Deoxyribonucleosides and Related Compounds. Part IV. The Configuration at the Glycosidic Centre in Deoxyadenosine and Deoxycytidine.*

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Toluene-*p*-sulphonylation of 3′-acetyl-2′-deoxyadenosine yields 3′-acetyl-2′-deoxy-5′-toluene-*p*-sulphonyladenosine, which is readily converted into a *cyclo*nucleoside salt in hot acetone. It follows that deoxyadenosine has the β-configuration at the glycosidic centre, *i.e.*, it is 9-(2-deoxy-β-D-ribofuranosyl)-adenine. 5′-Acetyl-2′-deoxy-3′-toluene-*p*-sulphonyladenosine does not yield a *cyclo*nucleoside salt when heated.

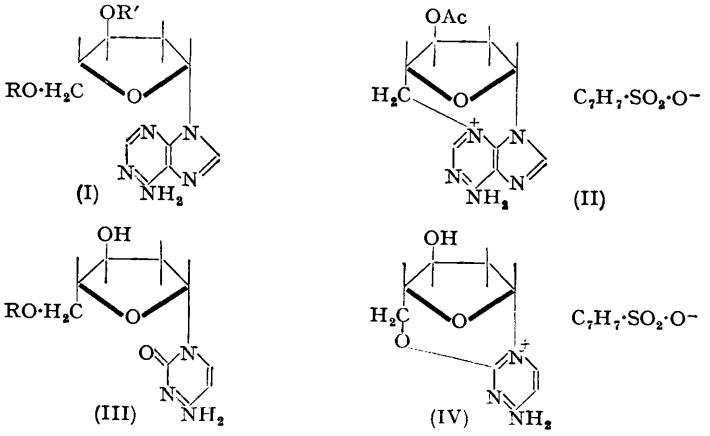
When 2′-deoxy-5′-toluene-*p*-sulphonylcytidine is heated in acetone, it appears also to form a *cyclo*nucleoside salt, but the latter is so unstable that it could not be isolated. The evidence of *cyclo*nucleoside salt formation is taken to indicate that deoxycytidine is also a β-glycoside.

The structure of the ribonucleosides (adenosine, guanosine, uridine, and cytidine) derived from natural ribonucleic acids is fully established. They are all β-D-ribofuranosides of the respective purine or pyrimidine bases (Davoll, Lythgoe, and Todd, *J.*, 1946, 833; Clark, Todd, and Zussman, *J.*, 1951, 2952) and their synthesis has been effected (Howard, Lythgoe, and Todd, *J.*, 1947, 1052; Davoll, Lythgoe, and Todd, *J.*, 1948, 967, 1685; Kenner, Taylor, and Todd, *J.*, 1949, 1620). Less is known with certainty about the natural deoxyribonucleosides (deoxyadenosine, deoxyguanosine, thymidine, deoxycytidine, and 5-methyldeoxycytidine). That they are 2-deoxy-D-ribofuranosides of the corresponding purine and pyrimidine bases has been established (Brown and Lythgoe, *J.*, 1950, 1990; Dekker and Elmore, *J.*, 1951, 2864), and the attachment of the sugar residue at $N_{(9)}$ in the purine and at $N_{(3)}$ in the pyrimidine nucleosides has been inferred from spectroscopic evidence (Gulland and Story, *J.*, 1938, 259, 262). No evidence bearing on the stereochemistry at the glycosidic centre has been advanced, nor has any one of them as yet been synthesised.

* Part III, *J.*, 1951, 2230.

The periodate oxidation method used to establish the β-configuration at the glycosidic carbon atom in the ribonucleosides (Davoll, Lythgoe, and Todd, *J.*, 1946, 833) cannot be applied to the deoxyribonucleosides since these substances contain no α-glycol system. Clark, Todd, and Zussman (*loc. cit.*) provided independent proof of the β-configuration of the ribonucleosides by showing that the 5′-*p*-toluenesulphonyl derivatives of adenosine and cytidine yield *cyclo*nucleoside salts when heated in acetone solution. Since the cyclisation can only occur with a β-glycoside, and involves only interaction between the 5′-position in the sugar residue and $N_{(3)}$ in adenosine, or the oxygen attached to $C_{(2)}$ in cytidine, it seemed reasonable to expect that this method would apply equally to deoxyadenosine (I; R = R′ = H) and deoxycytidine (III; R = H) provided that the sugar residue in these compounds is attached respectively to $N_{(9)}$ in the purine and $N_{(3)}$ in the pyrimidine nucleus.

As a first step in seeking to apply this method to deoxyadenosine it was necessary to prepare a 3′-substituted deoxyadenosine so that a toluene-*p*-sulphonyl group could be introduced unambiguously into position 5′. This was not altogether easy, since the manipulation of deoxyadenosine derivatives is complicated by the extreme lability of the glycosidic linkage. Treatment of deoxyadenosine with slightly more than 1 molar proportion of triphenylmethyl chloride in pyridine yielded a monotrityl derivative which we formulate as 2′-deoxy-5′-trityladenosine, substitution of the primary hydroxyl group under such conditions being a general phenomenon in carbohydrate derivatives. Acetylation of this product gave *N* : 3′-diacetyl-2′-deoxy-5′-trityladenosine which could not be crystallised. Attempts to remove the trityl group with acetic acid of varying strengths, or with ammonium chloride in ethanol, failed, the glycosidic linkage being largely hydrolysed. Hydrogenation in ethanol in presence of a mixture of palladium and palladised charcoal proved the only practicable method. This was far from satisfactory; not only was it extremely slow and incomplete, but the labile *N*-acetyl group was largely removed and extensive fission of the glycosidic linkage also occurred. In this way a small amount of the crystalline 3′-acetyl-2′-deoxyadenosine was obtained, sufficient for purposes of identification, but it was clear that the route to it was useless for preparative purposes. Recourse was therefore had to partial acetylation of deoxyadenosine. The nucleoside was allowed to react with acetic anhydride in pyridine at room temperature for three hours, and the product subjected to countercurrent distribution with an ethyl acetate–water system in an automatically operated 100-stage apparatus. In this way three acetylated nucleosides were



isolated in addition to unchanged deoxyadenosine. The major product, a diacetyldeoxyadenosine, accounted for some 55% of the acetylated material, while the remainder was made up of two monoacetyldeoxyadosines (32% and 13%). The monoacetyl compound obtained in smaller amount was identical with 3′-acetyl-2′-deoxyadenosine prepared *via* the 5′-trityl derivative. This being so it is reasonable to conclude that the other monoacetate was 5′-acetyl-2′-deoxyadenosine and the diacetate 3′ : 5′-diacetyl-2′-deoxyadenosine.

Toluene-*p*-sulphonylation of 3′-acetyl-2′-deoxyadenosine gave a resinous 3′-acetyl-2′-

deoxy-5′-toluene-*p*-sulphonyladenosine (I; R = $C_7H_7{\cdot}SO_2$, R′ = Ac) which was insoluble in water and readily soluble in organic solvents. When this product was heated in acetone a colourless crystalline compound separated, which analysed correctly for a toluene-*p*-sulphonyl derivative of 3′-acetyl-2′-deoxyadenosine but had the properties of a salt. It was freely soluble in water and insoluble in chloroform, and on paper chromatography it gave two spots, one showing ultra-violet absorption (purine) and the other having an acidic reaction (toluene-*p*-sulphonic acid); moreover, determination of molecular weight by the cryoscopic method gave values approximately half that expected for the covalent toluene-*p*-sulphonate. These properties correspond closely to those of the *cyclo*nucleoside salt obtained in similar fashion from 2′ : 3′-*iso*propylidene-5′-toluene-*p*-sulphonyladenosine (Clark, Todd, and Zussman, *loc. cit.*) and we therefore formulate our product as 3′-acetyl-2-′deoxy-3 : 5′-*cyclo*adenosine toluene-*p*-sulphonate (II). As in the case of the corresponding adenosine derivatives, cyclisation caused a marked shift of the ultra-violet absorption maximum to longer wave-length.

The formation of a *cyclo*nucleoside salt in this way indicates that deoxyadenosine (I) is a 2′-deoxy-β-D-ribofuranoside since examination of molecular models shows that only in the β-configuration is a cyclisation of this nature possible. Toluene-*p*-sulphonylation of 5′-acetyl-2′-deoxyadenosine gave a crystalline covalent 5′-acetyl-2′-deoxy-3′-toluene-*p*-sulphonyladenosine which could not be converted into an ionic isomer. This fact adds further confirmation to our formulation of the isomeric monoacetyl derivatives of deoxyadenosine.

Attention was now turned to deoxycytidine (III; R = H). Toluene-*p*-sulphonylation of *N* : 3′-diacetyl-2′-deoxycytidine (Michelson and Todd, *J.*, 1954, 34) gave a resinous substance converted by treatment with methanolic ammonia into crystalline 2′-deoxy-5′-toluene-*p*-sulphonylcytidine (III; R = $C_7H_7{\cdot}SO_2$). Deacetylation was carried out in this instance since, the formation of *cyclo*nucleoside salts depending on the basic nature of the purine or pyrimidine residue in the nucleoside, *N*-acylation of the cytosine residue might well prevent its occurrence. When an acetone solution of 2′-deoxy-5′-toluene-*p*-sulphonylcytidine was heated a gum was precipitated which, however, was not completely soluble in water. Examination by paper chromatography indicated that it was a mixture in which cytosine and toluene-*p*-sulphonate ion, as well as sugar derivatives of unidentified nature, were present. An attempt to cyclise *N* : 3′-diacetyl-2′-deoxy-5′-toluene-*p*-sulphonylcytidine gave mainly unchanged starting material, the remainder being converted into a similar mixture of decomposition products. Despite the failure of all efforts to isolate the expected 2′-deoxy-O^2 : 5′-*cyclo*cytidine *p*-toluenesulphonate (IV) after heating of 2′-deoxy-5′-toluene-*p*-sulphonylcytidine, the observation that toluene-*p*-sulphonate ion is present in the product makes it almost certain that the *cyclo*nucleoside is indeed formed by this process. We are at present unable to give a full explanation for its evident very ready decomposition. It may be recalled, however, that 2′ : 3′-*iso*propylidene-O^2 : 5′-*cyclo*cytidine toluene-*p*-sulphonate is not very stable; it is hydrolysed readily with acid, yielding cytidine, and decomposes partially on paper chromatography in presence of bases (Clark, Todd, and Zussman, *loc. cit.*). It would be expected that the deoxy*cyclo*cytidine would be even less stable since, as pointed out by Dr. V. M. Clark, the presence of a positive charge on $N_{(3)}$ in such a deoxyriboside derivative would lead to instability of the $N_{(3)}$–$C_{(1')}$ as well as of the O^2–$C_{(5')}$ linkage. Elimination of the sugar residue and production of cytosine might thus be expected, and indeed was observed in our experiments. We therefore consider that our results support the conclusion that 2′-deoxy-5′-toluene-*p*-sulphonylcytidine does undergo conversion into an unstable *cyclo*nucleoside salt, and that deoxycytidine (III; R = H) is accordingly to be regarded as a 2′-deoxy-β-D-ribofuranoside.

In the ribonucleoside series, determination of the β-configuration at the glycosidic centre in adenosine and cytidine suffices to prove the β-configuration for all four natural nucleosides, since guanosine and adenosine can be synthesised from a common precursor and cytidine can be converted into uridine. No such interrelationship exists between deoxyadenosine and deoxycytidine on the one hand and the remaining deoxyribonucleosides (deoxyguanosine, thymidine, 5-methyldeoxycytidine) and so, strictly, each individual should be examined separately. Nevertheless, it seems virtually certain that if deoxy-

adenosine and deoxycytidine are β-glycosides then all the natural deoxyribonucleosides are β-glycosides. This follows from *X*-ray studies carried out on deoxyribonucleic acid; the regularities observed in the packing of nucleoside residues in the macromolecule demand that the individual nucleosides must all have the same configuration (α or β) at the glycosidic centre (cf. Astbury, *Symp. Soc. Exp. Biol.*, 1947, **1**, 66).

Experimental

2′-Deoxy-5′-trityladenosine.—Anhydrous deoxyadenosine (prepared by drying 10·9 g. of the monohydrate overnight at 100°/0·1 mm.) was dissolved in warm dry pyridine (300 c.c.). The solution was cooled to room temperature, and triphenylmethyl chloride (12·45 g., 1·1 mol.) was added and dissolved by shaking. The mixture was set aside for 1 week, then poured into ice-cold aqueous sodium carbonate (8 g. in 1200 c.c.). The whole was evaporated to dryness under reduced pressure, the residue shaken with acetone (500 c.c.) at 35°, and the acetone solution filtered and evaporated *in vacuo*, giving a solid foam which readily disintegrated to a fine powder. The powder was shaken for 2 hr. at room temperature with benzene (250 c.c.), the mixture filtered, and the residue washed with benzene and air-dried; it consisted of a mixture of the required trityl derivative and unchanged deoxyadenosine. The latter (3·2 g.) was removed by stirring with water (200 c.c.) at 60° and the residual powder (11·5 g.) was dried and recrystallised by dissolving it in hot acetone (250 c.c.) and adding light petroleum (500 c.c.; b. p. 60—80°), giving *2′-deoxy-5′-trityladenosine* (8·86 g.), m. p. 195—197° (Found: C, 70·8; H, 5·8; N, 14·4. $C_{29}H_{27}O_3N_5$ requires C, 70·6; H, 5·5; N, 14·2%). The yield of trityl compound was 62% after allowance for recovered starting material.

N : *3′-Diacetyl-2′-deoxy-5′-trityladenosine.*—2′-Deoxy-5′-trityladenosine (2 g.) was dissolved in dry pyridine (50 c.c.), acetic anhydride (20 c.c.) was added, and the mixture kept overnight at room temperature. The solution was poured into ice-water (600 c.c.) and left overnight and the precipitate (2·3 g.) was collected, dried, and then freed from pyridine by evaporation with benzene (500 c.c.). The residue was dissolved in ethanol, and the solution filtered and evaporated. N : *3′-Diacetyl-2′-deoxy-5′-trityladenosine* was thus obtained as a solid foam which did not crystallise (Found: C, 68·4; H, 5·5; N, 12·2. $C_{33}H_{31}O_5N_5$ requires C, 68·6; H, 5·4; N, 12·1%).

3′-Acetyl-2′-deoxyadenosine.—(a) *From* N : *3′-diacetyl-2′-deoxy-5′-trityladenosine.* The above diacetyl trityl compound (3·4 g.) was hydrogenated in ethanol (150 c.c.) at 40—50° in presence of palladised charcoal (0·5 g. of 10%) and palladium oxide (1·7 g.). Uptake of hydrogen was exceedingly slow and even after several days only about half of the theoretical amount had been absorbed. Despite numerous trials under various conditions we were unable to improve this procedure and the time of hydrogenation varied from 3 to 10 days. The solution was filtered and evaporated, and the residue shaken with light petroleum (100 c.c., b. p. 40—60°) at room temperature overnight. The insoluble portion was then dissolved in hot ethanol (30 c.c.). On cooling, a small white precipitate separated and was filtered off; paper chromatography showed that it was mainly adenine. The filtrate was concentrated to smaller bulk (15 c.c.) and set aside. Colourless crystals (170 mg.) separated and were collected; examined by paper chromatography they appeared to consist largely of one compound (R_F 0·50—0·55 in *n*-butanol–water; 0·50 with 5% aqueous disodium hydrogen phosphate) mixed with adenine. Examination of the mother-liquors showed that they contained more adenine but little of the other substance. The crystalline material was twice recrystallised from ethanol and then gave colourless prisms of *3′-acetyl-2′-deoxyadenosine* (61 mg.), m. p. 211—212·5° (Found: C, 49·0; H, 5·3; N, 24·1. $C_{12}H_{15}O_4N_5$ requires C, 49·1; H, 5·2; N, 23·9%).

(b) *By partial acetylation of deoxyadenosine.* Anhydrous deoxyadenosine (8·7 g.; dried for 12 hr. at 80°/0·5 mm.) was dissolved in dry pyridine (310 c.c.). Acetic anhydride (18·1 c.c., 6 mols.) in dry pyridine (310 c.c.) was added, the mixture left for 3 hr. at room temperature and then poured into ice-water (100 c.c.), and the whole was evaporated under reduced pressure (bath-temp. $< 40°$), a process which ensured removal of *N*-acyl groups. The residual resin was twice evaporated with ethanol (50 c.c.), then dissolved in water previously saturated with ethyl acetate (41 c.c.). This solution was placed in the first two stages of an automatically operated 100-stage countercurrent distribution apparatus. With ethyl acetate–water the distribution process was continued until one hundred withdrawal stages had been completed. Determination of the optical density at 260 mμ in each of the hundred fractions removed from the apparatus after completion of the distribution showed three distinct peaks; on this basis the following groups were bulked: fractions 3—16, 18—56, and 58—83. The 100 withdrawal stages were similarly bulked.

Fractions 3—16 on evaporation yielded deoxyadenosine (0·7 g.). Fractions 58—83 on evaporation gave 3′-acetyl-2′-deoxyadenosine (0·9 g.), hexagonal prisms from ethyl acetate (Found: C, 49·3; H, 5·5; N, 23·9%). The m. p. of the purified 3′-acetyl derivative [216—217° (softens 214—216°)] was slightly higher than that of the material prepared as above *via* the trityl compound, but was not depressed when the two were mixed, and both materials showed the same behaviour on paper chromatograms and had identical infra-red spectra. Light absorption in ethanol: max. at 260 mμ (ε 14,300).

5′-Acetyl-2′-deoxyadenosine. The bulked fractions 18—56 from the above countercurrent distribution experiment were evaporated and the residue recrystallised from ethanol. *5′-Acetyl-2′-deoxyadenosine* (2·3 g.) was thus obtained as colourless needles, m. p. 140—141° (Found: C, 49·5; H, 5·3; N, 23·9%). Light absorption in ethanol: max. at 260 mμ (ε 14,400).

3′ : 5′-Diacetyl-2′-deoxyadenosine.—The combined withdrawal stages from the above countercurrent distribution were evaporated and the residue recrystallised from ethyl acetate–light petroleum (b. p. 40—60°). *3′ : 5′-Diacetyl-2′-deoxyadenosine* (4·0 g.) formed needles, m. p. 151—152° (Found, in material dried at 120°/10^{-3} mm. for 15 hr.: C, 50·3; H, 4·9; N, 20·4. $C_{14}H_{17}O_5N_5$ requires C, 50·2; H, 5·1; N, 20·9%). Light absorption in ethanol: max. at 259 mμ (ε 14,800).

3′-Acetyl-2′-deoxy-5′-toluene-p-*sulphonyladenosine.*—3′-Acetyl-2′-deoxyadenosine (240 mg.; dried for 12 hr. at 100°/0·1 mm.) was dissolved in dry pyridine (25 c.c.) and cooled in ice. Toluene-*p*-sulphonyl chloride (235 mg., 1·3 mols.) was added and the solution was set aside overnight at room temperature. Ice-cold saturated aqueous sodium hydrogen carbonate (10 c.c.) was added and the mixture was extracted with ice-cold chloroform. Evaporation of the dried chloroform extract gave a resin which was redissolved in chloroform, then filtered, and light petroleum (b. p. 60—80°) was added to opalescence. After 12 hr. unchanged 3′-acetyl-2′-deoxyadenosine (40 mg.) separated and was filtered off. The filtrate was further diluted with light petroleum (1·5 vols.) and left overnight. The resin which separated was washed with light petroleum and converted into a solid foam (250 mg.) by removal of traces of solvent under reduced pressure (0·01 mm.). This material, which did not crystallise, was essentially the required toluene-*p*-sulphonyl derivative, although paper chromatography in *n*-butanol–water showed that it contained small amounts of deoxyadenosine and 3′-acetyl-2′-deoxyadenosine (Found: C, 50·2; H, 4·7; N, 14·6. Calc. for $C_{19}H_{21}O_6N_5S$: C, 51·0; H, 4·7; N, 15·7). Light absorption in ethanol: max. at 257 mμ.

*3′-Acetyl-2′-deoxy-3 : 5′-cyclo**adenosine** Toluene*-p-*sulphonate.*—3′-Acetyl-2′-deoxy-5′-toluene-*p*-sulphonyladenosine (240 mg.) was heated in dry acetone (10 c.c.) in a sealed tube at 100° during 1¼ hr. Colourless crystals (70 mg.) of the cyclo*nucleoside* salt, m. p. 195·5°, separated. A further quantity was obtained by evaporation of the mother-liquors, dissolution of the residue in dioxan (10 c.c.), and refluxing for 2 hr.; the salt (90 mg.) which separated again had m. p. 195·5° [Found: C, 50·8; N, 4·6; N, 15·8%; *M* (Rast), 262, 244. $C_{19}H_{21}O_6N_5S$ requires C, 51·0; H, 4·7; N, 15·7%; *M*, 447] (total yield of crystalline salt was 67%). It was extremely soluble in water and insoluble in boiling chloroform, acetone, or dioxan. Light absorption in ethanol: max. at 274—275 mμ (ε 14,900).

Comparison of Covalent and Ionic Toluene-p-*sulphonates from 3′-Acetyl-2′-deoxyadenosine.*—The covalent isomer ran as a single spot on paper chromatography whereas the ionic compound gave a purine spot detectable by ultra-violet absorption and an acidic spot detectable by spraying with B.D.H. Universal Indicator, starch–iodide–iodate, or bromophenol-blue. Paper chromatographic comparisons are summarised below in Table 1.

5′-Acetyl-2′-deoxy-3′-toluene-p-*sulphonyladenosine.*—To an ice-cold solution of 5′-acetyl-2′-deoxyadenosine (255 mg.; dried for 12 hr. at 80°/0·1 mm.) in dry pyridine (3 c.c.) toluene-*p*-sulphonyl chloride (180 mg., 1·1 mols.) was added; the resulting orange solution was set aside overnight at room temperature. Ice-cold saturated aqueous sodium hydrogen carbonate (10 c.c.) was added, the mixture extracted with ice-cold chloroform, and the extract dried and evaporated. The yellowish solid residue (330 mg.) was recrystallised from chlorofrom–light petroleum (b. p. 60—80°), giving *5′-acetyl-2′-deoxy-3′-toluene*-p-*sulphonyladenosine* as plates (100 mg.), m. p. 147—148° (Found: C, 51·1; H, 4·8; N, 15·6. $C_{19}H_{21}O_6N_5S$ requires C, 51·0; H, 4·7; N, 15·7%). The compound was quite unaffected by refluxing it in dry dioxan or by heating its acetone solution in a sealed tube at 100°.

2′-Deoxy-5′-toluene-p-*sulphonylcytidine.*—Toluene-*p*-sulphonyl chloride (60 mg.) was added to a solution of *N* : 3′-diacetyl-2′-deoxycytidine (75 mg.; Michelson and Todd, *J.*, 1954, 34) in dry pyridine (2 c.c.), and the mixture left at room temperature for 20 hr., then cooled to 0°. Water (0·2 c.c.) was added, and after 1 hr. at 0° the mixture was poured into ice-water (25 c.c.)

and extracted with chloroform. The chloroform extract was washed successively with ice-cold dilute sulphuric acid, sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated under reduced pressure. The crude *N* : 3′-diacetyl-2′-deoxy-5′-toluene-*p*-sulphonylcytidine (85 mg.) thus obtained as a solid foam was not further purified. A quantity of it (75 mg.) was dissolved in cold half-saturated methanolic ammonia (2 c.c.) and after 20 hr. at 0° the mixture was evaporated under reduced pressure. A small amount of water was added to the

TABLE 1. *Paper chromatography of deoxyadenosine derivatives.*

Whatman No. 1 paper. Ascending R_F values.
Solvent systems: I, *n*-butanol–water (86 : 14); II, *tert.*-butanol–acetic acid–water (5 : 4 : 1); III, ethanol–ammonia–water (80 : 16 : 4); IV, 5% aqueous disodium phosphate–*iso*amyl alcohol (3 : 2).

	I	II	III	IV
Deoxyadenosine	0·40	0·75	0·47	0·55
3′ : 5′-Diacetyl-2′-deoxyadenosine	0·66	0·90	0·59	0·62
5′-Acetyl-2′-deoxyadenosine	0·56	0·82	0·51	0·62
3′-Acetyl-2′-deoxyadenosine	0·58	0·83	0·53	0·52
5′-Acetyl-2′-deoxy-3′-toluene-*p*-sulphonyladenosine	0·86	0·91	0·70	Streak at origin
3′-Acetyl-2′-deoxy-5′-toluene-*p*-sulphonyladenosine	0·82	0·84	0·67	Streak at origin
3′-Acetyl-2′-deoxy-3 : 5′-*cyclo*adenosine toluene-*p*-sulphonate :				
purine moiety	—	0·62	0·49	0·79
toluene-*p*-sulphonate ion	—	0·49	0·67	—

residue, and the crystalline deposit of *2′-deoxy-5′-toluene*-p-*sulphonylcytidine* (50 mg.) was collected. Recrystallised from water it had m. p. 120° (Found, in material dried for 12 hr. at 90°/0·1 mm.: C, 50·5; H, 5·0. $C_{16}H_{19}O_6N_3S$ requires C, 50·4; H, 5·0%).

Action of Heat on 2′-Deoxy-5′-toluene-p-*sulphonylcytidine and* N : *3′-Diacetyl-2′-deoxy-5′-toluene*-p-*sulphonylcytidine.*—A solution of 2′-deoxy-5′-toluene-*p*-sulphonylcytidine (40 mg.) in acetone (3 c.c.) was heated in a sealed tube at 100° for 2 hr., during which a gum slowly separated. Acetone was removed and the residue, which was only in part soluble in water, was examined by paper chromatography. Several spots were observed, indicating extensive decomposition of the *cyclo*nucleoside salt. Application of the same heating process to crude *N* : 3′-diacetyl-2′-deoxy-5′-toluene-*p*-sulphonylcytidine gave similar results; the product was for the most part insoluble in water and it contained a large amount of unchanged material. The results of these studies are given in Table 2, ascending R_F values of appropriate known substances as well as starting materials being recorded for comparison.

TABLE 2. *Paper chromatography of deoxycytidine derivatives.*

Whatman No. 1 paper. Ascending R_F values.
Solvent systems: I—III, see Table 1; IV, *iso*propanol–water–ammonia (7 : 2 : 1).

	I	II	III	IV
Cytosine	0·13	0·54	0·46	0·47
Acetylcytosine	0·28	0·73	0·52	0·53
Deoxycytidine	0·12	0·61	0·55	0·58
N : 3′-Diacetyl-2′-deoxycytidine	0·57	0·93	0·66	0·80
N : 3′-Diacetyl-2′-deoxy-5′-toluene-*p*-sulphonylcytidine	0·76	0·95	0·80	0·88
2′-Deoxy-5′-toluene-*p*-sulphonylcytidine	0·53	0·90	0·78	0·85
Toluene-*p*-sulphonate ion	0·27	0·50	0·75	0·75
Rearranged 2′-deoxy-5′-toluene-*p*-sulphonylcytidine	0·53	0·91	0·79	0·85
Ultra-violet absorbing spots	0·28	0·83	0·72	0·68
	0·13	0·47	0·55	0·47
	0·08	0·63	0·46	0·38 and 0·28
		Fluorescent		Fluorescent
Acid spots	—	0·50	0·78	—
Basic spots	—	0·83	—	—
Cysteine spray	—	—	—	streak 0·3 to 1·0

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