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105. Morio Ikehara, Nobuhiko Nakazawa, and Hiroshi Nakayama : Potential Antimetabolites. III.[*1] Synthesis of 5-Aminoimidazole-4-carboxthioamide and Nitro-1-β-D-ribofuranosylimidazoles.

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In order to obtain effective metabolite antagonists, various types of compounds have been synthesized. Many of these compounds exert their activity by interference with the synthetic pathway of inosinic acid *de novo*.[1] For example 6-mercaptopurine seems to inhibit the coures of inosinic acid to adenylic or guanylic acid by deactivation of the enzymes working in this step.[2,3]

Because of interest in this type of antagonists, attempt was made to synthesize 5-amino-4-imidazolecarboxthioamide (I) and 4-substituted 1-β-D-ribofuranosyl-5-nitroimdazoles (IIa, b). Compound (I) is expected to antagonize inosinic acid synthesis by inhibiting the enzyme of this step or by direct conversion to 6-mercaptopurine. Compound (II) may become the antagonist of 5-aminoimidazole- or 5-amino-4-imidazolecarboxamide ribotide synthesis.

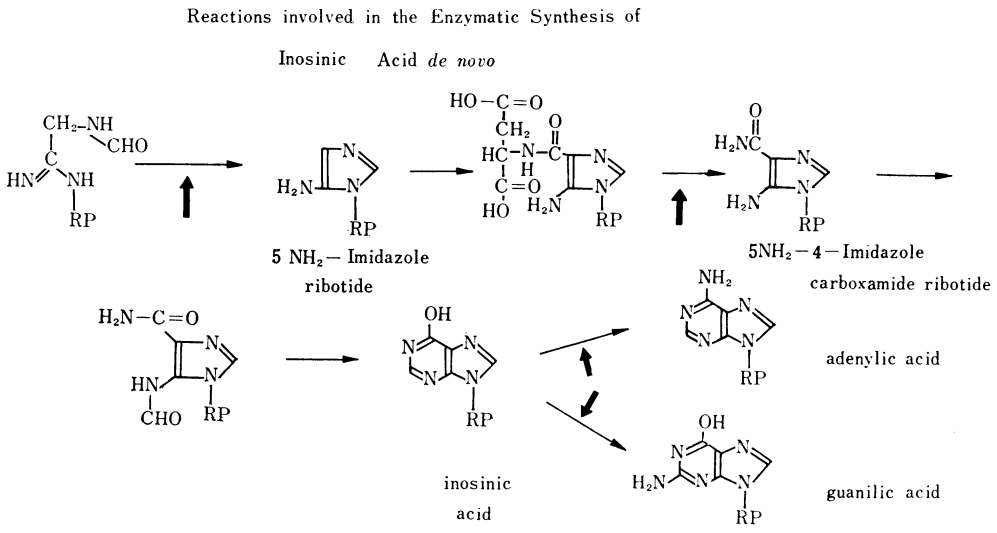

Chart 1.

Although Shaw[4] synthesized (I) by the reaction of α-amino-α-cyanothioacetamide and isopentylformimidate, no definite nature of this compound was given. Hitchings and Elion[5] postulated this compound as the intermediate of 6-mercaptopurine synthesis, but they did not isolate this compound as such.

*1 Part II : Y. Mizuno, M. Ikehara, and F. Ishikawa : This Bulletin, **10**, 653(1962).

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1) "The Chemistry and Biology of Purines," CIBA Foundation Symposium, 243, (1957) Churchill, London.
2) *Ibid.*, 286(1957).
3) J. S. Salser, D. H. Hutchison, M. E. Balis : J. Biol. Chem., **235**, 429 (1960).
4) G. Shaw, D. N. Butler : J. Chem. Soc., **1959**, 4040.
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As to the thiolation of both hydroxy-purine[6] and -pyrimidine,[7] phosphorus pentasulfide in pyridine was proved to be an excellent reagent and was utilized in thiolation of 5-aminoimidazole-4-carboxamide[8] (III) to compound (I).

The hydrochloride of (III) was treated with phosphorus pentasulfide in pyridine at reflux temperature for 2 hours. Because of the high solubility of resulting product in water and its hygroscopic nature, its isolation was quite troublesome. This was achieved by successive removal of hydrogen sulfide and phosphoric acid with barium hydroxide. Resulting product melted at 180~185° with decomposition and had ultraviolet absorption maxima at 324 and 287 mμ, which indicates the presence of thiocarbonyl group in the imidazole ring. Paper chromatography showed a spot at Rf 0.70, higher than that of starting material (0.58) and gave a different color of orange-red from that of (III) (blue) by the Pauly's test.[9] Grote's reagent[10] gave no marked reaction. (I) was hydrolyzed to (III) by the treatment with *N* hydrochloric acid and cyclized to hypoxanthine (IV), rather than 6-mercaptopurine (V), by the reaction with formic acid-acetic anhydride mixture, followed by alkaline cyclization. This was observed by the shift of ultraviolet absorption maximum from 324 to 288 mμ in the first step and appeared to 250 mμ by alkaline cyclization. This might be consonant with the formylation reaction and hydrolysis of thiocarbonyl group prior to cyclization.

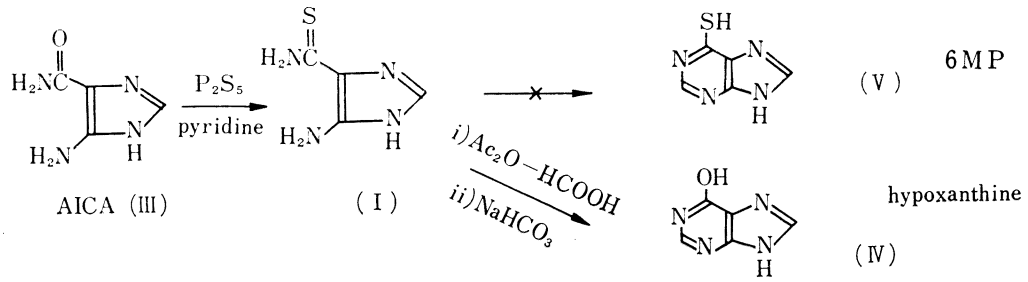

Chart 2.

These facts together with the elementary analysis data, confirmed the structure of this substance as 5-aminoimidazole-4-carboxthioamide. (I) was further transformed to an unknown compound with absorption maximum at 286 mμ resembling that of 2,4-diaminothiazole-5-carboxamide synthesized by the known procedure, and did not cyclized to hypoxanthine by treatment analogous to that mentioned above.

The synthesis of 1-β-D-ribofuranosyl-4-methyl-5-nitroimidazole (II) was attemted next from 4-methylimdazole zinc salt,[11] which was nitrated with nitric acid-sulfuric acid mixture. Resulting nitro derivative[12] (VI) was converted to mercury chloride complex (VII) and condensed with 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl chloride[13] in boiling xylene. Orientation of the attachment of riboside residue would be predicted by analogous reactions[14,15] as directing both to N_1 and N_3. Resulting protected riboside (VIII) was purified by chromatography on alumina. Only one crystalline substance, m.p.

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164~165°, was obtained from the middle fraction, preceded by the recovery of some sugar, in a yield of 44%. Considering relatively low yield of usual ribosidation reaction, this substance may be the main product. (Ⅷ) was debenzoylated by the action of methanol saturated with ammonia at 0° for 4 days. Riboside (Ⅱa) thus obtained was purified by paper chromatography and had ultraviolet absorption maxima at 304 (neutral), 303 (HCl), and 309 mμ (NaOH). Compared with those of known imidazole derivatives in the Table I, the optical behavior of (Ⅱa) suggests that this compound is the desired 5-nitro-4-methylimidazole riboside.

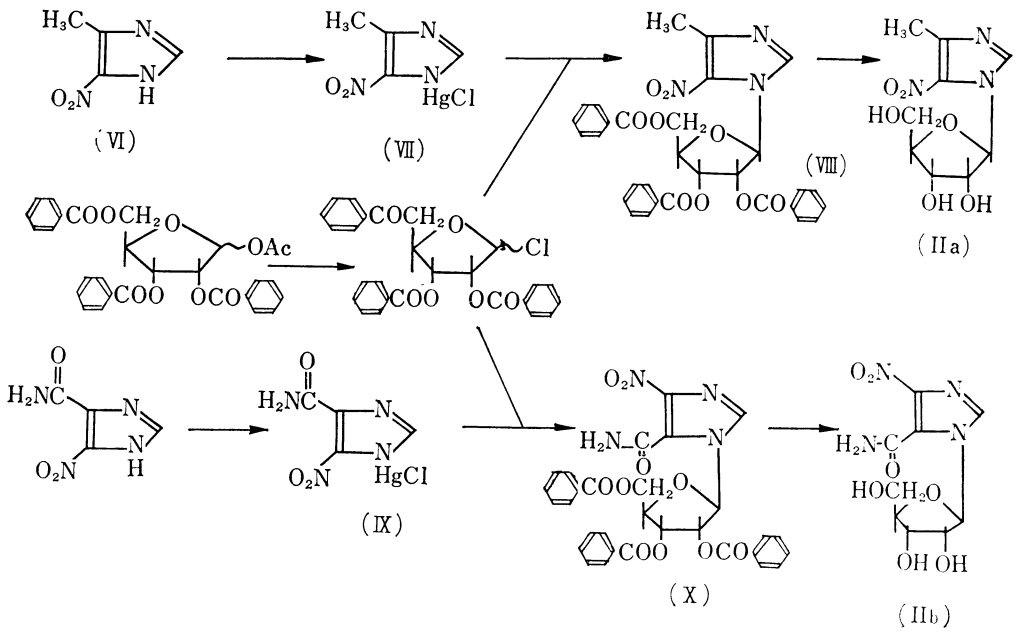

Chart 3.

This result leads to a postulation that the ribosidation reaction proceeded at least by the mechanism other than those described in alkaline methylation of imidazoles.[15]

The chloromercury salt of 4(5)-nitro-5(4)-methoxycarbonylimidazole (Ⅸ) with benzoyl-ribofuranosyl chloride gave 1-(2′,3′,5′-tri-O-benzoyl)-β-D-ribofuranosyl-4-nitro-5-methoxycarbonylimidazole (X) as the main product. This was deprotected by a treatment analogous to that of (Ⅱa) to afford ribofuranosyl-4-nitro-5-methoxycarbonylimidazole (Ⅱb), 148~150°, and identified by comparison of ultraviolet absorption maximum at 292 mμ with the data in the literature.[14] In this case the comparable electron-attracting tendencies of nitro and carbonyl groups attached to 4(5)-position of imidazole ring made the orientation of the attack of ribosyl moiety reversed.

Table I.

Compound	Ultraviolet absorption maximum (mμ)		
	pH 1	pH 7	pH 10
1-CH_3-4-NO_2-5-$CONH_2$[14]	300	299	300.5
1-CH_3-5-NO_2-4-$CONH_2$[14]	303.5	303.5	304.5
1-Rf-4-NO_2-5-$CONH_2$[14]	295	294	295.5
1-Rf-5-NO_2-4-$CONH_2$[14]	304	301	301
1-CH_3-5-NO_2[15]	266	303	
1-CH_3-4-NO_2[15]	300	269	
1-Rf-5-NO_2-4-CH_3	303	304	309

Considering these facts, together with the results obtained in benzimidazole series, Grimison's studies[15] on the orientation of imidazole ring are valid in chloromercury derivatives by assuming an alternate mechanism of substitution.

None of the products described above was effective against Ehrlich ascites tumor.

Experimental

5-Aminoimidazole-4-carboxthioamide (I)——A solution of 500 mg. of 5-aminoimidazole-4-carboxamide hydrochloride[8] dissolved in 50 cc. of dehyd. pyridine, by the addition of 2.5 g. of P_2S_5, was refluxed for 3 hr. with stirring. The whole was poured in a stream into 300 cc. of boiling H_2O in order to decompose unreacted P_2S_5. After 20 min. of boiling, the solution was filtered and the filtrate was evaporated in a reduced pressure. The residue was a red heavy oil, which was dissolved in dil. NH_4OH and extracted with Et_2O (50 cc. × 3) to remove contaminated Pyridine. Aqueous layer was neutralized with a equivalent amount of $Ba(OH)_2$ solution (containing 10 g. of $Ba(OH)_2$) and barium phosphate precipitated. The supernatant and washings were collected by centrifugation and evaporated *in vacuo*. Yellow glassy substance thus obtained was taken up in dehyd. MeOH and decolorized with active charcoal. Evaporation of MeOH gave a glassy residue which was triturated with dehyd. Et_2O to give orange amorphous powder, m.p. 180~185°(decomp.). This was found to have N and S by qualitative test and gradually converted to orange glass by atmospheric moisture. *Anal.* Calcd. for $C_4H_6N_4 \cdot \frac{1}{2}BaS$: C, 34.63; H, 4.22; N, 26.88. Found : C, 35.48; H, 4.25; N, 28.03. UV $\lambda_{max}^{H_2O}$ mμ : 324, 272; $\lambda_{min}^{H_2O}$ 287 mμ. Paper chromatography (solvent; PrOH-0.2*N* NH_3=2:1) Rf 0.70 (Pauly's test, orange). Cochromatography of 5-amino-4-imidazolecarboxamide : Rf 0.58 (blue). Grote's reagent gave no reaction. The optical behavior of (I) eluted from the spot was identical with the above data. Picrate; Recrystallized from EtOH-H_2O, m.p. 235~237°. *Anal.* Calcd. for $C_{10}H_9N_7O_7S$: C, 30.07; H, 2.26. Found : C, 29.44; H, 2.48.

Acid Hydrolysis of 5-aminoimidazole-4-carboxthioamide——Small amount of (I) was dissolved in *N* HCl and heated on a boiling water bath for 30 min. UV : λ_{max} at 324 mμ disappeared and λ_{max} at 207 mμ increased. Pauly's test gave a red color. These evidences suggested the hydrolysis of CS group to CO.

Attempted Cyclization of (I) to Hypoxanthine——Ten miligrams of (I) was refluxed in Ac_2O-HCOOH (98%) mixture (1:1) for 5 hr. ultraviolet absorption maximum at 304 mμ shifted to 288 mμ during this reaction. The solvent was evaporated in a reduced pressure, the residue was redissolved in 0.1*N* $KHCO_3$ (5 cc.), and refluxed for 30 min. ultraviolet absorption maximum at 250 mμ increased, showing the cyclization to hypoxanthine.

4(5)-Nitro-5(4)-methylimidazole (VI)——Suspension of 30 g. of methylimidazole zinc salt[11] in 200 cc. of H_2O was acidified with conc. H_2SO_4 to obtain a clear solution. The addition of saturated Na_2S produced precipitation of ZnS, which was removed by centrifugation. The supernatant was evaporated to dryness at 60~65° in a reduced pressure and the residue was dissolved in 120 cc. of conc. H_2SO_4. Nitration was carried out by the dropwise addition of 70 cc. of fuming HNO_3 (*d* 1.50) at 39~40.° After 3 hr., the whole was poured into 200 cc. of ice-water and neutralized with Na_2CO_3. Yellow precipitate was collected and the filtrate was evaporated to dryness. Extracts from the residue were combined with the above precipitate and crystallized from H_2O; m.p. 242~243° (Yield, 7 g.). UV : λ_{max} 305 mμ. *Anal.* Calcd. for $C_4H_5O_2N_3$: C, 37.90; H, 3.94; N, 33.07. Found : C, 38.72; H, 3.97; N, 33.43.

Chloromercury Salt of 4(5)-Nitro-5(4)-methylimidazole (VII)——To a solution of 2.0 g. of nitromethylimidazole (VI) dissolved in 200 cc. of warm EtOH containing 0.7 g. of NaOH, 4.70 g. of $HgCl_2$ (1.1 mole) in 50% EtOH, was added. Cooling of this solution gave a pale yellow precipitate, which was washed with H_2O, EtOH, and Et_2O, and dried. Yield, 4.4 g. (73%). *Anal.* Calcd. for $C_4H_4O_2N_3 \cdot HgCl$: N, 11.60. Found : N, 11.75.

1-(2′, 3′, 5′-Tri-O-benzoyl)-β-D-ribofuranosyl-4-methyl-5-nitroimidazole (VIII)——From 3.6 g. of 4(5)-methyl-5(4)-nitroimidazole chloromercury salt suspended in 150 cc. of xylene, 50 cc. was evaporated off. 2,3,5-Tri-O-benzoyl-D-ribofuranosyl chloride[13] (from 5.0 g. of 1-O-acetate) in 100 cc. of xylene was added and refluxed for 45 min. with vigorous stirring. The reaction mixture once became clear and precipitate appeared again. Filtered solution was evaporated below 60° and a glassy residue was purified by chromatography on alumina (acid treated). Elution with benzene-$CHCl_3$ (3:1) gave 2.4 g. of N-containing substance, which was crystallized from MeOH, m.p. 164~165°. *Anal.* Calcd. for $C_{30}H_{25}O_9N_3$: C, 63.01; H, 4.38; N, 7.35. Found : C, 62.50; H, 4.51; N, 7.05.

1-β-D-Ribofuranosyl-4-methyl-5-nitroimidazole (IIa)——A solution of 500 mg. of the above protected nucleoside (VIII) dissolved in 70 cc. of dehyd. MeOH was saturated with NH_3. Further bubbling of NH_3 gave a clear solution, which was set aside for 4 days at 0°. Some precipitated material was filtered off and the filtrate was evaporated *in vacuo*. Resulting yellow oil was dissolved in H_2O and

extracted with $CHCl_3$ to remove benzamide. H_2O-layer was evaporated in a reduced pressure below 60°. The residual glassy material was tested by paper chromatography (solvent, dil. NH_4OH), which gave a single spot at Rf 0.7. The positive test by IO_4^--benzidine spray[17] of the spot visualized by ultraviolet lamp showed the presence of cis-glycol system in the molecule of imidazole derivative. Elution of this spot with H_2O gave following absorption : UV : $\lambda_{max}^{pH\,1}$ 303, $\lambda_{max}^{pH\,7}$ 304, and $\lambda_{max}^{pH\,13}$ 309 mμ. This was identical with those taken from above glass (Yield, 120 mg.). Despite many efforts this substance could not be crystallized as yet.

4(5)-Nitro-5(4)-methoxycarbonylimidazole Chloromercury Salt (IX)——To a suspension of 4(5)-nitro-5(4)-methoxycarbonylimidazole[18] in 300 cc. of 50% MeOH, 1.85 g. of Na_2CO_3 dissolved in 200 cc. of H_2O was added. Into this clear solution 20 cc. of MeOH containing 4.73 g. of $HgCl_2$ was also added. Precipitation occurred after some stimulation on the wall of vessel by glass rod. Centrifugation, washing with H_2O, and drying gave 4.25 g. (70%) of the chloromercury salt.

1-(2′,3′,5′-Tri-O-benzoyl)-β-D-ribofuranosyl-4-nitro-5-methoxycarbonylimidazole (X)——A suspension of 6.0 g. of the above powdered Hg salt in 300 cc. of xylene were azeotropically dried by distillation of xylene. 2,3,5-Tri-O-benzoylribofuranosyl chloride (from 6.3 g. of 1-O-acetyl compound) in 73 cc. of xylene was added and refluxed for 2 hr. Solvent was evaporated off and the residue was taken up in $CHCl_3$. Resulting glassy residue obtained by evaporation of $CHCl_3$ was dissolved in benzene (15 cc.) and applied to alumina chromatography. After the recovery of 1.0 g. of protected sugar, a crystalline substance, m.p. 109~111° (MeOH), was obtained. Recrystallization from MeOH gave colorless prisms, m.p. 116~118° (250 mg.). UV λ_{max}^{EtOH} mμ : 232, 274. *Anal.* Calcd. for $C_{31}H_{25}O_7N_3 \cdot 3H_2O$: C, 61.15; H, 5.16; N, 6.90. Found : C, 60.67; H, 4.26; N, 6.86.

1-β-D-Ribofuranosyl-4-nitro-5-methoxycarbonylimidazole (IIb)——A solution of 100 mg. of above protected nucleoside (X) dissolved in 10 cc. of MeOH previously saturated with NH_3 at 0° was kept for 2 days at room temperature, solvent was removed by vacuum distillation and the residue was taken up in H_2O. This was washed with $CHCl_3$ and evaporated to give 30 mg. of residue, which was recrystallized from EtOH to white amorphous solid, m.p. 148~150°. This did not coincide with Baddiley's description.[14] UV : λ_{max}^{EtOH} 292 mμ, which indicates this is 4-nitro isomer.

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Summary

5-Aminoimidazole-4-carboxthioamide was synthesized by the phosphorus pentasulfide-pyridine treatment of 5-aminoimidazole-4-carboxamide. The structure was elucidated both by chemical and physical means. 1-β-D-Ribofuranosyl-4-methyl-5-nitroimidazole and 1-β-D-ribofuranosyl-4-nitro-5-methoxycarbonylimidazole were synthesized by the condensation of appropriate imidazole chloro-mercury salt with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride followed by the removal of protecting groups.

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