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The Synthesis of 2-Nitro-1-β-D-ribofuranosylimidazole (Azomycin Riboside) (1)

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Sir:

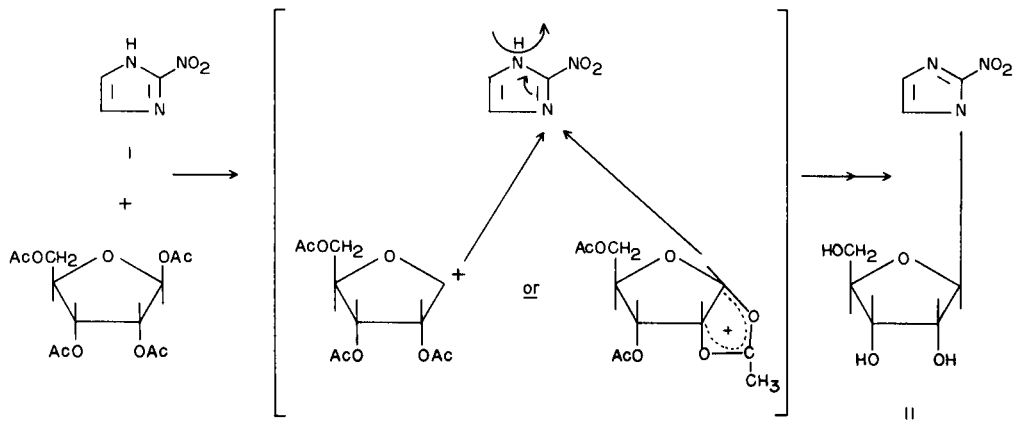
We wish to report the preparation of Azomycin riboside (II) *via* the fusion procedure (2,3). The antibiotic azomycin (I) was first isolated (4) from a microbial strain resembling *Norcardia mesenterica* and was later characterized (5) as 2-nitroimidazole. Azomycin has subsequently demonstrated (6) significant biological activity and has only recently been prepared chemically (7-9). It has been recently reported (10) that utilization of [2-^{14}C]2-aminoimidazole as a substrate for *Streptomyces strain LE* 13342 has furnished labelled azomycin. This would seem to indicate biosynthesis by the direct enzymatic oxidation of an amino group to a nitro group. The possibility that azomycin [like zeatin (11), 5-aminoimidazole (12) and 5-aminoimidazole-4-carboxamide (13)] may occur naturally in the form of a riboside prompted the present investigation.

Previous synthetic procedures (14) for the preparation of imidazole nucleosides leave much to be desired. However, a new general method of imidazole glycosidation has recently (15) been accomplished and utilized in the present work for the preparation of azomycin riboside (II).

A mixture of 2-nitroimidazole (7) (11.4 g.) and tetra-*O*-acetyl-β-D-ribofuranose (32.6 g.) was fused at 210° with a catalytic amount of chloroacetic acid for seven minutes *in vacuo*. The dark syrup was dissolved in 250 ml. of benzene and the solution filtered to collect 5.5 g. of unreacted I. Crystallization of the tri-*O*-acetyl nucleoside proved to be unfruitful. The benzene solution was evaporated to a syrup which was treated directly with methanolic ammonia to yield 4.3 g. (33.6%) of II (based on the recovery of unreacted I). Recrystallization from water furnished light yellow needles of azomycin riboside (II), m.p. 192-193°, $[\alpha]_D^{16}$ + 15.8 (C = 1, H_2O). *Anal.* Calcd. for $C_8H_{11}N_3O_6$: C, 39.2; H, 4.49; N, 17.14. Found: C, 39.45; H, 4.35; N, 16.99. The ultraviolet absorption spectra exhibited λ max (*p*H, 1), 325 (ε, 7,980); λ max (*p*H, 11), 325 (ε, 7,980) and 238 (ε, 2,820); λ min (*p*H, 1), 262 (ε, 2,000); λ min (*p*H, 11), 262 mμ (ε, 2,000).

The site of glycosidation was readily established since the ultraviolet absorption spectra of II was very similar to the ultraviolet absorption spectra reported (16) for 1-methyl-2-nitroimidazole and the p.m.r. spectra also definitely established that *N*-glycosidation had occurred.

The assignment of anomeric configuration for imidazole nucleosides has, in the past, consisted (14,15) primarily of chemical transformations which furnished either imidazole or purine nucleosides of established anomeric configuration. Since these methods were not applicable for the anomeric assignment of II, an alternate method of anomeric assignment was required. A correlation of the



chemical shift exhibited by the anomeric proton in the p.m.r. spectra has been recently (17) utilized for the assignment of anomeric configuration, however, this requires a comparison between the α and β anomers especially since it has been demonstrated that the chemical shift is affected (18) by various diverse factors. It has been established (19) that in a five membered ring the dihedral angle between neighboring *cis*-hydrogens and neighboring *trans*-hydrogens can vary from 0-45° and 75-165° which can produce coupling constants, using the Karplus equation (20) in a range of approximately 3.5-8.0 c.p.s. and 0-8.0 c.p.s., respectively. Therefore, an assignment of anomeric configuration, excluding conformational changes, can be made only if the coupling constant is less than about 3.5 c.p.s. but preferably a smaller coupling constant is desirable. In fact it has been stated (21) that this assignment should be applied only when the coupling constant for neighboring *trans*-hydrogens is less than approximately 1.0 c.p.s., *e.g.*, Neomycin B and Neomycin C (22). A visual inspection of the p.m.r. spectrum of II in the 6.5-6.6 δ region revealed a coupling constant of 1.0 c.p.s. or less for the anomeric proton of II. Therefore, II possesses a *trans* configuration between the neighboring hydrogens residing at C-1' and C-2' and on this basis has been assigned the beta configuration and the total structure of azomycin riboside is established as 2-nitro-1-(β-D-ribofuranosyl)imidazole (II).

The *N*-substitution of imidazoles has been proposed (23) to occur *via* an S_E2, S_E2_CB or S_E2' mechanism by analogy with other systems. Since it is visualized that the attacking carbohydrate moiety does so as a carbonium or acetoxonium ion, the most likely mechanism for glycosidation *via* the fusion method in this case would appear to be S_E2'. However, this presumption must await experimental substantiation by further studies of ratio distribution on glycosidation of a variety of imidazoles. The glycosidation of additional imidazoles *via* the fusion method is currently in progress and the spectrum of imidazole precursors and factors involved in a successful fusion reaction will be communicated at a later date.

REFERENCES

(1) This work was supported by Research Contract PH 43-65-1041 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.
(2) T. Sato, T. Simadate and Y. Ishida, *Nippon Kagaku Zasshi*, 81, 1440 (1960) and subsequent papers.
(3) R. J. Rousseau, L. B. Townsend and R. K. Robins, *Biochemistry*, 5, 756 (1966).
(4) K. Maeda, T. Osato and H. Umezawa, *J. Antibiotics (Tokyo)*, 6A, 182 (1953).
(5) S. Nakamura, *Chem. Pharm. Bull. (Tokyo)*, 3, 379 (1955).
(6) E. Grunberg and E. Titsworth, *Antimicrobial Agents and Chemotherapy*, 478 (1965); C. Cosar and L. Julon, *Ann. Inst. Pasteur*, 96, 238 (1959).
(7) A. G. Beaman, W. Tautz, T. Gabriel and R. Duschinsky, *J. Am. Chem. Soc.*, 87, 389 (1965).
(8) G. C. Lancini and E. Lazzari, *Experientia*, 15, 83 (1965).
(9) A. G. Beaman, W. Tautz, T. Gabriel, O. Keller, and R. Duschinsky, *Antimicrobial Agents and Chemotherapy*, 469 (1965).
(10) G. C. Lancini, D. Kluepfel, E. Lazzari and G. Sartori, *Biochim. Biophys. Acta*, 130, 37 (1966).
(11) C. O. Miller, *Proc. Nat. Acad. Sci., U. S.*, 54, 1052 (1965).
(12) B. Levenberg and J. M. Buchanan, *J. Biol. Chem.*, 224, 1005 (1957).
(13) G. R. Greenberg, *J. Am. Chem. Soc.*, 74, 6307 (1952).
(14) L. B. Townsend, *Chem. Rev.*, 67, in press (1967).
(15) R. J. Rousseau, L. B. Townsend and R. K. Robins, *Chem. Commun.*, 265 (1966).
(16) G. G. Gallo, C. R. Pasqualucci, P. Radaelli and G. C. Lancini, *J. Org. Chem.*, 29, 862 (1964).
(17) K. R. Darnall and L. B. Townsend, *J. Heterocyclic Chem.*, 3, 371 (1966).
(18) L. D. Hall, *Tetrahedron Letters*, 1457 (1964); T. Ledaal, *ibid.*, 1653 (1966) and references therein.
(19) K. S. Pitzer and W. E. Donath, *J. Am. Chem. Soc.*, 81, 3213 (1959).
(20) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959).
(21) R. U. Lemieux and D. R. Lineback, *Ann. Rev. Biochem.*, 32, 155 (1963).
(22) K. L. Rinehardt, Jr., W. S. Chilton, M. Hickens and W. J. von Phillipsborn, *J. Am. Chem. Soc.*, 84, 3216 (1962).
(23) A. Grimson, J. H. Ridd and B. V. Smith, *J. Chem. Soc.*, 1352 (1962).
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