Nucleotides. Part XXX. Mononucleotides derived from Deoxyadenosine and Deoxyguanosine.*

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The 3′ and the 5′ phosphates of deoxyadenosine and deoxyguanosine have been synthesised by unambiguous routes. Deoxyadenosine-5′ phosphate and deoxyguanosine-5′ phosphate correspond in their properties to the deoxyadenylic and deoxyguanylic acid previously obtained by enzymic degradation of natural deoxyribonucleic acids.

In previous papers of this series syntheses of the mononucleotides derived from the natural pyrimidine deoxyribonucleosides thymidine and deoxycytidine have been reported. Thymidine-3′ and -5′ phosphate (Part XX; Michelson and Todd, *J.*, 1953, 951) and deoxycytidine-3′ and -5′ phosphate (Part XXIII; Michelson and Todd, *J.*, 1954, 34) were prepared by phosphorylation of suitably protected nucleosides, and the 5′-phosphates were found to be identical respectively with the thymidylic and deoxycytidylic acids obtained by enzymic hydrolysis of natural deoxyribonucleic acids. As an extension of these studies we have now synthesised the 3′- and the 5′-phosphate of the natural purine deoxyribonucleosides deoxyadenosine and deoxyguanosine. The methods adopted (*viz.*, phosphorylation of suitably acylated nucleosides of known structure) were essentially similar to those used for the pyrimidine deoxyribonucleotides but the problem was complicated by the extreme lability of the purine deoxyribonucleosides and their derivatives to acid, and, in the case of deoxyguanosine, by low reactivity of the nucleoside towards acylating agents; these difficulties necessitated certain modifications in the synthetic procedures.

Andersen, Hayes, Michelson, and Todd (*J.*, 1954, 1882) in the course of determining the configuration at the glycosidic centre in deoxyadenosine prepared 3′- and 5′-acetyldeoxy-

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adenosine. These acetyl derivatives, the starting materials for the preparation of the deoxyadenosine phosphates, were found to be more easily prepared by partial deacetylation of 3′ : 5′-diacetyladenosine. Treatment of 3′-acetyldeoxyadenosine with dibenzyl phosphorochloridate in pyridine gave 3′-acetyldeoxyadenosine-5′ dibenzyl phosphate in good yield. After model experiments on the effect of ammonia on 2′ : 3′-*iso*propylideneadenosine-5′ dibenzyl phosphate and on deoxyadenosine, it was found that treatment of 3′-acetyldeoxyadenosine-5′ dibenzyl phosphate with methanolic ammonia under suitable conditions yielded deoxyadenosine-5′ benzyl phosphate in satisfactory yield. Removal of the benzyl group from this compound was effected by hydrogenolysis in aqueous solution buffered to pH 7 with sodium acetate; maintenance of neutral or alkaline pH during hydrogenolysis is essential to avoid fission of the glycosidic linkage. The deoxyadenosine-5′ phosphate so obtained was purified by ion-exchange chromatography and isolated as its calcium salt.

Phosphorylation of 5′-acetyldeoxyadenosine with dibenzyl phosphorochloridate was most unsatisfactory. True, on working up the product and removing protecting groups in the usual way deoxyadenosine-3′ phosphate was obtained but the amount (10 mg. from 2 g. of acetylnucleoside) was so low as to make the process virtually useless. Recourse was had to *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride, a powerful reagent for the preparation of phosphites (Corby, Kenner, and Todd, *J.*, 1952, 3669). This mixed anhydride reacted readily with 5′-acetyldeoxyadenosine in methyl cyanide in presence of 2 : 6-lutidine, giving a reasonable yield (55%) of 5′-acetyldeoxyadenosine-3′ benzyl phosphite. The phosphite was chlorinated with *N*-chlorosuccinimide in chloroform (methyl cyanide could not be used as a vehicle for the reaction), and the crude phosphorochloridate hydrolysed directly with aqueous pyridine, yielding impure 5′-acetyldeoxyadenosine-3′ benzyl phosphate as a brownish gum. Without further purification this product was deacetylated and hydrogenolysed in the manner described for the corresponding 5′-phosphate. Deoxyadenosine-3′ phosphate was formed and, after purification by ion-exchange chromatography, it was isolated as its calcium salt.

The two deoxyadenosine phosphates are readily distinguishable by paper and ion-exchange chromatography. The infra-red spectra of the calcium salts differ from one another but the differences (mainly in the region 8—10 μ) are rather small and the spectra are thus not very useful for characterisation. Their ultra-violet absorption spectra are very similar although a small difference was observed in that a slight inflection at 259 mμ in the spectrum of calcium deoxyadenosine-5′ phosphate was not observed in that of the 3′-phosphate; a similar small difference was observed in the spectra of the two monoacetyl derivatives of deoxyadenosine. Natural deoxyadenylic acid was conclusively identified with deoxyadenosine-5′ phosphate : they were indistinguishable in paper chromatographic and ion-exchange behaviour and in absorption spectrum. Mixtures of the two could not be separated on ion-exchange columns, whereas mixtures of the two synthetic nucleotides and of natural deoxyadenylic acid and deoxyadenosine-3′ phosphate each gave two distinct peaks. Natural deoxyadenylic acid, synthetic deoxyadenosine-5′ phosphate, and deoxyadenosine-5′ benzyl phosphate were all smoothly dephosphorylated by rattlesnake venom (*Crotalus atrox*) whereas deoxyadenosine-3′ phosphate and its benzyl ester were quite unaffected. The deoxyadenosine phosphates and their benzyl esters were unaffected by either ribonuclease or deoxyribonuclease.

For the synthesis of the isomeric deoxyguanosine phosphates it was necessary first to prepare 3′- and 5′-acetyldeoxyguanosine. As in the case of the acetyldeoxyadenosines, mixtures of these compounds were most readily prepared by the partial acetylation of deoxyguanosine or by partial deacetylation of 3′ : 5′-diacetyldeoxyguanosine; the latter procedure had the advantage that it gave much better yields of the 3′-acetyl derivative than the former, which yielded mainly the 5′-isomer. The isomeric acetyl derivatives were separated by countercurrent distribution but their orientation was more difficult than that of the monoacetyldeoxyadenosines, since no reference substance corresponding to 3′-acetyl-5′-trityldeoxyadenosine was available and a less direct method was employed. An investigation of the acid hydrolysis of the acetylated purine deoxyribonucleosides, in which the glycosidic linkage is extremely labile, showed that the acetates of 2-deoxy-D-

ribose were not decomposed under conditions which hydrolysed the glycosidic link completely. It was also possible to differentiate 3- and 5-acetyl-2-deoxy-D-ribose clearly by paper chromatography in several solvent systems; the differences in R_F values were small but adequate. Hydrolyses were therefore carried out side by side on the monoacetyl derivatives of deoxyadenosine and deoxyguanosine, and the two latter were oriented by the correspondence of the products with those from the deoxyadenosine derivatives whose orientation was already known. Neither of the monoacetyldeoxyguanosines has a proper melting point but they behave rather differently when heated, since 5'-acetyldeoxyguanosine crystallises from aqueous ethanol with 1 mol. of water which it loses at 125—130° whereas 3'-acetyldeoxyguanosine crystallises from the same solvent in anhydrous form. The two isomers are most readily distinguished by paper chromatography.

Since, in experiments directed to other ends, we had failed to bring about reaction between the monoacetyl derivatives of deoxyguanosine and toluene-*p*-sulphonyl chloride or methanesulphonyl chloride no attempt was made to phosphorylate them with dibenzyl phosphorochloridate. Instead they were phosphorylated by using *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride, and the acetyldeoxyguanosine benzyl phosphites, which were produced in *ca.* 30% yield, were converted into the deoxyguanosine nucleotides as described above for deoxyadenosine-3' phosphate. Deoxyguanosine-3' and -5' phosphate, which can be distinguished by paper chromatography, were isolated as their barium salts. No specimen of pure natural deoxyguanylic acid was available; there is, however, no doubt that the natural acid is identical with deoxyguanosine-5' phosphate since the latter and its benzyl ester, like the natural product, were readily dephosphorylated by rattlesnake venom whereas the 3'-phosphate was unaffected. Like the corresponding deoxyadenosine derivatives, neither the deoxyguanosine phosphates nor deoxyguanosine-5' benzyl phosphate were affected by ribonuclease or deoxyribonuclease.

Experimental

3' : 5'-Diacetyldeoxyadenosine.—Acetic anhydride (10 c.c.) was added to a solution of anhydrous deoxyadenosine (4 g.) in pyridine (20 c.c.), and the mixture left overnight at room temperature, then cooled to 0° and poured into ice–water (200 c.c.) with vigorous stirring. The aqueous solution was extracted twice with chloroform, and the combined extracts were washed with cold dilute sodium hydrogen carbonate solution, then with water, dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue of *3' : 5'-diacetyldeoxyadenosine* crystallised from ethyl acetate–light petroleum (b. p. 40—60°) as needles, m. p. 151—152° (4·6 g.) (Found, in material dried for 15 hr. at 120°/10^{-3} mm.: C, 50·4; H, 5·2; N, 20·9. $C_{14}H_{17}O_5N_5$ requires C, 50·2; H, 5·1; N, 20·9%).

3'-Acetyldeoxyadenosine and 5'-Acetyldeoxyadenosine.—Saturated methanolic ammonia (75 c.c.) was added to a solution of 3' : 5'-diacetyldeoxyadenosine (2·5 g.) in ethanol (250 c.c.), and the mixture left at room temperature for 1½ hr. Solvent was then removed under reduced pressure and the residue separated into its components by countercurrent distribution, using an ethyl acetate–water system as previously described (Anderson, Hayes, Michelson, and Todd, *loc. cit.*), to give deoxyadenosine (0·38 g.), 5'-acetyldeoxyadenosine (0·64 g.), 3'-acetyldeoxyadenosine (0·33 g.), and unchanged 3' : 5'-diacetyldeoxyadenosine (0·82 g.).

3'-Acetyldeoxyadenosine-5' Dibenzyl Phosphate.—A solution of 3'-acetyldeoxyadenosine (2 g., 1 mol.; dried for 24 hr. at 100°/0·5 mm.) in anhydrous pyridine (150 c.c.) was cooled to its f. p. (acetone–carbon dioxide). Dibenzyl phosphorochloridate (from 7·2 g., 4 mols., of dibenzyl phosphite) was added and the mixture kept at, or just above, its m. p. for 4 hr., then at room temperature overnight.

Aqueous sodium carbonate (6 g. in 40 c.c. of water) was added and the mixture filtered. The filtrate was evaporated to dryness under reduced pressure (temp. $\not>$ 35°) and the residue evaporated several times with ethanol (25 c.c.), after which it was dissolved in chloroform (25 c.c.). The chloroform solution was washed successively with saturated sodium hydrogen carbonate solution (3 × 50 c.c.) and water (2 × 50 c.c.) and evaporated at 0·01 mm. The residue (4·2 g.) was dissolved in dry acetone (10 c.c.); ether (20 c.c.) followed by light petroleum (70 c.c.; b. p. 60—80°) was added, and the mixture was set aside overnight. The supernatant liquid was decanted and solvent removed from the residue at room temp./0·01 mm. The brownish gum (3·0 g.) so obtained was redissolved in chloroform, the solution washed with sodium hydrogen carbonate and water and evaporated under reduced pressure, and the residue treated as before

with acetone, ether, and light petroleum. The precipitated resin was washed with light petroleum and converted into an amber-coloured solid foam (2·4 g., 63%) by evacuation to 0·01 mm. Paper chromatography in *n*-butanol–water (86 : 14) showed that this product was essentially a single substance (R_F 0·83) contaminated with small amounts of other slower-running substances. The crude 3′-acetyldeoxyadenosine-5′ dibenzyl phosphate was further purified by refluxing it with dry ether (1 c.c. of ether for 10 mg.) for 2 hr., decanting the supernatant liquid, and leaving the residue at 10^{-4} mm. It still contained some impurities (Found : C, 57·1; H, 5·3; N, 12·7%. Calc. for $C_{26}H_{28}O_7N_5P$: C, 56·4; H, 5·1; N, 10·6%).

Deoxyadenosine-5′ Benzyl Phosphate.—The above crude 3′-acetyldeoxyadenosine-5′ dibenzyl phosphate (2·3 g.) was dissolved in saturated methanolic ammonia (50 c.c.), and the solution left at room temperature for 100 hr. before evaporation to dryness at room temp./0·1 mm. The solid foam (2·2 g.) contained some unhydrolysed dibenzyl ester (paper chromatography); it was therefore dissolved in water (100 c.c.), and the solution washed with ethyl acetate (5 × 100 c.c.), the aqueous solution and the ethyl acetate washings being evaporated separately. The ethyl acetate washings on evaporation gave a gum (0·5 g.) which was, on the basis of its paper chromatographic behaviour, probably in the main deoxyadenosine-5′ dibenzyl phosphate; treatment of this residue with methanolic ammonia (10 c.c.) as before yielded a further amount of material identical with the product obtained by evaporating the aqueous solution; the combined yield was 1·53 g. There is no doubt that this product was essentially a single nucleotide but it behaved on paper chromatography like a salt. In the *n*-butanol–water (86 : 14) system it gave two spots (R_F 0·15 and 0·41). When these spots were eluted and the eluates run separately on fresh chromatograms, with the same system, it was found that (*a*) the material of R_F 0·15 gave only one spot (R_F 0·15) and (*b*) the material of R_F 0·41 gave two spots (R_F 0·15 and 0·41). This behaviour is presumably due to dissociation of a salt. In acidic or basic solvent systems only one spot is observed and in these systems the original product as well as both the materials into which it was resolved by chromatography in *n*-butanol–water gave the same single spot at the same R_F.

A solution of the product (150 mg.) in water (0·5 c.c.) was applied as a band on a strip of Whatman No. 3 paper (19 × 36 cm.), and the chromatogram developed with *n*-butanol–water (86 : 14); it showed two bands (by ultra-violet absorption) of R_F 0·1—0·14 and 0·34—0·4. The latter band was cut out and eluted with water, and the eluate evaporated. The pale brownish glass (85 mg.) appeared to be mainly deoxyadenosine-5′ benzyl benzylammonium phosphate (Found : C, 52·2; H, 6·3; N, 14·7; P, 5·4%; ratio N : P, 2·72. Calc. for $C_{24}H_{29}O_6N_6P$: C, 54·5; H, 5·5; N, 15·9; P, 5·9%; ratio N : P, 2·71).

For further stages in synthesis the crude product obtained directly from the washed aqueous solution of the ammonia-treated starting material was used directly since it was presumably a mixture of the ammonium and the benzylammonium salt of the desired phosphate.

Deoxyadenosine-5′ Phosphate.—The above monobenzyl ester (1·27 g.) was dissolved in distilled water (50 c.c.). Sodium acetate ("AnalaR;" 1·2 g.) and 10% palladised charcoal (100 mg.) were added and the mixture was shaken with hydrogen at room temperature/1 atm. for 20 hr., fresh catalyst (100 mg.) being added after 2 and 14 hr. The filtered solution was brought to pH 10 with ammonia and applied to a column of anion-exchange resin (Dowex 2, chloride form, 400 mesh; 6 cm. × 7 sq. cm.). The column was washed successively with aqueous 0·01M-ammonium chloride (500 c.c.) and hydrochloric acid (500 c.c. of 0·001N, followed by 7 l. of 0·003N). The eluate was collected in fractions of 10 c.c., the optical density of each at 260 mμ being determined. The desired product was located in the 0·003N-acid eluates; the bulked eluates containing it (4400 c.c.) were neutralised (pH 7·5) with carbonate-free lime water, and the solution was evaporated to small bulk (200 c.c.) under reduced pressure at $\not> 35°$. Ethanol (600 c.c.) was added, the mixture left overnight, and the precipitated calcium salt (0·95 g.) collected by centrifugation. The precipitate was warmed to 40° with distilled water and filtered through Hyflo Supercel, and the filtrate diluted with ethanol (900 c.c.) and set aside at 0° overnight. Calcium deoxyadenosine-5′ phosphate (700 mg.) separated and was collected by centrifugation.

Final purification was effected by reprecipitation from aqueous solution with ethanol, and the white amorphous salt dried at 120°/1 mm. during 24 hr.; its analyses presumed a *dihydrate* (Found : C, 29·6; H, 3·7; P, 7·9. $C_{10}H_{12}O_6N_5PCa,2H_2O$ requires C, 29·6; H, 4·0; P, 7·7%). The salt had $[\alpha]_D^{19}$ −26° (*c*, 0·38 in H_2O); Klein and Thannhauser (*Z. physiol. Chem.*, 1934, **224**, 252) record $[\alpha]_D^{19}$ −38° (*c*, 0·23 in H_2O) for natural deoxyadenylic acid. Light absorption : In N/100-HCl, max. at 257·5 mμ (ε 13,580), min. at 227·5 mμ (ε 2710), optical density ratios 250/260 = 0·84 and 280/260 = 0·23. In H_2O, max. at 260 mμ (ε 13,600), min. at 227 mμ

(ε 1850), optical density ratios 250/260 = 0·78 and 280/260 = 0·16. In N/100-NaOH, max. at 260 mμ (ε 14,140), min. at 227·5 mμ (ε 2554), optical density ratios 250/260 = 0·79 and 280/260 = 0·18.

The synthetic nucleotide was directly compared with natural deoxyadenylic acid and was identical with it in ultra-violet and infra-red spectrum, and in paper chromatographic and ion-exchange characteristics (see below). Like the natural nucleotide it was completely converted into deoxyadenosine and inorganic phosphate by rattlesnake venom in 3 hr. at 37°.

5'-Acetyldeoxyadenosine-3' Benzyl Phosphite.—5-Acetyldeoxyadenosine (1 g., 1 mol.; dried for 24 hr. at 60°/1 mm.) and anhydrous 2 : 6-lutidine (0·73 g., 2 mols.) were dissolved in anhydrous methyl cyanide (150 c.c.). *O*-Benzylphosphorous *OO*-diphenylphosphoric anhydride (2·75 g., 2 mols.) (Corby, Kenner, and Todd, *loc. cit.*) was added and the mixture left overnight at room temperature. Solvents were removed at room temperature under reduced pressure and the residue was dissolved in chloroform (75 c.c.). The chloroform solution was washed successively with water (4 × 20 c.c.), saturated aqueous sodium hydrogen carbonate (5 × 20 c.c.), and water (5 × 20 c.c.), dried, and evaporated, and the residue was twice evaporated with ethanol, giving an almost colourless resin (1·37 g.). Paper chromatography in *n*-butanol–water (86 : 14) showed that this resin contained two substances, one the desired phosphite (R_F 0·64) and the other (R_F 0·9) probably dibenzyl phosphite (cf. Corby, Kenner, and Todd, *loc. cit.*). The product was freed from the faster-moving component by dissolution in chloroform (5 c.c.), pouring the solution into *cyclo*hexane (200 c.c.), and setting the whole aside overnight. The supernatant liquid was decanted and the residual *5'-acetyldeoxyadenosine-3' benzyl phosphite* (0·83 g., 55%) converted into a yellowish solid foam by evacuation to 0·1 mm. (Found : C, 50·1; H, 5·0; N, 16·1; P, 6·5%; ratio N : P, 2·46. $C_{19}H_{21}O_6N_5P,0{\cdot}5H_2O$ requires C, 50·1; H, 4·9; N, 15·4; P, 6·8%; ratio N : P, 2·26). The same product was obtained in 40% yield when dimethylformamide was used in place of methyl cyanide in the above preparation.

Deoxyadenosine-3' Phosphate.—5'-Acetyldeoxyadenosine-3' benzyl phosphite (0·5 g., 1 mol.) was dissolved in " AnalaR " chloroform (20 c.c.), and *N*-chlorosuccinimide (0·165 g., 1·1 mols.) was added. The mixture was left for 5 hr. at room temperature, then aqueous pyridine (20 c.c.; pyridine : water = 8 : 1) was added, and the whole shaken for 12 hr. Solvents were removed under reduced pressure at room temperature and the residue was twice evaporated with ethanol, giving crude 5'-acetyldeoxyadenosine-3' benzyl phosphate as a brownish gum (0·9 g.).

This product was dissolved in half-saturated methanolic ammonia (40 c.c.) and after 12 hr. at room temperature the solution was evaporated at $\not>40°$, the residue dissolved in distilled water (20 c.c.), and crystalline sodium acetate (0·7 g.; " AnalaR ") added. The solution was then hydrogenated at room temperature and pressure by use of 10% palladised charcoal (100 mg.) during 20 hr., fresh catalyst being added twice during the operation. The solution was filtered, brought to pH 10 with ammonia, and applied to a column of anion-exchange resin (Dowex-2, chloride form, 400 mesh; 5 cm. × 2·2 sq. cm.). Elution was carried out successively with aqueous ammonium chloride (3700 c.c.; 0·01M) and hydrochloric acid (950 c.c. of 0·001N, followed by 2200 c.c. of 0·003N). The eluate was collected in 10-c.c. fractions, their optical density at 260 mμ being determined as usual. The desired phosphate collected in fractions 435—490 which were bulked, brought to pH 7·5 with carbonate-free lime water, and evaporated under reduced pressure at $\not>45°$ to 30 c.c. The solution was filtered and *calcium deoxyadenosine-3' phosphate* (88 mg.) precipitated as a white amorphous solid by ethanol (90 c.c.). For further purification the salt was twice reprecipitated by dissolving it in water (10 c.c.) and adding ethanol (30 c.c.); thus obtained it appeared to be hydrated (Found, in material dried for 24 hr. at 100°/1 mm. : C, 28·4; H, 4·2; N, 15·2; P, 6·8%; ratio N : P, 2·24. $C_{10}H_{12}O_6N_5PCa,3H_2O$ requires C, 28·3; H, 4·3; N, 16·5; P, 7·3%; ratio N : P, 2·26). The substance had $[\alpha]_D^{19}$ −10·8° (*c*, 0·46 in H_2O). Light absorption : In N/100-HCl, max. at 257—258 mμ (ε 14,650), min. at 229 mμ (ε 3390), optical density ratios 250/260 = 0·83 and 280/260 = 0·23. In H_2O, max. at 259—260 mμ (ε 14,450), min. at 226 mμ (ε 1915), optical density ratios 250/260 = 0·77 and 280/260 = 0·16. In N/100-NaOH, max. at 259—260 mμ (ε 15,090), min. at 227 mμ (ε 2050), optical density ratios 250/260 = 0·76 and 280/260 = 0·16. The synthetic nucleotide was recovered unchanged after incubation with rattlesnake venom at 37°.

Paper Chromatography of Deoxyadenosine Phosphates.—Ascending chromatograms, Whatman No. 1 paper. Solvent systems : I, *n*-butanol–water (86 : 14); II, *n*-butanol–acetic acid–water (4 : 1 : 5); III, *tert.*-butanol–acetic acid–water (5 : 4 : 1); IV, 5% aqueous disodium hydrogen phosphate–*iso*pentyl alcohol (3 : 2); V, ethanol–ammonia–water (80 : 4 : 16); VI, *iso*propanol–ammonia–water (7 : 1 : 2). Spots were detected on the chromatograms by (*a*) ultra-violet absorption, (*b*) cysteine hydrochloride spray for 2-deoxyribose derivatives (Stumpf, *J. Biol.*

Chem., 1947, **169**, 367), and (*c*) perchloric acid–ammonium molybdate spray for phosphorus (Hanes and Isherwood, *Nature*, 1949, **174**, 1107). The R_F obtained are tabulated.

System :	I	II	III	IV	V	VI
Deoxyadenosine-3′ phosphate	0	0·10	—	0·64	—	0·08
Deoxyadenosine-5′ phosphate	0	0·10	0·12	0·70	0	0·08
Natural deoxyadenylic acid	0	0·10	—	0·70	—	0·08
Deoxyadenosine-3′ benzyl phosphate	0·14	0·44—46	0·46	0·55	0·37—44	0·54
Deoxyadenosine-5′ benzyl phosphate	0·15	0·40	0·46	0·64	0·40—44	0·52

Ion-exchange Chromatography of Deoxyadenosine Phosphates.—The samples of calcium salts (1—1·5 mg.) were dissolved in 0·0075N-ammonia (0·5 c.c.) and absorbed on a column (7 cm. × 1 cm. diam.) of Dowex-2 resin (400 mesh) in the chloride form. Elution was carried out with 0·03M-formic acid, eluates being collected in 10·c.c. fractions with a flow rate of 0·6—1 c.c./min., and optical density at 260 mμ was determined on each fraction. Number of fractions to peak : Deoxyadenosine-3′ phosphate, 95; deoxyadenosine-5′ phosphate, 56; natural deoxyadenylic acid, 56. The last two materials were indistinguishable and could not be separated on the column when mixed.

3′ : 5′-*Diacetyldeoxyguanosine.*—Acetic anhydride (10 c.c.) was added to a solution of anhydrous deoxyguanosine (4 g.; dried for 12 hr. at 100/1 mm.) in dimethylformamide (50 c.c.) and pyridine (20 c.c.), and the mixture left overnight at room temperature, then cooled to 0° and poured into ice–water (350 c.c.) with vigorous stirring. After 1 hr. the crystalline precipitate of 3′ : 5′-*diacetyldeoxyguanosine* (4·14 g.) was collected, washed with water, and dried. Recrystallised from 90% ethanol it forms colourless needles, m. p. 222° (decomp.) (Found, in material dried for 15 hr. at 120°/10^{-3} mm. : C, 48·1; H, 5·0; N, 20·1. $C_{14}H_{17}O_6N_5$ requires C, 47·9; H, 4·8; N, 19·9%). The substance had R_F 0·49 when run on paper with *n*-butanol–water (86 : 14) as solvent system, and $[\alpha]_D^{18}$ −38° [*c*, 0·3192 in ethanol–water (1 : 9)].

3′-*Acetyldeoxyguanosine and* 5′-*Acetyldeoxyguanosine by Partial Hydrolysis of* 3′ : 5′-*Diacetyldeoxyguanosine.*—The above diacetyl derivative (3·2 g.) was dissolved in aqueous ethanol (600 c.c., containing 12% of water by vol.) and saturated methanolic ammonia (150 c.c.) was added. After 3¼ hr. at room temperature the mixture was evaporated to dryness under reduced pressure, first at room temperature until the ammonia had been removed and then at ≯35°. The product was separated into its components by countercurrent distribution in an automatically operated 100-stage apparatus. The separation was similar to that described for the acetyldeoxyadenosines (Anderson, Hayes, Michelson, and Todd, *loc. cit.*), but the solvent system (empirically selected) was ethyl acetate–*n*-butanol–water (100 : 25 : 125 by vol.), and the process was continued until 165 withdrawal stages had been completed. Determination of the optical density at 255 mμ of suitably spaced fractions gave a distribution curve showing three distinct peaks. Fractions corresponding to each of these peaks were separately bulked, as were the withdrawal stages as follows.

Fractions 0—32. Evaporation and recrystallisation of the residue gave deoxyguanosine (0·5 g.).

Fractions 33—56. Evaporation followed by recrystallisation from 40% ethanol gave 5′-*acetyldeoxyguanosine* (0·8 g.) as large clusters of colourless hydrated needles. It frothed at 125—130°, then set to a colourless glass which slowly became opaque above 170° and charred on further heating (Found, in material dried at room temperature : C, 44·5; H, 5·2; N, 21·5. $C_{12}H_{15}O_5N_5,H_2O$ requires C, 44·0; H, 5·2; N, 21·4. Found, in material dried for 24 hr. at 120°/0·1 mm. : C, 46·6; H, 5·1; N, 22·5. $C_{12}H_{15}O_5N_5$ requires C, 46·6; H, 4·9; N, 22·7%). On paper chromatography in *n*-butanol–water (86 : 14) it had R_F 0·32. It had $[\alpha]_D^{18·5}$ −33° [*c*, 0·4212 in ethanol–water (1 : 9)].

Fractions 62—98. The residue left on evaporation was recrystallised from 90% ethanol, giving 3′-*acetyldeoxyguanosine* (0·45 g.) as colourless plates which charred without melting above 240° (Found, in material dried for 12 hr. at 100°/0·5 mm.: C, 46·5; H, 5·2; N, 22·5%), R_F 0·38 in *n*-butanol–water (86 : 14), $[\alpha]_D^{19·5}$ −12·5° [*c*, 0·3448 in ethanol–water (1 : 9)].

Withdrawal stages 0—165. From the combined withdrawal stages 3′ : 5′-diacetyldeoxyguanosine (0·85 g.), m. p. and mixed m. p. 221—222°, was obtained by evaporation and recrystallisation.

3′-*Acetyldeoxyguanosine and* 5′-*Acetyldeoxyguanosine by Partial Acetylation of Deoxyguanosine.*—Deoxyguanosine (10 g.; dried for 24 hr. at 100°/1 mm.) was dissolved in dry dimethylformamide (1 l.), and a solution of freshly redistilled acetic anhydride (41·8 c.c., 12 mols.) in anhydrous pyridine (300 c.c.) added. The mixture was left for 9 hr. at room temperature, then water (300 c.c.) was added, and the resulting solution was evaporated to dryness under reduced

pressure at $>60°$. The residue (12·1 g.) was twice evaporated with water (100 c.c.), and the product then separated into its components by countercurrent distribution as described in the previous experiment. The yields obtained were: deoxyguanosine, 1 g.; 5′-acetyldeoxyguanosine 5·5 g.; 3′-acetyldeoxyguanosine, 0·57 g.; 3′ : 5′-diacetyldeoxyguanosine, 3 g.

Orientation of the Monoacetyldeoxyguanosines.—The acid hydrolysis of the unsubstituted nucleosides as well as of the two monoacetyldeoxyguanosines and of 3′-acetyldeoxyadenosine and 5′-acetyldeoxyadenosine was studied first under varying conditions, and the following procedure was finally adopted. The nucleosides and their acetyl derivatives (5 mg.) were treated with 0·02N-hydrochloric acid (2·5 c.c.). All the compounds dissolved completely in the cold, without decomposition, in 10—15 min. The solutions were then heated at 100°, the degree of hydrolysis and the nature of the products being examined by paper chromatography at intervals. Chromatograms with appropriate controls were run in various solvent systems, and the hydrolysis products were detected on chromatograms by ultra-violet absorption (purine derivatives) and by the cysteine hydrochloride (Stumpf, *loc. cit.*) and aniline phthalate (Partridge, *Nature*, 1949, **174**, 443) sprays (for 2-deoxy-D-ribose derivatives). The results may be summarised as follows: (1) The nucleosides are completely hydrolysed to purine and sugar after 2—5 min. at 100°. (2) The glycosidic linkage in the monoacetylnucleosides is completely hydrolysed after 5 min. at 100° (10 min. required for the 3′ : 5′-diacetyl derivatives). (3) The monoacetyl derivatives of 2-deoxy-D-ribose produced from the monoacetylnucleosides begin to lose acetyl groups only after 10 min. at 100°. (4) The isomeric 3′- and 5′-acetyl-2-deoxy-D-ribose can be distinguished chromatographically in three solvent systems.

The monoacetyl derivatives of deoxyadenosine and deoxyguanosine (5 mg.) were therefore separately heated at 100° for 5 min. with 0·02N-hydrochloric acid (2·5 c.c.), and the hydrolysis products were run (ascending chromatograms, Whatman No. 1 paper) in the following solvent systems: I, *n*-butanol–water (86 : 14); II, pyridine–ethyl acetate–water (1 : 2 : 2); III, *n*-butanol–acetic acid–water (4 : 1 : 5); IV, *n*-butanol–pyridine (Chargaff *et al.*, *J. Biol. Chem.*, 1949, **177**, 405). The results are tabulated, the abbreviations used being U.V. = ultra-violet spot, D = cysteine spot, AP = aniline phthalate spot.

Substance hydrolysed	R_F with staged solvent system and method of detection											
	I			II			III			IV		
	UV	D	AP	UV	D	AP	UV	D	AP	UV	D	AP
Acetyldeoxyadenosine:												
3′-	0·40	0·58	0·60	0·75	0·93	0·93	0·50	0·69	0·68	0·57	0·79	0·82
5′-	0·40	0·63	0·64	0·75	0·94	0·93	0·50	0·72	0·70	0·57	0·82	0·84
Acetyldeoxyguanosine:												
3′-	0·16	0·59	0·60	0·59	0·92	0·89	0·32	0·69	0·67	0·39	0·79	0·82
5′-	0·16	0·63	0·64	0·59	0·93	0·90	0·32	0·72	0·69	0·40	0·83	0·85

Small differences in the R_F found for the same product in the same solvent system when detected by different reagents are due to the fact that each vertical column in the Table summarises the results from a separate paper strip.

5′-Acetyldeoxyguanosine-3′ Benzyl Phosphite.—5′-Acetyldeoxyguanosine (1·98 g., 1 mol.; dried for 12 hr. at 120°/1 mm.) was suspended in anhydrous methyl cyanide (200 c.c.). Dry 2 : 6-lutidine (1·38 g., 2 mols.) and *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (5·2 g., 2 mols.) were added and the suspension was shaken at room temperature for 50 hr. Chromatographic study of the solution showed that the major product was the desired phosphite but that a considerable amount of unchanged starting material was also present. After removal of methyl cyanide at room temperature the residue was dissolved in chloroform, and the solution washed successively with saturated aqueous sodium hydrogen carbonate (5 × 20 c.c.) and water (5 × 20 c.c.) and then evaporated to dryness at room temperature. The gummy residue (2·2 g.) was dissolved in chloroform (5 c.c.), and the solution poured into dry *cyclo*hexane (200 c.c.). The white amorphous precipitate of *5′-acetyldeoxyguanosine-3′ benzyl phosphite* (0·85 g., 28%) was collected and dried *in vacuo* at room temperature. Chromatography in *n*-butanol–water (86 : 14) showed that the product contained traces of two phosphorus-free substances (probably guanine and 5′-acetyldeoxyguanosine) (Found, in material dried for 48 hr. at 60°/2 mm.: C, 49·2; H, 5·8; N, 14·4; P, 6·3%; ratio N : P, 2·29. $C_{19}H_{22}O_7N_5P$ requires C, 49·3; H, 4·7; N, 15·1; P, 6·7%; ratio N : P, 2·26). The acetonitrile used as vehicle in this preparation could be replaced with dimethylformamide (in which the starting material is more soluble) without material alteration in the yield.

Deoxyguanosine-3′ Phosphate.—The above phosphite (0·5 g., 1 mol.) was treated in chloroform

(20 c.c.) containing 2% ethanol by volume with *N*-chlorosuccinimide (167 mg., 1·1 mols.). The mixture was shaken and set aside at room temperature. After 1 hr. a white solid began to separate and after 5 hr. aqueous pyridine (20 c.c. containing 10% water by vol.) was added and the mixture set aside overnight. Solvents were removed under reduced pressure and the residue was twice evaporated with ethanol (20 c.c.), giving crude 5′-acetyldeoxyguanosine-3′ benzyl phosphate as a yellow resin (0·9 g.).

This resin was now dissolved in half-saturated methanolic ammonia (40 c.c.), and the solution left overnight at room temperature. It was then evaporated and the residue taken up in distilled water (20 c.c.) and hydrogenated in presence of sodium acetate (0·7 g.) in presence of 10% palladised charcoal (100 mg.). The solution obtained was worked up exactly as described above for the corresponding deoxyadenosine nucleotide, purification being effected by anion-exchange chromatography. The nucleotide was concentrated in fractions 456—654 (*i.e.*, 0·003N-hydrochloric acid eluate). These fractions were combined, brought to pH 7·5 with carbonate-free aqueous barium hydroxide, concentrated to small bulk (120 c.c.) ***in vacuo*** at $\not>40°$, and filtered, and *barium deoxyguanosine-3′ phosphate* (120 mg.) was precipitated by ethanol (360 c.c.). The salt, a white amorphous powder, was purified by two further precipitations from water with ethanol (Found, in material dried for 24 hr. at 100°/1 mm.: C, 23·5; H, 3·7; N, 12·9; P, 5·8%; ratio N : P, 2·25. $C_{10}H_{12}O_7N_5PBa,2H_2O$ requires C, 23·2; H, 3·1; N, 13·5; P, 6·0%; ratio N : P, 2·26). The nucleotide was quite unaffected by rattlesnake venom at 37°. Light absorption: In N/100-HCl, max. at 254—256 mμ (ε 11,610), min. at 227—228 mμ (ε 3750), inflection at 274 mμ (ε 8220), optical density ratios 250/260 = 1·01 and 280/260 = 0·72. In water, max. at 252—253 mμ (ε 12,150), min. at 225 mμ (ε 4090), inflection at 265 mμ (ε 9202), optical density ratios 250/260 = 1·15 and 280/260 = 0·72. In N/100-NaOH, max. at 262—265 mμ (ε 11,240), min. at 231 mμ (ε 5380), optical density ratios 250/260 = 0·90 and 280/260 = 0·67. The salt had $[\alpha]_D^{19\cdot5}$ −8·5° (*c*, 0·412 in H_2O).

3′-Acetyldeoxyguanosine-5′ Benzyl Phosphite.—3′-Acetyldeoxyguanosine (1·03 g., 1 mol.; dried for 12 hr. at 120°/1 mm.) was suspended in dry methyl cyanide (100 c.c.). Dry 2 : 6-lutidine (0·71 g., 2 mols.) and *O*-benzylphosphorous *OO*-diphenylphosphoric anhydride (2·26 g., 2 mols.) were added. The mixture was shaken at room temperature for 50 hr. and worked up as described for the 3′-isomer (above). *3′-Acetyldeoxyguanosine-5′ benzyl phosphite* (460 mg., 30%) was obtained in slightly impure condition as a white solid by precipitation from chloroform with *cyclo*hexane (Found, in material dried for 60 hr. at 60°/2 mm.: C, 46·1; H, 5·0; N, 14·8; P, 6·6%; ratio N : P, 2·23. $C_{19}H_{22}O_7N_5P$ requires C, 49·3; H, 4·7; N, 15·1; P, 6·7%; ratio N : P, 2·26).

Deoxyguanosine-5′ Phosphate.—The preparation of this substance from 3′-acetyldeoxyguanosine-5′ benzyl phosphite (0·53 g.) was carried out as described above for the 3′-isomer. The nucleotide was isolated as its hydrated *barium salt* (33 mg.) by precipitation from aqueous solution with ethanol (Found, in material dried for 24 hr. at 100°/1 mm.: C, 21·9; H, 3·7; N, 12·1; P, 5·7%; ratio N : P, 2·11. $C_{10}H_{12}O_7N_5PBa,4H_2O$ requires C, 21·7; H, 3·6; N, 12·6; P, 5·6%; ratio N : P, 2·26). The salt had $[\alpha]_D^{19\cdot5}$ −18·6° (*c*, 0·366 in H_2O); Klein and Thannhauser (*Z. physiol. Chem.*, 1933, **218**, 173) reported $[\alpha]_D^{19}$ −31° (*c*, 0·46 in H_2O). Light absorption: In N/100-HCl, max. at 254—256 mμ (ε 13,650), min. at 227 mμ (ε 3970), inflection at 273 mμ (ε 9810), optical density ratios 250/260 = 1·01 and 280/260 = 0·69. In H_2O, max. at 252—255 mμ (ε 15,680), min. at 225 mμ (ε 5170), inflection at 265 mμ (ε 11,270), optical density ratios 250/260 = 1·09 and 280/260 = 0·68. In N/100-NaOH, max. at 263—264 mμ (ε 12,870) min. at 230—231 mμ (ε 5440), optical density ratios 250/260 = 0·88 and 280/260 = 0·66. Like natural deoxyguanylic acid the synthetic product was completely dephosphorylated by rattlesnake venom in 3½ hr. at 37°.

Paper Chromatography of Deoxyguanosine Phosphates.—Ascending chromatograms on Whatman No. 1 paper. Solvent systems: I, *n*-butanol–acetic acid–water (4 : 1 : 5); II, aqueous disodium hydrogen phosphate (5%)–*iso*pentyl alcohol (3 : 2); III, *iso*propanol–ammonia–water (7 : 1 : 2); IV, aqueous potassium dihydrogen phosphate (5%)–*iso*pentyl alcohol (3 : 2).

	I	II	III	IV
Deoxyguanosine-3′ phosphate	0·07	0·76	0·03	0·60
Deoxyguanosine-5′ phosphate	0·07	0·78	0·03	0·65

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