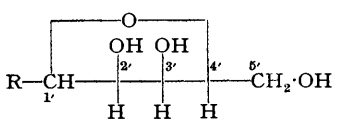
12. *Nucleotides. Part IX.* The Synthesis of Adenylic Acids* a *and* b *from 5′-Trityl Adenosine.*

By D. M. Brown and A. R. Todd.

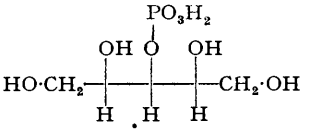
Phosphorylation of 5′-trityl adenosine with dibenzyl chlorophosphonate, followed by removal of protecting groups, yields two adenylic acids, identical in all respects with the isomeric adenylic acids *a* and *b* derived from ribonucleic acids, and evidence is presented for their formulation as adenosine-2′ phosphate and adenosine-3′ phosphate, although not necessarily respectively. Their interconversion under acidic conditions is explained by facile phosphoryl migration, a concept which clarifies some other properties of the acids and their derivatives.

Earlier work in these and other laboratories has been directed to the final structural elucidation and total synthesis of the natural ribonucleosides adenosine, guanosine, uridine, and cytidine obtained by hydrolysis of ribonucleic acids. They can now be represented by the general formula (I) in which R represents the purine (adenine or guanine) or pyrimidine (uracil or cytosine) residue, D-ribose being linked in the β-orientation to $N_{(9)}$ in the case of the purine and to $N_{(3)}$ in the case of the pyrimidine nucleosides. In the present series of papers, attention has been directed to, *inter alia*, the synthesis of their monophosphates, the ribonucleotides, using, if possible, methods which would define without ambiguity the location of the phosphoryl group in the synthetic products. To date, unambiguous syntheses have been recorded for adenosine-5′ phosphate (muscle adenylic acid) (Baddiley and Todd, *J.*, 1947, 648; Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131; Bredereck, Berger, and Ehrenberg, *Ber.*, 1940, **73**, 269), for uridine-5′ phosphate (Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113; Michelson and Todd, *J.*, 1949, 2476), and for guanosine-5′ phosphate and cytidine-5′ phosphate (Michelson and Todd, *loc. cit.*).

For many years it was generally accepted that only four mononucleotides could be isolated from chemical hydrolysates of ribonucleic acids. The location of the phosphoryl group in these substances was studied by Levene and Harris (*J. Biol. Chem.*, 1932, **98**, 9; 1933, **101**, 419) who, on the basis of certain degradative experiments on yeast adenylic and guanylic acids, concluded that these nucleotides were the 3′-phosphates of the corresponding nucleosides. The stability of the glycosidic linkage in the pyrimidine nucleotides rendered similar degradative procedures impracticable but it was assumed by analogy that they too were 3′-phosphates. Michelson and Todd (*loc. cit.*), using a synthetic route which, on available evidence, seemed unambiguous, synthesised nucleoside-3′ phosphates and did in fact obtain adenylic and uridylic acids which were identical with the natural products and gave identical derivatives. Their synthetic route involved as



(I)



(II)

key intermediates the benzylidene nucleosides whose formulation as 3′ : 5′-benzylidene derivatives appeared secure from the degradative work on benzylidene guanosine carried out by Gulland and Overend (*J.*, 1948, 1380; cf. also Bredereck and Berger, *Ber.*, 1940, **73**, 1124). The same benzylidene derivatives were used to prepare the so-called 2′-phosphates of the nucleosides by direct phosphorylation and removal of the benzylidene group (Gulland and Smith, *J.*, 1947, 338; 1948, 1527; Michelson and Todd, *loc. cit.*).

* Part VIII, *J.*, 1951, 2952.

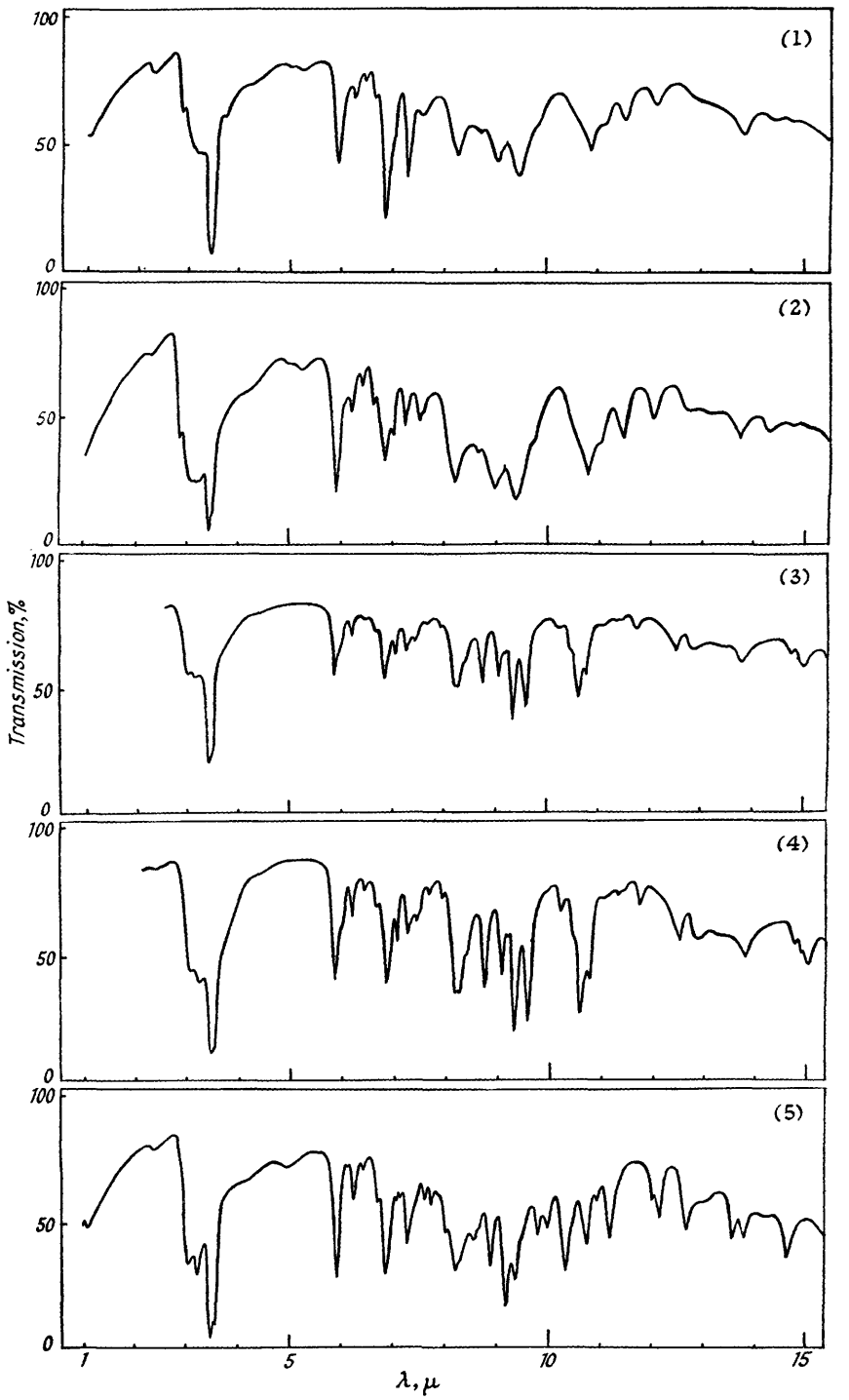
Subsequent work (Brown, Haynes, and Todd, *J.*, 1950, 2299) showed that the benzylidene nucleosides used were in fact 2′ : 3′-benzylidene derivatives; it followed that the "2′-phosphates" were in fact 5′-phosphates and that the syntheses of the natural nucleotides by Michelson and Todd (*loc. cit.*), although they gave the natural products, were ambiguous in that they proved only that the phosphoryl group was either at $C_{(2')}$ or at $C_{(3')}$.

An important advance in nucleic acid chemistry was made by Carter and Cohn (*Fed. Proc.*, 1949, **8**, 190) when they succeeded in separating from alkaline hydrolysates of yeast ribonucleic acid two isomeric adenylic acids which they termed *a* and *b*. This was followed by the separation into two isomers of crude guanylic, uridylic, and cytidylic acids in similar hydrolysates (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471, 2811; Loring, Luthy, Bortner, and Levy, *ibid.*, p. 1471). Carter and Cohn (*loc. cit.*; see also Carter, *J. Amer. Chem. Soc.*, 1950, **72**, 1466) degraded the isomeric adenylic acids *a* and *b* to adenine and adenosine and suggested that they were adenosine-2′ and adenosine-3′ phosphates, adenosine-5′ phosphate being excluded as a possible structure for either on enzymic evidence and on chemical grounds, including their stability to periodic acid (Brown, Haynes, and Todd, *loc. cit.*). Other types of isomerism, however, were not excluded. Their view that adenylic acid *b* was adenosine-3′ phosphate rested on its identity with the previously known and synthetically available yeast adenylic acid; as has already been mentioned, however, the synthetic route to this material did not in fact prove the location of the phosphoryl group. It seemed that a solution of the constitutional problem might be most directly obtained by unambiguous synthesis of either or both of the isomeric nucleotides. It may be stated at once that this objective has not been reached. With the demonstration that the condensation product of adenosine with benzaldehyde is 2′ : 3′-benzylidene adenosine exactly analogous to the condensation product with acetone, the problem of differentiating the $C_{(2')}$ and the $C_{(3')}$ positions became much more difficult. Many examples are known in carbohydrate chemistry in which benzaldehyde condenses with 1 : 3-glycol systems to produce cyclic benzylidene compounds (cf. Haworth and Hirst, *Ann. Rev. Biochem.*, 1936, **5**, 82), and its failure to do so in the case of the nucleosides made it probable that no better results would be obtained with other reagents. Moreover, in alkylation and acylation no preferential reaction at $C_{(2')}$ or $C_{(3')}$ has been observed. Nevertheless, many experiments were carried out with a variety of reagents in the hope of preparing a 3′ : 5′-substituted adenosine; since no success attended them they are not described here. It is apparent from a consideration of the stereochemistry of the ribofuranose residue present in the nucleosides that the $C_{(2')}$ and the $C_{(3')}$ positions are very similar and that the relation between the hydroxyl groups at $C_{(3')}$ and $C_{(5')}$ is not favourable to the formation of 3′ : 5′-cyclic derivatives; further evidence pointing in the same direction has come from a study of the phosphoryl migration in adenylic acid derivatives discussed in some detail in the following paper.

Attention was therefore directed to the products obtained from the phosphorylation of 5′-substituted nucleosides, since Brown, Haynes, and Todd (*loc. cit.*) had already noticed that on phosphorylating $N_{(6)}$: 5′-dityrityl adenosine and removing protecting groups a product was obtained which on paper chromatography showed two spots corresponding to adenylic acids *a* and *b*. Treatment of 5′-trityl adenosine (Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131) with dibenzyl chlorophosphonate in the usual manner gave a product having the composition of 5′-trityl adenosine dibenzyl phosphate in excellent yield. This was hydrogenated to remove benzyl groups, then converted into its lead salt, and the latter suspended in water and decomposed with hydrogen sulphide, a process which simultaneously removed the 5′-trityl group (cf. Michelson and Todd, *loc. cit.*). From the filtered solution obtained, free nucleotides could be isolated but the yield was always low. The solution was, however, shown to contain only two components, corresponding to adenylic acids *a* and *b* which could be separated in crystalline form after ion-exchange chromatography.

The low yields obtained above made this isolation procedure unattractive and for large-scale preparation the following method was adopted. The 5′-trityl adenosine dibenzyl phosphate was treated with 80% acetic acid, causing removal of the trityl group

Spectra of solids in Nujol.



1, ***Synthetic adenylic acid*** a. 2, *Natural adenylic acid* a. 3, *Synthetic adenylic acid* b. 4, *Natural adenylic acid* b. 5, *Adenosine-5′ phosphate.*

and partial debenzylation. Hydrogenation then gave the mixed adenylic acids, isolated as a barium salt in an overall yield of some 50%. On the evidence of paper chromatography, this material contained no nucleotides other than adenylic acids *a* and *b*. Application of anion-exchange chromatography by the method used by Cohn (*loc. cit.*) for the separation of the natural acids separated the synthetic mixture into two crystalline isomeric adenylic acids. These were rigidly identified as adenylic acid *a* and adenylic acid *b* by comparison with specimens of the natural acids (kindly supplied by Drs. Carter, Cohn, and Doherty) by comparing their ion-exchange and paper-chromatographic behaviour, *X*-ray powder photographs, and infra-red absorption spectra (figure). The synthetic adenylic acid *b* was also obtained as a beautifully crystalline hydrate which gave a different *X*-ray powder photograph but was otherwise similar to the anhydrous acid. In view of the complexity of the *X*-ray diffraction photographs we have not indexed the lines and have relied on visual comparison. Clark (*Arch. Biochem. Biophys.*, 1951, **31**, 18) has recorded data from *X*-ray powder photographs of yeast adenylic acid without, however, indicating which isomer was examined. Blout and Fields (*J. Biol. Chem.*, 1949, **178**, 335) have also published an infra-red spectrum of yeast adenylic acid; our spectra (figure) show considerably greater resolution, and for completeness we have included also the spectrum of adenosine-5′ phosphate (muscle adenylic acid).

Having established that the phosphorylation of 5′-trityl adenosine yields the isomeric adenylic acids *a* and *b*, it is necessary to enquire whether these acids are indeed the 2′- and 3′-phosphates of adenosine. It is clear from the stability of both acids to periodate that they contain no free α-glycol system. Again, it may be argued that in general monoesterification of a molecule containing two similar hydroxyl groups would be expected to yield isomers differing in the location of the acyl group. In the present instance, the production of only two isomeric acids is strong presumptive evidence for their formulation as the 2′- and 3′-phosphates. Carter (*loc. cit.*) suggested the alternative possibility that both acids might be 3′-phosphates and that they might differ by virtue of α-β-isomerism at the glycosidic linkage. Doherty (Abstr. 118th Meeting Amer. Chem. Soc., 1950, 56c) has carried out degradative experiments which at first sight appear to favour this view. The original work of Levene and Harris (*locc. cit.*) on yeast adenylic acid rested on removal of the sugar phosphate residue from a nucleotide under acid conditions followed by its reduction to a ribitol phosphate; since their product was optically inactive, they formulated it as ribitol 3-phosphate (II) and hence drew the conclusion that the phosphoryl group in adenylic acid was at position 3′. Doherty (*loc. cit.*) treated both the adenylic acids *a* and *b* with dry hydrogen chloride in benzyl alcohol and then hydrogenated the resulting benzyl riboside phosphate to a ribitol phosphate. In each case he obtained optically inactive products and this he held to indicate that they were derived from riboside-3′ phosphates, *i.e.*, that *a* and *b* were α- and β-adenosine-3′ phosphates. There are, however, a number of objections to this conclusion. Only one adenosine has been obtained from natural sources, including ribonucleic acids, and this has been shown to have the β-configuration (Davoll, Lythgoe, and Todd, *J.*, 1946, 833; Clark, Todd, and Zussman, *J.*, 1951, 2952); no example of α-β-isomerism among any of the natural nucleosides has ever been observed. Moreover, we observed that adenylic acids *a* and *b* undergo ready interconversion in dilute acidic solutions to an equilibrium mixture containing approximately equal amounts of the two isomers. A similar interconversion occurs in the case of the isomeric pyrimidine nucleotides from ribonucleic acid (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). This interconversion is inherently unlikely to result from an α-β-isomerisation since in that case one form, being more stable, would be expected to predominate (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 861) and isomerisation would be accompanied by furanose–pyranose changes. Since such α-β-interconversions depend on the electron availability at the nitrogen atom attached to $C_{(1)}$ of the sugar residue (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) it is very unlikely on theoretical grounds that they should occur in adenosine derivatives where $N_{(9)}$ in the purine residue has a very low basicity; this argument also applies to other natural and synthetic nucleosides and their derivatives.

All the properties of adenylic acids *a* and *b* (and equally those of the other isomeric

pairs) are, however, easily explained on the basis that they are 2′- and 3′-phosphates and that their interconversion is due to facile phosphoryl migration. That a migration of this type is to be expected in acid solution is clear from the analogous acid-catalysed phosphoryl migration in the monophosphates of glycerol (Bailly, *Compt. rend.*, 1938, **206**, 1902; 1939, **208**, 443, 1820; Verkade, Stoppelenburg, and Cohen, *Rec. Trav. chim.*, 1940, **59**, 886). Verkade *et al.* (*loc. cit.*) have also shown that the glycerol monophosphates are stable without rearrangement in alkaline solution, an observation which we have also made with adenylic acids *a* and *b*. This phosphoryl migration suggests a probable explanation for the lack of optical activity in the ribitol phosphates obtained by Levene and Harris (*locc. cit.*) and by Doherty (*loc. cit.*) in their degradations of adenylic acids, since if at any time the phosphoryl group becomes attached to the symmetrical $C_{(3)}$ position (cf. II) further migration will lead to the production of racemates. On this view, the evidence obtained from such degradative experiments is unreliable and the conclusion must be drawn that in no case has the position of the phosphoryl residue in any of the natural nucleotides (other than the 5′-nucleotides) been established. In our view, the weight of evidence indicates that the isomeric adenylic acids *a* and *b* are the 2′- and 3′-phosphates of adenosine, although which is the 2′- and which the 3′- remains to be determined. The other pairs of isomeric nucleotides have almost certainly analogous structures.

In the course of the synthesis of adenylic acids *a* and *b* described above, the initially prepared 5′-trityl adenosine dibenzyl phosphate was treated with 80% acetic acid, and the water-soluble product hydrogenated to yield the nucleotides. In one experiment hydrogenation was slow and incomplete, and after conversion of the products into barium salts there was obtained, in addition to the barium salt of adenylic acids *a* and *b*, a crude barium salt of what was evidently a mixture of the monobenzyl esters of these acids. By paper-chromatographic methods it was found that acid hydrolysis of this material yielded adenine, whereas hydrogenation converted it into a mixture of adenylic acids *a* and *b*. Moreover, by treating the mixed adenylic acids *a* and *b* with phenyldiazomethane in dimethylformamide and neutralisation of the product with barium hydroxide, it was possible to isolate a barium salt having the analytical composition of a monobenzyl ester of adenylic acid and showing the same paper-chromatographic behaviour as that of the material obtained above. Two components were present in the barium salt prepared by either route, corresponding to the *a* and *b* isomers, and it was possible to relate them to their corresponding adenylic acid. We have not isolated the separate isomeric esters, since they appear to undergo interconversion reactions very readily during the working up of the phenyldiazomethane reaction product. In the phenyldiazomethane reaction, small amounts of the dibenzyl esters are also produced but we have not studied them further.

The monobenzyl esters of adenylic acids *a* and *b* obtained above are very readily and completely debenzylated by both acids and alkali, with simultaneous phosphoryl migration (*i.e.*, production of a mixture of the two nucleotides). This interesting behaviour in which diesters of phosphoric acid show a remarkable instability towards both acid and alkali has a counterpart in the behaviour of the esters of glycerol monophosphates (Bailly and Gaumé, *Bull. Soc. chim.*, 1935, **2**, 354; Baer and Kates, *J. Biol. Chem.*, 1948, **175**, 79). Its mechanism will be discussed more fully elsewhere (Brown and Todd, following paper), together with its important implications with regard to the hydrolytic behaviour and structure of the nucleic acids.

Experimental

M. p.s are uncorrected. *X*-Ray powder photographs were taken by using a Metropolitan-Vickers Raymax crystallographic unit and a Unicam standard powder camera (19-cm. diam.; Cu-K_α radiation). The infra-red absorption spectra were determined by using a Perkin–Elmer Model 21, self-recording, double-beam instrument with sodium chloride prisms.

Paper Chromatography of Some Adenine Derivatives.—For the paper-chromatographic analysis of the products of the reactions described here two solvent systems were used. The 5% disodium hydrogen phosphate–*iso*amyl alcohol (hereafter, sodium phosphate) solvent of Carter (*loc. cit.*) gave readily reproducible R_F values within small limits. The values quoted in the

annexed Table are slightly lower than those given by Carter for those substances which he studied. In the butanol–acetic acid–water (butanol–acetic acid) solvent system (Partridge, *Biochem. J.*, 1948, **42**, 238) R_F values varied widely in different chromatograms, particularly when run downwards; those values quoted were typical (not average) for upward-run chromatograms. This solvent had the advantage of separating the adenylic acids widely from their benzyl esters (which were well resolved) and from non-phosphorylated derivatives. In chromatograms using either solvent standard compounds were always run simultaneously. Adenylic acid *a* is not distinguished from adenosine-5′ phosphate in the sodium phosphate solvent (Carter, *loc. cit.*), nor are the three adenylic acids characteristically resolved by butanol–acetic acid. For the detection of spots, chromatograms were photographed on Ilford Reflex Document Paper No. 50 in ultra-violet light (Markham and Smith, *Biochem. J.*, 1949, **45**, 294).

Substance	R_F values: 5% Na_2HPO_4–*iso*amyl alcohol	R_F values: Butanol–acetic acid–water (4 : 1 : 5)
Adenine	0·36	0·57
Adenosine	0·50	0·48
Adenylic acid *a*	0·69	0·15
Adenylic acid *b*	0·62	—
Adenosine-5′ phosphate	—	0·15
Adenosine benzyl hydrogen phosphate *a*	0·67	0·44
Adenosine benzyl hydrogen phosphate *b*	0·56	0·50
Adenosine-5′ benzyl hydrogen phosphate	0·65	0·41

5′-Trityl Adenosine Dibenzyl Phosphate.—A solution of 5′-trityl adenosine (3·1 g.) in dry pyridine (60 c.c.) at a temperature slightly above the m. p. of the solution was treated with dibenzyl chlorophosphonate (from 4·5 g. of dibenzyl hydrogen phosphite) with exclusion of moisture. The solution was kept at this temperature, with occasional shaking, for 4 hours and then left at room temperature overnight. A solution of sodium carbonate (3·0 g.) in water (15 c.c.) was added, sodium chloride removed by filtration, and the filtrate evaporated to dryness under reduced pressure. The residue was evaporated under reduced pressure several times with ethanol to remove pyridine completely. The residual pale brown gum was shaken with chloroform and sodium hydrogen carbonate solution, and the chloroform layer further washed with sodium hydrogen carbonate and then water and dried (Na_2SO_4). After evaporation to dryness, the residue was dissolved in ethanol (15 c.c.) and set aside for 48 hours, whereupon unchanged 5′-trityl adenosine (0·24 g.) separated. After filtration, the solution was evaporated to dryness and the resulting solid foam refluxed with dry ether (25 c.c.) for 2 hours. The granular *phosphate* (4·7 g.) was hygroscopic but free-flowing when dry (Found, in material dried at 55° *in vacuo* over phosphoric oxide: C, 65·6; H, 5·5; N, 8·8. $C_{43}H_{40}O_7N_5P$ requires C, 67·2; H, 5·2; N, 9·1%. $C_{43}H_{40}O_7N_5P,H_2O$ requires C, 65·6; H, 5·3; N, 8·9%).

Adenylic Acids a *and* b *from 5′-Trityl Adenosine Dibenzyl Phosphate.*—(a) *Lead salt method.* The above dibenzyl ester (1·0 g.) was dissolved in ethanol (50 c.c.) and water (5 c.c.) and hydrogenated at room temperature and pressure over palladised charcoal (70 mg.) and palladium oxide (30 mg.). Hydrogenation was slow (85 c.c. of hydrogen absorbed in 24 hours). After removal of catalyst, the solution was evaporated to dryness under reduced pressure, and the residual glass was dissolved in chloroform and poured into light petroleum (b. p. 40—60°). The crude 5′-trityl adenosine phosphate was precipitated as a hygroscopic solid (0·72 g.) (Found: C, 60·9; H, 5·5; N, 9·0. Calc. for $C_{29}H_{28}O_7N_5P$: C, 59·2; H, 4·8; N, 11·7%). This material (0·6 g.) was converted into the lead salt (0·55 g.) by addition of lead acetate solution to its neutralised solution in aqueous ethanol (Found: C, 45·7; H, 3·9; N, 7·4. Calc. for $C_{29}H_{26}O_7N_5PPb$: C, 44·0; H, 3·3; N, 8·8%). The lead salt was suspended in water containing a little ethanol and saturated with hydrogen sulphide. Lead sulphide was filtered off and extracted with hot water, and the combined filtrate and extracts were evaporated to small bulk. After filtration from a little triphenylcarbinol, the mixed nucleotides (43 mg.) were precipitated by addition of four volumes of ethanol. Presence of only two substances, corresponding to adenylic acids *a* and *b*, in this product was disclosed on paper chromatograms. Extraction of the separated substances from the paper and quantitative determination by measurement of the optical density at 260 mμ showed that they were present in approximately equal amount. A portion (13 mg.) of the mixture was separated into two components by ion-exchange chromatography (Amberlite IRA-400 resin) on a small column, as described below. An elution diagram was obtained identical with that given by a mixture of natural

adenylic acids *a* and *b* on the same column. The products were isolated and crystallised from water. Paper-chromatographic analysis showed that complete separation had been achieved and that the two products were indistinguishable from adenylic acids *a* and *b*.

(b) *Preparative method.* A solution of the dibenzyl phosphate (0·41 g.) in 80% acetic acid (4 c.c.), was boiled under reflux for 20 minutes. On cooling, crystalline triphenylcarbinol separated. Water and chloroform were added and the aqueous phase was separated, washed with more chloroform, and evaporated. The product was freed from acetic acid by evaporation with ethanol under reduced pressure. The residual gum (0·22 g.) was dissolved in water and hydrogenated over palladium charcoal–palladium oxide (38 c.c. absorbed). Catalyst was filtered off and the solution evaporated. The product (0·187 g.) was converted into the barium salt which was purified by precipitation several times from its aqueous solution by addition of ethanol (3 vols.) and formed a white powder (0·126 g., 49%) (Found: C, 24·5; H, 3·1; N, 13·3. Calc. for $C_{10}H_{12}O_7N_5PBa$: C, 24·9; H, 2·5; N, 14·5%). A paper-chromatographic study of these reactions showed that the product obtained after the acetic acid hydrolysis contained traces of adenine and adenosine, adenylic acids *a* and *b* (which were in one case isolated), the monobenzyl esters of these acids (see below), and an unidentified component (R_F 0·83 in sodium phosphate and 0·70 in butanol–acetic acid). The last substance, which contained phosphorus, was not present in the solution after hydrogenation, only adenylic acids *a* and *b* and traces of adenosine and adenine being observed.

Separation of Adenylic Acids a *and* b *from the Mixture of Synthetic Barium Salts of the Acids.*—The barium salt (414 mg.) described above was dissolved in water (90 c.c.) and run slowly on to a column (15 cm. × 4 sq. cm.) of Dowex-2 anion-exchange resin (mesh size 200—400) in the formate form. The column was water-washed until barium ion was no longer present in the effluent (150 c.c.). Elution was continued with 0·096N-formic acid at a flow rate of 3 c.c./minute and the eluate collected in 25-c.c. fractions in an automatically operated fraction collector. The progress of elution of the products was followed by the optical density of the fractions at 260 and 280 mμ. After the first component (adenylic acid *a*) had been eluted (volume to peak, *ca.* 3·5 l.), the second component (adenylic acid *b*) was removed from the column with N-formic acid.

The fractions containing adenylic acid *a* were united and concentrated, below room temperature under reduced pressure, to small bulk (50 c.c.), and the product was isolated by freeze-drying this solution. It was recrystallised three times from water (charcoal), giving *adenylic acid* a as a colourless crystalline solid (67·2 mg.), m. p. 187° (decomp.) undepressed by a sample of natural adenylic acid *a* [m. p. 187° (decomp.)] (Found, in material dried at 115°/0·1 mm.: C, 34·9; H, 4·3; N, 19·8. $C_{10}H_{14}O_7N_5P$ requires C, 34·6; H, 4·1; N, 20·2%. Uptake of periodate: 0·0 mol./mol.). The *X*-ray powder photograph and infra-red spectrum (figure) of this material and of natural adenylic acid *a* were identical. The compounds were indistinguishable on paper chromatograms, the synthetic compound travelling as one component.

The fractions containing adenylic acid *b* were set aside at 0° overnight. From the peak fraction *adenylic acid* b separated in long, fine colourless needles (56·3 mg.), m. p. 197° (decomp.) and mixed m. p. 195° (decomp.) with natural adenylic acid *b* [m. p. 193° (decomp.)] (Found, in air-dried material: C, 34·1; H, 4·3; N, 20·5%). The *X*-ray powder photograph and infra-red spectrum of this compound were identical in all respects with those of a recrystallised sample of natural adenylic acid *b*. By freeze-drying these fractions a further quantity of adenylic acid *b* was obtained which crystallised from water as a *hydrate* in prismatic needles (63·7 mg.) (Found, in material dried at room temp.: C, 31·9; H, 4·5; N, 18·5. $C_{10}H_{14}O_7N_5P,1{\cdot}5H_2O$ requires C, 31·9; H, 4·5; N, 18·7%. Found, in material dried at 110°: C, 35·0; H, 4·1; N, 20·0%. Periodate uptake: 0·0 mol./mol.). The hydrate was identical with the natural material on paper chromatograms but gave a different *X*-ray powder diagram. The infra-red spectrum was, however, the same as that of the uncrystallised natural compound in the position of the absorption bands, which differed only slightly in relative intensity.

Adenylic acids *a* and *b*, when separately heated under reflux in 80% acetic acid for 10 minutes, were both converted into a mixture of approximately equal parts of each. They were not interconverted by sodium hydroxide solution (0·25N.) at 28° or by aqueous ammonia (*d* 0·880) in a sealed tube at 100° during 24 hours. In the latter case some degradation to adenine and adenosine occurred.

Barium Salts of Adenosine Benzyl Hydrogen Phosphates a *and* b.—(i) 5′-Trityl adenosine dibenzyl phosphate (2·0 g.) was heated with acetic acid (8 c.c. of 80%) for 10 minutes and the product hydrogenated as before. In this case, hydrogenation was slow and incomplete. The

mixed barium salt of adenylic acids *a* and *b* was precipitated (0·363 g., 29%) by ethanol, and from the aqueous-ethanolic mother-liquors there was obtained, by concentration and further addition of ethanol, a crude barium salt. Further purification was effected by repeated precipitation from water with ethanol, but analytically pure material was not obtained. The product was a white hygroscopic powder (0·073 g.). Hydrolysis of this material with 0·1N-hydrochloric acid gave adenine, and micro-hydrogenation yielded a mixture of adenylic acids *a* and *b*, identified by paper chromatography; the material, on paper chromatograms, showed two components (R_F values, 0·56 and 0·67 in sodium phosphate and 0·44 and 0·50 in butanol–acetic acid : cf. Table).

(ii) To a suspension of adenylic acid *a* (0·016 g.) in pure, dry dimethylformamide (1·0 c.c.), phenyldiazomethane (0·23 c.c.) was added. The acid dissolved slowly with effervescence, and next morning the solution was examined on paper chromatograms. A component was present having similar R_F values in both sodium phosphate and butanol–acetic acid (0·67 and 0·44 respectively) to one of the components of the above benzyl esters. It is therefore designated adenosine benzyl hydrogen phosphate *a*. A similar reaction with adenylic acid *b* led to the identification of the other component (R_F 0·56 in sodium phosphate, 0·50 in butanol–acetic acid) as adenosine benzyl hydrogen phosphate *b*. Ether was added to the above dimethylformamide solutions, and the precipitated reaction products were washed with ether. In addition to the monobenzyl esters, small amounts of the dibenzyl esters also appeared to be present from a consideration of chromatograms. During the working up, some interconversion of the *a* and *b* monobenzyl esters also occurred.

The ether-washed product from a larger-scale preparation was shaken with chloroform and water, and the aqueous phase separated, washed with more chloroform, and then neutralised with barium hydroxide solution. After evaporation of the mixture to small bulk and filtration, ethanol precipitated the mixed barium salt of adenosine benzyl hydrogen phosphates *a* and *b* as a white hygroscopic powder (Found, in material dried at 113°/0·1 mm. over phosphoric oxide : C, 39·2; H, 4·1; N, 12·3. Calc. for $C_{17}H_{19}O_7N_5PBa_{\frac{1}{2}}$: C, 40·4; H, 3·8; N, 13·8. Calc. for $C_{17}H_{19}O_7N_5PBa_{\frac{1}{2}},H_2O$: C, 39·1; H, 4·0; N, 13·4%).

Hydrolysis of Adenosine Benzyl Hydrogen Phosphates a *and* b *with Acid and Alkali.*—Adenosine benzyl hydrogen phosphates *a* and *b* (2·7 mg.; from 5′-trityl adenosine dibenzyl phosphate) were dissolved in 80% acetic acid (0·2 c.c.), and the solution was heated at 100°. Paper chromatography using butanol–acetic acid showed that debenzylation to the adenylic acids was complete in 30 minutes. Some degradation to adenine also occurred. No debenzylation occurred when adenosine-5′ benzyl hydrogen phosphate was treated under the same conditions.

The mixed barium adenosine benzyl phosphates *a* and *b* (5·4 mg.; from 5′-trityl adenosine dibenzyl phosphate) was dissolved in sodium hydroxide solution (0·2 c.c. of 0·5N.) and set aside at 30° for 20 hours. Chromatographic analysis of this solution in both sodium phosphate and butanol–acetic acid solvents showed that complete conversion into a mixture of adenylic acids *a* and *b* had been effected.

In a similar reaction, the ether-precipitated and washed product (7·4 mg.) from the action of phenyldiazomethane on adenylic acid *b* was treated with sodium hydroxide solution (0·5 c.c. of 0·5N.; 50% aqueous ethanol). The product, characterised chromatographically in both solvents, was a mixture of adenylic acids *a* and *b*. An identical result was obtained on using the benzylated product from adenylic acid *a*.

Adenosine-5′ benzyl hydrogen phosphate (3 mg.) was treated as above with sodium hydroxide (0·3 c.c. of 0·5N.). Chromatography of the solution in butanol–acetic acid showed that the starting material was unaffected; no adenosine-5′ phosphate was produced.

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UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, August 15th,* 1951.]