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Inhibition of Guanine Deaminase with Derivatives of 5-Amino-4-imidazolecarboxamide[1]

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Antitumor activity of 8-azaguanine was elevated by simultaneous administration of 5-amino-4-imidazolecarboxamide (AICA).[3] This effect was suggested to be due to the inhibition of guanine deaminase (3.5.4.3) by AICA.[3,4]

Of the AICA derivatives known to have antitumor activity,[4–7] 5-monomethyltriazeno-imidazole-4-carboxamide was also found to inhibit the enzyme.[4]

In this report, attempts were made to search for more inhibitory compounds against guanine deaminase than AICA and to establish possible relationship between chemical structure and inhibitory activity.

It was suggested that the amino group at position 5, the hydrogen at position 1 and the oxygen of carboxamide group at position 4 of AICA were necessary for the inhibition of the enzyme activity.

Materials and Methods

Assay of Guanine Deaminase Activity——Guanine deaminase of rat liver was prepared by Kalckar's method.[8] Guanine (11.33 mg) was dissolved in 0.25 ml of 1N KOH, and diluted to 25 ml with distilled water to the concentration of 3×10^{-3}M. Test compounds were dissolved in 0.25 ml of 1N KOH if soluble or of dimethylsulfoxide if insoluble, and diluted to 25 ml with 0.1M phosphate buffer (pH 7.0) at the concentration of 6×10^{-3}M. The assay procedure was as follows: In a flask were placed 1.3 ml of the test solution or of 0.1M phosphate buffer (pH 7.0) and 0.5 ml of enzyme solution (0.03 unit/ml). The reaction was started by the addition of 0.2 ml of guanine (3×10^{-3}M) to the mixture. It was then incubated with shaking in a water bath at 37° for 30 min in an air atmosphere. The residual guanine after the incubation was determined at 245 mμ by the method of Raush, *et al.*[9] The concentration for 50% inhibition, $V_0/V_I=2$, was obtained, by plotting V_0/V_I against the inhibitor concentration [I], where V_0 is the velocity without inhibitor and V_I is the velocity with inhibitor. Further, the ratio of inhibitor to substrate concentrations giving 50% inhibition, $([I]/[S])_{0.5}$, was determined.

Materials——Four derivatives of AICA, 5-Formamido-4-imidazolecarboxamide (FAICA), 1-β-D-Ribofuranosyl-5-amino-4-imidazolecarboxamide (AICAR), 5-amino-4-imidazolethiocarboxamide (TAICA), and 1-β-D-ribofuranosyl-5-amino-4-imidazolethiocarboxamide (TAICAR), were used and their chemical formula are shown in Fig. 1.

1) Presented in part at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
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3) H.G. Mandel and L.M. Law, *Cancer Res.*, 14, 808 (1954); H.G. Mandel, J.L. Way and P.K. Smith, *Biochem. Biophys. Acta*, 23, 402 (1957).
4) H. Nishio, I. Yamamoto, K. Kariya and K. Hano, *Chem. Pharm. Bull.* (Tokyo), **17**, 539 (1969).
5) K. Hano, A. Akashi, I. Yamamoto, S. Narumi and Z. Horii, *Gann*, **56**, 417 (1965).
6) Y.F. Shealy, J.A. Montgomery and W.R. Laster, *J. Biochem. Pharmacol.*, **11**, 674 (1962).
7) A. Hoshi, K. Kumagai and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **16**, 2080 (1968); A. Hoshi, F. Kanzawa, K. Kumagai and K. Kuretani, *Cancer Chemother. Rept.*, **53**, 165 (1969).
8) H.M. Kalckar, *J. Biol. Chem.*, **167**, 461 (1947).
9) A. Raush and E.R. Norris, *Arch. Biochem.*, **29**, 124 (1950).
10) B.R. Baker, W.W. Lee, W.A. Skinner, A.P. Martinez and E. Tong, *J. Med. Chem.*, **2**, 633 (1960).
11) B.R. Baker, *J. Med. Chem.*, **10**, 59 (1967).

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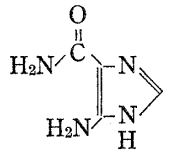
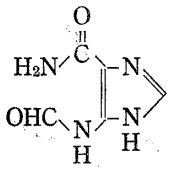
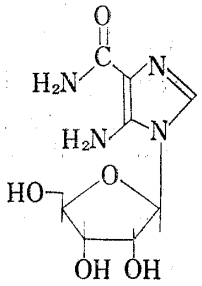
5-Amino-4-imidazolecarboxamide (AICA)



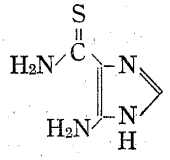
5-Formamido-4-imidazolecarboxamide (FAICA)



1-β-D-Ribofuranosyl-5-amino-4-imidazolecarboxamide (AICAR)



5-Amino-4-imidazolethiocarboxamide (TAICA)



1-β-D-Ribofuranosyl-5-amino-4-imidazolethiocarboxamide (TAICAR)

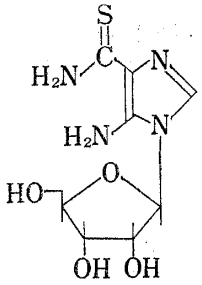


Fig. 1. Chemical Formulae of Test Compounds

Detection of Decomposed Products——Products under incubation conditions as above were determined as described earlier[12] by thin-layer chromatography with plates of Silicagel F_{254} and the solvent system; $CHCl_3$–MeOH–28% NH_4OH (240:80:1).

Results and Discussion

Effects of Derivatives of 5-Amino-4-imidazolecarboxamide

Inhibiting activity of FAICA and AICAR was compared with that of AICA. As shown in Fig. 2, guanine deaminase was inhibited with AICA and $([I]/[S])_{0.5}$ was 1.9, which almost coincided with that of Baker's value; 2.1.[11] FAICA and AICAR were weakly active and $([I]/[S])_{0.5}$ were 4.9 and 5.0 respectively.

FAICA was labile in acidic pH and it was decomposed to AICA which was the active inhibitor. Furthermore, FTAICA and TAICAR were metabolized and excreted in urine as TAICA.[12] FAICA and AICAR would be thus decomposed to AICA in the incubation mixture, if desired enzymes were presence. On the other hand, TAICAR and AICAR would be changed in part to the corresponding nucleotides by nucleoside phosphorylase which might be contami-

12) A. Hoshi, Y. Ohsaka and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **17**, 1720 (1969).

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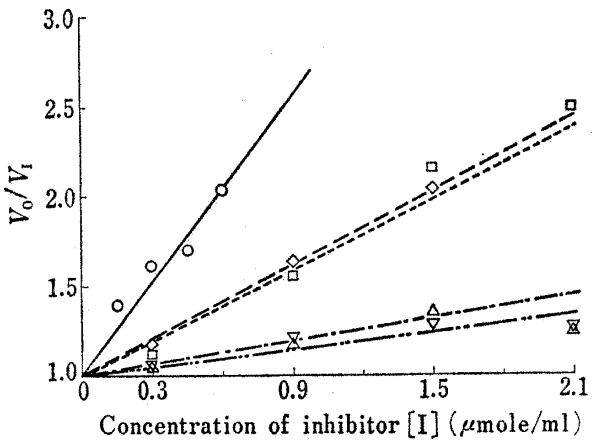

Fig. 2. Effect of Derivatives of AICA on the Activity of Guanine Deaminase

-○-: AICA -◇-: FAICA -□-: AICAR
-▽-: TAICA -△-: TAICAR

nated in the enzyme preparation.[8] But none of the above compounds was detected in the mixture after 30 min incubation. The activity of AICA was reduced by the introduction with either formyl to the amino group at position 5 or ribofuranosyl to the nitrogen at position 1.

Effects of Derivatives of 5-Amino-4-imidazolethiocarboxamide

Two mercapto derivatives having antitumor activity were tested. TAICA and TAICAR were almost inactive against guanine deaminase. 5-Formamido-4-imidazolethiocarboxamide, which had potent antitumor activity, could not be examined because of its insolubility in the incubation mixture. When the oxygen of the carboxamide group at position 4 of AICA was substituted with sulfur, the inhibitory activity was reduced.

To conclude, an inhibitor more active than AICA for guanine deaminase (3.5.4.3) was not found among some derivatives of AICA tested in this investigation, and these results suggested that the amino group at position 5, the hydrogen at position 1 and the oxygen of carboxamide group at position 4 of AICA were necessary for the inhibition of the enzyme activity.

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The Effects of DBM on Rat Ascites Tumors and Antibody Production

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DBM is 1,6-dibromo-1,6-dideoxy-D-mannitol (dibromomannitol, myelobromol, NSC-94100) as shown in Fig. 1. The effectiveness of DBM in chronic myeloid leukemia was first reported by Eckhardt, *et al.*[2,3] Szentkláray[4] attained complete remission in patients suffering from polycythemia vera. In experimental tumor this compound has an excellent effect against solid strain of Yoshida sarcoma on transplantation subcutaneously.[5] Several cross-resistant tests have been carried out also with various types of antitumor agents with DBM-

1) Location: *1–3, Ukima, Kita-ku, Tokyo.*
2) S. Eckhardt, C. Sellei, I.P. Honváth and L. Institoris, *Cancer Chem. Rep.*, 33, 57 (1963).
3) C. Selfei and S. Ekhardt, *Rev. Franc. Etude. Clin. Biol.*, **8**, 483 (1963).
4) J. Szentkláray, *Orv. Hetil.*, **107**, 2182 (1966).
5) E. Csányi, *Arzneim.-Forsch.*, **14**, 668 (1964).

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