A Synthesis of 3-(β-D-Ribofuranosyl)xanthine (3-Isoxanthosine)

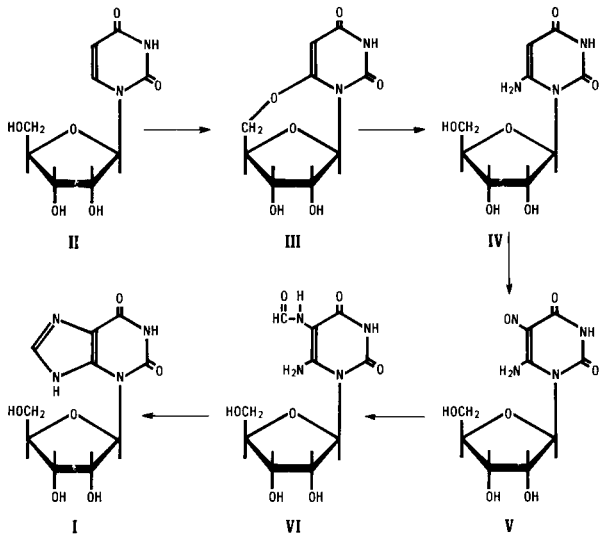
David Lipkin, C. T. Cori and Jaime A. Rabi

Department of Chemistry, Washington University (St. Louis)

Sir:

In recent years there has been an increased interest in isopurine nucleosides and nucleotides because of their potential biological activity (1) and activity in enzyme catalyzed reactions (2,3). Compounds of this type have been synthesized by several methods. A common procedure is alkylation of the appropriately substituted halo sugar with the required aglycon (4-11). Another method involves the modification of existing pyrimidine nucleosides (12,13) and in two cases such compounds have been formed enzymatically (2,14).

We wish to report the unambiguous synthesis of 3-(β-D-ribofuranosyl)xanthine (3-isoxanthosine) (I). The first two steps in the synthesis involve the conversion of uridine (II) to O^6,5′-cyclouridine (III) (15,16). Compound III then is treated with liquid ammonia 0.4 *M* in ammonium chloride (60°, 25 hours) to give (60%) 6-aminouridine (IV) (11,17,18) [m.p. 190-191° dec.; λ max (*p*H 7) 272 mμ (ε 2.48 x 10^4); pK_a 10.1 ± 0.1; $[\alpha]_D^{25}$ + 6.18° (*c* 0.42, water)]. The structure of IV was confirmed by the following observations: (a) cleavage of the *N*-glycosidic bond by treatment with 1 *M* ammonium hydroxide (reflux, 40 minutes) to yield 6-aminouracil and ribose; (b) deamination by treatment with 0.01 *M* sulfuric acid (room temperature, 20 hours) to yield the known compound 6-hydroxyuridine (15b,20); (c) nitrosation followed by deamination (by treatment first with nitrous acid followed by hydrolysis with sulfuric acid) to yield the known compound 6-hydroxy-5-nitrosouridine (15b); (d) positive periodate (21) and positive (light red) Ehrlich's (22) spray tests; (e) comparison of the uv spectra and pK_a of IV with those of 6-amino-1-methyluracil (23) [λ max (*p*H 7) 266.5 mμ (ε 2.18 x 10^4); pK_a 10.9 ± 0.1], 6-amino-3-methyluracil (24) [λ max (*p*H 7) 263.5 mμ (ε 2.12 x 10^4); pK_a 8.7 ± 0.1], and the N^3-isomer of IV, 6-hydroxycytidine (15b) [λ max (*p*H 7) 267 mμ (ε 1.98 x 10^4); pK_a 8.50 ± 0.05]; and (f) the PMR spectrum. Some features of this spectrum are a doublet centered at δ 6.20 assigned to H_1' ($J_{1'2'}$ 6.7 cps) (25); a singlet at δ 4.63 assigned to H_5; and the correct integration for six broad NH and OH resonances which rapidly exchanged upon the addition of deuterium oxide. Atom H_5 also exchanged upon the addition of deuterium oxide ($t_{1/2}$ ~ 0.5 hour) by what is presumed to be an amino-imino tautomerism involving the 6-amino group. Analogous exchange reactions (26) have been observed for 6-hydroxycytidine and 6-hydroxyuridine (20c) and the corresponding bases.



6-Aminouridine was treated with aqueous nitrite and hydrochloric acid at *p*H 5 (0°, 1 hour) (27) to yield (90%) 6-amino-5-nitrosouridine (V) [m.p. 199-200° dec.; λ max (*p*H 7) 213 mμ (ε 1.18 x 10^4) and 223 mμ (ε 1.48 x 10^4)]. Compound V then was reduced with zinc and aqueous formic acid (27) to give 6-amino-5-formamidouridine (VI) [λ max (*p*H 7) 270 mμ], which was then converted, without isolation, by treatment with 2 *N* aqueous sodium hydroxide (80°, 15 minutes) (27) to the desired I [m.p. 203-205°; λ max (*p*H 5) 267 mμ (ε 1.17 x 10^4); pK_a 8.0 ± 0.1; $[\alpha]_D^{25}$ - 26.5° (*c* 0.355, water)]. The yield of I was 50% based on 6-aminouridine.

The structure of I was confirmed by the following observations: (a) cleavage of the *N*-glycosidic bond by treatment with anhydrous hydrogen fluoride (28) (room temperature, 12 hours) to yield xanthine; (b) a positive periodate spray test (21); (c) comparison of the uv spectra and pK_a of I with those of 3-methylxanthine (28,30) [λ max (*p*H 5) 271 mμ (ε 1.08 x 10^4); pK_{a_1}

8.32 ± 0.05]; and (d) the PMR spectrum. Some important features of this are a doublet centered at δ 6.22 assigned to H_1' ($J_{1'2'}$ 6.7 cps); a singlet at δ 8.09 assigned to H_8; and the correct integration for five broad NH and OH resonances which rapidly exchanged upon the addition of deuterium oxide (31). It was demonstrated, furthermore, that I is identical with the 3-(ribosyl)xanthine synthesized enzymatically (2) in chromatographic behavior; spectral properties; and its relative stability toward acid hydrolysis when compared with 9-(β-D-ribofuranosyl)-xanthine.

Work is now in progress to synthesize other isopurine nucleosides and nucleotides employing similar methods.

Acknowledgment.

This investigation was supported, in part, by Public Health Service Research Grant No. CA-03870 from the National Cancer Institute and by National Science Foundation Grant No. GB-3980.

REFERENCES

(1) N. J. Leonard and R. A. Laursen, *Biochemistry*, **4**, 365 (1965).

(2) D. Hatfield and J. B. Wyngaarden, *J. Biol. Chem.*, **239**, 2587 (1964).

(3) F. Bergmann, H. Kwietny, G. Levin and D. J. Brown, *J. Am. Chem. Soc.*, **82**, 598 (1960).

(4) L. Birkofer, A. Ritter and H. P. Kuhlthau, *Angew. Chem.*, 75, 209 (1963).

(5) N. J. Leonard and R. A. Laursen, *Biochemistry*, **4**, 354 (1965).

(6) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, 87, 5442 (1965).

(7) N. J. Leonard and R. A. Laursen, *ibid.*, **85**, 2026 (1963).

(8) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, **5**, 741 (1968).

(9) M. Asai, M. Miyaki and B. Shimizu, *Agr. Biol. Chem.* (Tokyo), 31, 319 (1967).

(10) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, **5**, 303 (1968).

(11) R. Lohrmann, J. M. Lagowski and H. S. Forrest, *J. Chem. Soc.*, 451 (1964).

(12) J. J. Fox and D. Van Pragg, *J. Org. Chem.*, **26**, 526 (1961).

(13) R. E. Holmes and R. K. Robins, Abstracts, 144th ACS Meeting, Los Angeles, California, 1963, p. 2C.

(14) D. Hatfield and J. B. Wyngaarden, *J. Biol. Chem.*, **239**, 2580 (1964).

(15a) D. Lipkin, F. Howard, D. Nowotny and M. Sano, Abstr. 6th Int. Congr. Biochem., N.Y., paper no. 1-117 (1964). (b) D. Lipkin, C. T. Cori and M. Sano, *Tetrahedron Letters*, 5993 (1968).

(16) B. A. Otter, E. A. Falco and J. J. Fox [*J. Org. Chem.*, **34**, 1390 (1969)] refer to III as 5′,6-anhydro-6-hydroxyuridine. It is our present opinion that the prefix "6-hydroxy" is redundant.

(17) Satisfactory elemental analyses were obtained for compounds with melting points reported herein. PMR spectra (with DMSO-d_6 as the solvent and relative to TMS) were determined on a 60 MHz spectrometer. For the exchange of NH and OH of I and the NH, OH, and H_5 of IV, 25 λ of deuterium oxide was added to the 300 λ of solution in the PMR tube.

(18) A large number of ring opening reactions of the cyclonucleoside structure in O^6,5′-cyclouridine and O^6,5′-cyclocytidine by means of nucleophiles were investigated [D. Lipkin and C. T. Cori, unpublished results]. Only in the case of the acid catalyzed ammonolysis of III to IV is it unequivocally demonstrated that the ring opening takes place by attack of nucleophile on C_6 of the heterocyclic ring rather than on C_5' of the sugar. O^2,5′-cyclouridine behaves in similar fashion on ammonolysis [D. M. Brown, A. R. Todd and S. Varadarjan, *J. Chem. Soc.*, 868 (1957)].

(19) The difference in properties of IV and those reported by Lohrman, *et al.*, in ref. 11 can be attributed, in part, to a typographical error in ref. 11 for the value of the λ max of 6-amino-1-(D-ribosyl)uracil (268 mμ rather than 248 mμ). J. Lagowski to D. Lipkin, personal communication.

(20a) T. Ukita, M. Yoshida, A. Gamada and Y. Kato, *Chem. Pharm. Bull.* (Tokyo), 12, 459 (1964). (b) B. A. Otter, E. A. Falco and J. J. Fox, *Tetrahedron Letters*, 2967 (1968). (c) B. A. Otter, E. A. Falco and J. J. Fox, *J. Org. Chem.*, **34**, 1390 (1969).

(21) M. Visconti, D. Hoch and P. Karrer, *Helv. Chim. Acta.*, 38, 642 (1955).

(22) R. M. Fink, R. E. Cline, C. McGaughey and K. Fink, *Anal. Chem.*, 28, 4 (1956).

(23) V. Papesch and E. F. Schroeder, *J. Org. Chem.*, **16**, 1879 (1951).

(24) W. Pfleiderer, *Chem. Ber.*, **90**, 2272 (1957).

(25) The β-configuration of I is based upon the known configuration of uridine and the improbability that any of the subsequent reactions would involve epimerization of C_1'. The large coupling constant of H_1' and H_2' in I and in IV, is also observed in xanthosine ($J_{1'2'}$ 6.5 cps) and isoadenosine ($J_{1'2'}$ 5.2 cps) [N. J. Leonard and J. A. Laursen, *J. Am. Chem. Soc.*, **85**, 2026 (1963)]. It indicates that the ribofuranose ring is twisted. The most reasonable conformation of I and IV is the one in which C_2' is pushed toward the heterocyclic base, thus making the angle between H_1' and H_2' greater than 90°.

(26) D. Lipkin and C. T. Cori, unpublished results.

(27) K. A. Chkhikvadz and O. Yu. Magidson, *Chem. Abstr.*, 53, 16146c (1959).

(28) D. Lipkin, B. E. Phillips and J. W. Abrell, *J. Org. Chem.*, 34, 1539 (1969).

(29) L. F. Cavalieri, J. J. Fox, A. Stone and N. Chang, *J. Am. Chem. Soc.*, 76, 1119 (1954).

(30) J. M. Gulland, E. R. Holiday, and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).

(31) By contrast, it required refluxing for 2 hours in deuterium oxide to exchange 23% of the H_8 proton, analogous to the exchange of H_8 previously observed in various purine bases [F. J. Bullock and O. Jardetzty, *J. Org. Chem.*, **28**, 1988 (1964)].

Received September 18, 1969 St. Louis, Missouri 63130