous pyridine (24 c.c.; 50%) was added during 30 minutes and then ice-water (96 c.c.). The mixture was now made alkaline to thymolphthalein with saturated barium hydroxide solution, and about 100 c.c. of solvent were removed under reduced pressure. The precipitate was centrifuged off and the supernatant liquid evaporated to dryness under reduced pressure. The white solid obtained was dissolved in water, and the solution filtered and combined with the hot-water extracts of the first precipitate. When this solution was concentrated to 250 c.c. and an equal volume of ethanol was added, the *barium salt* of 2′ : 3′-iso*propylidene guanosine*-5′ *phosphate* separated; it was centrifuged off, washed with ethanol and ether, and dried (1·5 g., 33%) (Found, in material dried for 4 hours at 120°/1 mm.: C, 29·3; H, 4·0; N, 12·5. $C_{13}H_{16}O_8N_5PBa$ requires C, 29·0; H, 3·0; N, 13·0%). The lead salt prepared from the supernatant liquors and worked up in the usual way gave a small amount of guanosine-5′ phosphate (0·21 g., 7%) (Found, in material dried at room temperature: C, 30·2; H, 4·8; N, 17·6. Calc. for $C_{10}H_{14}O_8N_5P,2H_2O$: C, 30·1; H, 4·5; N, 17·5%).

Guanosine-5′ *Phosphate*.—(*a*) Phosphoryl chloride (1·58 c.c., 1 mol.) in anhydrous pyridine (20 c.c.) was added dropwise during 15 minutes to a vigorously stirred solution of 2′ : 3′-*iso*propylidene guanosine (5·5 g.; dried for 24 hours at 120°/1 mm.) in anhydrous dimethylformamide (40 c.c.) and pyridine (60 c.c.) at −10°, and stirring was continued for a further 2 hours. Ice-cold aqueous pyridine (50 c.c.; 50%) was now added during 30 minutes, followed by ice-water (190 c.c.) and cold 0·35N-barium hydroxide (245 c.c.) to pH 8·7 (colour change in the solution), and the mixture was then evaporated to dryness. The residue was dissolved in water and filtered through Hyflo supercel, barium was precipitated with sulphuric acid, and enough water and sulphuric acid were added to bring the solution to a volume of 1000 c.c. with an acid concentration of 0·1N. After 2 days at room temperature the solution was neutralised with barium hydroxide and filtered hot. 20% Lead acetate solution (35 c.c.) was added and the lead salt centrifuged off, washed with water, suspended in hot water, and decomposed with hydrogen sulphide. The solution so obtained was filtered from lead sulphide, aërated, and evaporated to small volume under reduced pressure, and the nucleotide precipitated by adding acetone. The crude phosphate was then redissolved in a minimum of water, and acetone slowly added to the filtered solution. *Guanosine*-5′ *phosphate* separated as a colourless mass of micro-crystals (1 g., 20%), m. p. 190—200° (decomp.), which tenaciously retained water of crystallisation (Found, in material dried for 24 hours at 100°/1 mm.: C,

32·5; H, 4·1; N, 18·5; P, 8·4. $C_{10}H_{14}O_8N_5P,\frac{1}{2}H_2O$ requires C, 32·3; H, 4·0; N, 18·8; P, 8·3%). The *dibrucine* salt crystallised from water in small clusters of squat needles which on heating began to turn brown and shrink from 190° and melted with decomposition at 210—220°; on further heating to 225° the melt effervesced (Found, in air-dried material: C, 54·2; H, 6·3; N, 9·8. $C_{10}H_{14}O_8N_5P,2C_{23}H_{26}O_4N_2,5H_2O$ requires C, 54·2; H, 6·1; N, 10·1%. Found, in material dried for 7 hours at 140°/1 mm.: N, 11·2. $C_{10}H_{14}O_8N_5P,2C_{23}H_{26}O_4N_2$ requires N, 11·0%). The acridine salt was amorphous.

(*b*) Barium 2′ : 3′-*iso*propylidene guanosine-5′ phosphate was hydrolysed with 0·1N-sulphuric acid at room temperature and neutralised with barium hydroxide and barium carbonate, and the filtered solution concentrated to small volume under reduced pressure. On cooling, *barium guanosine-5′ phosphate* separated and was centrifuged off; on being dissolved in hot water and allowed to cool it separated as a white powder showing no definite crystalline form (Found, in material dried for 4 hours at 120°/1 mm.: N, 14·2. $C_{10}H_{12}O_8N_5PBa$ requires N, 14·1%).

3′ : 5′-*Benzylidene Guanosine*.—Prepared by a method analogous to that employed for 3′ : 5′-benzylidene adenosine (see above), and recrystallised from 70% ethanol, this substance had m. p. 296° (decomp.), $[\alpha]_D^{18}$ −98·5° (*c*, 2·08 in pyridine), −92·5° (*c*, 1·54 in dimethylformamide) (Found: C, 54·7; H, 4·3; N, 18·7. Calc. for $C_{17}H_{17}O_5N_5$: C, 54·9; H, 4·6; N, 18·9%). Gulland and Overend (*J.*, 1948, 1380) record m. p. 296°.

Guanosine-2′ Phosphate.—The preparation of this compound from 3′ : 5′-benzylidene guanosine was unsatisfactory, low yields being obtained by all methods investigated. Phosphorylation in dimethylformamide–pyridine with dibenzyl chlorophosphonate, followed by removal of the benzylidene residue with 0·01N-sulphuric acid at 80° for 45 minutes and conversion into the *barium* salt, gave an impure amorphous product in low yield (Found: C, 26·3; H, 6·1; N, 11·9. $C_{10}H_{12}O_8N_5PBa$ requires C, 24·1; H, 2·4; N, 14·1%). This salt was dissolved in hot water and treated with 20% lead acetate solution. The lead salt which separated was decomposed with hydrogen sulphide in the usual way, and the filtered solution concentrated to small bulk under reduced pressure. On gradual addition of acetone to the solution, *guanosine-2′ phosphate* separated as a white powder, m. p. 192° (decomp.) (Found, in material dried for 12 hours at 110°/1 mm.: P, 8·2. $C_{10}H_{14}O_8N_5P$ requires P, 8·5%).

Phosphorylation of 3′ : 5′-benzylidene guanosine with phosphoryl chloride, as in the case of 2′ : 3′-*iso*propylidene guanosine (see above), followed by removal of the benzylidene residue and isolation *via* the lead salt, gave a very low yield of impure guanosine-2′ phosphate which gave unsatisfactory analytical values and could not be purified (Found, in material dried at room temperature: C, 29·0; H, 4·0; N, 15·1. Calc. for $C_{10}H_{14}O_8N_5P,2H_2O$: C, 30·1; H, 4·5; N, 17·5%).

Uridine-5′ Phosphate.—A solution of 2′ : 3′-*iso*propylidene uridine (4·5 g.; dried for 18 hours at 110°/1 mm.; Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113) in dry pyridine (60 c.c.) at −50° was treated with dibenzyl chlorophosphonate (from 10 g. of dibenzyl phosphite) and kept just above the m. p. of the mixture (*i.e.*, between −40° and −30°) for 3 hours and then at room temperature overnight, and the product worked up in the usual way. The reddish gum so obtained was dissolved in ethanol, reprecipitated with ether, and dissolved again in aqueous ethanol (charcoal), and the filtered solution hydrogenated at room temperature and atmospheric pressure with a mixture of palladium and palladised charcoal as catalyst. Absorption was rapid, 315 c.c. of hydrogen being taken up in 1 hour, indicating a 44% yield of phosphorylated product (theory, 710 c.c.). This apparently low yield is probably due to solubility of the initially formed 2′ : 3′-*iso*propylidene uridine-5′ dibenzyl phosphate in ether. Catalyst was removed, the colourless solution concentrated to small volume under reduced pressure, N-sulphuric acid (25 c.c.) added, and the solution kept at 75° for 1½ hours. Barium hydroxide was added until neutral, and the warm solution filtered and evaporated to dryness *in vacuo*. The residual colourless glass was dissolved in water (20 c.c.) and filtered into absolute ethanol (25 c.c.), and the *barium* salt centrifuged off, washed with ethanol and ether, and dried (3·1 g., 43% overall yield from 2′ : 3′-*iso*propylidene uridine). Recrystallisation from water gave a mass of fine colourless needles which, when kept overnight at 0°, changed to large hexagonal plates, sparingly soluble in hot water (Found, in material dried for 15 hours at 110°/1 mm.: C, 22·3; H, 3·1; N, 5·7; P, 6·7. $C_9H_{11}O_9N_2PBa,H_2O$ requires C, 22·6; H, 2·7; N, 5·9; P, 6·5%. Found, in material dried for a further 18 hours: N, 6·3. $C_9H_{11}O_9N_2PBa$ requires N, 6·1%).

Dibrucine salt. The above precipitated crude barium salt (1 g.) was dissolved in warm water (15 c.c.), N-sulphuric acid (4·35 c.c.; end point checked with rhodizonic acid) rapidly added, and the precipitated barium sulphate centrifuged off. Brucine (2·03 g.) in methanol was added (to neutrality) and the solution evaporated to dryness under reduced pressure. Recrystallisation from aqueous methanol (30%) gave the pure dibrucine salt (2·2 g.) as colourless needles. When heated it softened very slightly at 165°, gave clear globules at 185—190°, and was completely molten at 202°; at 225—230° it decomposed with slight effervescence (Found, in material dried at 110°/1 mm. for 15 hours: N, 7·5; P, 2·8. Calc. for $C_9H_{13}O_9N_2P,2C_{23}H_{26}O_4N_2$: N, 7·6; P, 2·8%), $[\alpha]_D^{18}$ −70·4° (*c*, 1·2 in pyridine). Levene and Tipson (*J. Biol. Chem.*, 1934, **106**, 113) give m. p. (with foaming) at 200° after softening at 163—165° and $[\alpha]_D^{24}$ −68·8° (in pyridine). Gulland and Hobday (*J.*, 1940, 749) give m. p. 163—164° and decomposition with frothing at 198—200°, and $[\alpha]_D^{20}$ −69·7° (in pyridine).

2′-*Acetyl* 3′ : 5′-*Benzylidene Uridine*.—A solution of anhydrous 3′ : 5′-benzylidene uridine (13 g.) (Gulland and Smith, *J.*, 1947, 338) in pyridine (75 c.c.) and acetic anhydride (50 c.c.) was kept at room temperature overnight and then poured into ice-water (1 l.) with stirring. The somewhat gummy solid was filtered off, washed with water, dried, and dissolved in cold acetone (40 c.c.), the solution was filtered, and the filtrate was evaporated to dryness, finally under reduced pressure, giving a colourless glass (13 g.), which could not be crystallised (Found, in material dried for 48 hours at 40°/1 mm.: C, 57·9; H, 4·6; N, 7·6. $C_{18}H_{18}O_7N_2$ requires C, 57·8; H, 4·8; N, 7·5%).

2′-*Acetyl Uridine*.—A solution of 2′-acetyl 3′ : 5′-benzylidene uridine (11·5g.) in 20% acetic acid (350 c.c.) was heated under reflux for 3 hours. The solvent was removed under reduced pressure, final traces of acetic acid being removed by drying over potassium hydroxide *in vacuo*. The gummy residue was then dissolved in warm water (*ca.* 50 c.c.), filtered from insoluble material, and evaporated to dryness as before, giving a residue which was extracted with hot ethanol and again evaporated to dryness under

reduced pressure. *2′-Acetyl uridine* was thus obtained as a slightly yellow glass (7 g., 80%) which did not crystallise (Found, in material dried for 48 hours at 50°/1 mm.: C, 46·3; H, 5·0; N, 10·1. $C_{11}H_{14}O_7N_2$ requires C, 46·2; H, 4·9; N, 9·8%), $[\alpha]_D^{16}$ +9·1° (*c*, 1·98 in water).

2′-Acetyl 5′-Trityl Uridine.—A solution of 2′-acetyl uridine (5 g. anhydrous) and triphenylmethyl chloride (5·5 g., 1·11 mols.) in dry pyridine (80 c.c.) was set aside at room temperature overnight, heated at 100° for 3 hours, cooled to 0°, and poured into ice–water (800 c.c.) with vigorous stirring. The precipitated gum was well washed with water, dissolved in aqueous ethanol, and boiled with charcoal. The solution was evaporated to dryness, the residue redissolved in ethanol, and the solution again evaporated to dryness *in vacuo*, giving a brownish glass. The glass was taken up in a small volume of acetone and filtered into light petroleum (1000 c.c.; b. p. 60—80°) with vigorous stirring, and the amorphous solid (6·6 g., 72%) collected, washed with light petroleum and a mixture of this solvent with ether, and dried. The *compound* could not be crystallised; on heating it softened and swelled at 107—108° and melted with decomposition at *ca.* 130° (Found, in material dried for 48 hours at 60°/1 mm.: C, 69·0; H, 6·0; N, 5·6. $C_{30}H_{28}O_7N_2$ requires C, 68·2; H, 5·3; N, 5·3%).

Uridine-3′ Phosphate.—The above 2′-acetyl 5′-trityl uridine (5 g.; dried for 24 hours at 80°/1 mm.) was phosphorylated in the usual way in pyridine (60 c.c.) with dibenzyl chlorophosphonate (from 10 g. of dibenzyl phosphite), the mixture being kept for 6 hours at −40° and then at room temperature overnight. The resulting gum was precipitated from ethanol by addition of ether, dissolved in aqueous ethanol (charcoal) and, evaporated to dryness, giving a yellowish glass (6·3 g.). This glass (5·5 g.) was dissolved in 50% ethanol (200 c.c.) and hydrogenated (catalyst: palladium and palladised charcoal). Absorption of hydrogen was very slow, the theoretical amount for removal of two benzyl groups being absorbed in 15 hours. The filtrate was neutralised with sodium hydroxide and treated with 20% lead acetate solution, the lead salt centrifuged off, washed with water, and decomposed with hydrogen sulphide. Triphenylmethyl alcohol and lead sulphide were removed by filtration, and the filtrate was maintained at pH 10 by the addition of barium hydroxide, kept at 30° for 1 hour, neutralised with carbon dioxide, filtered hot, and concentrated under reduced pressure to *ca.* 100 c.c. Barium was quantitatively removed with sulphuric acid; the solution was evaporated to dryness *in vacuo* below 30° and dissolved in methanol, and the *nucleotide* (0·6 g., 30%) precipitated as a white powder by addition of acetone. When heated it softened and shrank at 167° and melted to an opaque melt at 192°; when further heated it effervesced slightly between 192° and 200° (Found: C, 30·9; H, 3·8; N, 7·9; P, 9·1. $C_9H_{13}O_9N_2P,1\frac{1}{2}H_2O$ requires C, 30·8; H, 4·6; N, 8·0; P, 8·8%). The dibrucine salt, prepared in the usual way, had an indefinite m. p.; when heated it shrank at 182° and became molten but opaque at 182—187°; the melt became clear at 189° and effervesced slightly at 195° (identical behaviour was shown by an authentic specimen of the dibrucine salt of uridylic acid prepared from yeast ribonucleic acid, and a mixture of the two materials behaved in the same way) (Found, in air-dried material: C, 53·6; H, 6·6; N, 6·7. Calc. for $C_9H_{13}O_9N_2P,2C_{23}H_{26}O_4N_2,7H_2O$: C, 53·3; H, 6·4; N, 6·8%), $[\alpha]_D^{16}$ −57·5° (*c*, 1·09 in pyridine). For the dibrucine salt of uridylic acid, Levene (*J. Biol. Chem.*, 1919, **40**, 395) gives m. p. 195° with effervescence and previous formation of an opaque mass at 185°, and $[\alpha]_D^{24}$ −55·9° (in pyridine). Gulland and Smith (*loc. cit.*) state that it swells to an opaque mass at 175—185° and becomes transparent at 188—190°, and has $[\alpha]_D$ −55·0° (in pyridine).

Uridine-3′ Phosphate by Direct Phosphorylation of 5′-Trityl Uridine.—Anhydrous 5′-trityl uridine (Levene and Tipson, *J. Biol. Chem.*, 1934, **104**, 385) (3·1 g.) was phosphorylated in the usual way with dibenzyl chlorophosphonate (from 6 g. of dibenzyl phosphite) in dry pyridine (40 c.c.) at −40° for 6 hours and then at room temperature overnight. Thereafter the procedure was analogous to that described above for the preparation from 2′-acetyl 5′-trityl uridine. The crude nucleotide (1·1 g., 53%) had m. p. *ca.* 180° (decomp.) (Found, in material dried for 20 hours at 110°/1 mm.: C, 29·6; H, 4·3; N, 8·1; P, 9·2%). The dibrucine salt was identical with that prepared from authentic uridylic acid (Found, in air-dried material: N, 7·0. Calc. for $C_9H_{13}O_9N_2P,2C_{23}H_{26}O_4N_2,7H_2O$: N, 6·8%), $[\alpha]_D^{16}$ −60·1° (*c*, 0·97 in pyridine). The crystalline *lead* salt was prepared from a solution of the ammonium salt of the acid (Levene, *J. Biol. Chem.*, 1919, **40**, 395) (Found, in material dried for 20 hours at 120°/1 mm.: C, 20·2; H, 2·5; N, 5·0. $C_9H_{11}O_9N_2PPb$ requires C, 20·4; H, 2·1; N, 5·3%).

The acid-hydrolysis curves of the free uridylic acid from both syntheses of the nucleotide were identical with that of natural uridylic acid from yeast ribonucleic acid.

*2′ : 3′-iso**Propylidene Cytidine.*—Cytidine (4 g.; dried for 12 hours at 110°/1 mm.) was dissolved in a solution of anhydrous zinc chloride (10 g.) in dry acetone (100 c.c.), and the mixture heated under reflux for 7 hours with exclusion of moisture. The clear solution was set aside overnight at room temperature, acetone then removed under reduced pressure, and dry ether (250 c.c.) cautiously added to the residue, with shaking. The fine precipitate of the zinc chloride–2′ : 3′-*iso*propylidene cytidine double salt was filtered off, washed with ether, dried, and added to a slight excess of warm barium hydroxide solution. The mixture was neutralised with carbon dioxide and filtered warm, the filter residue being extracted thrice with hot water. The combined filtrate and extracts were evaporated to dryness under reduced pressure, and the dry solid residue was extracted with hot ethanol. Removal of the solvent under reduced pressure gave a colourless glass. This was dissolved in water and filtered through Hyflo supercel to remove a slight trace of semi-colloidal impurity, the solution evaporated, and the residue redissolved in hot absolute ethanol, filtered, and again evaporated under reduced pressure, whereupon the iso*propylidene* compound was left as a glass (4·4 g., 95%). Addition of acetone to a solution of the glass in ethanol gave a white, very hygroscopic, powder (Found, in material dried for 48 hours at 55°/1 mm.: C, 49·4; H, 6·4; N, 14·1. $C_{12}H_{17}O_5N_3,\frac{1}{2}H_2O$ requires C, 49·4; H, 6·2; N, 14·4%).

Cytidine-5′ Phosphate.—Dibenzyl chlorophosphonate (from 10 g. of dibenzyl phosphite) was added to a solution of 2′ : 3′-*iso*propylidene cytidine (4·0 g.; dried for 18 hours at 60°/1 mm.) in dry pyridine (60 c.c.) at −40°, and the solution maintained at −40° for 3 hours and then set aside at room temperature overnight. When the solution was worked up in the usual way, a gum was obtained which was evaporated twice with ethanol and then precipitated from concentrated ethanolic solution by ether. The resinous product was hydrogenated in aqueous ethanol (catalyst: palladium and palladised charcoal). Absorption of hydrogen was fairly rapid, 435 c.c. being absorbed in 1 hour, indicating a 65—70% yield

of phosphorylated product (theoretical uptake, 635 c.c.). Catalyst was removed by filtration, the filtrate concentrated to small volume under reduced pressure, and N-sulphuric acid (20 c.c.) added; the mixture was kept at 70—75° for 1½ hours, then neutralised with barium hydroxide and barium carbonate, and filtered hot, the residue being washed with hot water. The combined filtrate and washings gave on evaporation a glass which was dissolved in hot water (20 c.c.) and filtered. Ethanol (30 c.c.) was added to the solution. The precipitated granular *barium* salt was collected, washed with ethanol and then ether, and dried (2·6 g., 38% overall yield from cytidine). For analysis a sample was reprecipitated from water by adding ethanol, whereupon it formed a readily filterable granular white powder (Found, in material dried for 20 hours at 120°/1 mm.: C, 24·4; H, 3·4; N, 9·8. $C_9H_{12}O_8N_3PBa$ requires C, 23·6; H, 2·6; N, 9·2%), $[\alpha]_D^{16}$ +11·4° (*c*, 0·33 in water). N-Sulphuric acid (*ca.* 3·8 c.c.) was added to a solution of the barium salt (1 g.) in water till all the barium had been precipitated (rhodizonic acid). Barium sulphate was removed by filtration through Hyflo supercel, and the filtrate evaporated to *ca.* 10 c.c. under reduced pressure. To the hot solution, hot ethanol (20 c.c.) was added, yielding *cytidine-5′ phosphate* (0·65 g., 90%) as colourless plates, m. p. 233° (decomp. with vigorous effervescence), unchanged by further recrystallisation (Found: C, 31·4; H, 4·5; N, 12·4; P, 9·4. $C_9H_{14}O_8N_3P,H_2O$ requires C, 31·6; H, 4·7; N, 12·3; P, 9·1%), $[\alpha]_D^{14}$ +27·1° (*c*, 0·54 in water).

The *dibrucine* salt, prepared from the barium salt by removal of barium and neutralisation with brucine, was recrystallised thrice from water. When heated it softened at *ca.* 185°, formed transparent globules at *ca.* 195°, and decomposed with effervescence at 215° (Found, in air-dried material: C, 49·6; H, 7·1; N, 7·1. $C_9H_{14}O_8N_3P,2C_{23}H_{26}O_4N_2,12H_2O$ requires C, 49·7; H, 6·8; N, 7·4%).

Cytidine-2′ Phosphate.—Dibenzyl chlorophosphonate (from 9 g. of dibenzyl phosphite) was added to a solution of 3′:5′-benzylidene cytidine (3·5 g.; Gulland and Smith, *J.*, 1948, 1527) in dry pyridine (50 c.c.) at −40°, and the solution maintained at −40° for 5 hours and then set aside at room temperature for 2 hours. Working up in the usual way gave a gum which was hydrogenated in aqueous ethanol (catalyst: palladium and palladised charcoal). The catalyst was removed by filtration and extracted with hot aqueous ethanol, and the combined filtrate and extracts concentrated to small volume under reduced pressure, made up to 250 c.c. with sulphuric acid (final strength 0·25N.), and heated under reflux for 2 hours. Benzaldehyde was extracted with ether, barium hydroxide solution added to pH 10 (no ammonia was detected), and the solution then neutralised with carbon dioxide, filtered hot, and concentrated to small bulk (*ca.* 100 c.c.) under reduced pressure. Ethanol (100 c.c.) was added, and the precipitated barium salt centrifuged off, washed with ethanol, and redissolved in water. The barium was then removed quantitatively. The aqueous solution was evaporated under reduced pressure to small volume, hot absolute ethanol (2 volumes) added to the boiling solution, and the mixture allowed to cool slowly. The crystalline nucleotide (1·0 g., 30%) was collected and recrystallised from aqueous ethanol (70%), giving pure cytidine-2′ phosphate as colourless irregular plates, m. p. 235° (with vigorous decomposition) after previous softening and darkening (Found, in material dried for 24 hours at room temperature over phosphoric anhydride: C, 33·1; H, 4·7; H, 13·0; P, 9·4. Calc. for $C_9H_{14}O_8N_3P$: C, 33·4; H, 4·4; N, 13·0; P, 9·6%), $[\alpha]_D^{16}$ +45·1° (*c*, 0·83 in water). Gulland and Smith (*loc. cit.*) give "m. p. 240—242° (placed in bath at 230°, m. p. tube 1 mm. diam., rate of heating 4° per minute)" and $[\alpha]_D^{18}$ +21·4° (*c*, 1·05 in water).

D-*Ribofuranose-5 Phosphate.*—A solution of 2′:3′-*iso*propylidene methyl D-ribofuranoside (4·5 g.; Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 299) in dry pyridine (50 c.c.) was cooled to −40°, and treated with dibenzyl chlorophosphonate (from 16 g. of dibenzyl phosphite). The mixture was kept at −40° for 3 hours and then at room temperature for a further 3 hours. Sodium carbonate (7 g.) and water (30 c.c.) were added, and the mixture was evaporated to dryness under reduced pressure. The residue was extracted with chloroform, the chloroform extract washed with aqueous sodium hydrogen carbonate and then water, dried (Na_2SO_4), and evaporated *in vacuo*. The residual mobile syrup was dissolved in aqueous ethanol and hydrogenated (catalyst: palladium, palladised charcoal, or platinum). Absorption of hydrogen was very rapid (*ca.* 40 c.c. per minute). The catalyst was removed by filtration, and the filtrate evaporated to small volume under reduced pressure, diluted with water, filtered from a small amount of oil, and made up to 400 c.c. with sulphuric acid (final strength N./3). The solution was set aside at room temperature for 18 hours, then neutralised with barium hydroxide and barium carbonate, and filtered through Hyflo supercel. The filtrate was concentrated to small volume (*ca.* 40 c.c.) *in vacuo*. The clarified solution was poured into absolute ethanol (200 c.c.), and the precipitated salt washed with ethanol and ether, and dried (6·9 g., 86%). The barium D-ribofuranose-5 phosphate, recrystallised from water, formed colourless hexagonal plates, was free from inorganic impurities, and readily reduced Fehling's solution (Found, in material dried for 2 hours at 80°/1 mm.: P, 8·3. Calc. for $C_5H_9O_8PBa$: P, 8·5%). The free acid had $[\alpha]_D^{14}$ +16·5° (0·15475 g. of the anhydrous barium salt dissolved in 1·05 ml. of N-hydrochloric acid and made up to 5 ml. with water). Levene and Stiller (*J. Biol. Chem.*, 1934, **104**, 299) give $[\alpha]_D^{26}$ +16·09°, +16·54°.

Hydrolysis of Nucleotides with 0·1N-*Sulphuric Acid.*—A weighed amount of nucleotide (*ca.* 20 mg.) was dissolved in water, and the solution made up to 20 ml. 2-Ml. portions of this solution were mixed with 0·2N-sulphuric acid (2 ml.), sealed in glass tubes, and immersed in a boiling water-bath. Single tubes were removed at suitable intervals, the contents washed into a standard flask and made up to 25 ml. with water, and free phosphoric acid was determined colorimetrically by the method of Allen (*Biochem. J.*, 1940, **34**, 858). The results are shown graphically in the figure, which also includes curves for guanosine-3′ phosphate taken from the data of Yamagawa (*J. Biol. Chem.*, 1920, **43**, 339) and for uridine-5′ phosphate, uridine-2′ phosphate, and cytidine-3′ phosphate from those of Gulland and Smith (*J.*, 1947, 341, 1527).

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