# 197. *A Synthesis of Cytidine.*

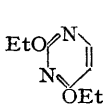
By G. A. Howard, B. Lythgoe, and A. R. Todd.

Hydrogenolysis of 1 : 2 : 3-triacetyl 5-trityl *d*-ribofuranose gives a syrupy 1 : 2 : 3-triacetyl *d*-ribofuranose converted on acetylation into the crystalline 1 : 2 : 3 : 5-*tetra-acetyl* d-*ribofuranose*. Treatment of the latter with liquid hydrogen bromide gave crude acetobromoribofuranose which was used directly to prepare *theophylline-7-β-d-ribofuranoside*. By reaction of the acetobromoribofuranose with 2 : 6-diethoxypyrimidine, followed by treatment of the product with methanolic ammonia, 3-*β*-*d*-ribofuranosidocytosine has been synthesised identical with the natural nucleoside, cytidine.

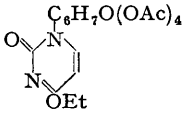
Methods for the synthesis of nucleosides have been under investigation in this laboratory for some time with a variety of objects in view. On the one hand it was hoped that a successful outcome of the work would render available for biological examination a range of structural analogues of the natural nucleosides as a possible means of elucidating the mode of action of the latter in organised systems; on the other, study of the simpler compounds seemed a necessary preliminary to broader synthetic investigations relating to the co-enzymes and polynucleotides in the molecules of which nucleoside structures are preformed.

In a series of papers in this *Journal* ("Experiments on the Synthesis of Purine Nucleosides," Parts I—XVI, reviewed in *J.*, 1946, 647) we have reported progress made in the development of methods for the synthesis of analogues of adenosine, perhaps the most important of the natural nucleosides. General methods have been made available for the synthesis of 9-glycopyranosido- and 9-glycofuranosido-adenine derivatives, and answers have been provided to certain structural questions on which at the outset there was either no evidence or on which available evidence was not completely rigid. With the major problems of this part of the field approaching, as we hope, a satisfactory solution, it seemed desirable to broaden the scope of the investigations to include compounds of the type of guanosine, the pyrimidine ribosides, and the pyrimidine and purine deoxyribosides. The present communication records experiments which have led to the synthesis of the natural pyrimidine riboside, cytidine.

The problems of synthesis of pyrimidine glycosides have been examined by a number of previous workers. The route which has received most attention depends on the direct introduction of an acetylated glycosyl residue into the nucleus of an appropriate pyrimidine derivative by use of an acetohalogeno-sugar, a method essentially similar to Fischer and Helferich's synthesis of purine glycosides (*Ber.*, 1914, **47**, 210). The early investigators (Fischer and Helferich, *loc. cit.*; Fischer, *Ber.*, 1914, **47**, 1377; Levene and Sobotka, *J. Biol. Chem.*, 1925, **65**, 469) employed, for glycosidisation, pyrimidine derivatives containing substituents such as hydroxyl, to which, as a result of their capacity for prototropic change, the glycosyl residue became attached, so that they were not successful in obtaining heterocyclic *N*-glycosides. This difficulty was overcome by Hilbert and his collaborators, employing 2 : 6-dialkoxypyrimidines, in which tautomerisation possibilities are excluded. Thus interaction of 2 : 6-diethoxypyrimidine (I) and acetobromoglucose gave the tetra-acetyl glucoside (II) which could be converted by methanolic hydrogen chloride into a 3-*d*-glucosidouracil (III; R = OH), or by methanolic ammonia into a 3-*d*-glucosidocytosine (III; R = $NH_2$) (Hilbert and Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 4489; Hilbert and Jansen, *ibid.*, 1936, **58**, 60).



(I.)



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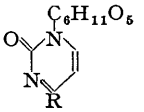


(III.)

Structural analogues of (III) carrying various glycosyl residues have been obtained by this method (Hilbert, *ibid.*, 1937, **117**, 331) including a 3-*d*-ribosidouracil (Hilbert and Rist, *J. Biol. Chem.*, 1937, **117**, 331); all these compounds must, from their mode of preparation, be pyranosides.

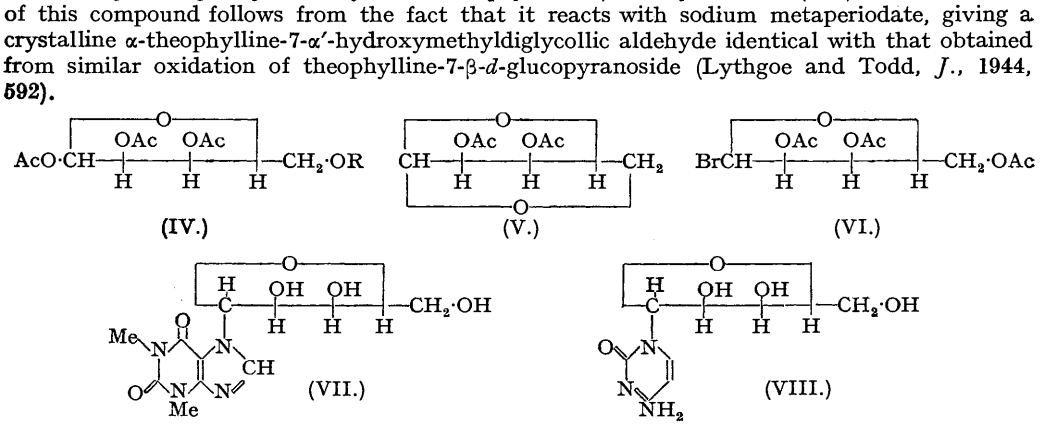
An alternative synthesis of pyrimidine nucleosides may be envisaged involving cyclisation of an appropriate glycosidamino-compound; such a method would be analogous in principle to our method for synthesis of purine-9-glycosides from 5-amino-4-glycosidaminopyrimidine derivatives. If developed, such a method might offer, in comparison with the synthesis from acetohalogeno-sugars, advantages such as the demonstration of the position of the sugar residue in the final product. It was in part this consideration that influenced us in our choice of a route for the synthesis of purine-9-glycosides, but it seemed of less importance in the case of the

pyrimidine nucleosides, since the $N_3$ location of the sugar residue in uridine has been fully established by Levene and Tipson (*J. Biol. Chem.*, 1934, **104**, 385), and we have shown (Davoll, Lythgoe, and Todd, *J.*, 1946, 833) that in the uracil-*d*-glucoside obtained by Hilbert's method the glucose residue does in fact, as formerly assumed from analogy (Hilbert and Johnson, *loc. cit.*), occupy the same position. We therefore decided in the first instance to attempt the synthesis of cytidine by extending Hilbert's method.

The acetohalogeno-ribofuranose required for this purpose belongs to a class of compounds which in spite of their potential synthetic value have been little explored, mainly because of the inaccessibility of suitable furanose intermediates. In the pentose series no true representative of this class has been obtained, and amongst the hexoses only those derived from galactose (Schlubach and Wagenitz, *Z. physiol. Chem.*, 1932, **213**, 87). Aniline-*d*-ribofuranoside (Berger and Lee, *J. Org. Chem.*, 1946, **11**, 75) seemed at first sight to be a suitable starting material for the preparation of acetobromoribofuranose. It has been stated (*idem, ibid.*; Berger, Solmssen, Leonard, Wenis, and Lee, *ibid.*, p. 91) that this compound can be acetylated to a 2 : 3 : 5-triacetyl *d*-ribosidoaniline which yields on careful acidic hydrolysis 2 : 3 : 5-triacetyl *d*-ribofuranose. However, as already recorded by us (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) the acetylation is accompanied by a change to the isomeric pyranoside, so that in fact the product of the above reaction series is 2 : 3 : 4-triacetyl *d*-ribopyranose.

It was therefore decided to study in more detail the method used by Bredereck, Köthnig, and Berger (*Ber.*, 1940, **73**, 956) in their attempt to obtain acetobromoribofuranose. These investigators prepared 1 : 2 : 3-triacetyl 5-trityl *d*-ribose (IV; R = $CPh_3$), but attempts at selective removal of the trityl group from this compound were unsuccessful, giving instead of the desired 1 : 2 : 3-triacetyl *d*-ribofuranose (V; R = H) a diacetyl anhydroribose <1 : 5> <1 : 4> (V) owing to simultaneous loss of the acetyl group at $C_1$. In these experiments hydrogen bromide in acetic acid or aqueous acetic acid was used for fission of the trityl ether linkage, but it is known (*e.g.*, Micheel, *Ber.*, 1932, **65**, 262) that the fission may be effected by hydrogenolysis. By using the latter method we were successful in preparing from (IV; R = $CPh_3$) a syrupy 1 : 2 : 3-triacetyl *d*-ribofuranose (IV; R = H), in which the terminal location of the free hydroxyl group was shown by its conversion into a syrupy tosyl derivative which readily exchanged its tosyl group for iodine in the Oldham–Rutherford reaction. Further acetylation of (IV; R = H) gave in good yield a crystalline 1 : 2 : 3 : 5-*tetra-acetyl* d-*ribofuranose* (IV; R = Ac). In order to convert this into acetobromoribofuranose we adopted the method used by Schlubach and Wagenitz (*loc. cit.*) for the preparation of acetobromogalactofuranose. Brief treatment of (IV; R = Ac) with liquid hydrogen bromide gave a syrup consisting essentially of the desired acetobromo-compound (VI); on account of its evident lability, isolation of the pure compound was not attempted, the crude product being used directly in glycosidisation experiments.

When allowed to react with a suspension of theophylline silver in dry xylene, triacetyl theophylline-*d*-ribofuranoside was obtained as a gum, which on treatment with methanolic ammonia gave in good yield a crystalline *theophylline*-7-β-d-*ribofuranoside* (VII). The structure of this compound follows from the fact that it reacts with sodium metaperiodate, giving a crystalline α-theophylline-7-α′-hydroxymethyldiglycollic aldehyde identical with that obtained from similar oxidation of theophylline-7-β-*d*-glucopyranoside (Lythgoe and Todd, *J.*, 1944, 592).




These reactions provided satisfactory evidence of the nature of the crude acetobromoribofuranose and we could now proceed to the synthesis of cytidine (VIII). Interaction of 2 : 6-diethoxypyrimidine with (VI) at 65° gave a gummy product which on treatment with methanolic ammonia yielded a mixture of bases, from which cytidine was isolated by recrystallisation of the

picrates and finally of the sulphate. The cytidine sulphate so obtained agreed in composition and physical constants with that isolated from yeast nucleic acid. The Molisch test was negative, but after prolonged heating with the perchloric acid–tryptophan reagent (Cohen, *J. Biol. Chem.*, 1944, **156**, 691) the green colour characteristic of pentoses was slowly developed; natural cytidine sulphate behaved in the same manner. Final confirmation of the identity of the natural and synthetic products was obtained by comparison of their *X*-ray crystal photographs, for which we are indebted to Dr. Clews and Mr. Nicol of the Department of Crystallography.

The work described above represents the first synthesis of a naturally occurring nucleoside. Since it is known that cytidine can be deaminated to give uridine, the work also constitutes a synthesis of uridine, and provides confirmation of the degradative evidence relating to the structures of these two nucleosides.

## Experimental.

**1 : 2 : 3 : 5-*Tetra-acetyl* d-*Ribofuranose*.**—1 : 2 : 3-Triacetyl 5-trityl *d*-ribofuranose (21 g.; Bredereck, Köthnig, and Berger, *loc. cit.*) dissolved in glacial acetic acid (250 c.c.) was hydrogenated at 35° and atmospheric pressure in presence of Adams's palladium oxide (1 g.). After 3 hours 900 c.c. of hydrogen had been absorbed and the filtered solution was evaporated to dryness under reduced pressure. Crystallisation of the residue from alcohol gave triphenylmethane (8 g.), and the mother liquors after evaporation were dissolved in a little chloroform, light petroleum (b. p. 100—120°) was added, and the chloroform was removed by evaporation. The petroleum layer was removed, and the insoluble 1 : 2 : 3-triacetyl *d*-ribose acetylated in the usual way by acetic anhydride in pyridine. The acetylated material, isolated in the usual way, was dissolved in chloroform, washed with aqueous sodium bicarbonate and then with water, and the chloroform was removed from the dried solution under reduced pressure. Distillation of the residue at 100—110° (bath temp.)/$10^{-4}$ mm. gave 1 : 2 : 3 : 5-*tetra-acetyl* d-*ribofuranose* as a syrup which crystallised on standing; m. p. 58°, $[\alpha]_D^{15°}$ +20° (±1°) (*c*, 0·645 in chloroform). Yield, 9 g. (70%) (Found: C, 49·1; H, 5·7. $C_{13}H_{18}O_9$ requires C, 49·2; H, 5·2%).

*Theophylline-7-β-*d*-ribofuranoside*.—Pure anhydrous liquid hydrogen bromide (5 c.c.) was distilled into a Carius tube containing 1 : 2 : 3 : 5-tetra-acetyl *d*-ribose (1·5 g.) and, after being sealed, the tube was kept at room temperature for 10 minutes; dissolution was then complete. The tube was then opened and hydrogen bromide allowed to evaporate at room temperature, and the brown residue was evaporated twice with benzene under reduced pressure at 30° and finally heated to 35° in a high vacuum to remove hydrogen bromide as completely as possible. The crude acetobromoribofuranose so obtained was dissolved in a little xylene and the solution refluxed with a suspension of dry theophylline silver (1·5 g.) in dry xylene (50 c.c.) for 1 hour with exclusion of moisture. Silver bromide was removed from the hot solution, which was allowed to cool and filtered from a little theophylline which had separated. The filtrate was evaporated to dryness under reduced pressure and set aside for 2 days at 0° with methanolic ammonia. Removal of solvents and crystallisation of the residue from water gave *theophylline-7-β-*d*-ribofuranoside* as colourless needles, m. p. 189°, $[\alpha]_D^{15°}$ +27° (±5°) (*c*, 0·257 in water) (Found in material dried at 100° and 0·1 mm.: C, 45·1; H, 5·5; N, 19·7. $C_{12}H_{16}O_6N_4$ requires C, 45·0; H, 5·0; N, 20·0%). Yield, 0·88 g. (60%).

*Periodate Oxidation*.—The above glycoside (209·2 mg.) was treated with a solution of sodium meta-periodate (208 mg. per 6 c.c. water) and set aside for 24 hours. The crystalline deposit of *α*-theophylline-7-*α'*-hydroxymethyldiglycollic aldehyde was collected (140 mg.), and had m. p. 205—206° (decomp.), $[\alpha]_D^{18°}$ −38° (*c*, 0·21 in water) (Found: C, 43·7; H, 4·9. Calc. for $C_{12}H_{14}O_6N_4,H_2O$: C, 43·9; H, 4·9%). Lythgoe and Todd (*loc. cit.*) record for the same compound obtained from theophylline-7-*β*-*d*-glucopyranoside, m. p. 207—208° (decomp.), $[\alpha]_D^{19°}$ −42°.

In one experiment on the preparation of theophylline-*d*-ribofuranoside by the method described, there was isolated as sole product a *substance* separating from aqueous alcohol as colourless needles, m. p. 193°, $[\alpha]_D^{16°}$ +110° (*c*, 0·254 in water) (Found: C, 45·8; H, 5·0; N, 17·9. $C_{12}H_{16}O_6N_4$ requires C, 46·2; H, 5·1; N, 17·9%). This substance required 1·06 mols. of periodate per mol. for complete oxidation, the oxidised product having $[M]_D^{14°}$ 1·03° (±0·5°) × $10^4$. It seems possible that it may be theophylline-7-*d*-*α*-ribofuranoside.

*Cytidine Sulphate*.—Crude syrupy acetobromoribofuranose prepared in the manner described above from 1 : 2 : 3 : 5-tetra-acetyl *d*-ribose (5 g.) was dissolved in 2 : 6-diethoxypyrimidine (15 g.; Hilbert and Jansen, *J. Amer. Chem. Soc.*, 1935, **57**, 553) and the solution maintained at 65° for 24 hours with exclusion of moisture. After filtration from a little resin the solution was freed from excess of diethoxypyrimidine by evaporation at 100°/$10^{-2}$ mm. The residual 9 g. were dissolved in methanolic ammonia (40 c.c., saturated at 0°) and the solution was maintained at 80° for 4 days and then evaporated under reduced pressure. To a solution of the residue in hot alcohol (10 c.c.) hot alcoholic picric acid (5 g. in 50 c.c.) was added. The picrate separating on cooling was recrystallised from water, and then had m. p. *ca.* 150°. It was decomposed with dilute sulphuric acid, picric and sulphuric acids were removed in the usual manner, and the solution was concentrated to 5 c.c. After addition of concentrated sulphuric acid (0·7 c.c.) and alcohol (25 c.c.), inoculation with a trace of cytidine suphate caused crystallisation. The product after recrystallisation from alcohol containing dilute sulphuric acid had m. p. 224° (decomp.) alone and in admixture with authentic material. $[\alpha]_D^{16°}$ +35° (*c*, 0·366 in 1% aqueous sulphuric acid). Yield, 350 mg. [Found in material dried at 140°/0·1 mm.: C, 37·2; H, 5·2; N, 14·0. Calc. for $(C_9H_{13}O_5N_3)_2,H_2SO_4$: C, 37·0; H, 4·8; N, 14·2%].

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