655. *Nucleotides. Part VIII.** cyclo*Nucleoside Salts. A Novel Rearrangement of Some Toluene*-p-*sulphonylnucleosides.*

By V. M. Clark, A. R. Todd, and J. Zussman.

Toluene-*p*-sulphonylation of 2′ : 3′-*iso*propylidene adenosine (I) yields two isomeric products, one the normal toluene-*p*-sulphonyl ester (II) and the second an ionic compound containing the toluene-*p*-sulphonate anion. The ester (II), on being heated in acetone solution, is rapidly converted into the ionic isomer which yields an ionic iodide on treatment with sodium iodide. Chemical evidence, coupled with *X*-ray crystallographic analysis, shows that these ionic compounds are salts of 2′ : 3′-*iso*propylidene 3 : 5′-*cyclo*-adenosine (III and IV) in which a new ring has been formed by intramolecular alkylation. *cyclo*-Derivatives are not produced from the toluene-*p*-sulphonyl derivatives of inosine or uridine, but 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl cytidine rearranges readily to the ionic 2′ : 3′-*iso*propylidene O^2 : 5′-*cyclo*cytidine toluene-*p*-sulphonate (V). The formation of these *cyclo*nucleoside salts affords independent proof of the β-configuration of the natural ribonucleosides.

During investigations into the synthesis of nucleotides and related phosphates of carbohydrate derivatives, we have devoted some attention to the condensation of halogeno-derivatives of carbohydrates and nucleosides with silver salts of phosphoric and polyphosphoric acids. The reaction between a silver diaryl or diaralkyl phosphate and a halogeno-sugar was first studied by Zervas (*Naturwiss.*, 1939, **27**, 317), and the method has been used for the preparation of glucose-1 phosphate (Zervas, *loc. cit.*; Wolfrom, Smith, Pletcher, and Brown, *J. Amer. Chem. Soc.*, 1942, **64**, 23) and galactose-1 phosphate (Reithel, *ibid.*, 1945, **67**, 1056). For the synthesis of simple nucleotides, condensation of the iodo-derivative of a nucleoside (*e.g.*, 5′-iodo-5′-deoxyadenosine) with silver dibenzyl phosphate seemed a feasible alternative to the route using a suitably protected nucleoside and dibenzyl chlorophosphonate (Baddiley and Todd, *J.*, 1947, 648). The method seemed particularly attractive for, if successful, it would open the way to the synthesis of dinucleotides by condensation of halogeno-nucleosides with the silver salts of nucleotide monobenzyl esters, for which convenient methods of preparation are now available (Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815; Clark and Todd, *J.*, 1950, 2023, 2030).

The displacement of the toluene-*p*-sulphonyl residue from carbohydrate derivatives by anionic attack, particularly by the iodide ion (Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366) is well known, and it was hoped to obtain the requisite iododeoxynucleosides in this way from the corresponding toluene-*p*-sulphonylated nucleosides. Thus, 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl uridine reacts with sodium iodide (Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113) to give the corresponding 5′-iodo-5′-deoxy-compound, which can be condensed with silver dibenzyl phosphate to yield the 5′-(dibenzyl phosphate); this, after hydrogenolysis of the benzyl groups and hydrolysis of the *iso*propylidene residue, gives uridine-5′ phosphate (experiment by Dr. J. Davoll), identical with the product prepared by an alternative route (Michelson and Todd, *J.*, 1949, 2476).

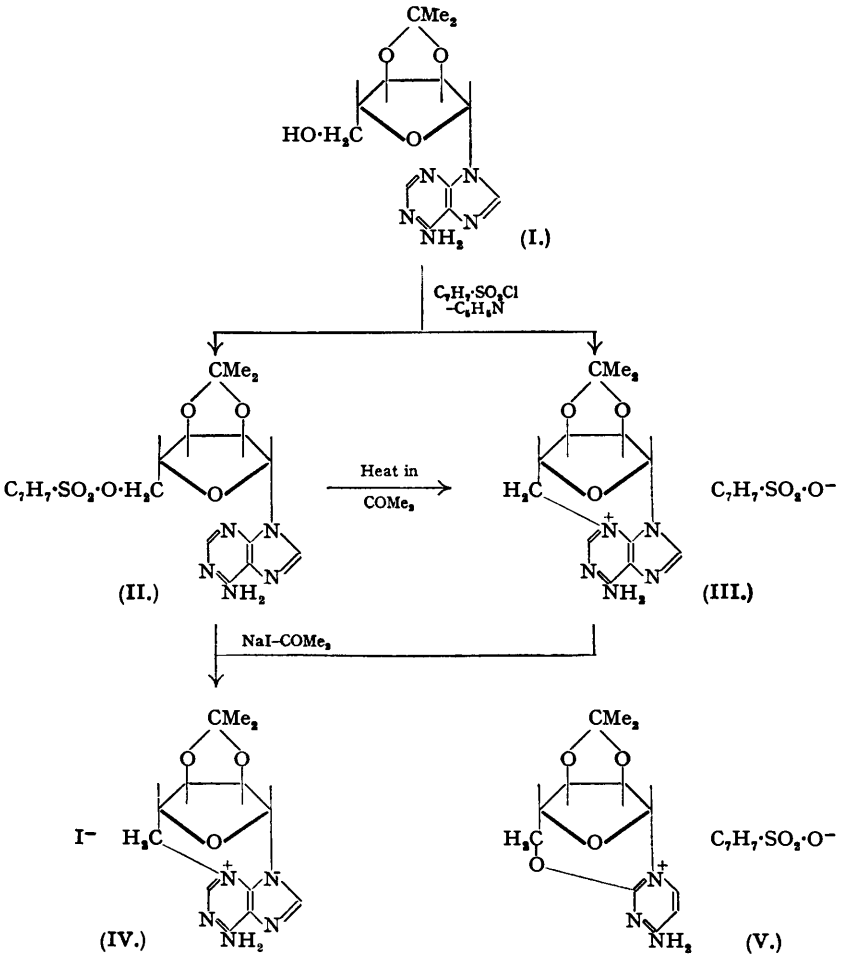
It was expected that the performance of an analogous series of reactions with adenosine in place of uridine would yield muscle adenylic acid, but this has not proved to be the case. Several groups of workers (*e.g.*, Satoh and Makino, *Nature*, 1951, **167**, 238; Baddiley, Trauth, and Weygand, *ibid.*, p. 360) have claimed the preparation of 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl adenosine but the product has yet to be adequately characterised and the work described herein shows that those structural proofs based on this intermediate leave not a little to be desired.

After the reaction between 2′ : 3′-*iso*propylidene adenosine (I) and toluene-*p*-sulphonyl chloride in pyridine solution it was found possible to isolate two products, each having the expected elementary composition, the one behaving as a covalent compound and the other as an ionic compound. When heated with sodium iodide in acetone, the two toluene-*p*-sulphonyl derivatives gave the same iodo-derivative, which contained ionic iodine. That the transformation from the covalent compound to the ionic one was associated with the toluene-*p*-

* Part VII, *J.*, 1951, 1867.

sulphonate was shown by heating the covalent compound, 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl adenosine, in acetone solution, whereupon it was converted into the isomeric ionic toluene-*p*-sulphonate.

The covalent toluene-*p*-sulphonate can only be obtained under rigidly controlled conditions. The rearrangement to the ionic product proceeds slowly at room temperature (62% conversion after 1 week in acetone solution) but rapidly when heated (68% after 1½ hours in acetone solution



at 100°; almost quantitative after 1 hour in dioxan solution under reflux). Paper chromatography established the essential homogeneity of the covalent toluene-*p*-sulphonate, photography in ultra-violet light disclosing a single spot ($R_F = 0{\cdot}86$ in ethanol–ammonia–water). Under the same conditions, the ionic toluene-*p*-sulphonate gave a single purine spot ($R_F = 0{\cdot}68$) together with a spot corresponding to the anion of toluene-*p*-sulphonic acid ($R_F = 0{\cdot}83$), detected by the iodide–iodate–starch spray developed in this laboratory (Long, Quayle, and Stedman, *J.*, 1951, 2197). Similar observations were made in two other solvent systems. It seemed clear that this rearrangement was bound up with the basic character of the adenine nucleus present in adenosine, for 2′ : 3′-*iso*propylidene inosine, which contains a hypoxanthine (6-hydroxypurine) nucleus, gives a normal toluene-*p*-sulphonyl ester (Levene and Tipson, *J. Biol. Chem.*, 1935, **111**, 313) which does not appear to undergo rearrangement to an ionic compound. This suggests that the toluene-*p*-sulphonylated adenosine derivative, being at once a base and the ester of a strong acid, can undergo rearrangement involving either alkylation (which could be intramolecular or intermolecular) or elimination of toluene-*p*-sulphonic acid to give the salt of a riboseen derivative in which there would be a double bond

between $C_{(4')}$ and $C_{(5')}$ in the adenosine molecule. The latter possibility can be ruled out on spectroscopic ground. The infra-red spectrum of the ionic compound showed no band indicative of the grouping $^{+}O\!>\!C\!=\!CH_2$. From the Table it can be seen that the ultra-violet absorption maxima of the ionic toluene-*p*-sulphonate and iodide differ markedly in their position from that of adenosine itself or the covalent toluene-*p*-sulphonate.

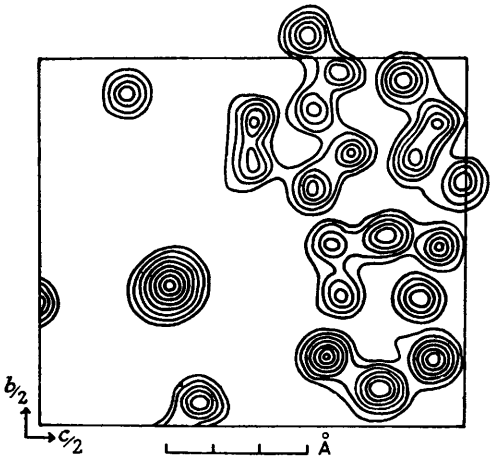
Ultra-violet absorption maxima.

Compound	Solvent	$\lambda_{max.}$ (mμ.)	$\epsilon_{max.}$
9-Methyladenine	N/20-HCl	260	14,140
9-Methyladenine toluene-*p*-sulphonate (salt)	N/20-HCl	260	13,790
2′ : 3′-*iso*Propylidene adenosine	Water	259	14,800
2′ : 3′ : 5′-Triacetyl adenosine	EtOH	260	14,180
Covalent toluene-*p*-sulphonate (II)	EtOH	263	12,350
Ionic toluene-*p*-sulphonate (III)	N/20-HCl	272	16,310
Ionic iodide (IV)	N/20-HCl	272	14,900

It is well known from the work of Gulland and Holiday (*J.*, 1936, 768) that substitution in the group attached to $N_{(9)}$ in adenine derivatives usually has little effect on either the position or the intensity of maximum absorption, so that the ultra-violet absorptions of the ionic compounds

FIG. 1.

Electron density projected on (100) *plane.*



recorded in the Table strongly suggest that they are substituted in the purine nucleus at some position in addition to $N_{(9)}$, *i.e.*, that they have been formed by an alkylation process. A cryoscopic determination of the molecular weight of the ionic toluene-*p*-sulphonate showed that the alkylation must be intramolecular; the value obtained was 240, which compares with a calculated formula weight of 461 for 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl adenosine (II). It follows that the ionic toluene-*p*-sulphonate is formed as a result of an intramolecular alkylation and an examination of space models suggests that the alkylation would be most likely to occur on $N_{(3)}$ in the pyrimidine nucleus since this atom can come into close proximity to $C_{(5')}$ in the sugar residue. That this is indeed the case, and that in these ionic derivatives $C_{(5')}$ is bonded to $N_{(3)}$, *i.e.*, that the ionic toluene-*p*-sulphonate is (III) and the iodide (IV), has been conclusively established by *X*-ray crystallographic analysis. The *X*-ray study was carried out on the ionic iodide which formed needles of approximately 0·03-mm. diameter. From oscillation and Weissenberg photographs, the space group and cell dimensions were found to be $P2_12_12_1$; $a = 5{\cdot}4$, $b = 15{\cdot}4$, $c = 18{\cdot}1$ Å. The specific gravity of the crystals was 1·81, which corresponds to a unit cell weight of 1640. This confirms that the re-arrangement is not intermolecular for, if it were, the weight of the structural unit would be *ca.* 800 and this would require but two asymmetric units per cell which is impossible in this particular space group.

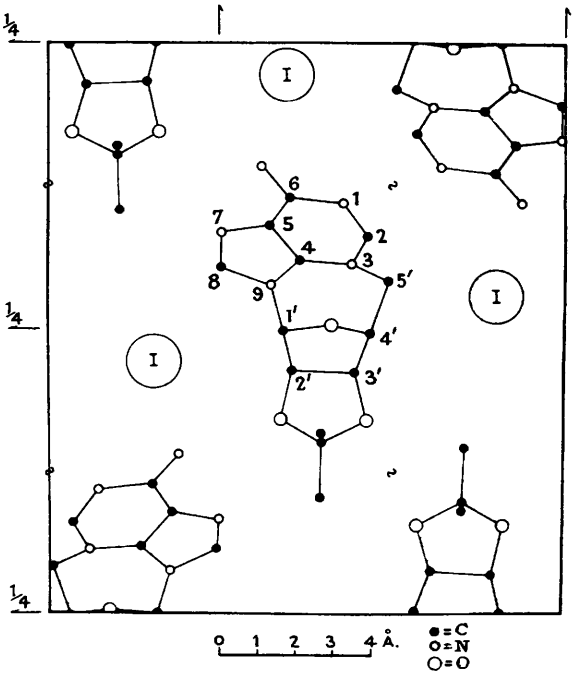
Intensities of " zero-layer " reflections were estimated visually on Weissenberg photographs, the crystal being rotated about the " *a* " crystallographic axis (the needle axis). The normal

" heavy atom " method of structural analysis was applied and this resulted in the projected electron-density map (Fig. 1). This shows clearly the general configuration of the molecule, and a three-dimensional scale model constructed on the basis of the approximately known bond lengths and angles fits the projection almost exactly. The model consistent with the projection is that of the iodide of 2′ : 3′-*iso*propylidene 3 : 5′-*cyclo*adenosine (IV) * in which (*a*) $C_{(1')}$ is coplanar with the purine ring, (*b*) the plane of the furanose ring is at an angle of *ca.* 100° with that of the purine ring, and (*c*) the approach of $C_{(5')}$ to $N_{(3)}$ is sufficiently close to indicate normal bond formation.

From the calculated distances $C_{(1')}$–$C_{(5')}$, $C_{(1')}$–$N_{(3)}$, based on observed distances in cytidine (Furberg, *Acta Cryst.*, 1950, **3**, 325) and adenine hydrochloride (Broomhead, *ibid.*, 1948, **1**, 324), the separation between $C_{(5')}$ and $N_{(3)}$ in the direction of the " *a* " axis is calculated to be

FIG. 2.

Structure as viewed along the " a " axis, showing one molecule and its environment.



approximately 1·0 Å. As the projected $C_{(5')}$–$N_{(3)}$ separation is 1·15 Å, the actual approach distance of $C_{(5)}$ and $N_{(3)}$ is approximately 1·5 Å.

Further *X*-ray analysis, together with refinement of this projection, is in progress in order to complete the structural determination.

This remarkable rearrangement of a nucleoside toluene-*p*-sulphonyl ester is not restricted to the case of adenosine. Although, as mentioned above, the 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonates of inosine and uridine behave as normal alkyl toluene-*p*-sulphonates and do not rearrange, isomerisation to an ionic species is observed in the case of the corresponding cytidine ester. Here as in adenosine the presence of the amino-group at $C_{(6)}$ in the pyrimidine ring is the decisive factor. Experimentally the cytidine case is more difficult to study, since toluene-*p*-sulphonylation of 2′ : 3′-*iso*propylidene cytidine yields a mixture of covalent $O_{(5')}$ and *N*-toluene-*p*-sulphonyl derivatives. However, the mixture can be used satisfactorily for further work since only the $O_{(5')}$-derivative can undergo the rearrangement involved. The mixed toluene-*p*-sulphonates dissolved readily in acetone and when the solution was heated at 100° for

* The nomenclature of such substances as (III) and (IV) presents considerable difficulty and it is proposed to refer to compounds of this type in which an additional ring has been formed as salts of " *cyclo*nucleosides," the atoms joined together to form the new ring being specified. This allows precise definition of the compounds and as far as can be seen avoids confusion.

15 minutes a crystalline isomeric compound separated which was ionic in character and which corresponded in amount to some 60% of the mixed esters. As in the adenosine case, this substance was a product of intramolecular alkylation since ebullioscopic determination of the molecular weight gave a value 205, the formula weight of 2′ : 3′-*iso*propylidene 5′-toluene-*p*-sulphonyl cytidine being 437. On stereochemical grounds it seemed hardly possible that the alkylation could have involved either of the ring-nitrogen atoms, but the proximity of $C_{(5')}$ to the oxygen atom attached to $C_{(2)}$ of the pyrimidine cycle suggested that in this instance alkylation had occurred on oxygen, *i.e.*, that the ionic compound was the toluene-*p*-sulphonate of 2′ : 3′-*iso*propylidene O^2 : 5′-*cyclo*cytidine (V).

It is well known that quaternary salts of 2-alkoxypyrimidines [to which class the compound (V) belongs] are readily dealkylated by acids, yielding the corresponding 2-hydroxypyrimidines (cf. Hilbert *et al.*, *J. Amer. Chem. Soc.*, 1930, **52**, 2001, 4489; 1936, **58**, 60). Pyrimidine derivatives alkylated on nitrogen, however, are very stable substances, and regeneration from them of the parent pyrimidines is impossible by this method. These facts offered a convenient method of establishing the nature of our ionic cytidine derivative; on being heated with dilute sulphuric acid it yielded cytidine sulphate, identified by comparison with an authentic specimen. It follows that structure (V) correctly represents the compound.

In addition to clarifying the position regarding 5′-toluene-*p*-sulphonyl esters of the ribonucleosides, the results described in this paper have a further implication. Examination of molecular models shows that formation of the *cyclo*nucleosides is possible only if the original nucleosides have the β-configuration at the glycosidic carbon atom. That the natural ribonucleosides have this configuration has already been established by Davoll, Lythgoe, and Todd (*J.*, 1946, 833) by a method involving periodate titration, the conclusion resting on the reasonable assumption that α-acetobromoglucose reacts with the silver salt of 2 : 8-dichloroadenine to produce a β-glucoside. The present work provides independent proof, since cytidine can be converted into uridine (Levene and Jacobs, *Ber.*, 1910, **43**, 3159), and adenosine into inosine (*Idem, ibid.*, p. 3161), and guanosine has been synthesised from the same 2 : 8-dichloroadenine D-ribofuranoside as adenosine (Davoll, Lythgoe, and Todd, *J.*, 1948, 1685).

It is possible that the type of rearrangement here described may have some counterpart in the chemistry of the nucleotides and polynucleotides. This possibility and other applications of the *cyclo*nucleosides are under investigation.

Experimental.

2′ : 3′-iso*Propylidene* 5′-*Toluene*-p-*sulphonyl Adenosine*.—2′ : 3′-*iso*Propylidene adenosine (2·322 g.; dried at 110°/0·1 mm. over phosphoric oxide for 12 hours) was dissolved in anhydrous pyridine (25 c.c.) at 40°, and the solution cooled in ice. Toluene-*p*-sulphonyl chloride (1·60 g., 1·1 equivs.) was added and the mixture shaken as the acid chloride rapidly dissolved. There was no appreciable rise in temperature but the initially colourless solution rapidly became deep yellow. The reaction mixture was kept at room temperature overnight, whereupon a semi-crystalline sludge separated; water (10 c.c.) was then added, followed almost immediately by ice-cold saturated sodium hydrogen carbonate solution (100 c.c.). The opalescent aqueous pyridine solution was extracted with ice-cold chloroform (3 × 100 c.c.), and the extract washed with ice-cold saturated sodium hydrogen sulphate solution (2 × 200 c.c.) and then with ice-water (2 × 200 c.c.). Chloroform, pyridine, and water were removed under reduced pressure at room temperature; subsequent evacuation to 0·01 mm. left a pale yellow resin (2·645 g., 76%) which was taken up in dry chloroform (50 c.c.) and filtered from insoluble material (50 mg.). Light petroleum (100 c.c.; b. p. 60—80°) was added and the solution left at 0° overnight; a colourless resin was deposited. The mother-liquor was decanted, the resin washed with light petroleum and the flask evacuated, whereupon the resinous 2′ : 3′-iso*propylidene* 5′-*toluene*-p-*sulphonyl adenosine* formed a foam which crumbled to an off-white amorphous powder (1·509 g., 43%). This product softened between 210° and 230° and decomposed with effervescence at 252° (Found, in material dried at 78°/15 mm. for 5 hours : C, 52·2; H, 4·9; N, 15·0. $C_{20}H_{23}O_6N_5S$ requires C, 52·1; H, 5·0; N, 15·2%).

2′ : 3′-*iso*Propylidene 5-toluene-*p*-sulphonyl adenosine dissolved in cold dilute hydrochloric acid and was reprecipitated on addition of sodium carbonate solution. Passage of gaseous hydrogen chloride into its ethereal solution resulted in the formation of a gel; from chloroform, the hydrochloride separated as a hygroscopic resin which rapidly became pink in air.

Paper chromatography (Whatman No. 1), with an ethanol–ammonia–water system (80 : 4 : 16), indicated that the product was homogeneous, a single spot (R_F = 0·86) being detectable by photography in ultra-violet light. When a *tert.*-butanol–acetic acid–water (5 : 4 : 1) solvent mixture was used, the R_F value was 0·86.

Evaporation of the mother-liquor left after precipitation of the toluene-*p*-sulphonyl derivative (above) yielded a yellow resin (0·299 g.), decomposing at 242° (Found, in material dried for 8 hours at 78°/15 mm. : N, 14·2. Calc. for monotoluene-*p*-sulphonyl derivative : N, 15·2; Calc. for a ditoluene-*p*-sulphonyl derivative : N, 11·4%).

Rearrangement of 2′ : 3′-iso*Propylidene* 5′-*Toluene*-p-*sulphonyl Adenosine*.—2′ : 3′-*iso*Propylidene 5 -toluene-*p*-sulphonyl adenosine (0·190 g.) was dissolved in pure dry acetone (15 c.c.), and the clear

solution sealed in a Carius tube. On being placed in a bath at 100°, the solution rapidly became opalescent and a crystalline solid separated in quantity within 20 minutes. After 1½ hours at 100° the solid (130 mg.) was filtered off and dried. The product, 2′ : 3′-iso*propylidene* 3 : 5′-cyclo*adenosine toluene*-p-*sulphonate*, was extremely soluble in cold water, and insoluble in hot acetone and chloroform. Recrystallised from aqueous ethanol it formed colourless monoclinic prisms, m. p. 296° (decomp.) (Found, in material dried at 78°/15 mm. for 4 hours : C, 52·2; H, 4·7; N, 15·5. $C_{20}H_{23}O_6N_5S$ requires C, 52·1; H, 5·0; N, 15·2%).

The same product was obtained in almost quantitative yield by heating the original toluene-*p*-sulphonyl derivative under reflux in dioxan for 1 hour or by keeping it (163 mg.) in dry acetone at room temperature for 1 week (104 mg. deposited).

Paper chromatography (Whatman No. 1) of the product with ethanol–ammonia–water (80 : 4 : 16) gave a single purine spot (R_F = 0·68), detected by photography in ultra-violet light, together with a spot corresponding to the anion of toluene-*p*-sulphonic acid (R_F = 0·83) detected by the iodide–iodate–starch spray. In *tert.*-butanol–acetic acid–water (5 : 4 : 1) the purine component had R_F = 0·75, and the acid component R_F = 0·50.

Cryoscopic determination in water gave the molecular weight as 240 ($C_{20}H_{23}O_6N_5S$ requires *M*, 461); the Van't Hoff factor is therefore 2, as required by a uni-univalent ionic species.

Interaction of 2′ : 3′-iso*Propylidene* 5′-*Toluene*-p-*sulphonyl Adenosine and Sodium Iodide.*—2′ : 3′-*iso*Propylidene 5′-toluene-*p*-sulphonyl adenosine (1·2 g.), anhydrous sodium iodide (1·0 g.), and dry acetone (20 c.c.), were kept (sealed tube) at 100° for 2 hours. The solution, initially clear, rapidly became opalescent and a mixture of glistening needles and a micro-crystalline product separated. This was collected (1·55 g.) and shown to consist of sodium toluene-*p*-sulphonate (theor., 0·503 g.) and the iodide of the *cyclo*purine glycoside. The mixed solids were dissolved in water (50 c.c.), and the acetone mother-liquors added. This solution was extracted once with chloroform (25 c.c.), boiled with charcoal, filtered, evaporated to a small bulk (5 c.c.), and left at 0° overnight. 2′ : 3′-iso*Propylidene* 3 : 5′-cyclo-*adenosine iodide* separated as colourless needles (0·55 g.) and, recrystallised from aqueous ethanol, had m. p. 277° (decomp.) (Found, in material dried at 78°/15 mm. for 5 hours : C, 37·5; H, 3·6; N, 17·0; I, 30·9. $C_{13}H_{16}O_3N_5I$ requires C, 37·4; H, 3·9; N, 16·8; I, 30·4%).

The aqueous solution of the iodide, on addition of silver nitrate solution, gave an immediate precipitate of silver iodide in quantitative yield. 2′ : 3′-*iso*Propylidene 3 : 5′-*cyclo*adenosine iodide could also be obtained by adding an equivalent of sodium iodide to a cold aqueous solution of the corresponding toluene-*p*-sulphonate and concentrating the solution to a small bulk at room temperature.

Toluene-p-*sulphonylation of* 2′ : 3′-iso*Propylidene Cytidine.*—2′ : 3′-*iso*Propylidene cytidine (1·75 g.; dried for 12 hours at 78°/0·1 mm.; Michelson and Todd, *J.*, 1949, 2476) was dissolved in dry pyridine (50 c.c.), and the solution cooled in ice. Toluene-*p*-sulphonyl chloride (1·18 g., 1 equiv.) was added, and the solution agitated until homogeneous and then left at room temperature overnight, whereupon a partly crystalline sludge separated; to this, water (10 c.c.) was added, followed by ice-cold saturated sodium hydrogen carbonate solution (100 c.c.). After effervescence ceased, the solution was extracted with ice-cold chloroform (3 × 100 c.c.), and the extract washed with ice-cold saturated sodium hydrogen sulphate solution (2 × 200 c.c.) and then with ice-water (2 × 200 c.c.). Solvents were removed under reduced pressure at room temperature, leaving a colourless resin which was dissolved in chloroform (40 c.c.); light petroleum (b. p. 60—80°) was then added to opalescence. During 36 hours at 0° a colourless oil separated. The supernatant liquid was decanted, the oil washed with light petroleum, and the flask evacuated, giving the product as a cream-coloured foam (0·146 g.), which decomposed slowly and indefinitely between 205° and 224° (Found, in material dried at 78°/15 mm. for 6 hours : C, 52·4; H, 5·2; N, 9·5. Calc. for $C_{19}H_{23}O_7N_3S$: C, 53·3; H, 5·3; N, 9·6%).

Paper chromatography (Whatman No. 1) with ethanol–ammonia–water (80 : 4 : 16) resolved the product into two pyrimidine components, detected by photography in ultra-violet light, having R_F values of 0·85 and 0·61. No free toluene-*p*-sulphonic acid was detectable and it is presumed that the product was a mixture of 5′-toluene-*p*-sulphonyl and *N*-toluene-*p*-sulphonyl compounds. This product (137 mg.) was dissolved in dry acetone (20 c.c.) and heated in a sealed tube at 100° for 45 minutes. Within a few minutes a colourless fibrous solid separated. The solid (83 mg.) was filtered off and washed with dry acetone. This product, 2′ : 3′-iso*propylidene* O^2 : 5′-cyclo*cytidine toluene*-p-*sulphonate*, was extremely soluble in water; recrystallised from ethanol–acetone it formed long flat prisms, m. p. 242° (decomp.) (Found, in material dried at 78°/15 mm. for 6 hours : C, 52·3; H, 5·4; N, 9·2. $C_{19}H_{23}O_7N_3S$ requires C, 52·2; H, 5·3; N, 9·6%).

The supernatant mother-liquors of the mixture of covalent toluene-*p*-sulphonyl compounds, when kept at room temperature for 24 hours, deposited a water-soluble solid (713 mg.), m. p. 140° (decomp.) undepressed on admixture with the product of rearrangement in acetone solution.

Paper chromatography (Whatman No. 1) of the crystalline rearrangement product with ethanol–ammonia–water (80 : 4 : 16) gave a large diffuse pyrimidine spot (R_F = 0·71), detected by photography in ultra-violet light, together with an acidic spot (R_F = 0·77) corresponding to the anion of toluene-*p*-sulphonic acid, detected as above. In *tert.*-butanol–ammonia–water (80 : 4 : 16) the pyrimidine moiety was resolved, with trailing into two spots having R_F = 0·74 and 0·42 respectively. From these observations it was apparent that the *cyclo*cytidine product underwent some decomposition in the presence of weak bases.

Ebullioscopic determination, in ethanol, of the molecular weight of the crystalline rearrangement product gave a value of 205 ($C_{19}H_{23}O_7N_3S$ requires *M*, 437); hence the Van't Hoff factor is 2, as required by a uni-univalent ionic species.

Acid Hydrolysis of 2′ : 3′-iso*Propylidene* O^2 : 5′-cyclo*Cytidine Toluene*-p-*sulphonate*.—2′ : 3′-*iso*-Propylidene O^2 : 5′-*cyclo*cytidine toluene-*p*-sulphonate (24·6 mg.) was dissolved in water (1 c.c.) and sulphuric acid added (1 c.c.; N.). The clear solution was heated on the steam-bath for 2 hours and ethanol (10 c.c.) added. The solution was evaporated to a small bulk (1 c.c.), and acetone (5 c.c.) added. When the mixture was kept at 0° overnight, colourless needle-like prisms separated (10·3 mg.). These were collected and washed with acetone; they had m. p. 218—219° (Kofler block), undepressed on admixture with an authentic specimen of cytidine sulphate.

Paper chromatography (Whatman No. 1) of the hydrolysis product with *tert.*-butanol–ammonia–water (80 : 4 : 16) confirmed its homogeneity and established the presence of sulphate ion and cytidine (R_F = 0·25; reference cytidine spot, R_F = 0·25).

We record our indebtedness to Dr. R. N. Haszeldine for some of the absorption spectra, to Mr. F. C. Baker for molecular-weight determinations, and to Dr. J. Davoll for some of the initial work on adenosine. Grateful acknowledgment is made to the Department of Scientific and Industrial Research for a Maintenance Allowance (to J. Z.) and to Roche Products Ltd. for grants and gifts of material.

UNIVERSITY CHEMICAL LABORATORY AND CAVENDISH LABORATORY,
CAMBRIDGE. [*Received, May 21st* 1951.]