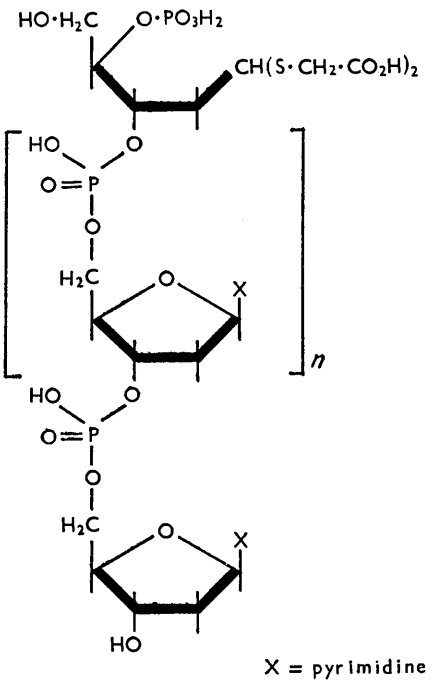
507. *The Nucleotide Sequence in Deoxypentosenucleic Acids. Part III.* The Nature of the End Groups Produced by the Alkaline Hydrolysis of Calf-thymus* aldehydo*Apurinic Acid Di(carboxymethyl) Dithioacetal.*

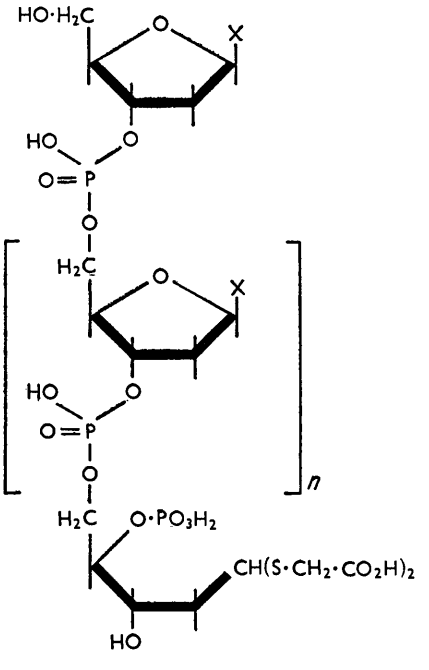
By A. S. Jones, D. S. Letham, and M. Stacey.

The sulphur-containing components produced by the alkaline hydrolysis of calf-thymus *aldehydo*apurinic acid di(carboxymethyl) dithioacetal appear to exist in two forms, *viz.*, (II) in which treatment with phosphomonoesterase would produce a 4′ : 5′-dihydroxy-compound, and (III) in which the enzyme would produce a 3′ : 4′-dihydroxy-compound. It is tentatively suggested that these components carry a terminal 4′-phosphate group.

Degradation of calf-thymus *aldehydo*apurinic acid di(carboxymethyl) dithioacetal (I of preceding paper) with dilute alkali gave pyrimidine oligonucleotides, some of which were linked to a sulphur-containing end group.* For these sulphur-containing components, two structures (II) and (III) were possible, of which only the former would give formaldehyde when oxidised with periodate after treatment with phosphomonoesterase.



(II)



(III)

Certain of the components isolated from the alkaline hydrolysate of the dithioacetal (I) were treated with phosphomonoesterase and then with sodium periodate under conditions which effected the maximum liberation of formaldehyde from the total alkaline hydrolysate. About 25% of the formaldehyde expected from (II) was obtained. The enzyme used in these experiments was free from deoxyribonuclease but contained appreciable ribonuclease. It was unlikely that the latter enzyme would have degraded the components, but even if this were the case, no compounds would be produced which would give

* Part II, preceding paper.

rise to formaldehyde on treatment with periodate. These results indicated that the components were mixtures of the compounds (II) and (III), with the latter predominating.

Support for this view has been obtained by studying the products obtained by the action of snake-venom diesterase. Components 3A and 3C (II and III, where $n = 0$, and the pyrimidine was thymine and cytosine respectively) were treated with the enzyme. Since snake-venom diesterase hydrolyses 3′-phosphate linkages in deoxyribonucleic acids and ribonucleic acids to give 5′-phosphates,[1] thymidylic acid and deoxycytidylic acid would be produced from 3A and 3C respectively if (II) were present, and thymidine and deoxycytidine respectively from (III). The fact that both the nucleotide and the nucleoside were produced from both 3A and 3C, with the nucleoside predominating, indicated a mixture of (II) and (III) in these components. Similarly component 2A (II and III; $n = 1$, pyrimidine = thymine) gave thymidylic acid and thymidine, and component 2C (II and III; $n = 1$, pyrimidine = 1 mol. of cytosine and 1 mol. of thymine) gave thymidine, thymidylic acid, deoxycytidine, and deoxycytidylic acid. These results indicated the presence of (III) but did not exclude the presence of (II). Component 2B (II and III; $n = 2$, pyrimidine = 2 mols. of thymine and 1 mol. of cytosine) gave thymidine, thymidylic acid, and deoxycytidylic acid, which indicated the presence of structures of type (III) but excluded the presence of a sequence with deoxycytidine, as the end group. The presence of the sequence, thymidine–phosphate–thymidine in this component has already been demonstrated.[2]

It appeared therefore that the components were heterogeneous with respect to the end group. Compounds of structure (II) and (III) were so similar as to make their separation improbable by the methods used in this work.

The exact location of the terminal phosphate group has not been proved. The production of nucleosides by the action of snake-venom diesterase showed that it must have been on the 2-deoxy-*aldehydo*-D-ribose di(carboxymethyl) dithioacetal group. There remained the possibility of its being at position 3′, 4′, or 5′. Since the components were stable to alkali it appeared that cyclic phosphate formation was not possible under alkaline conditions: this condition is fulfilled if the phosphate group is at position 4′, to which it has been tentatively assigned.

Experimental

Phosphomonoesterase.—This enzyme was prepared from human prostate glands (21 g.) by the method of Markham and Smith.[3] A sample (0·1 ml.) of the enzyme solution (acetate buffer, ionic strength, 0·1; pH 5·2; total volume, 26 ml.) completely dephosphorylated thymidylic acid (1·5 mg.) and deoxycytidylic acid (1·5 mg.) in 1 hr. at 37° and pH 5·2. The enzyme preparation did not hydrolyse diphenyl hydrogen phosphate or deoxyribonucleic acid at pH 5·2 but did hydrolyse ribonucleic acid at this pH.

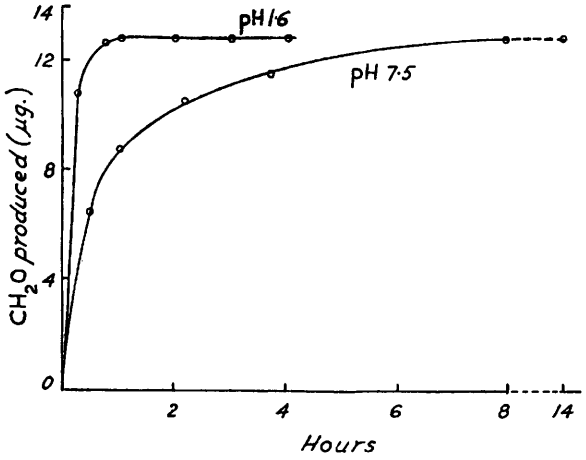
Snake-venom Diesterase.—This enzyme was prepared from Russell viper venom (kindly supplied by the Wellcome Research Laboratories) by the method of Hurst and Butler.[4] The enzyme hydrolysed diphenyl hydrogen phosphate and was free from 5′-nucleotidase.

Treatment of the Alkaline Hydrolysate of the Dithioacetal (I) *with Phosphomonoesterase and Subsequent Oxidation with Periodate.*—A solution of the freeze-dried alkaline hydrolysate of the dithioacetal (I) (27 mg.) in acetate buffer (ionic strength, 0·1, pH 5·2; 5 ml.) was treated with phosphomonoesterase solution (0·5 ml.) at 37° for 2 hr. Sodium metaperiodate reagent[5] (0·015M; pH 7·5; 5·5 ml.) was then added and the oxidation allowed to proceed at room temperature. Samples (1·5 ml.) were removed at intervals, oxidation was arrested by the addition of lead dithionate (0·15 g.), the solutions were centrifuged, and formaldehyde was determined in the supernatant liquids by the use of the chromatropic acid reagent as described by O'Dea and Gibbons.[5] Control determinations carried out on the phosphomonoesterase gave only small amounts of formaldehyde. A similar experiment was then performed, but the oxidation with periodate was carried out at pH 1·6. The results of the two reactions are shown in

[1] G. Schmidt, "Nucleic Acids," Academic Press Inc., New York, 1955, Vol. I., p. 585.
[2] Jones, Letham, and Stacey, preceding paper.
[3] Markham and Smith, *Biochem. J.*, 1952, **52**, 558, 565.
[4] Hurst and Butler, *J. Biol. Chem.*, 1951, **193**, 91.
[5] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.

the Figure. Since the reaction at pH 1·6 was the more rapid and there was less chance of diesterase action, these conditions were used for the investigation of the reaction on individual components of the hydrolysate.

Eluates from blank paper (Whatman No. 3) gave appreciable amounts of formaldehyde when oxidised with periodate. Hence electrophoretic zones bearing individual components were



Liberation of formaldehyde by the action of periodate on phosphomonoesterase-treated, alkaline hydrolysate of calf-thymus aldehydo*apurinic acid di(carboxymethyl) dithioacetal.*

eluted with acetate buffer (ionic strength, 0·1; pH 5·2); each eluate was halved; one half was oxidised with periodate, and the other was treated with phosphomonoesterase and then oxidised. The differences between the two values gave the amount of formaldehyde produced from the component in question. These results are shown in the Table.

Properties of components of the alkaline hydrolysate of the dithioacetal (I).

Component	Proposed structure *	Moles of CH_2O per atom of P ‡: Theoretical for structure III	Found	Products in snake-venom diesterase digests
3A	T–P–S–P	0·50	0·13	Thymidine, thymidylic acid
3C	C–P–S–P	0·50	0·16	Deoxycytidine, deoxycytidylic acid
2A	T–P–T–P–S–P	0·33	0·09	Thymidine, thymidylic acid
2B	T–P–T–P–C–P–S–P †	0·25	0·08	Thymidine, thymidylic acid, deoxycytidylic acid
2C	T–P–C–P–S–P †	0·33	0·07	Thymidine, thymidylic acid, deoxycytidine, deoxycytidylic acid

* T = thymidine residue; C = deoxycytidine residue; S = 2-deoxy-*aldehydo*-D-ribose di(carboxymethyl)dithioacetal residue; P = phosphate residue.
† These represent only one of the possible structures.
‡ Produced by treatment with phosphomonoesterase followed by periodate oxidation.

Treatment of the Components of the Alkaline Hydrolysis of the Dithioacetal (I) *with Snake-venom Diesterase.*—Each of the components shown in the Table was hydrolysed with the diesterase at pH 9·0 and 37°. Controls with thymidylic acid were not dephosphorylated by this treatment. The digests were chromatographed with propan-2-ol–water–ammonia (*d* 0·88) (70 : 30 : 6), and the products identified in the usual way. The results are summarised in the Table.

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