Synthesis of 5-Ribosyluracil and of 5-Ribosyluridine

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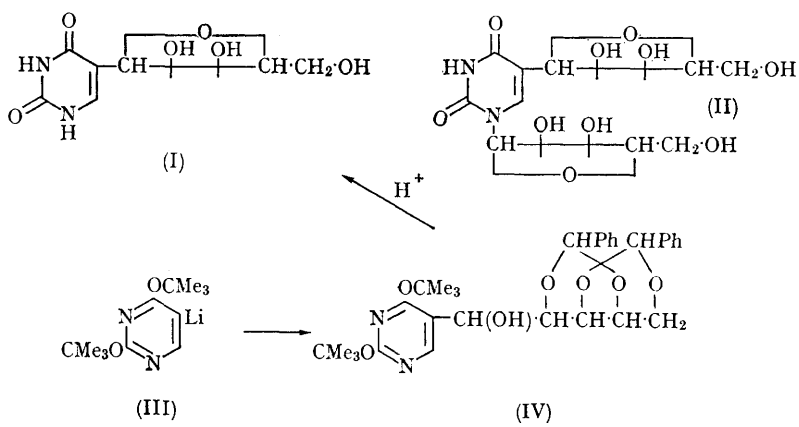
INTEREST has been shown recently in the biosynthesis of pseudouridine (5-β-D-ribofuranosyluracil) (I), the *C*-glycosidic nucleoside present in transfer ribonucleic acid. 3,5-Diribosyluracil (II) has been suggested[1,2] as an intermediate in the enzymic interconversion of uridine and pseudouridine in some organisms. Pollak and Arnstein[3] isolated a substance from an *E. coli* strain in very small amount with properties consistent with (II). Lis and Lis[4] obtained a substance, presumed to be (II), from commercial uridine and while studying nucleoside synthesis with enzyme preparations



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from a *Penicillium* and an *E. coli* strain. While the evidence given does suggest that 3,5-diribosyluracil (hereafter called 5-ribosyluridine) occurs naturally it does not lead conclusively to that structure. We record here a synthesis of 5-β-D-ribosyluracil and of 5-β-D-ribosyluridine, so that the latter is now available for comparative purposes.

A synthesis of pseudouridine in very low yield has already been described[5] involving coupling of 5-lithio-2,6-dimethoxypyrimidine with 2,3,5-tri-*O*-benzoylribosyl chloride. Our synthesis utilised the reaction of 5-lithio-2,6-di-t-butoxypyrimidine (III) (from the 5-bromo-compound) with 2,4 : 3,5-di-*O*-benzylidene-D-ribose.[6] In this case, the intermediate (IV) was not isolated, and removal of protecting groups and ring-closure of the sugar residue was effected directly by aqueous-methanolic hydrochloric acid at room temperature. Separation of nucleosidic material by borate gradient ion-exchange chromatography gave pseudouridine (18%) and an isomer (4%) corresponding to the α-anomer of pseudouridine (isomer B).[7] No appreciable amounts of the pyranose isomers were formed under these mild conditions of ring-closure. The crystalline pseudouridine, m.p. 222°, was identical in every way with the natural substance.

By similar means, using 5-bromo-2′,3′-di-*O*-isopropylideneuridine as starting material, two substances having chromatographic properties expected of ribofuranosyluridine were obtained (electrophoretic mobilities respectively 1·25 and 1·45 times that of uridine in 0·1M-borate, pH 9·2). Warming with N-hydrochloric acid converted the latter essentially quantitatively into the former which was isolated as needles (5%), m.p. 241—242°, $\lambda_{max.}$ 256 mμ at pH 1 and 12 (cf. refs. 3, 4) and periodate uptake 1·9 moles/mole. Elementary analysis, the n.m.r. spectrum in D_2O (no C-5 proton), together with its conversion by yeast nucleosidase[8] into pseudouridine confirmed its structure as 5-β-D-ribofuranosyluridine (II). The other substance formed in the reaction is still under investigation; it does not appear to be the α-anomer.

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