# *Nucleotides. Part XXIII.* Mononucleotides derived from Deoxycytidine. Note on the Structure of Cytidylic Acids* a *and* b.

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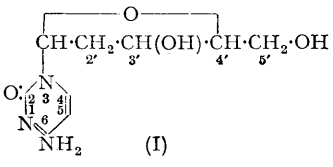
Starting from the parent nucleoside, deoxycytidine-3′ and -5′ phosphates have been synthesised. The latter compound is identical with the deoxycytidylic acid obtained by enzymic hydrolysis of deoxyribonucleic acids. Comparison of the infra-red spectra and other properties of the cytidine and deoxycytidine phosphates provides strong evidence for the view that cytidylic acid *b* is cytidine-3′ phosphate and hence that uridylic acid *b* is uridine-3′ phosphate.

In Part XX (Michelson and Todd, *J.*, 1953, 951) we described the synthesis, from the natural deoxyribonucleoside thymidine, of thymidine-3′ and -5′ phosphates, and showed that the latter is identical with the thymidylic acid obtained by enzymic hydrolysis of deoxyribonucleic acids by Klein and Thannhauser (*Z. physiol. Chem.*, 1933, **218**, 173; 1934, **224**, 252; 1935, **231**, 96). The present paper describes an extension of this work to the mononucleotides derived from deoxycytidine (I).

The methods employed were analogous to those used for the corresponding thymidine derivatives. With triphenylmethyl chloride in pyridine deoxycytidine yielded mainly a

* Part XXII, *J.*, 1953, 2947.

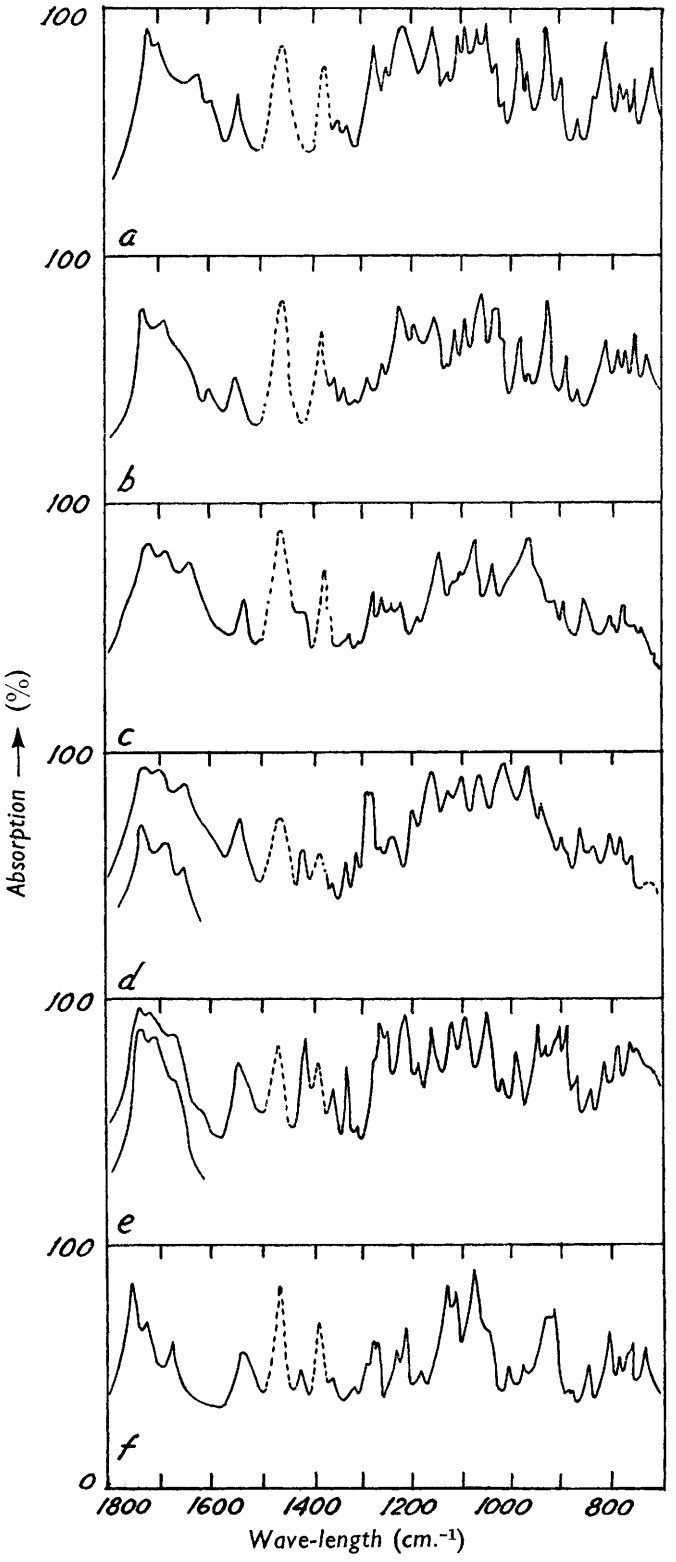
monotrityl derivative formulated as 5′-*O*-trityldeoxycytidine by analogy with 5′-*O*-tritylthymidine (Levene and Tipson, *J. Biol. Chem.*, 1935, **109**, 623), accompanied by a small amount of a ditrityl derivative. When acetylated, 5′-*O*-trityldeoxycytidine yielded a diacetyl derivative, clearly an $N : O^{3'}$-diacetyl-$O^{5'}$-trityldeoxycytidine, although no definite evidence exists as to which of the two possible nitrogen atoms is substituted [$N_{(6)}$ in (I) or $N_{(1)}$ in the tautomeric form]. From this $N : O^{3'}$-diacetyldeoxycytidine was obtained by short treatment with hot 80% acetic acid; even under these conditions some hydrolysis of the labile *N*-acetyl group and of the glycosidic linkage occurred so that a substantial amount of 3′-*O*-acetyldeoxycytidine was also formed, together with some sugar-free material. Phosphorylation of $N : O^{3'}$-diacetyldeoxycytidine with dibenzyl phosphorochloridate, followed by monodebenzylation and removal of acetyl groups, yielded deoxycytidine-5′ benzyl phosphate, converted by catalytic hydrogenation into deoxycytidine-5′ phosphate. This synthetic nucleotide was shown to be identical with deoxycytidylic acid obtained from deoxyribonucleic acid by the method of Klein and Thannhauser (*loc. cit.*); the natural and synthetic materials were identified by comparison of m. p., optical rotation, infra-red spectrum, *X*-ray powder photography, and by paper chromatographic and ion-exchange behaviour.


(I)

When 5′-*O*-trityldeoxycytidine was phosphorylated with dibenzyl phosphorochloridate in the usual manner, and the crude product heated with aqueous acetic acid to remove simultaneously the trityl residue and one benzyl group, deoxycytidine-3′ benzyl phosphate was the major product. It was, however, accompanied by traces of deoxycytidine-3′ phosphate and larger amounts of another substance which appeared to be a cytosine-*N* benzyl phosphate from its analytical composition, and the facts that it was sugar-free and that it yielded cytosine on catalytic hydrogenation. The formation of this by-product was not unexpected, since *N*-acylation occurs readily in cytosine derivatives and such acylation in the case of cytidine and deoxycytidine labilises the glycosidic linkage, giving them a stability similar to that of the corresponding purine nucleosides. Catalytic hydrogenation of deoxycytidine-3′ benzyl phosphate furnished the free nucleotide deoxycytidine-3′ phosphate.

With the completion of these syntheses it is now definitely established that the pyrimidine deoxyribonucleotides obtained from natural sources by Klein and Thannhauser (*loc. cit.*) are the 5′-phosphates of the respective nucleosides. It is hoped to provide similar synthetic proof of the nature of the natural purine deoxyribonucleotides although it is to be expected that they, too, are 5′-phosphates by analogy with their pyrimidine congeners in the light of the general structure of the deoxyribonucleic acids (Brown and Todd, *J.*, 1952, 52), and in view of the evidence obtained by Carter (*J. Amer. Chem. Soc.*, 1951, **73**, 1537) from experiments on the action of purified 5′-nucleotidase. Volkin, Khym, and Cohn (*ibid.*, p. 1535) compared the ion-exchange behaviour of adenosine-2′, -3′, and -5′ phosphates with that of natural deoxyadenylic acid and concluded on this basis that the latter was deoxyadenosine-5′ phosphate. Although the conclusion may well be correct, an examination of the ion-exchange characteristics of the cytidine and deoxycytidine phosphates shows that the differences between the 3′- and 5′-phosphates are such as to make general application of this type of argument rather dangerous unless supported by other evidence.

The properties of the deoxycytidine phosphates are of considerable interest because of their bearing on the question of the true orientation of the *a* and *b* ribonucleotides. These substances, which are obtained by the alkaline hydrolysis of ribonucleic acids, are undoubtedly the 2′- and the 3′-phosphates of their respective nucleosides (Brown and Todd, *J.*, 1952, 44) but until recently it was impossible to say which was the 2′- and which the 3′-isomer in any case. Although direct synthetic evidence is not yet available on this point, indirect evidence for the view that cytidylic acid *b* is cytidine-3′ phosphate has been advanced on the basis of solubility, ultra-violet absorption, and acid strength (Loring, Hammell, Levy, and Bortner, *J. Biol. Chem.*, 1952, **196**, 821; Cavalieri, *J. Amer. Chem. Soc.*, 1952, **74**, 5804), while Khym, Doherty, Volkin, and Cohn (*ibid.*, 1953, **75**, 1262) have produced evidence from hydrolytic studies for the view that the adenylic acids *a* and *b* are respectively the 2′- and




*Infra-red spectra :*

(*a*) *cytidine-5′ phosphate.*

(*b*) *deoxycytidine-5′ phosphate.*

(*c*) *cytidylic acid* b (form "$b_{H_2O}$" of Harris *et al., loc. cit.*).

(*d*) *deoxycytidine-3′ phosphate.*

(*e*) *cytidylic acid* b (form" $b_{alc.}$" of Harris *et al., loc. cit.*).

(*f*) *cytidylic acid* a (form "$a_{H_2O}$" of Harris *et al., loc. cit.*).

(Photocopies of these spectra, on a larger scale, may be obtained from the General Secretary, The Chemical Society; price 3*s.* 0*d.* each. The following numbers must be cited when ordering: *a*, C.S. 61; *b*, C.S. 60; *c*, C.S. 63; *d*, C.S. 62; *e*, C.S. 59; *f*, C.S. 58.)

3′-phosphates of adenosine. Strong evidence for the view that cytidylic acid *b* is cytidine-3′ phosphate is provided by a comparison of the infra-red spectra of the phosphates of cytidine and deoxycytidine (see Fig.). The spectra of the two 5′-phosphates are closely similar, as also are those of deoxycytidine-3′ phosphate (curve *d*) and one form of cytidylic acid *b* (curve *c*). The infra-red spectrum of cytidylic acid *a* in either of its isomorphic modifications (Harris, Orr, Roe, and Thomas, *J.*, 1953, 489) is quite different from any of them. The conclusion that cytidylic acid *b* is cytidine-3′ phosphate, and hence that cytidylic acid *a* is cytidine-2′ phosphate, although not valid on this evidence alone, is further supported by comparison of optical rotation and ultra-violet absorption data in the cytidine and deoxycytidine series (see Experimental section). Accepting this evidence, it also follows that uridylic acid *b* is uridine-3′ phosphate, since the former has been prepared from cytidylic acid *b* by deamination under conditions which preclude phosphoryl migration (Brown, Dekker, and Todd, *J.*, 1952 2715).

The action of several enzymes on the synthetic nucleotides and nucleotide esters described in this paper as well as (for comparison) on several other nucleotides has been examined. Rattlesnake venom (*Crotalus atrox*) dephosphorylated deoxycytidine-5′ phosphate (natural and synthetic) and -5′ benzyl phosphate, and cytidine-5′ phosphate; it had no effect on deoxycytidine-3′ phosphate, -3′ benzyl phosphate, or -3′ : 5′ diphosphate, or on cytidylic acid *a* or *b*. As was to be expected from its mode of action on esters of ribonucleotides (Brown and Todd, *J.*, 1953, 2040) crystalline pancreatic ribonuclease had no effect on the benzyl esters of deoxycytidine-3′ or -5′ phosphate or thymidine-5′ phosphate. More surprising is the fact that the same compounds were all unaffected by deoxyribonuclease, since they can be regarded structurally as the simplest analogues of the polydeoxyribonucleotides; whether this means that deoxyribonuclease needs substrates more complex than simple nucleotide esters or whether it merely indicates a specificity for purine nucleotides remains to be determined. In this connection it is of interest that deoxyribonuclease is also without action on apurinic acids (Tamm, Shapiro, and Chargaff, *J. Biol. Chem.*, 1952, **199**, 313), degradation products of deoxyribonucleic acids in which purine residues are absent.

In Part XIX (Dekker, Michelson, and Todd, *J.*, 1953, 947) we described the preparation of deoxycytidine-3′ : 5′ diphosphate by a process involving direct phosphorylation of deoxycytidine with dibenzyl phosphorochloridate and mentioned the simultaneous formation of much mononucleotidic material. This has now been identified as deoxycytidine-5′ phosphate; part of it seems to be produced directly and part by partial hydrolysis of an $N : O^{5'}$-diphosphate during the working up.

## Experimental

5′-O-*Trityldeoxycytidine*.—Triphenylmethyl chloride (14 g.) was added to a suspension of anhydrous deoxycytidine (5·9 g.) in dry pyridine (160 c.c.), and the mixture shaken vigorously at room temperature until a clear solution was obtained (approx. 2 hr.), then set aside at room temperature for 1 week, with exclusion of moisture. The solution was then cooled to 0° and poured into ice-water (1200 c.c.) with vigorous stirring, and the precipitate collected, washed with water, and dried. The product was next dissolved in acetone containing a little methanol, and the solution filtered; on cooling, the filtrate deposited 5′-O-*trityldeoxycytidine* as small needles (10·8 g., 89%), m. p. 239° (Found, in material dried for 8 hr. at 150°/1 mm.: C, 71·0; H, 6·0; N, 8·8. $C_{28}H_{27}O_4N_3$ requires C, 71·6; H, 5·8; N, 8·9%). The *picrate* crystallised from absolute ethanol in short needles, m. p. 166—167° (decomp.) (Found, in material dried for 8 hr. at 120°/1 mm.: C, 58·0; H, 4·9; N, 12·2. $C_{28}H_{27}O_4N_3,C_6H_3O_7N_3$ requires C, 58·4; H, 4·3; N, 12·0).

A small amount of a *ditrityldeoxycytidine* could be isolated from the acetone mother-liquors of the crude 5′-*O*-trityldeoxycytidine. This material crystallised from acetone–ethanol in needles or colourless laths, m. p. 172—173° (decomp.) (Found, in material dried for 12 hr. at 100°/1 mm.: C, 77·4; H, 6·2; N, 5·6. $C_{47}H_{41}O_4N_3,C_2H_5{\cdot}OH$ requires C, 77·7; H, 6·2; N, 5·6%).

$N : O^{3'}$-*Diacetyl*-$O^{5'}$-*tritydeoxycytidine*.—A solution of anhydrous 5′-*O*-trityldeoxycytidine (2·9 g.) in dry pyridine (40 c.c.) and acetic anhydride (10 c.c) was kept at room temperature for

*ca.* 20 hr., then cooled to 0° and poured into ice water (500 c.c.) with vigorous stirring. The colourless precipitate was collected, washed with water, and dried. Recrystallised from methanol, N : O$^{3'}$-*diacetyl*-O$^{5'}$-*trityldeoxycytidine* formed long needles (3·0 g., 88%), m. p. 196° (Found, in material dried for 8 hr. at 120°/1 mm. : C, 69·4; H, 5·7; N, 7·6. $C_{32}H_{31}O_6N_3$ requires C, 69·4; H, 5·6; N, 7·6%).

N : O$^{3'}$-*Diacetyldeoxycytidine*.—A solution of *N* : *O*$^{3'}$-diacetyl-*O*$^{5'}$-trityldeoxycytidine (4·05 g.) in acetic acid (15 c.c. of 80%) was heated under reflux for 5 min., then the acetic acid was evaporated under reduced pressure below 30°. The residue was triturated with ether (100 c.c.), the mixture kept at 0° for 1 hr., and the ether decanted off. Paper chromatography with *n*-butanol–water (86 : 14) showed that the gummy solid contained two main components, corresponding to a mono- and a di-acetate, as well as traces of cytosine and *N*-acetylcytosine. Purification was effected by countercurrent separation with ethyl acetate–water. The fractions containing the diacetate were combined and evaporated to dryness under reduced pressure. The residue crystallised from acetone–light petroleum (b. p. 40—60°) as rosettes of small needles (1·04 g.), m. p. 170°. Recrystallised from water, N : O$^{3'}$-*diacetyldeoxycytidine* formed long thin needles, m. p. 171° (Found, in material dried at 90°/10$^{-3}$ mm. for 5 hr. : C, 50·2; H, 5·7; N, 13·6. $C_{13}H_{17}O_6N_3$ requires C, 50·2; H, 5·5; N, 13·5%), $\lambda_{max.}$ 247, 296 mμ; $\lambda_{min.}$ 227, 270 mμ. and optical ratio 280/260 mμ, 0·776 in 0·015M-H·$CO_2$H. The fractions containing monoacetyldeoxycytidine were evaporated to dryness under reduced pressure, and the residue was dissolved in water (10 c.c.), filtered, and treated with aqueous picric acid. The *picrate* of 3′-*O*-acetyldeoxycytidine separated in rosettes of very long needles, which, recrystallised from water (0·48 g.), had m. p. 173° (decomp.) (Found, in material dried for 8 hr. at 120°/1 mm. : C, 41·3; H, 3·8; N, 16·5. $C_{11}H_{15}O_5N_3$,$C_6H_3O_7N_3$ requires C, 41·0; H, 3·6; N, 16·9%).

*Deoxycytidine-5′ Benzyl Phosphate*.—Dibenzyl phosphorochloridate (from 4 g. of dibenzyl phosphite) was added to a solution of anhydrous *N* : *O*$^{3'}$-diacetyldeoxycytidine (0·90 g.) in anhydrous pyridine (10 c.c.) at −30°, and the mixture kept just above its m. p. for 6 hr. and then left at 0° overnight. Water (20 c.c.) and sodium carbonate (3 g.) were added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform, washed with aqueous sodium hydrogen carbonate and then with water, and dried ($Na_2SO_4$); removal of the solvent under reduced pressure gave a thick oil (2·3 g.) which was dissolved in a mixture of dry benzene (10 c.c.) and 4-methylmorpholine (20 c.c.) and kept at 100° for 2 hr. to effect monodebenzylation. Solvent was removed under reduced pressure, the residue dissolved in water (50 c.c.), the deep yellow solution was extracted three times with chloroform, and the chloroform extracts were discarded. The aqueous solution was then adjusted to pH 10 with aqueous ammonia, kept at this pH at room temperature for 12 hr., then run on to a column (10 cm. × 5 sq. cm.) of Dowex-2 anion-exchange resin (mesh size 200—400) in the formate form, and the column washed well with water. Elution was continued with 0·025M-formic acid (approx. 2 c.c./min.), and the eluate collected in 20-c.c. fractions in an automatically operated fraction-collector. The appropriate fractions (optical density ratio 280 mμ/260 mμ = 2·1) were combined and evaporated to small volume under reduced pressure (bath-temp. below 30°) and finally freeze-dried. The residue was dissolved in water and filtered and the filtrate evaporated to dryness under reduced pressure to give *deoxycytidine-5′ benzyl phosphate* as a colourless glass (0·73 g.) (Found, in material dried at 50°/10$^{-3}$ mm. for 24 hr. : N, 10·5; P, 7·7. $C_{16}H_{20}O_7N_3P$ requires N, 10·6; P, 7·8%).

*Deoxycytidine-5′ Phosphate*.—A solution of deoxycytidine-5′ benzyl phosphate (0·4 g.) in aqueous ethanol (100 c.c. of 50%) was hydrogenated with a palladium catalyst at room temperature and pressure. Catalyst was removed by filtration, the filtrate was concentrated to small bulk under reduced pressure and filtered, and two volumes of ethanol were added to the filtrate. Deoxycytidine-5′ phosphate crystallised as small needles (0·30 g., 97%), m. p. 183—184° (decomp.) undepressed on admixture with natural deoxycytidylic acid (Found, in material dried at 120°/10$^{-3}$ mm. for 6 hr. : C, 35·2; H, 4·9; N, 13·7; P, 10·0. $C_9H_{14}O_7N_3P$ requires C, 35·2; H, 4·6; N, 13·7; P, 10·1%), $[\alpha]_D^{17}$ +38·5° (*c*, 1·2 in $H_2O$). Klein and Thannhauser (*Z. physiol. Chem.*, 1935, **231**, 96) give m. p. 183—187° and $[\alpha]_D^{21}$ +35°. Infra-red spectra of the natural and synthetic nucleotides were identical, as were their *X*-ray powder photographs.

*Deoxycytidine-3′ Benzyl Phosphate*.—A solution of 5′-*O*-trityldeoxycytidine (10 g.) in dry pyridine (80 c.c.) was cooled to just above its m. p. and dibenzyl phosphorochloridate (from 20 g. of dibenzyl phosphite) was added. The mixture was kept at *ca.* −30° for 6 hr., then left at 0° overnight. Water (60 c.c.) and sodium carbonate (12 g.) were added, the mixture was evaporated under reduced pressure, and the residue shaken with chloroform and water. The chloroform extract was further washed with water, dried ($Na_2SO_4$), and evaporated to a yellow

glass which was dissolved in acetic acid (150 c.c. of 80%), and the solution was gently boiled for 7 min. Acetic acid was removed under reduced pressure, and water and chloroform were added to the residue together with sufficient ammonia to bring the pH to 8·5. After extraction of the aqueous layer 3 times with chloroform (chloroform extracts were discarded) the solution was adjusted to pH 9 and run on to a column (10 cm. × 5 sq. cm.) of Dowex-2 anion-exchange resin (formate form). The column was eluted with water (deoxycytidine removed), then 0·02M-formic acid (mononucleotide material removed; shown to be deoxycytidine-3′ phosphate by enzyme experiments), and finally 0·15M-formic acid. Two peaks were obtained with the last solvent: the first, corresponding to deoxycytidine-3′ benzyl phosphate, had an optical density ratio 280 mμ/260 mμ of 1·9, while the second, corresponding to cytosine-*N* benzyl phosphate, had an optical density ratio 280 mμ/260 mμ of 2·6 (in 0·15M-formic acid). Appropriate fractions containing deoxycytidine-3′ benzyl phosphate were combined, taken to small volume under reduced pressure, and finally freeze-dried. The residue, recrystallised from water, gave *deoxycytidine-3′ benzyl phosphate* (0·85 g.) as clusters of hydrated needles, m. p. 100—101°, m. p. after drying 150—151° (Found, in material dried at 95°/$10^{-3}$ mm. for 24 hr.: C, 47·9; H, 5·0; N, 10·5; P, 7·8. $C_{16}H_{20}O_7N_3P$ requires C, 48·3; H, 5·0; N, 10·6; P, 7·8%).

The fractions containing cytosine-*N* benzyl phosphate were combined, taken to small volume, and freeze-dried, and the residue was recrystallised from water (yield, 0·27 g.). *Cytosine-*N *benzyl phosphate* crystallised as small needles which contracted at 180° and melted at 187° (Found: C, 43·8; H, 4·4; N, 13·9; P, 10·3. $C_{11}H_{12}O_4N_3P,H_2O$ requires C, 44·2; H, 4·7; N, 14·0; P, 10·4%), $\lambda_{max.}$ 287 mμ, $\lambda_{min.}$ 248 mμ. Optical density ratio 280/260 mμ = 2·45 in 0·015M-$H{\cdot}CO_2H$. Hydrogenation of this material yielded cytosine, identical with an authentic sample in ultra-violet absorption and paper-chromatographic behaviour.

*Deoxycytidine-3′ Phosphate.*—An aqueous-ethanolic solution of deoxycytidine-3′ benzyl phosphate (0·15 g.) was hydrogenated in the usual manner with a mixture of palladium and palladised charcoal catalysts. *Deoxycytidine-3′ phosphate* crystallised from aqueous ethanol in clusters of needles (0·105 g.), m. p. 196—197° (decomp.), $[\alpha]_D^{17}$ +57·0° (*c*, 1·35 in $H_2O$) (Found, in material dried at 100°/$10^{-3}$ mm. for 6 hr: C, 35·2; H, 4·4; N, 13·9; P, 10·0. $C_9H_{14}O_7N_3P$ requires C, 35·2; H, 4·6; N, 13·7; P, 10·1%).

*Phosphorylation of Deoxycytidine.*—A solution of deoxycytidine (1 g.) in anhydrous pyridine (55 c.c.) was phosphorylated in the usual manner with dibenzyl phosphorochloridate (from 5 g. of dibenzyl phosphite). The crude gum was dissolved in ethanol (20 c.c.), and ether (200 c.c.) added to precipitate the phosphorylated deoxycytidine as a gum (1·5 g.) which was hydrogenated in aqueous ethanol (200 c.c. of 50%) over palladium and palladised charcoal catalysts. After removal of the catalyst the solution was neutralised with aqueous ammonia and taken to dryness under reduced pressure. The residue was dissolved in water (200 c.c.) adjusted to pH 9 with dilute ammonia, and run on to a column (10 cm. × 5 sq. cm.) of Dowex-2 (chloride form) ion-exchange resin. After being washed with water, the column was connected to an automatic fraction-collecting device, and 20-c.c. fractions were collected. Elution was commenced with 0·0025N-hydrochloric acid to remove a peak corresponding to deoxycytidine-5′ phosphate. Elution with 0·004N-acid then removed the major component, presumably deoxycytidine-*N* : 5′ diphosphate. Appropriate fractions were combined in each case, taken to small volume under reduced pressure, and finally freeze-dried. Crystallisation from 70% ethanol gave deoxycytidine-5′ phosphate (20 mg.), m. p. 183—184° (decomp.), from the first peak (Found: N, 13·7. Calc. for $C_9H_{14}O_7N_3P$: N, 13·7%), and the same phosphate from the second peak (100 mg.), m. p. 183° (decomp.) (Found, in material dried at 100°/$10^{-3}$ mm. for 6 hr.: C, 35·2; H, 5·0; N, 13·7. Calc. for $C_9H_{14}O_7N_3P$: C, 35·2; H, 4·6; N, 13·7%). That both specimens were deoxycytidine-5′ phosphate was shown by enzyme experiments, infra-red spectra and *X*-ray powder photographs and behaviour on ion-exchange columns. The most probable explanation of the two peaks is that the second crop of 5′-phosphate arises from decomposition of an *N* : 5′-diphosphate during elution. Further elution of the column with 0·01N-hydrochloric acid removed a small amount of deoxycytidine-3′ : 5′ diphosphate, isolated as its dibarium salt (20 mg.).

*Action of Rattlesnake* (Crotalus atrox) *Venom on Deoxycytidine Phosphates.*—To each nucleotide derivative (*ca.* 1 mg.) were added glycine buffer (0·3 c.c. of 0·25M; pH 9), magnesium chloride (0·1 c.c. of 0·1N), and rattlesnake venom (0·1 c.c. of a solution containing 20 mg. of dried *Crotalus atrox* venom in 1 c.c. of 0·1M-potassium chloride). The mixture was in each case incubated at 37° for 3 hr. and then run on paper chromatograms with appropriate controls; the solvent system were *n*-butanol–water (86 : 14), *iso*propanol–ammonia–water (70 : 10 : 20), and *n*-propanol–2N-hydrochloric acid (3 : 1).

*Paper Chromatography of Deoxycytidine Derivatives.*—Solvent systems used: I, *n*-butanol–

water (86 : 14); II, *iso*propanol–ammonia–water (70 : 10 : 20); III, *n*-propanol–2N-hydrochloric acid (3 : 1).

| | Ascending $R_F$ values | | |
|---|---|---|---|
| | I | II | III |
| Deoxycytidine | 0·19 | 0·59 | 0·33 |
| $O^{5'}$-Trityldeoxycytidine | 0·88 | — | — |
| $N$ : $O^{3'}$-Diacetyl-$O^{5'}$-trityldeoxycytidine | 0·92 | — | — |
| $N$ : $O^{3'}$-Diacetyldeoxycytidine | 0·65 | — | — |
| $O^{3'}$-Acetyldeoxycytidine | 0·40 | — | — |
| Deoxycytidine-3′ phosphate | 0 | 0·07 | 0·33 |
| Deoxycytidine-5′ phosphate | 0 | 0·09 | 0·33 |
| Natural deoxycytidylic acid | 0 | 0·09 | 0·33 |
| Deoxycytidine-3′ : 5′ diphosphate | 0 | 0 | 0·36 |
| Deoxycytidine-3′ benzyl phosphate | 0·054 | 0·54 | 0·67 |
| Deoxycytidine-5′ benzyl phosphate | 0·057 | 0·51 | 0·65 |
| Cytosine-$N$ benzyl phosphate | 0·037 | 0·47 | — |

*Separation of Mixtures of Deoxycytidine-3′ and Deoxycytidine-5′ Phosphates.*—Separation was achieved with an ion-exchange column (Dowex-2; formate form) and elution with 0·015M-formic acid. The elution diagram showed two peaks, the first corresponding to deoxycytidine-5′ phosphate and the second to deoxycytidine-3′ phosphate.

*Comparison of the Cytidine and Deoxycytidine Phosphates.*—See Table.

| | $[\alpha]_D$ | Optical density ratio 280/260 mμ in 0·015M-H·$CO_2$H | No. of fractions to peak on standard Dowex-2 column with 0·015M-H·$CO_2$H |
|---|---|---|---|
| Deoxycytidine-3′ phosphate | +57° | 2·0 | 19 |
| Deoxycytidine-5′ phosphate | +38·5 | 2·1 | 13 |
| Cytidine-5′ phosphate | +27·1 | 2·1 | 15 |
| Cytidylic acid *b* | +50 [1] | 2·0 | 41 |
| Cytidylic acid *a* | +18 [1] | 1·82 | 31 |

[1] Loring, Hammell, Levy, and Bortner, *loc. cit.*

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