406. *Deoxyribonucleosides and Related Compounds. Part II. A Proof of the Furanose Structure of the Natural 2-Deoxyribonucleosides.*

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Ion-exchange methods have been used with advantage in isolating the pyrimidine deoxyribonucleosides from hydrolysates of deoxyribonucleic acid. The behaviour towards periodate of the 2′-deoxyribosides of guanine, hypoxanthine, cytosine, and thymine affords proof of their furanose structures.

The widely-held view that the naturally occurring deoxyribonucleosides have furanose structures is based partly on the analogy with the related ribonucleosides, for which such structures are well established, and partly on the results of chemical studies of the compounds themselves. Levene and Tipson (*J. Biol. Chem.*, 1935, **109**, 623) found that the behaviour of certain triphenylmethyl and toluene-*p*-sulphonyl derivatives of thymidine indicated a furanose structure, and in agreement with this the conductivity of a boric acid solution was not increased by the presence of the nucleoside, showing the absence of a *cis*-1 : 2-glycol system (*idem*, *Z. physiol. Chem.*, 1935, **234**, v). The 2′-deoxyribosides of guanine and hypoxanthine were investigated by Makino (*Biochem. Z.*, 1935, **282**, 263), using the boric acid method, and found to behave similarly.

Attempts to synthesise 2′-deoxyribosides of purines and pyrimidines at present in progress in this laboratory made a more rigid proof of the ring-structures of the naturally occurring representatives desirable, and we have sought to obtain this by use of the periodate oxidation method which was found valuable in earlier work on natural and synthetic ribonucleosides (Lythgoe and Todd, *J.*, 1944, 592; Davoll, Lythgoe, and Todd, *J.*, 1946, 833). The 2′-deoxyribosides of guanine, hypoxanthine, thymine, and cytosine required for this work were obtained by enzymic hydrolysis of herring-sperm deoxyribonucleic acid. In agreement with Zittle (*Fed. Proc.*, 1947, **6**, 500), the phosphatase preparation from calf's intestinal mucosa which is usually used for this purpose (Klein, *Z. physiol. Chem.*, 1938, **255**, 82; Brady, *Biochem. J.*, 1941, **35**, 855) was found incapable of dephosphorylating the nucleic acid completely, and a preliminary treatment with a deoxyribonuclease preparation (McCarty, *J. Gen. Physiol.*, 1946, **29**, 123) was found advantageous. For the separation from the hydrolysate of the purine deoxyribosides a method recently described by Schindler (*Helv. Chim. Acta*, 1949, **32**, 979) was used successfully, but the chromatographic method which he employed to separate the pyrimidine derivatives was found to give only a partial resolution. Improved yields were obtained when this method was supplemented by that used by Elmore for the separation of cytidine and uridine (*Nature*, 1948, **161**, 931; cf. Harris and Thomas, *ibid.*; *J.*, 1948, 1936). This method which consists in the use of a column of a cation-exchange resin (" Zeo-Karb 215 ") gave a complete resolution of the two pyrimidine deoxyribosides and also allowed the cytosine deoxyriboside to be obtained pure directly, without the need for a preliminary isolation of the picrate as in Schindler's method.

If the natural deoxyribonucleosides have a furanoside structure they should be resistant to oxidation with sodium metaperiodate solution. In accord with this thymidine and cytosine deoxyriboside both consumed only a very small amount of the reagent ($<0{\cdot}1$ mol. per mol. of glycoside) in 20 hours, after which reaction ceased. A possible explanation of this small but real initial uptake of oxidant may be that there was present in the periodate solution small amounts of other oxidising species which are non-specific for 1 : 2-glycol systems (cf. Head, *Nature*, 1950, **165**, 236). The purine deoxyribosides behaved rather unexpectedly, for, whilst

both of them showed a very small initial uptake of the oxidant during the first 20 hours, the reaction continued further, instead of terminating as with the pyrimidine compounds. The secondary phase of the oxidation of guanine deoxyriboside was rather slow, less than 0·5 mol. of oxidant per mol. of glycoside having been reduced after 400 hours, but the further oxidation of hypoxanthine deoxyriboside proceeded more rapidly as time went on and after 142 hours 2·4 mols. of oxidant per mol. of glycoside had been reduced. The autocatalytic nature of this reaction is probably attributable to the very labile character of the glycosidic link in hypoxanthine deoxyriboside; presumably a slow hydrolysis of this link takes place in the acidic reaction medium; the oxidation of the sugar set free in this way would then give rise to formic acid, thus leading to an acceleration of the hydrolysis and oxidation. It is significant that this phenomenon was not observed at all with the pyrimidine deoxyribosides, where the glycosidic link is known to be much more stable to hydrolysis than in the purine compounds.

These results afford confirmation of the furanoside nature of the natural deoxyribosides.

Experimental.

Isolation of the Deoxyribonucleosides.—Commercial herring-sperm deoxyribose nucleic acid was purified by the method of Sevag, Lackmann, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425), and then had only a faintly positive biuret reaction and contained P, 7%. Its sodium salt (20 g.), dissolved in water (200 c.c.), was treated with a preparation of deoxyribonuclease (30 mg.), kindly provided by Mrs. B. Holmes of the Radiotherapeutics Department, and kept for 18 hours. The hydrolysis was then completed by the action of the phosphatase of calf's intestinal mucosa as described by Klein (*loc. cit.*); after 44 hours 96% of the total phosphorus present had been liberated as inorganic phosphate. From the hydrolysate the purine nucleosides were isolated by Schindler's method (*loc. cit.*). Guanine deoxyriboside was obtained as needles (45 mg.) from water (Found, in material dried over phosphoric oxide at 55°/1 mm.: C, 42·1; H, 5·0. Calc. for $C_{10}H_{13}O_4N_5,H_2O$: C, 42·1; H, 5·3%). Hypoxanthine deoxyriboside was obtained as small needles (600 mg.) from water (Found: C, 47·6; H, 5·2; N, 22·7. Calc. for $C_{10}H_{12}O_4N_4$: C, 47·6; H, 4·8; N, 22·2%).

Chromatography of the pyrimidine nucleoside-containing fraction on aluminium oxide by Schindler's method gave thymidine as long needles (215 mg.), m. p. 186°, from methanol–ether (Found: C, 49·7; H, 5·7. Calc. for $C_{10}H_{14}O_5N_2$: C, 49·6; H, 5·8%), and cytosine deoxyriboside as prisms (85 mg.), m. p. 210°, from methanol–ether (Found: C, 47·6; H, 5·9. Calc. for $C_9H_{13}O_4N_3$: C, 47·6; H, 5·8%).

Separation of Thymidine and Cytosine Deoxyriboside by Cation-exchange Column.—The residues from the fractions containing thymidine and cytosine deoxyriboside were combined, dissolved in distilled water, and allowed to percolate through a column (10 × 1 cm.) of "Zeo-Karb 215" resin (The Permutit Co. Ltd.) which had been activated by treatment with 3N-hydrochloric acid. The thymidine passed directly through the column and was obtained pure by evaporation of the effluent. After crystallisation from methanol–ether it was obtained as needles (1·5 g.), m. p. 186°. Elution of the column with 2% aqueous pyridine, and concentration of the eluate under reduced pressure gave cytosine deoxyriboside, which after crystallisation from methanol–ether formed needles (0·6 g.), m. p. 210°. Treatment of the mother-liquors with picric acid gave the picrate (0·27 g. of recrystallised material), from which the free nucleoside was conveniently regenerated as follows. A solution of the picrate (100 mg.) in water (50 c.c.) was allowed to percolate through a column (10 × 1 cm.) of "De-Acidite B" resin which had been activated by treatment with 2N-sodium hydroxide solution. The column was washed with water, and the effluent evaporated, crystallisation of the residue from methanol–ether giving cytosine deoxyriboside (37 mg., 75%) as prisms, m. p. 210°.

Sodium Metaperiodate Titrations.—Analytically pure specimens of the nucleosides (*ca.* 0·20 millimol.) were treated with 0·248M-sodium metaperiodate (5 c.c.), and the solution was diluted to 25 c.c. with water and kept at room temperature, aliquots being withdrawn for titration at intervals. Thymidine and cytosine deoxyriboside consumed 0·08 mol. of oxidant per mol. of glycoside during 18 hours; this value was unchanged after 92 hours. For hypoxanthine deoxyriboside the following values were obtained: Mol. of oxidant consumed per mol. of glycoside: after 22 hours, 0·10; 44 hours, 0·33; 94 hours, 0·89; 142 hours, 2·4. The reaction between guanine deoxyriboside and sodium metaperiodate was carried out in a solution which was 0·012M. with respect to the oxidant; after 21 hours 0·08, after 70 hours 0·10, after 169 hours 0·22, and after 408 hours 0·48 mol. of oxidant had been reduced per mol. of glycoside.

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