643. *Nucleotides. Part VI. The Structure of the Synthetic Nucleotides prepared from the Benzylidene Ribonucleosides.*

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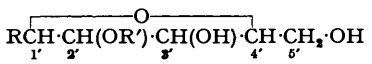
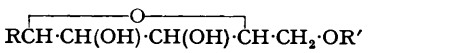
Re-examination of the synthetic compounds described by Gulland and Smith (*J.*, 1947, 338; 1948, 1527) as uridine-2′ phosphate and cytidine-2′ phosphate and by Michelson and Todd (*J.*, 1949, 2476) as adenosine-2′ phosphate and guanosine-2′ phosphate, has shown that they are in fact nucleoside-5′ phosphates. The reasons for the production of 5′-phosphates in syntheses hitherto believed to yield 2′-phosphates have been studied, and it is concluded that the condensation products of the nucleosides with benzaldehyde are 2′ : 3′- and not, as hitherto believed, 3′ : 5′-benzylidene derivatives. The implications of these findings and conclusions are discussed.

In Part III of this series, Michelson and Todd (*J.*, 1949, 2476) described the synthesis of a number of purine and pyrimidine mononucleotides by methods believed at the time to be unambiguous as regards the position of the phosphoryl group in the synthetic products. The validity of the syntheses described was confirmed in the cases of adenosine-3′ phosphate and uridine-3′ phosphate by direct comparison with nucleotides isolated from natural sources, but at the time no nucleoside bearing a phosphoryl group in the 2′-position was known to have been isolated from natural sources, so that no comparison could be made in the case of the synthetic adenosine-2′ and guanosine-2′ phosphates. That the latter compounds were indeed nucleoside-2′ phosphates rested wholly on their having been prepared by methods exactly analogous to those by which Gulland and Smith claimed to have prepared uridine-2′ phosphate (*J.*, 1947, 338) and cytidine-2′ phosphate (*J.*, 1948, 1527). As we have already briefly reported (Brown, Haynes, and Todd, *J.*, 1950, 408), subsequent work has shown that these claims to the synthesis of nucleoside-2′ phosphates must be withdrawn, and the present paper gives a detailed account of the investigations which have led to this conclusion.

In 1949, Carter and Cohn (*Fed. Proc.*, 1949, **8**, 190) reported the isolation from ribonucleic acid hydrolysates of two isomeric adenylic acids *a* and *b*, of which *b* was identical with the previously known yeast adenylic acid (adenosine-3′ phosphate). Adenylic acid *a* was not identical with the known adenosine-5′ phosphate (muscle adenylic acid) and, as it could be hydrolysed to adenosine, it appeared to be almost certainly adenosine-2′ phosphate (I; R = adenine; R′ = PO_3H_2). Through the kindness of Drs. Carter and Cohn, we were able to compare specimens of their adenylic acids with our synthetic materials. Periodate titration of these



(I).

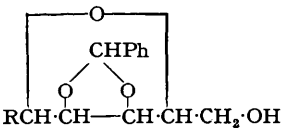


(II.)

acids showed that both were stable to this oxidant and that neither could be a 5′-phosphate. Direct comparison of the *X*-ray powder photographs of adenylic acid *a* and the synthetic " adenosine-2′ phosphate " of Michelson and Todd (*loc. cit.*), which had originally been described as amorphous but which by further solvent treatment was obtained in crystalline form by Dr. J.

Baddiley, showed that the two compounds were not identical. Surprisingly, however, the *X*-ray powder photograph of the synthetic product was identical with that of authentic adenosine-5′ phosphate (II; R = adenine; R′ = PO_3H_2) (Baddiley and Todd, *J.*, 1947, 648). The *X*-ray powder diagram of a sample of " cytidine-2′ phosphate " (Gulland and Smith, *loc. cit.*) prepared by Michelson and Todd (*loc. cit.*) similarly proved to be identical with that of cytidine-5′ phosphate (II; R = cytidine; R′ = PO_3H_2). These findings caused us to re-examine the alleged 2′-substituted ribonucleosides which had been prepared in this laboratory, using sodium metaperiodate as a diagnostic reagent, since only those nucleosides substituted in the 2′- or 3′-positions are stable towards it. The supposed adenosine-2′ phosphate, 2′-acetyl adenosine, and cytidine-2′ phosphate each consumed approximately 1 mol. of oxidant per mol.; all of them must therefore be substituted not in the 2′- but in the 5′-position. Through the courtesy of Dr. H. Smith, we were able to examine the original specimens of " cytidine-2′ phosphate," " dibrucine uridine-2′ phosphate," and " barium cytidine-2′ phosphate " described by Gulland and Smith (*locc. cit.*); each of these compounds on periodate titration consumed 1 mol. of oxidant per mol. The conclusion seems inescapable that the compounds described by Gulland and Smith (*locc. cit.*) and Michelson and Todd (*loc. cit.*) as ribonucleoside-2′ phosphates are in fact ribonucleoside-5′ phosphates. The failure of these authors to realise this fact was doubtless due in part to the rather indefinite physical criteria of purity employed and in part to too great reliance on the validity of the synthetic routes employed; it emphasises too the value of periodate titration and *X*-ray data in the characterisation of nucleotides.

The question now arose—why were 5′-substituted compounds obtained in these syntheses rather than the expected 2′-compounds? The validity of these syntheses rested first on the belief that the condensation product of benzaldehyde with guanosine was 3′ : 5′-benzylidene guanosine (IV; R = guanine) and secondly on the assumption that the benzylidene derivatives of the other nucleosides adenosine, uridine, and cytidine had a similar structure. This second assumption seemed reasonable and the evidence for the structure of benzylidene guanosine appeared to be adequate. Benzylidene guanosine was first described by Bredereck and Berger



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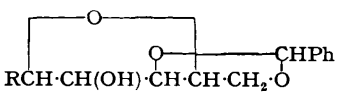

(IV.)

(*Ber.*, 1940, **73**, 1124), who prepared it by condensing guanosine with benzaldehyde in presence of fused zinc chloride. They formulated it as 3′ : 5′-benzylidene guanosine (IV; R = guanine) rather than the alternative 2′ : 3′-compound (III; R = guanine) because they were unable to prepare a trityl ether from it by reaction with trityl chloride, and they held that this indicated the absence of a primary alcoholic group. This formulation, too, was in agreement with the known tendency of benzylidene groups to be attached to 1 : 3-rather than 1 : 2-positions in carbohydrates (cf. Haworth and Hirst, *Ann. Rev. Biochem.*, 1936, **5**, 82). Further evidence for the 3′ : 5′-structure of benzylidene guanosine was adduced by Gulland and Overend (*J.*, 1948, 1380) who claimed that the acetyl guanosine obtained by acetylation of the benzylidene compound and subsequent removal of the benzylidene group was stable to periodate and could not therefore contain a free 1 : 2-glycol system (Lythgoe and Todd, *J.*, 1944, 592). They also methylated benzylidene guanosine, hydrolysed the product, and then hydrogenated the methyl ribose produced to a methyl ribitol; this methyl ribitol was optically active and hence could not be 3-methyl ribitol. The stability of the acetyl guanosine to periodate would eliminate a 2′ : 3′-structure for benzylidene guanosine, while the isolation of an optically active methyl ribitol would rule out the possibility of a 2′ : 5′-structure.

The apparent strength of this evidence for the structure of benzylidene guanosine led us to consider the possibility that the isolation of 5′-substituted compounds in the synthetic work already described might be due to acyl migrations. As a first step, a sample of adenylic acid *a*, which, on the evidence of Carter and Cohn and on the results of our periodate titrations, is almost certainly adenosine-2′ phosphate, was refluxed with mineral acid (N/100) under the conditions used by Michelson and Todd (*loc. cit.*) to remove the benzylidene group from the phosphorylation product of benzylidene adenosine. The only product isolated in crystalline form was unchanged starting material, but by means of paper chromatography, adenosine and adenine were detected in the crystallisation mother-liquors, together with adenylic acid *a* and two other substances, one of which travelled at the same speed as adenosine-3′ phosphate and may well have been that

compound in view of the reported isomerisation of pyrimidine nucleotides by mineral acid (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). The possibility of migration under these conditions cannot therefore be excluded. Benzylidene adenosine dibenzyl phosphate (Michelson and Todd, *loc. cit.*) when refluxed for 90 minutes with 10% aqueous acetic acid gave in high yield adenosine-5′ monobenzyl phosphate, identical in every respect with an authentic specimen prepared from 2′ : 3′-*iso*propylidene adenosine (Baddiley and Todd, *J.*, 1947, 648). As no evidence for the presence of any other product was obtained, it seems very unlikely that the 5′-phosphate owed its origin to the migration of the substituted phosphoryl residue from $C_{(2')}$ to $C_{(5')}$. Furthermore, repetition of the preparation of acetyl adenosine from benzylidene adenosine, in a manner designed to preclude acyl migration under alkaline conditions (Helferich and Klein, *Annalen*, 1926, **450**, 219; 1927, **455**, 173), gave the same product as has been described by Michelson and Todd (*loc. cit.*). This product again was found to be identical with 5′-acetyl adenosine prepared *via* 2′ : 3′-*iso*propylidene adenosine.

Our conclusion from these experiments was that, although some migration of a phosphate residue may occur in adenosine-2′ phosphate when heated with mineral acid, there is no evidence for, and much against, the view that the isolation of 5′-substituted compounds in syntheses starting from benzylidene adenosine is due to easy and complete migration of acyl groups from $C_{(2')}$ to $C_{(5')}$. It was therefore decided to re-examine the evidence upon which a 3′ : 5′-structure had been assigned to benzylidene guanosine (and hence by analogy to the other benzylidene nucleosides). Benzylidene guanosine, having the same melting point and other characteristics as the material described by Gulland and Overend (*loc. cit.*), when acetylated as described by these authors, yielded, in our hands, only amorphous heterogeneous products. Acetylation with acetic anhydride in pyridine solution, however, gave crystalline monoacetyl benzylidene guanosine which, on treatment with 30% aqueous acetic acid at 70°, furnished a crystalline monoacetyl guanosine similar in appearance to that of Gulland and Overend, but whose melting point was almost 20° higher than that recorded by these workers. This monoacetyl guanosine consumed approximately 1 mol. of periodate per mol. and was therefore clearly 5′-acetyl guanosine. This result is directly at variance with Gulland and Overend's statement that their acetyl guanosine was stable to sodium metaperiodate during 72 hours. The cause of this discrepancy remains unknown; it is true that our product had a higher melting point, but it is difficult to believe that it is an entirely different substance. In order to obtain further evidence on the structure of benzylidene guanosine which did not involve acylation procedures, benzylidene guanosine was methylated. The crystalline methylation product was not structurally investigated; from analytical values and its ultra-violet absorption spectrum it seems clear that during methylation the purine ring system had been broken and extensive *N*-methylation had occurred. The product was hydrolysed with sulphuric acid and, after working up, a yellow syrup was obtained which readily reduced Fehling's solution and was evidently a somewhat impure reducing sugar. Comparison of this material with syrupy 5-methyl ribose prepared from 2 : 3-*iso*propylidene methylribofuranoside (Levene, *J. Biol. Chem.*, 1934, **104**, 301) by methylation and subsequent hydrolysis was made on paper chromatograms. In three solvent systems, the main component of each product had the same R_F value and there was no evidence for the presence of any other reducing sugar. Dr. R. J. Anderson of Nottingham University has informed us that he found independently that the methyl ribose prepared from benzylidene guanosine showed the same behaviour as 5-methyl ribose on paper chromatography. That the product obtained from benzylidene guanosine is in fact 5-methyl ribose must on this evidence be regarded as highly probable, although the paper chromatographic evidence admittedly does not amount to absolute proof. The tritylation evidence upon which Bredereck and Berger (*loc. cit.*) based their structure of benzylidene guanosine was admittedly weak, but work carried out by Dr. P. E. Macey (private communication) has shown that it is, in any event, invalid, since he has been able to prepare a benzylidene trityl guanosine from benzylidene guanosine by reaction with trityl chloride.

To sum up then, the evidence presented in this paper indicates that all the synthetic nucleoside-2′ phosphates described by Gulland and Smith (*locc. cit.*) and by Michelson and Todd (*loc. cit.*) are, in fact, nucleoside-5′ phosphates. It further points to the conclusion that the condensation product of benzaldehyde with guanosine is 2′ : 3′-benzylidene guanosine (III; R = guanine) and not 3′ : 5′-benzylidene guanosine (IV; R = guanine); it would also follow that adenosine, uridine, and cytidine similarly form 2′ : 3′-benzylidene derivatives. These latter conclusions have important repercussions on the structures assigned to the intermediates described by Gulland and Smith (*locc. cit.*) and to a number of those described by Michelson and Todd (*loc. cit.*). In the work of the last-named authors, the supposed "2′-acetyl

nucleosides " derived from benzylidene adenosine and benzylidene uridine were employed as starting materials for the synthesis of the nucleoside-3′ phosphates. Since these acetyl derivatives are now known to be 5′-acetyl nucleosides it is clear that their tritylation products cannot have the structures previously assigned to them. Presumably tritylation occurs on nitrogen in these cases and the syntheses of adenosine-3′ phosphate and uridine-3′ phosphate from them are not unambiguous. There is no doubt, however, as to the identity of the adenosine derivative with Carter and Cohn's yeast adenylic acid *b* (*loc. cit.*) and with the previously known yeast adenylic acid, whose structure has been shown by degradative methods to be adenosine-3′ phosphate (Levene and Harris, *J. Biol. Chem.*, 1933, **101**, 419). There is also no doubt but that the synthetic uridine-3′ phosphate is identical with the long-known natural yeast uridylic acid. It should be pointed out, however, that no evidence has ever been adduced to prove that the phosphate residue in uridylic and cytidylic acids is at $C_{(3')}$; their accepted structures rest on the believed analogy between them and yeast adenylic acid. Michelson and Todd's synthesis (*loc. cit.*) certainly appears to support their current formulation in so far as it is probable that a process yelding from adenosine a product undoubtedly adenosine-3′ phosphate would similarly yield, from uridine, uridine-3′ phosphate. Further evidence of structure would, however, be desirable, especially in view of the recently reported heterogeneity in pyrimidine nucleotides from yeast ribonucleic acid (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811) and the isolation of an isomer of cytidylic acid from hydrolysates of yeast ribonucleic acid (Loring, Luthy, Bortner, and Levy, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). In the field of monoribonucleotides, unambigous syntheses are therefore available only for adenosine-5′ phosphate (Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131; Bredereck and Berger, *Ber.*, 1940, **73**, 269; Baddiley and Todd, *loc. cit.*), guanosine-5′ phosphate (Michelson and Todd, *loc. cit.*), cytidine-5′ phosphate (Michelson and Todd, *loc. cit.*), and uridine-5′ phosphate (Levene and Tipson, *J. Biol Chem.*, 1934, **106**, 113; Michelson and Todd, *loc. cit.*). The recorded syntheses of the nucleoside-3′ phosphates, although in some cases satisfactory in operation (Michelson and Todd, *loc. cit.*), do not of themselves offer proof of structure. Further investigations into the question of the structure of the pyrimidine ribonucleotides are now in progress, as also are studies on the synthesis of nucleoside-2′ phosphates, and will be reported upon in due course.

A final point may be mentioned. In their work on the phosphorylation of N^6 : 5′-ditrityl adenosine and 5′-trityluridine, Michelson and Todd (*loc. cit.*) reported a preferential phosphorylation at $C_{(3')}$ in each case, since they were able to isolate finally only adenosine-3′ phosphate and uridylic acid from these experiments. Re-examination of the phosphorylation of N^6 : 5′-ditrityl adenosine has shown however that attack at the 3′-position is not exclusive, since adenosine-2′ phosphate (adenylic acid *a*) can be detected in the crude product by paper chromatography.

Experimental.

X-Ray powder photographs were taken by using a Metropolitan-Vickers Raymax crystallographic unit and a Unicam standard powder camera (19 cm. diameter, Cu-α radiation).

Adenosine-5′ Phosphate from Benzylidene Adenosine.—Benzylidene adenosine (4 g.) (Michelson and Todd, *loc. cit.*) was phosphorylated with dibenzyl chlorophosphonate at −10°, rather than −30° as in the original procedure, to give benzylidene adenosine dibenzyl phosphate (3·6 g.). Hydrogenation followed by hydrolysis with dilute sulphuric acid (N/100), as described by Michelson and Todd, yielded adenosine-5′ phosphate, crystallising from water in needles, m. p. 186° (decomp.) (Found: C, 34·6; H, 4·7; N, 20·1. Calc. for $C_{10}H_{14}O_7N_5P$: C, 34·6; H, 4·1; N, 20·2%). This material—Michelson and Todd's " adenosine-2′ phosphate "—on periodate titration showed an uptake of 1·2 mols. of oxidant/mol. Its m. p. was undepressed in admixture with an authentic specimen of adenosine-5′ phosphate and it gave an identical *X*-ray powder photograph. This powder photograph differed from those given by adenylic acid *a* and adenylic acid *b*. The last named gave a photograph identical with that of adenosine-3′ phosphate. Adenylic acid *a* gave a *dibrucine* salt which crystallised from water as a pentahydrate, m. p. 165—175° (decomp.) (Found: C, 55·1; H, 6·0; N, 10·3. $C_{10}H_{14}O_7N_5P,2C_{23}H_{26}O_4N_2,5H_2O$ requires C, 54·8; H, 6·2; N, 10·3%). This compound did not depress the m. p. of the dibrucine salt of Michelson and Todd's " adenosine-2′ phosphate " nor did its *X*-ray powder photograph differ appreciably from that of the latter on visual inspection. It is evident, in view of the distinction between the free nucleotides, that both mixed m. p. and *X*-ray powder photograph evidence are liable to break down for complex salts of this type.

Adenosine-5′ Benzyl Phosphate.—Benzylidene adenosine dibenzyl phosphate (0·5 g.) was refluxed for 1½ hours with aqueous acetic acid (50 c.c.; 10%). The solution was evaporated to dryness and the residue crystallised by trituration with ethanol. Recrystallised from hot water adenosine-5′ benzyl phosphate was obtained as rectangular plates (0·3 g.), m. p. 235° undepressed by an authentic specimen of m. p. 235° (Baddiley and Todd, *loc. cit.*) (Found: C, 46·5; H, 4·4; N, 16·1. Calc. for $C_{17}H_{20}O_7N_5P$: C, 46·5; H, 4·6; N, 16·1%); *X*-ray powder photographs of the two materials were identical. This product on periodate titration showed an uptake of 1·0 mol. of oxidant/mol. The same material was also obtained by partial hydrogenation of benzylidene adenosine dibenzyl phosphate, a mixed palladium oxide–

palladised charcoal catalyst being used, followed by removal of the benzylidene group with aqueous acetic acid (10%).

" *Cytidine-2′ Phosphate.*"—" Cytidine-2′ phosphate " as prepared by Michelson and Todd (*loc. cit.*) had $[\alpha]_D^{14} = 23{\cdot}4°$ (*c*, 1·07 in water); the higher value quoted by these authors has not been confirmed. On periodate titration it absorbed 0·96 mol. of oxidant/mol., and it gave an *X*-ray diffraction photograph identical with that of the product described under the same name by Gulland and Smith (*loc. cit.*) and of authentic cytidine-5′ phosphate. A sample of Gulland and Smith's original " barium cytidine-2′ phosphate " showed an uptake of 1·1 mols. of periodate/mol.; in this experiment, the initially formed insoluble barium metaperiodate was dissolved before titration, by addition of a little dilute hydrochloric acid, and the barium was removed by adding ammonium sulphate.

" *Uridine-2′ Phosphate.*"—" Dibrucine uridine-2′ phosphate," prepared by Gulland and Smith (*loc. cit.*), on periodate titration showed an uptake of 1·1 mols./mol. and the same uptake was observed with a sample prepared by Michelson and Todd (*loc. cit.*). In these titrations brucine was removed by addition of excess of ammonia and several extractions with chloroform. The aqueous phase was reduced in volume, and then periodate was added and the titration carried out in the usual manner.

*5′-Acetyl 2′ : 3′-iso**Propylidene Adenosine.***—Acetic anhydride (3 c.c.) was added to a solution of *iso*propylidene adenosine (0·98 g.) in dry pyridine (25 c.c.), and the mixture set aside overnight. Ethanol (10 c.c.) was now added and after 1½ hours the solution was taken to dryness, the residue dissolved in chloroform, and the solution washed with sodium hydrogen carbonate and water and dried (Na_2SO_4). The dried solution was evaporated to small bulk and light petroleum (b. p. 60—80°) was cautiously added. *5′-Acetyl 2′ : 3′-iso**propylidene adenosine* (0·65 g.) separated. Recrystallised from chloroform–ether, it formed needles, m. p. 167° (Found: C, 51·7; H, 5·8; N, 20·2. $C_{15}H_{19}O_5N_5$ requires C, 51·7; H, 5·5; N, 20·1%).

5′-Acetyl Adenosine.—The acetyl *iso*propylidene derivative (0·45 g.) was refluxed for 1½ hours with aqueous acetic acid (10 c.c.; 10%). The solution was evaporated to dryness, and then re-evaporated with ethanol and the residue recrystallised from ethanol (5 c.c.). *5′-Acetyl adenosine* separated from ethanol or water as nacreous, hydrated plates; dried at 112° (P_2O_5) it had m. p. 143° after softening at 134°, but was very hygroscopic (Found: C, 46·2; H, 5·1. Calc. for $C_{12}H_{15}O_5N_5$: C, 46·6; H, 4·9%). On periodate titration it showed an uptake of 1·06 mols./mol. It formed a *picrate* which separated from water as small yellow plates, m. p. 194° (decomp.) (Found: C, 39·8; H, 3·5. $C_{12}H_{15}O_5N_5,C_6H_3O_7N_3$ requires C, 40·2; H, 3·4%).

5′-Acetyl Adenosine from Benzylidene Adenosine.—Michelson and Todd's *ON*-diacetylbenzylidene adenosine (*loc. cit.*) (0·75 g.) was warmed to 70° for two hours with aqueous acetic acid (50 c.c.; 10%). The solution was evaporated to dryness under reduced pressure, and the residue re-evaporated with ethanol and crystallised first from ethanol (5 c.c.) and then water, giving nacreous plates, which after careful drying had m. p. 143°, undepressed in admixture with authentic 5′-acetyl adenosine (see above) (Found: C, 46·3; H, 4·7; N, 22·2%). Michelson and Todd describe a dihydrate, m. p. 67—70°; we have found that by varying the period of drying, crystalline products of intermediate m. p. can be obtained. Periodate uptake: 0·9 mol./mol. It gave a picrate, m. p. 194° undepressed in admixture with an authentic specimen.

Effect of Mineral Acid on Adenylic Acid a.—Adenylic acid *a* (100 mg.) (supplied by Drs. Carter and Cohn) was dissolved in dilute sulphuric acid (30 c.c.; N/100), and the solution refluxed for one hour, then cooled and neutralised with barium hydroxide. Barium sulphate was removed by centrifugation and the clear solution concentrated to small bulk (3 c.c.). Adenylic acid *a* (30 mg.) crystallised on cooling; it was identical in every way with the starting material and gave the same *X*-ray powder photograph. A further small quantity was obtained by concentration of the mother-liquor. The final mother-liquor from this experiment was evaporated to dryness, and the residue redissolved in water and put on a paper chromatogram with appropriate reference compounds, 5% disodium hydrogen phosphate and 5% potassium dihydrogen phosphate solutions being used as developing solvents (Carter, *J. Amer. Chem. Soc.*, 1950, **72**, 1466). In this way, the presence in the mother-liquors of adenine, adenosine, adenosine-3′ phosphate (adenylic acid *b*), and adenylic acid *a*, together with an unidentified product giving a considerably faster moving spot (R_P 0·85 in 5% disodium hydrogen phosphate solution) was established. No evidence for the presence of adenosine-5′ phosphate, which travels at the same rate as adenylic acid *a* in these solvents, was found by the periodate spray described by Buchanan, Dekker, and Long (*J.*, 1950, 3162).

Acetyl Benzylidene Guanosine.—Benzylidene guanosine [4 g.; m. p. 296° (decomp.)] (Gulland and Overend, *loc. cit.*) was dissolved in boiling pyridine (100 c.c.), the solution cooled to *ca.* 30°, and acetic anhydride (15 c.c.) added. The mixture was set aside overnight, ethanol (25 c.c.) added, and after one hour the whole was evaporated under reduced pressure. The residue was twice re-evaporated with ethanol, and then dissolved in warm chloroform (100 c.c.) and set aside. The acetyl benzylidene guanosine (2·8 g.) which separated was recrystallised from aqueous ethanol and then had m. p. 253—254°. Bredereck and Berger (*loc. cit.*) give m. p. 263° and Gulland and Overend (*loc. cit.*) m. p. 260—262° for " 2′-acetyl 3′ : 5′-benzylidene guanosine " (Found: C, 54·6; H, 5·0; N, 17·1. Calc. for $C_{19}H_{19}O_6N_5$: C, 55·2; H, 4·6; N, 17·0%).

5′-Acetyl Guanosine.—The acetyl benzylidene compound (0·4 g.) was heated with aqueous acetic acid (15 c.c.; 30%) for two hours at 70°, and then cooled, diluted with water (30 c.c.), and left overnight. Unchanged starting material which separated was filtered off, and the filtrate evaporated to dryness and triturated with ethanol whereupon the product crystallised (0·14 g.). Recrystallised from ethanol it had m. p. 192—193° (Found: C, 43·9; H, 4·5; N, 21·2. Calc. for $C_{12}H_{15}O_6N_5$: C, 44·5; H, 4·6; N, 21·5%). The product which was hygroscopic is formulated as 5′-acetyl guanosine on the basis of periodate titrations which showed an uptake of 0·95, 1·01 mols./mol. Bredereck and Berger (*loc. cit.*) give m. p.

180° and Gulland and Overend (*loc. cit.*) 176—179° for their " 2'-acetyl guanosine " prepared by essentially similar methods.

Methylation of Benzylidene Guanosine (cf. Gulland and Overend, *loc. cit.*).—Methyl sulphate (4·3 c.c.) and aqueous sodium hydroxide (34 c.c.; 30%) were gradually added during two hours to a mechanically stirred suspension of acetyl benzylidene guanosine (2·7 g.) in acetone (30 c.c.). The mixture was heated at 75° for a further 2 hours then cooled, neutralised (phenolphthalein) with sulphuric acid, and evaporated almost to dryness. The entire methylation process was now repeated on the residue, and thereafter the product was extracted several times with chloroform. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The gummy residue crystallised on being boiled with methanol; washing it with further small amounts of this solvent removed a little purple material, leaving the methylation product as a solid (1·25 g.). Recrystallised from chloroform–ether, it was obtained as small prisms, m. p. 226—228° (Found: C, 57·0, 57·0; H, 6·0, 6·1; N, 15·2, 15·2; MeO, 8·2; NMe, 18·3%). The methylated compound was readily soluble in chloroform but virtually insoluble in ether, methanol, and dilute sodium hydroxide solution; on being boiled with mineral acid it liberated benzaldehyde and a reducing sugar. Ultra-violet absorption in alcohol: maxima at 2800—2810 A. ($E^{1\%}_{1\,cm.}$ = 267) and 2275—2295 A. ($E^{1\%}_{1\,cm.}$ = 532); minima at 2555 A. ($E^{1\%}_{1\,cm.}$ = 96) and 2195 A. ($E^{1\%}_{1\,cm.}$ = 500).

Hydrolysis of Methylated Benzylidene Guanosine.—The methylated material prepared as above (202 mg.) was heated for 4½ hours at 80° with dilute sulphuric acid (10 c.c.; N/10). During this operation the starting material gradually dissolved giving a yellow solution which was cooled, extracted with chloroform to remove benzaldehyde, and then left overnight with excess of barium carbonate to remove sulphate ions. The filtered solution was now evaporated to dryness under reduced pressure giving a reddish gum which was repeatedly extracted with chloroform (75 c.c. in all). The dried extract was evaporated yielding an orange gum which was dissolved in ethanol (1 c.c.) and dry ether (10 c.c.) was added. The clear solution was filtered from a trace of red semi-solid precipitate, and evaporated. The product was a yellowish syrup (81 mg.) which readily reduced Fehling's solution. A 2·5% aqueous solution of this product was prepared and compared with a similar solution of syrupy 5-methyl ribose on paper chromatograms. Both 5-methyl ribose and the degradation product gave a yellow spot when detected with the aniline oxalate reagent, in contrast to the cherry-red colour developed by ribose. The R_F values in five different solvent systems are given in the table, together with those of ribose itself (used as a marker). Hydrolysis of the methylated benzylidene guanosine with 87% formic acid gave, after working up, a dark gum containing a reducing sugar with the same chromatographic characteristics as the product of sulphuric acid hydrolysis. The slight spread of R_F values observable in the table for the systems containing collidine and pyridine we have at times observed with other compounds; it seems that in such solvents the R_F values are noticeably affected by the presence of inorganic impurities.

Solvent system.	*A*.	*B*.	*C*.	*D*.			*E*.		
5-Methyl ribose	0·52	0·43	0·45	0·73	0·77	0·69	0·77	0·77	0·8
Sulphuric acid hydrolysis product	0·52	0·43	0·45	0·75	0·77	0·74	0·81	0·79	0·84
Ribose	0·34	0·2	0·25	0·64	0·64	0·64	0·74	0·72	0·76
Mixed spot of 5-methyl ribose and hydrolysis product	—	—	—	—	—	0·72	—	—	0·82

Solvent systems: *A*, *n*-butanol–acetic acid [1]; *B*, *n*-butanol–water [1]; *C*, *n*-butanol–ethanol–1% aqueous ammonia [1]; *D*, collidine–water [1]; *E*, ethyl acetate–pyridine–water [2].

[1] Partridge, *Biochem. J.*, 1948, **42**, 238. [2] Jermyn and Isherwood, *ibid.*, 1949, **44**, 402.

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