Nucleotides. Part XXVII. The Structures of Adenylic Acids a and b.*

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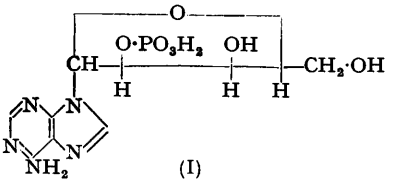
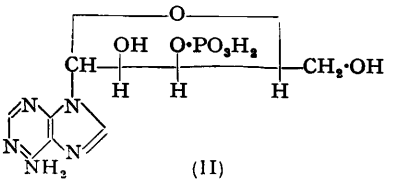
Adenosine-2′ phosphate has been synthesised by a route which avoids phosphoryl migration and is thus unambiguous. The synthetic product is identical with adenylic acid *a* obtained from ribonucleic acids. It follows that adenylic acid *b* is adenosine-3′ phosphate, a conclusion independently reached by *X*-ray crystallographic analysis.

The chemical hydrolysis of ribonucleic acids yields a mixture of eight mononucleotides consisting of 4 pairs of isomeric *a* and *b* nucleotides, each derived from one and the same nucleoside (Carter and Cohn, *Fed. Proc.*, 1949, **8**, 190, and subsequent papers). Distinguished from the nucleoside-5′ phosphates by their stability towards periodate, they were formulated as nucleoside-2′ and -3′ phosphates, although not necessarily respectively (Brown and Todd, Part IX, *J.*, 1952, 44). This conclusion was rendered more certain, in the case of the isomeric adenylic acids (I and II), by their simultaneous synthesis by phosphorylation of 5′-*O*-trityladenosine (Part IX, *loc. cit.*). Precise determination of the structures of the isomeric nucleotides is necessary, *inter alia*, for rigid definition of the position of the internucleotidic linkage-points in the polyribonucleotides.

In the pyrimidine nucleotide series, consideration of physical properties (density of aqueous solutions, p*K* values, and ultra-violet and infra-red absorption spectra) has led several investigators to the conclusion that cytidylic acid *a* and *b* are cytidine-2′ and -3′ phosphate respectively (Loring, Hammell, Levy, and Bortner, *J. Biol. Chem.*, 1952, **196**, 821; Cavalieri, *J. Amer. Chem. Soc.*, 1952, **74**, 5804; Fox, Cavalieri, and Chang, *ibid.*, 1953, **75**, 4315; Michelson and Todd, *J.*, 1954, 34). Cytidylic acid *b* has been related chemically to uridylic acid *b* (Brown, Dekker, and Todd, *J.*, 1952, 2715), so that, if the orientations reached for the cytidylic acids are accepted, the structures of the uridylic acids follow. Clearly, however, rigid chemical confirmation would be desirable, since the evidence quoted above is merely circumstantial.



(I)



(II)

The chemical properties of the isomeric acids severely limit the available means of structural determination by degradation. The *a* and the *b* nucleotides undergo interconversion in acidic but not in alkaline media (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811; Part IX, *loc. cit.*; Loring, Bortner, Levy, and Hammell, *J. Biol. Chem.*, 1952, **196**, 807), while their monoesters are labile in both acid and alkaline solution, phosphoryl migration and loss of the esterifying group occurring simultaneously (Brown and Todd, Part X, *J.*, 1952, 52). These interconversions proceed through the intermediate cyclic nucleoside-2′ : 3′ phosphate (Brown, Magrath, and Todd, *J.*, 1952, 2708).

Phosphoryl migration clearly invalidates earlier conclusions regarding the structures of yeast adenylic and guanylic acids (Levene and Harris, *J. Biol. Chem.*, 1932, **98**, 9; 1933, **101**, 419), since the degradations used depended on acid hydrolysis to remove the aglycone. Doherty (Abs. 118th Meeting Amer. Chem. Soc., 1950, 56c) obtained the *same* products from both adenylic acid *a* and *b* by acid-catalysed alcoholysis, a result undoubtedly to

* Part XXVI, preceding paper.

be ascribed to phosphoryl migration. Migration of the phosphoryl group was also observed when uridylic acid *b* was methylated with Purdie's reagent (Brown, Magrath, and Todd, preceding paper). This result was considered to depend on the action of the basic reagent on the methyl esters formed initially; the method, in any event, was not applicable to determining the location of the phosphoryl group.

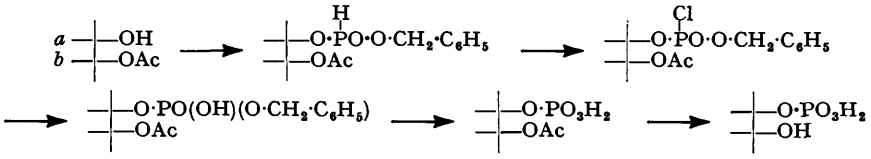
Khym, Doherty, Cohn, and Volkin have recently described in a preliminary communication (*J. Amer. Chem. Soc.*, 1953, **75**, 1262) the application of a modified hydrolytic procedure to the structural determination of adenylic acids *a* and *b*. Short treatment of either acid with the sulphonic acid resin Dowex-50 in the acid form gave a mixture of ribose-2 and ribose-3 phosphates which were separable by ion-exchange chromatography. The proportions of the two sugar phosphates differed in each case, and according to these authors " the amount of each is found to be proportional to the average amount of each resin-absorbed adenylic acid isomer existing during the hydrolysis period (*ca.* 30 seconds, 100°)." No detail of the experiments was given, but the conclusion drawn from them was that adenylic acids *a* and *b* are respectively adenosine-2′ and adenosine-3′ phosphates. Although this conclusion was probably justified, it clearly rested on arguments depending on relative rates of phosphoryl migration and hydrolysis. We have sought a solution of the structural problem using processes by which phosphoryl migration could be experimentally excluded. Only by imposing this condition could complete unambiguity be attained.

By using a synthetic route it was hoped that a general method might be evolved, applicable equally to the case of the other nucleotide isomers. Further, it was expected that the synthetic method would yield nucleosides oriented with respect to substituents in the 2′- and 3′-positions which would be of value in other nucleotide studies in progress in these laboratories. The results of the present chemical work have already been briefly reported, together with concomitant *X*-ray crystallographic studies (Brown, Fasman, Magrath, Todd, Cochran, and Woolfson, *Nature*, 1953, **172**, 1184). Both the chemical and the physical studies showed that adenylic acids *a* and *b* are adenosine-2′ and -3′ phosphate respectively, in agreement with the conclusions of Khym *et al.* In the present communication, the chemical studies are described in detail.

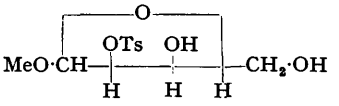
In principle, our aim was to obtain an *x* : 5′-di-*O*-substituted adenosine, which by phosphorylation and removal of protecting groups would yield only one of the two adenylic acids. By further substituting the same disubstituted adenosine with a group which would be incapable of migration during further chemical degradation it was hoped to show unambiguously the position of that group and hence the position of the phosphoryl group in the synthetic adenylic acid. Owing to the *trans*-arrangement of the hydroxyl groups at $C_{(3')}$ and $C_{(5')}$ in the ribofuranose residue of the nucleosides, the formation of 3′ : 5′-bridged (*e.g.*, benzylidene) derivatives has not been possible (Brown, Haynes, and Todd, *J.*, 1950, 3299). Recourse was had to acylation methods. Monoacetylation of 5′-*O*-trityladenosine gave a product which could not be purified, although removal of the trityl group and counter-current separation yielded a crystalline mono(2′ or 3′)-*O*-acetyladenosine. This material was not further studied. Acetylation of 5′-*O*-acetyladenosine (Brown, Haynes, and Todd, *loc. cit.*) in the same way led to a mixture of mono-, di-, and triacetyladenosines from which one, and only one, crystalline diacetyladenosine could be isolated by counter-current distribution.

The di-*O*-acetyladenosine was phosphorylated with *O*-benzyl phosphorous *OO*-diphenylphosphoric anhydride (Corby, Kenner, and Todd, *J.*, 1952, 3669), yielding a diacetyladenosine benzyl phosphite which, by the action of *N*-chlorosuccinimide (Kenner, Todd, and Weymouth, *J.*, 1952, 3675), followed by very mild hydrolysis with aqueous pyridine, gave diacetyladenosine benzyl hydrogen phosphate. Hydrogenation to remove the benzyl group, followed by deacetylation with methanolic ammonia, gave the nucleotide. The intermediates which were, as expected, gums or amorphous materials were not fractionated, so that loss of nucleotidic products was minimised. The final product was obtained in crystalline form and was identified as adenylic acid *a*, by comparison with authentic specimens in melting point, chromatographic and ion-exchange characteristics, *X*-ray powder photographs, and infra-red spectra (cf. Part IX, *loc. cit.*). Moreover, the solution

obtained at the deacetylation stage, but before crystallisation of the free acid, was shown by ion-exchange chromatography to contain *only* adenylic acid *a*, there being no trace of the *b* isomer. Individual steps in the synthetic method, which is set out in partial formulæ below, were designed to avoid phosphoryl migration. The production of one, and only one, adenylic acid indicates its success, since phosphoryl migration at any stage would necessarily have led to a mixture of two isomeric acids.



If the position of the phosphoryl group in the ribofuranose residue of adenylic acid *a* is denoted by *a* and that in the *b* isomer by *b*, then it follows that the diacetyladenosine used was *b* : 5′-di-*O*-acetyladenosine. This, with toluene-*p*-sulphonyl chloride, gave in high yield the crystalline *b* : 5′-di-*O*-acetyl-*a*-*O*-toluene-*p*-sulphonyladenosine, converted by the action of methanolic ammonia into *a*-*O*-toluene-*p*-sulphonyladenosine. It was intended to convert this toluene-*p*-sulphonyladenosine, or its diacetate, into a toluene-*p*-sulphonylribose, or a derivative of the latter, by hydrolysis or alcoholysis. In model experiments 2′ : 3′ : 5′-tri-*O*-acetyladenosine was treated with 1% methanolic hydrogen chloride at 65° and with 5% acid at 100°. In each case methyl ribopyranoside could be isolated in high yield, the ring size being determined by periodate titration. On use of the former conditions, the diacetyltoluene-*p*-sulphonyladenosine gave only toluene-*p*-sulphonyladenosine. The more vigorous conditions gave methyl *a*-toluene-*p*-sulphonylriboside. This substance was stable to periodate. Clearly it was either a 2- or 3-*O*-toluene-*p*-sulphonylribofuranoside (*viz.*, III) or else 3-*O*-toluene-*p*-sulphonylribopyranoside, but no further conclusions could be drawn from periodate titration results as to the location of the toluene-*p*-sulphonyl group.



(III)

(Ts = *p*-$C_6H_4Me\cdot SO_2$·)

Acid hydrolysis was next studied and it became clear that the diacetyltoluene-*p*-sulphonyladenosine was very stable. Conditions which readily yielded ribose from adenosine only deacetylated it. More vigorous acidic conditions did degrade the compound and gave solutions containing adenine and a reducing substance which had the expected chromatographic characteristics of a toluene-*p*-sulphonylribose. The material could not, however, be isolated in a pure state and, indeed, it appeared that considerable decomposition of the sugar had occurred. An attempt to oxidise the crude product with bromine and isolate the toluene-*p*-sulphonylribonic acid as its amide also failed. The methyl toluene-*p*-sulphonylriboside proved equally resistant to acid hydrolysis.

Several examples of increased stability of glycosides and glycosyl halides, toluene-*p*-sulphonylated in the 2- or the 3-position, have been recorded (Reynolds, *J.*, 1931, 2626; Bernoulli and Stauffer, *Helv. Chim. Acta*, 1940, **23**, 615; Percival and Percival, *J.*, 1938, 1585; Percival and Zobrist, *J.*, 1952, 4306). Reynolds (*loc. cit.*) noted the considerably enhanced stability of triacetyl-2-*O*-toluene-*p*-sulphonylglucosyl chloride over the corresponding triacetyl-3-*O*-toluene-*p*-sulphonylglucosyl bromide (Freudenberg and Ivers, *Ber.*, 1922, **55**, 929). We, ourselves, found that methyl 2-*O*-toluene-*p*-sulphonyl-α-glucoside was very stable toward aqueous sulphuric acid and we were unable by this means to convert it into the free sugar. Moreover, Percival and Zobrist (*loc. cit.*) noted that when methyl 3 : 5-*O*-*iso*propylidene-2-*O*-toluene-*p*-sulphonylxyloside was treated with 1% methanolic hydrogen chloride at 70° removal of the acetone residue occurred almost exclusively without ring expansion, only traces of pyranoside being formed.

The accumulated evidence seemed to point to the conclusion that we were dealing

with 2-toluene-*p*-sulphonyl derivatives, in which case the methyl *a*-toluene-*p*-sulphonylriboside must have retained the furanose form (III). This proved to be the case. Methylation with Purdie's reagent, removal of the toluene-*p*-sulphonyl group by reductive fission with sodium amalgam, and acid hydrolysis (which now proceeded smoothly), yielded a mixture of methylated sugars. Paper chromatography showed that the product was a mixture of mono- and di-methylriboses, in which the latter predominated; no trimethylribose was present. The mixture was fractionated by chromatography on a cellulose column. We have discussed elsewhere the identification of various methylated ribose derivatives by means of paper chromatographic and ionophoretic methods (preceding paper); the same techniques were applied to the present problem. The monomethylriboses were eluted from the column in two fractions. The first contained 5-*O*-methylribose, and the substance formulated (preceding paper) as 3-*O*-methylribose. The second, very small, fraction contained a sugar which could only be distinguished with difficulty from 2-*O*-methylribose on paper chromatograms in the usual solvent systems. The chromatographic method of Bayly and Bourne (*Nature*, 1953, **171**, 385), depending on conversion into *N*-benzylglycosylamines, however, readily distinguished the substance from 2-*O*-methylribose. We formulate it as 4-*O*-methylribose and attribute its formation to a very small amount of pyranoside present in the methyl toluene-*p*-sulphonylriboside (cf. Percival and Zobrist, *loc. cit.*). This accords well with the absence of this substance in the products of methylation and hydrolysis of *a*-toluene-*p*-sulphonyladenosine, the only discernible monomethylribose in that case being 3-*O*-methylribose.

The dimethylribose fraction appeared to be essentially 3 : 5-di-*O*-methylribose. 2 : 3- and 2 : 5-Di-*O*-methylribose were entirely absent and only a trace of another substance was present. This is formulated as 3 : 4-di-*O*-methylribose (cf. Percival and Zobrist, *loc. cit.*) on the basis of its ionophoretic characteristics and its absence in the methylated sugars derived from methylated *a*-*O*-toluene-*p*-sulphonyladenosine.

The dimethylribose fraction was treated with *p*-bromophenylhydrazine and yielded an osazone, in good yield, identical with an authentic specimen of 3 : 5-*O*-dimethylribose *p*-bromophenylosazone (preceding paper).

The isolation of 3 : 5-di-*O*-methylribose uncontaminated with any 2-substituted ribose proves that it was derived from methyl 2-toluene-*p*-sulphonylribofuranoside (III). It follows rigidly that the original toluene-*p*-sulphonylated diacetyladenosine was 3′ : 5′-di-*O*-acetyl-2′-*O*-toluene-*p*-sulphonyladenosine and hence that adenylic acid *a* is adenosine-2′ phosphate (I). It follows, further, that adenylic acid *b* is adenosine-3′ phosphate (II) (cf. Brown and Todd, Part IX); independent proof has also been obtained by *X*-ray crystallographic analysis of adenylic acid *b* (Brown, Fasman, Magrath, Todd, Cochran, and Woolfson, *loc. cit.*).

Convincing evidence has already been presented for the view that the ribonucleic acids are *b* : 5′-linked polynucleotides (Brown, Dekker, and Todd, *loc. cit.*; Brown and Todd, *J.*, 1953, 2040; Brown, Heppel, and Hilmoe, *J.*, 1954, 40). The evidence presented in this paper, coupled with the probable identification of the *b* isomers of the pyrimidine nucleotides with the corresponding nucleoside-3′ phosphates, makes it virtually certain that the ribonucleic acids are polynucleotides in which successive nucleoside residues are linked through the 3′ and the 5′ positions by phosphodiester groupings. Final proof is now being sought by extension of the method of structural determination here described to the pyrimidine nucleotides. From physical measurements of the type earlier applied to the cytidilic acids Cavalieri (*J. Amer. Chem. Soc.*, 1953, **75**, 5268) has recently obtained data on the isomeric adenylic acids from which he concluded that the acids have the structures demonstrated here.

Experimental

R_F values quoted, except where otherwise stated, are from paper chromatograms run in *n*-butanol–acetic acid–water (40 : 10 : 50) on Whatman No. 1 paper.

2′(or 3′)-O-*Acetyladenosine*.—5′-*O*-Trityladenosine (4·14 g.) was dissolved in hot, dry pyridine (105 c.c.), and the solution cooled to 0°. After addition of acetic anhydride (0·9 c.c., 1 mol.) with stirring, the solution was set aside at room temperature overnight. The solution

was poured into ice-water, and the gummy product collected and refluxed with 80% acetic acid for 20 min. Evaporation below 35° left a residue which was extracted with boiling water (250 c.c.) for ½ hr. The combined filtrates from this and two further similar extractions of the residue gave, on evaporation under reduced pressure, a colourless resin. This material was fractionated by counter-current distribution with ethyl acetate–water (23 transfers; 94-c.c. phase). The nature of the material present in each tube was determined by paper chromatography. In addition to adenosine (R_F 0·29) and 2′ : 3′-*O*-diacetyladenosine (R_F 0·94) a monoacetyladenosine (R_F 0·63) was present (in tubes 5—9). Evaporation of the monoacetyladenosine fraction yielded a gum (0·6 g.) which was dissolved in a minimum of ethanol. Careful addition of light petroleum (b. p. 80—100°) furnished colourless needles of 2′(or 3′)-O-*acetyladenosine*, m. p. 173—175° (Found, in material dried at 105°/1 mm.: C, 46·6; H, 5·0. $C_{12}H_{15}O_5N_5$ requires C, 46·7; H, 4·9%).

3′ : 5′-*Di*-O-*acetyladenosine*.—5′-*O*-Acetyladenosine (2·67 g.; Brown, Haynes, and Todd, *loc. cit.*), dried for 2 days at 50°/0·1 mm., was dissolved in warm, dry pyridine (254 c.c.) with exclusion of moisture, then cooled to 0°. Acetic anhydride (0·9 c.c., 1·1 mols.) was added with shaking, and the solution was set aside overnight. Removal of solvents below 50° followed by evaporation with added ethanol and toluene left a thick gum. This was dissolved in 80% acetic acid (267 c.c.), and the solution boiled under reflux for 20 min., in order to remove any *N*-acetyl groups. Removal of acetic acid below 50° left a brown gum which was separated into its components by counter-current distribution in an 11-stage, 95-c.c. phase machine, using 22 transfers. The solvents were ethyl acetate and water. 5′-*O*-Acetyl- and 2′ : 3′ : 5′-tri-*O*-acetyl-adenosine were recovered from the appropriate tubes. The contents of tubes 6—16 were combined and the solvent was removed under reduced pressure. The residual clear gum was dissolved in ethanol, and light petroleum (b. p. 80—100°) (or pentane) was added to opalescence. Slow crystallisation occurred when the solution was set aside in the ice-chest for several weeks. 3′ : 5′-*Di*-O-*acetyladenosine* formed colourless ball-like aggregates of needles (0·81 g., 23%), m. p. 146—147° (Found, in material dried at 0·1 mm.: C, 47·9; H, 5·2; N, 19·7. $C_{14}H_{17}O_6N_5$ requires C, 47·8; H, 4·9; N, 19·9%).

For a larger-scale preparation (from 21 g. of 5′-*O*-acetyladenosine) a 100-stage automatic counter-current apparatus was used (20·5-c.c. phase). The gum was dissolved in the bottom phase of the first 11 tubes, and 88 transfers were carried out. The diacetyladenosine was recovered from tubes 35—64. Outside these limits the product was contaminated with 5′-*O*-acetyl- and tri-*O*-acetyl-adenosine.

Adenosine-2′ Phosphate (*Adenylic Acid* a).—3′ : 5′-Di-*O*-acetyladenosine (0·1 g.) was dissolved in a mixture of dry benzene (15 c.c.) and dry methyl cyanide (3 c.c.). *O*-Benzyl-phosphorous *OO*-diphenylphosphoric anhydride (2 mols.; Corby, Kenner, and Todd, *loc. cit.*) in benzene was added, followed by 2 : 6-lutidine (0·31 c.c., 2 mols.), and the mixture was shaken vigorously. The materials dissolved slowly during 1·5 hr. After a further 30 min., solvents were removed under reduced pressure and the residue was dissolved in chloroform (25 c.c.). The solution was washed successively with water (5 c.c.), saturated sodium hydrogen carbonate solution (5 c.c.), and water (5 c.c.), and then evaporated, the last traces of water being removed by azeotropic distillation with benzene under reduced pressure. The colourless resin (0·15 g.) was dissolved in benzene (5 c.c.) and the solution poured into *cyclo*hexane (100 c.c.). The colourless, gummy 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphite which separated was dried *in vacuo* (0·139 g., 97%) and had R_F 0·78.

The above material was dissolved in dry methyl cyanide (10 c.c.), *N*-chlorosuccinimide (0·043 g., 1·1 mols.) was added, and the solution was set aside for 2 hr. Water (0·3 c.c.) and pyridine (1·0 c.c.) were added to the yellow solution, and the mixture was set aside overnight. Removal of solvents under reduced pressure, followed by re-evaporation with ethanol, yielded a gum which was dissolved in water (10 c.c.). The solution was brought to pH 1 with dilute hydrochloric acid and rapidly extracted with chloroform (5 × 10 c.c.), and the chloroform extract washed with water (5 c.c.). Evaporation of the chloroform left a gum from which the last traces of water were removed by azeotropic distillation with benzene. The crude 3′ : 5′-di-*O*-acetyladenosine-2′ benzyl phosphate (0·087 g., 59%) had R_F 0·70, but was contaminated with a small amount of unidentified impurity of R_F 0·36.

The above benzyl phosphate was dissolved in water (10 c.c.) and ethanol (2 c.c.), and the solution hydrogenated overnight in presence of platinum oxide (5 mg.) and palladium–charcoal (15 mg.). After being heated to 100°, the solution was filtered and the catalyst washed with more solvent. Evaporation to dryness yielded 3′ : 5′-di-*O*-acetyladenosine-2′ phosphate as a gum (0·067 g., 55%; R_F 0·33). The material was dissolved in half-saturated methanolic

ammonia (10 c.c.) and set aside overnight. Removal of solvents gave crude ammonium adenosine-2′ phosphate. It was dissolved in water (100 c.c.), and the solution brought to pH 8 and run on to a column (2·5 cm.^{2} × 3 cm.) of Dowex-2 resin (formate form). The column was washed with water (200 c.c.), and the product was eluted with N-formic acid (100 c.c.). The solution was evaporated to small volume under reduced pressure and finally freeze-dried, yielding a colourless foam (0·04 g.). Recrystallisation from a few drops of water gave pure adenosine-2′ phosphate (14 mg.), m. p. 183°, R_F 0·04 (Found, in material dried at 50° over P_2O_5: N, 18·7. Calc. for $C_{10}H_{14}O_7N_5P,1{\cdot}5H_2O$: N, 18·7%). The substance had an infra-red spectrum and an *X*-ray powder diagram identical with those of natural and synthetic adenylic acid *a*. It did not depress the m. p. (183°) of an authentic specimen and was unaffected by sodium metaperiodate (Part IX, *loc. cit.*). It had R_F 0·68 in Carter's sodium phosphate–*iso*amyl alcohol solvent system (*J. Amer. Chem. Soc.*, 1950, **72**, 1466) (authentic adenylic acid *a*, 0·69). The crude ammonium salt (3 mg.; above) was chromatographed on a Dowex-2 (formate) ion-exchange column which had been calibrated with respect to the elution positions of adenylic acids *a* and *b*. Only one peak was observed corresponding to adenylic acid *a*, so that in the product before crystallisation only one of the isomeric acids was present.

Adenosine-5′ Phosphate.—In initial experiments to test the feasibility of the above reaction scheme, adenosine-5′ phosphate was synthesised from 2′ : 3′-di-*O*-acetyladenosine (0·1 g.) by the same procedure. Crystallisation from water gave the pure acid (0·030 g., 30%) (Found, in material dried at 50°: N, 19·4. Calc. for $C_{10}H_{14}O_7N_5P,H_2O$: N, 19·2%). The synthetic acid had m. p. 192° and did not depress the m. p. (189°) of an authentic sample of adenosine-5′ phosphate. It gave an *X*-ray powder photograph identical with that of an authentic specimen. The infra-red spectrum of the anhydrous substance (dried at 110°) as a mull in Nujol was different from that recorded earlier for adenosine-5′ phosphate (Part IX) but was identical with that obtained for a recrystallised specimen of adenosine-5′ phosphate prepared *via* 3′ : 5′-*O*-benzylidene adenosine (Brown, Haynes, and Todd, *loc. cit.*) and dried in the same way. The infra-red absorption bands (μ) (w = weak, m = medium strength, *s* = strong) were: 3·03, 3·15 m (doublet), 5·91 s, 6·40 w, 6·66 m, 7·45 m, 7·65 w, 7·83, 7·93 w (doublet), 8·2, 8·46, 8·64 m, 8·90 s, 9·20 m, 9·45, 9·90, 10·55 s, 11·15, 11·45, 11·6, 11·85 w, 12·94, 13·43, 13·9 m. The difference between this spectrum and that recorded earlier is doubtless due to the fact that the earlier determination was carried out on hydrated material.

3′ : 5′-*Di*-O-*acetyl*-2′-O-*toluene*-p-*sulphonyladenosine.*—Dry 3′ : 5′-di-*O*-acetyladenosine (0·454 g.) was dissolved in anhydrous pyridine (11·1 c.c.) at 40°, and the solution cooled to 0°. Toluene-*p*-sulphonyl chloride (0·744 g., 1·1 mols.) was added with shaking, and the yellow solution set aside at room temperature overnight. Water (10 c.c.) and cold saturated sodium hydrogen carbonate solution (50 c.c.) were added and the solution was extracted rapidly with ice-cold chloroform (3 × 50 c.c.). This extract was washed with ice-cold saturated sodium hydrogen sulphate solution and then with water. Removal of solvent, together with remaining traces of water, by distillation with benzene under reduced pressure gave colourless crystals. Recrystallisation from chloroform gave the *product* (0·545 g., 84%) as hair-like needles, m. p. 78—81° (Found, in material dried at 50°/0·1 mm. over P_2O_5: C, 49·9; H, 4·9; N, 13·3; S, 6·2. $C_{21}H_{23}O_8N_5S$ requires C, 49·9; H, 4·6; N, 13·8; S, 6·3%). The compound was evidently dimorphous since crystallisation from a small amount of ethanol gave rosettes of needles, m. p. 144° (Found, C, 49·9; H, 4·6%). The substance had R_F 0·87.

2′-O-*Toluene*-p-*sulphonyladenosine.*—(*a*) The above diacetyl derivative (26 mg.) was dissolved in half-saturated methanolic ammonia (1 c.c.) and set aside at 0° overnight. Evaporation to dryness and crystallisation of the residue from ethanol gave the *product* in irregular transparent plates, m. p. 222—223° (Found, in material dried at 110°/0·1 mm. over P_2O_5: C, 48·8; H, 4·5. $C_{17}H_{19}O_6N_5S$ requires C, 48·5; H, 4·5%).

(*b*) The diacetyl derivative (0·2 g.) was boiled under reflux in 2% methanolic hydrogen chloride (20 c.c.) for 12 hr. The acid was neutralised with diazomethane and then the solution evaporated to dryness. The residual gum was dissolved in ether (10 c.c.) and methanol (1 c.c.) and filtered. Evaporation of the filtrate and crystallisation of the residue (0·133 g.) from ethanol gave 2′-*O*-toluene-*p*-sulphonyladenosine, m. p. 222—223°, undepressed in admixture with the material prepared by methanolic ammonia deacetylation (Found: C, 48·5; H, 4·5%). The substance had R_F, 0·74.

Methanolysis of 3′ : 5′-*Di*-O-*acetyl*-2′-O-*toluene*-p-*sulphonyladenosine.*—The tosyl derivative (0·5 g.) was dissolved in 5% methanolic hydrogen chloride solution (15 c.c.) and heated for 6 hr. at 100° in a sealed Pyrex tube. The solution was neutralised by addition of ethereal diazomethane. After evaporation of ether and dilution to 20 c.c. with dry methanol the

solution was passed through a column (6 × 1 cm. diam.) of Dowex-50 resin (acidic form) which had been washed with dry methanol. After washing with more methanol (25 c.c.), the eluate and washings, now free of adenine, were combined and evaporated. The gum was dissolved in methanol, and the solution filtered and evaporated. The gummy product (0·193 g., 61%) was dried for 8 hr. at 10^{-5} mm. and consisted of *methyl* 2-O-*toluene*-p-*sulphonylribofuranoside* contaminated with a trace of the pyranoside (Found: C, 48·6; H, 5·8. $C_{13}H_{18}O_7S$ requires C, 49·0; H, 5·7%). On periodate oxidation the substance consumed 0·02 mol. of oxidant per mol.

Methanolysis of 2′ : 3′ : 5′-*Tri*-O-*acetyladenosine*.—Under the conditions used above, triacetyladenosine (0·2 g.) and 5% methanolic hydrogen chloride (15 c.c.) yielded methylribopyranoside as an oil (0·082 g., 98%) (Found: C, 44·0; H, 7·6. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4%). It consumed 2·02 mols. of metaperiodate per mol.

The product (Found: C, 43·9; H, 7·1%) obtained by using 1% methanolic hydrogen chloride under reflux for 6 hr. consumed 1·6 mols. of periodate per mol.

Acid Hydrolysis of 3′ : 5′-*Di*-O-*acetyl*-2′-*toluene*-p-*sulphonyladenosine*.—A large variety of conditions was studied, with 4—10% aqueous hydrochloric acid at 100°. The degree of hydrolysis was followed by paper chromatography, by observing the disappearance of toluene-*p*-sulphonyladenosine and the formation of adenine together with a substance which gave a pink spot (R_F *ca.* 0·8) with the aniline hydrogen phthalate reagent, presumably 2-*O*-toluene-*p*-sulphonylribose. Working up by the methods used above for the methyl ribosides yielded dark oils giving variable analytical values. The product gave no *p*-bromophenylosazone or -hydrazone, nor could oxidation to the ribonolactone and conversion into the ribonamide be effected.

Methyl 2-O-*Toluene*-p-*sulphonyl*-α-*glucoside*.—The 4 : 6-*O*-benzylidene derivative (Robertson, *J.*, 1935, 1193) was boiled under reflux for 12 hr. with oxalic acid (15 g.) in acetone (450 c.c.) and water (50 c.c.). Barium carbonate was added and the precipitate removed by filtration. The filtrate was evaporated, to remove acetone, and the residual aqueous solution extracted with ether. Evaporation of the aqueous layer left a gum which crystallised from ethyl acetate in rosettes of needles. *Methyl* 2-O-*toluene*-p-*sulphonyl*-α-*glucoside* (1·9 g.) had m. p. 138—139° (Found: C, 48·5; H, 6·1. $C_{14}H_{20}O_8S$ requires C, 48·3; H, 5·8%). The substance was recovered unchanged (m. p. 136°) after being heated with 0·33N-sulphuric acid for 8 hr. at 70°.

Methylation of Methyl 2-O-*Toluene*-p-*sulphonylriboside*—Methyl 2-*O*-toluene-*p*-sulphonylriboside (0·19 g.) was shaken in methyl iodide (0·5 c.c.) with silver oxide (0·15 g.) for 17 hr. at 25°. Removal of silver salts by filtration and evaporation to dryness yielded a gum which was dissolved in methyl iodide (1·5 c.c.) and shaken with three further portions of silver oxide (2 × 0·07 g., and 0·15 g.) added at 12-hr. intervals. Evaporation of the filtered solution gave the methylated material as a colourless resin.

The methylation product was dissolved in methanol (6·6 c.c.) and water (3 c.c.), and 4% sodium amalgam (6 g.) added during 5 hr. with vigorous stirring at 35°. After being stirred for a further 6 hr., the solution was set aside overnight and then decanted through a filter. The residue was washed with more methanol, and filtrate and washings were neutralised with 2N-sulphuric acid. The solution was evaporated and the residue dried at 35°/0·1 mm. Extraction with chloroform (5 × 10 c.c.) and evaporation of the extract yielded the methylated methyl ribofuranoside as a colourless oil (0·115 g.).

This material was heated on the steam-bath in 0·5N-sulphuric acid (3 c.c.) for 5 hr. and the solution was then neutralised with saturated barium hydroxide solution. Barium sulphate was removed by filtration and washed with hot water. Filtrate and washings were reduced to small bulk, filtered, and evaporated to dryness. The pale orange syrup (0·103 g.) was dried at 35°/0·5 mm. Chromatographic examination of this material indicated that the syrup consisted largely of dimethylated ribose together with a smaller quantity of monomethylated ribose. No ribose or trimethylribose was present.

Separation and Identification of the Methylated Ribose Derivatives.—Chromatography using butanol–water as solvent on a column (6 cm. diam.) of cellulose (300 g.; 90-mesh), in accordance with the technique described in the preceding paper, permitted fractionation of the above methylated sugar mixture. Three fractions were obtained and are described below in order of elution.

Fraction 1 (*dimethylribose fraction*). The colourless syrup (0·064 g.) consisted almost entirely of a substance whose behaviour on paper chromatography and ionophoresis was identical with that of 3 : 5-di-*O*-methylribose. No 2 : 5-di-*O*-methylribose was present. A trace of a substance was observed with R_F 0·67 in pyridine–ethyl acetate–water and M_B

0·22 in 0·1M-sodium borate (3 : 5-di-*O*-methylribose has R_F 0·71 and M_R 0·90). The material is regarded as 3 : 4-di-*O*-methylribose (see Theoretical section).

A solution of the syrup (0·06 g.) in Methylcellosolve (2-methoxyethanol) (0·83 c.c.) and 2N-acetic acid (1·62 c.c.) was heated with *p*-bromophenylhydrazine (0·197 g.) in a stoppered tube at 100° for 4·5 hr. After cooling to room temperature, the product was collected and then triturated with cold 2N-acetic acid (3 × 2 c.c.) and then water. The dried solid (0·122 g.) was dissolved in benzene and purified by chromatography on a column of neutral alumina (1·7 cm. diam.; 15 g.) as described in the preceding paper. The crystalline product (0·106 g., 61%) separated from aqueous methanol in fine, yellow needles, m. p. 176—177°, undepressed on admixture with authentic 3 : 5-di-*O*-methylribose *p*-bromophenylosazone.

Fraction 2. The colourless syrup (18 mg.) consisted of a mixture of two sugars identified as 3- and 5-*O*-methylribose by their ionophoretic and paper-chromatographic characteristics in a number of solvents.

Fraction 3. This fraction (3·6 mg.) consisted of a colourless syrup with a trace of solid material. Chromatographic examination showed the presence of a single reducing sugar of slightly lower R_F (0·47 in pyridine–ethyl acetate–water) than 2-*O*-methylribose (0·49). On ionophoresis, it had M_R 0·49 in 0·1M-sodium borate, identical with that of 2-*O*-methylribose. The two sugars were clearly distinguished by applying the benzylamine technique (Bayly and Bourne, *loc cit.*). 2-*O*-Methylribose gave a spot, detected with the ninhydrin and aniline hydrogen phthalate spray reagents, with R_F 0·84 (pyridine–ethyl acetate–water), while the new substance, in common with 3- and 5-*O*-methylribose, had R_F 0·95. The sugar is tentatively regarded as 4-*O*-methylribose.

Methylation of 2′-O-*Toluene*-p-*sulphonyladenosine*.—2′-*O*-Toluene-*p*-sulphonyladenosine (from 0·11 g. of diacetyl derivative) was methylated by the technique used for the methylation of uridylic acid *b* (preceding paper). Fifteen treatments with silver oxide and methyl iodide failed to effect complete methylation. The pale resin obtained was reduced with 4% sodium amalgam (2 g.) in aqueous methanol, and the product hydrolysed with 0·5N-sulphuric acid, as above. The solution was neutralised with sodium hydrogen carbonate and evaporated to small bulk, much of the methyladenine separating as a brown oil. The aqueous phase was studied on chromatograms and by ionophoresis. Only two reducing substances appeared to be present. The first was identified as 3 : 5-di-*O*-methylribose by comparison with specimens prepared above and previously. The other reducing sugar, which constituted about 65% of the mixture, was readily distinguished from 5-*O*-methylribose and behaved identically with the substance formulated as 3-*O*-methylribose. Its presence, in association with 3 : 5-di-*O*-methylribose, is strong confirmation of the correctness of the structure ascribed to it in the preceding paper (p. 1443).

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