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THE SYNTHESIS OF OROTIDINE

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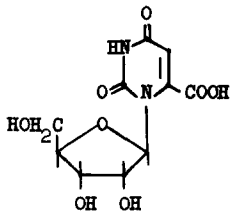
Michelson, Drell, and Mitchell[1] first reported the isolation and purification of a ribosyl derivative of orotic acid from the mycelia of a mutant of Neurospora. Lieberman et al.[2] subsequently converted this compound to orotidine-5'-phosphate and thence to uridine-5'-phosphate by enzymatic means, thereby providing good evidence that orotidine was 1-(β-D-ribofuranosyl)uracil-6-carboxylic acid (I). The position of the glycosyl linkage was confirmed by Fox et al.[3] through a comparison of the ultraviolet absorption spectra of orotidine with 1-methyl- and 3-methylorotic acids.



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1 A. M. Michelson, W. Drell, and H. K. Mitchell, Proc. Natl. Acad. Sci. 37, 396 (1951).

2 I. Lieberman, A. Kornberg, and E. S. Simms, J. Biol. Chem. 215, 403 (1955).

3 J. J. Fox, N. Yung, and I. Wempen, Biochim. et Biophys. Acta 23, 295 (1957).

The synthesis of this nucleoside by conventional procedures was contraindicated by the finding that orotic acid, when treated with excess dimethyl sulfate in hot, alkaline solution, afforded the 3-methyl derivative. Furthermore this 3-methyl compound, when subjected to the same treatment, gave only meager yields of 1,3-dimethylorotic acid.[3] These results and an examination of molecular models led Fox et al.[3] to conclude that the 6-carboxyl group causes a considerable amount of steric hindrance at the N-1 position of orotic acid. In contrast to this work we have found that similar treatment of orotic or 3-methylorotic acid at room temperature leads to the formation of 1,3-dimethylorotic acid as the principal product. These results show that the N-1 position is not nearly so hindered as previously indicated and prompted us to investigate a synthetic route to orotidine even though several unsuccessful attempts had been described.[4,5]

We have obtained orotidine in 14.5% yield (as the cyclohexylammonium salt) by condensing the monomercuri derivative of n-butyl orotate with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride, removing the protecting groups, and separating the desired nucleoside by gradient elution ion-exchange chromatography. This work provides a synthetic confirmation for the structure of orotidine (I); the mode of synthesis also supports the assignment of the β configuration at the anomeric center.[6]

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4 R. K. Ralph, G. Shaw, and R. N. Naylor, J. Chem. Soc. 1169 (1959) reported that in preliminary experiments they could find no evidence of condensation between metal derivatives of ethyl orotate and 2,3,5-tribenzoylribosyl chloride.

5 Michelson et al. in reference 1 stated that several unsuccessful attempts had been made to synthesize glycosides and especially a ribofuranoside of orotic acid.

6 B. R. Baker in Ciba Foundation Symposium on the Chemistry and Biology of Purines (Edited by G. E. W. Wolstenholme and C. M. O'Connor) p. 120., J. and A. Churchill Ltd., London, England, 1957.

n-Butyl orotate[7] was converted to the monomercuri derivative (95%) using methanolic mercuric acetate: (Calcd. for $C_9H_{11}N_2O_4$: C, 26.3; H, 2.7; N, 6.8. Found: C, 25.9; H, 2.9; N, 6.6). This product was refluxed in xylene with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride for three hours, cooled, filtered from some unreacted mercury derivative, and the filtrate poured into a large volume of petroleum ether. The resulting sirup was treated with methanolic sodium methoxide, then aqueous base to remove the protecting groups. This material was applied to a Dowex 1- X8 (Cl) column and eluted with a linear water-0.1M ammonium bicarbonate gradient.[8] The orotidine-containing fractions (as determined by 280/260 mμ ratios) were combined and the desired nucleoside separated from chloride and bicarbonate ions by adsorption on acid-washed Norit A. The product was eluted with ethanolic-ammonia, converted to the cyclohexylammonium salt by passage through Dowex 50 W- X4 (cyclohexylammonium form) and crystallized from ethanol-ethyl acetate. The yield was 557 mg. (14.5%), m.p. 180-182°, $[\alpha]_D$ = + 15 ± 5 (c = 1 in water), (Calcd. for $C_{16}H_{25}N_3O_8$: C, 49.6; H, 6.5; N, 10.9. Found: C, 49.5; H, 6.5; N, 10.7). The identity of this material with authentic orotidine cyclohexylammonium salt[9] was confirmed by mixture melting point, paper chromatography, and ultraviolet and infrared absorption spectra.

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7 L. O. Ross, L. Goodman, and B. R. Baker, J. Org. Chem. 25, 1950 (1960).

8 We are indebted to Dr. T. R. Breitman for valuable advice on ion-exchange and charcoal adsorption procedures.

9 Commercially available from California Corporation for Biochemical Research, Los Angeles 63, California.