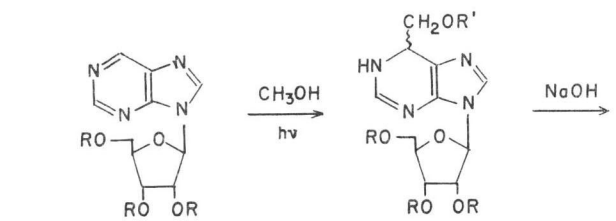
SYNTHESIS OF ISOCOFORMYCIN, AN ADENOSINE DEAMINASE INHIBITOR OF SYNTHETIC ORIGIN

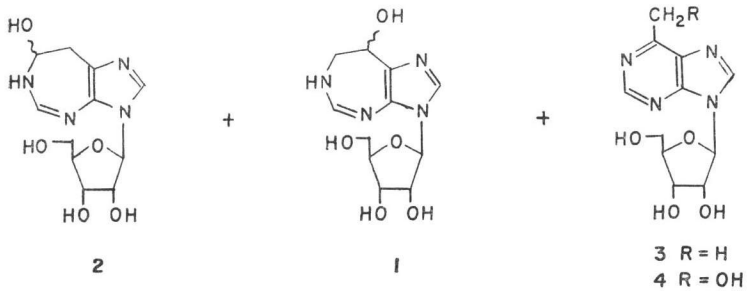
Sir:

Since the structure and the interesting biological activity of coformycin (**1**) were disclosed[1,2], a considerable amount of attention has been focused on such unusual nucleosides of microbial origin[3~7]. Such modification of the base moiety by nature has led us to approach various seven-membered nucleosides by chemical synthesis and to investigate structure-activity relationships of such unusual nucleosides. In this communication, we describe a synthesis of an isomer of coformycin, 3-β-D-ribofuranosyl-3,6,7,8-tetrahydroimidazo-[4,5-d][1,3]-diazepin-7-ol (**2**) named isocoformycin, which has also been found to possess significant biological activity.

Previously we reported a synthesis of coformycin starting from 9-β-D-ribofuranosylpurine (**5**) or nebularine, but later it became apparent that the products formed in each step were very sensitive to reaction conditions, requiring more careful analysis such as with tlc and high pressure liquid chromatography. The present approach also centered on two key synthetic operations: (a) photoaddition of methanol to **5** and (b) ring expansion of a major product **7** to seven-membered compounds. Thus, the photoaddition of methanol to **6** was able to be carried out in a stereoselective manner by using Vycor filter (30 W low-pressure mercury lamp, 0~5°C, Argon atmosphere), predominantly affording one of the diastereomers of **7** (EtOAc - MeOH, 5: 1; Rf 0.10, 8% and Rf 0.20, 75%).* The major adduct (mp 58~61°C, $[\alpha]_D^{23}$ −30.0° (*c* 3.0, MeOH)) was separated by silica gel chromatography (benzene-acetone eluent) and subjected to mesylation with mesyl chloride in the presence of potassium carbonate.

The unstable mesylate (**8**) was immediately treated with dilute sodium hydroxide in aqueous 1,2-dimethoxyethane at room temperature for 2 hours. After neutralizing the solution with acetic acid, the solution was subjected to the following column chromatography using ion exchange resins. Treatment with Amberlite IR-120 resin (NH_4^+: H^+ = 1: 1, v/v) and development with 1 N NH_4OH afforded fractions having λ_{max} at 280 nm. The condensed fractions were again treated with Amberlite CG-50 resin (Type I, NH_4^+: H^+ = 1: 1, v/v) and eluted simply with water. Four products were obtained from the fractions. 9-β-D-Ribofuranosyl-6-methylpurine (**3**)[9~10], 9-β-D-ribofuranosyl-6-hydroxymethyl purine (**4**)[11] and an epimeric mixture of coformycin were obtained in 20, 10, and 6% yields, respectively. The fourth compound was obtained in 30% yield from the last fractions and proved to be 3-β-D-ribofuranosyl-3,6,7,8-tetrahydroimidazo-[4,5-d][1,3]-diazepin-7-ol named isocoformycin based on the chemical and spectroscopic evidences. ^{1}H-NMR and ^{13}C-NMR are consistent with structure **2**, but ^{13}C-NMR shows doublet of each peak demonstrating that **2** is an epimeric mixture of alcohol at C-7. Isocoformycin **2** has the formula $C_{11}H_{16}N_4O_5$ (M^+ 284): mp 185~190°C; $[\alpha]_D^{23}$ −58.0° (*c* 1.0, H_2O); UV_{max} (H_2O)





* The photoaddition is very sensitive to reaction conditions and the details of this interesting photoaddition will be mentioned in a separate paper.

280 nm (ε 8200). The hydroxyl group at C-7 is slowly replaced by a methoxyl group in methanol at room temperature and regenerated in hot water, which is characteristic of α-hydroxyamino group[12]. Amazingly, isocoformycin is also a strong inhibitor to adenosine deaminase which will be the subject of a subsequent paper, showing the biological significance of seven-membered nucleosides*.

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References

1) NAKAMURA, H.; G. KOYAMA, Y. IITAKA, M. OHNO, N. YAGISAWA, S. KONDO, K. MAEDA & H. UMEZAWA: Structure of coformycin, an unusual nucleoside of microbial origin. J. Am. Chem. Soc. 96: 4327, 1974
2) SAWA, T.; Y. FUKAGAWA, H. HOMMA, T. TAKEUCHI & H. UMEZAWA: Mode of inhibition of coformycin on adenosine deaminase. J. Antibiotics, Ser. A 20: 227~231, 1967
3) HARRAP, K. R. & R. M. PAINE: Adenosine metabolism in cultured lymphoid cells. Adv. Enzyme Regulation 15: 169~193, 1977
4) SHANNON, W. M.: Third conference on antiviral substances. Ann. New York Acad. Sci. 284: 3~5, 1977
5) AGARWAL, R. P. & R. E. PARKS, Jr.: Potent inhibition of muscle 5'-AMP deaminase by the nucleoside antibiotics coformycin and deoxycoformycin. Biochem. Pharmacol. 26: 663~666, 1977
6) CARON, H.; S. H. LEE & A. F. KIMBALL: Effect of 2'-deoxycoformycin, 9-β-D-arabinofuranosyladenine 5'-phosphate, and 1-β-D-arabinofuranosylcytosine triple combination therapy on intracerebral leukemia 1210. Cancer Res. 37: 3274~3279, 1977
7) CHA, S.; R. P. AGARWAL & R. E. PARKS, Jr.: Non-steady state nature of inhibition of milk xanthine oxidase by allopurinol and alloxanthine and of human erythrocytic adenosine deaminase by coformycin. Biochem. Pharmacol. 24: 2187~2197, 1975
8) OHNO, M.; N. YAGISAWA, S. SHIBAHARA, S. KONDO, K. MAEDA & H. UMEZAWA: Synthesis of coformycin. J. Amer. Chem. Soc. *96: 4326*, 1974
9) GARDON, M. P.; V. S. WELSKY & G. B. BROWN: A study of the action of acid and alkali on certain purines and purine nucleosides. J. Amer. Chem. Soc. 79: 3245~3250, 1957
10) MONTGOMERY, J. A. & K. HEWSON: Analogs of 6-methyl-9-β-D-ribofuranosylpurine. J. Med. Chem. 11: 48~52, 1968
11) EVANS, B. & R. WOLFENDEN: A potential transition state analog for adenosine deaminase. J. Amer. Chem. Soc. 92: 4751~4752, 1972
12) MIYAMOTO, M.; S. KONDO, H. NAGANAWA, K. MAEDA, M. OHNO & H. UMEZAWA: Structure and synthesis of neothramycin. J. Antibiotics 30: 340~343, 1977

* The reaction products containing coformycin and isocoformycin were carefully checked by high pressure liquid chromatography (detector 280 nm UV. Jascorex CV-01 cation exchange resin).