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# **Morio Ikehara**: Studies on Coenzyme Analogs. II. A Convenient Synthesis of "Thioinosine."

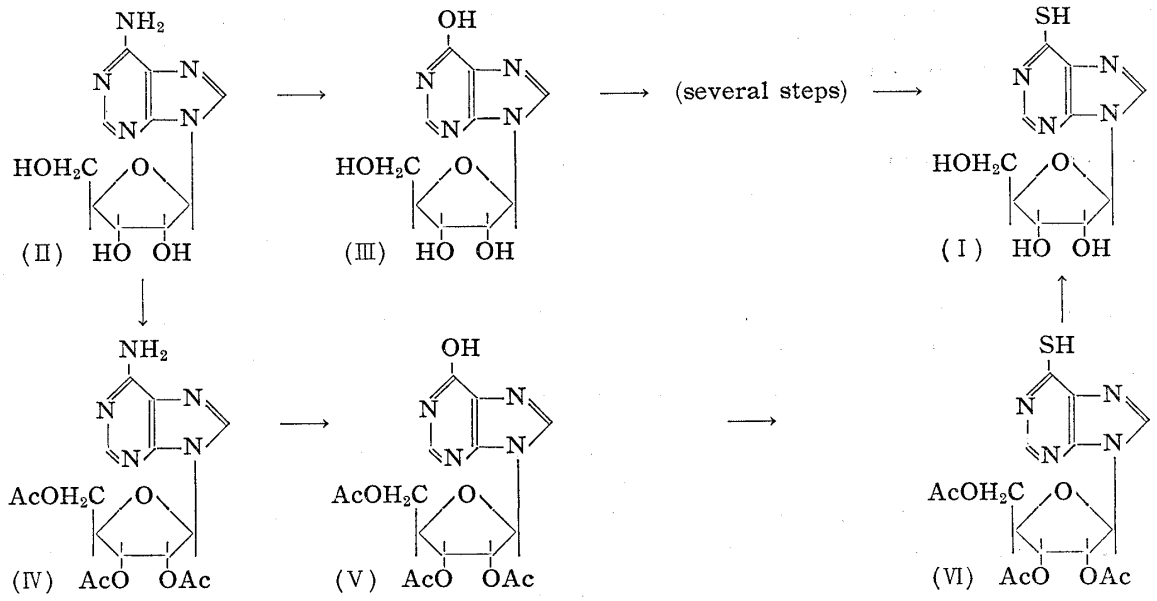
(*Faculty of Pharmacy, Medical School, University of Hokkaido*[*1])

It is important in nucleotide synthesis to obtain the key intermediate, 6-mecapto-9-β-D-ribofuranosylpurine (thioinosine) (I), by easily accessible procedure.

Originally, adenosine (II) was converted to inosine (III) in hydrous acetic acid with the aid of sodium nitrite or barium nitrite,[1] and after protection of the OH group by acylation, thiolated to thioinosine.[2] It is quite tedious to separate inosine from inorganic salts in this procedure, because of the same solubility of both in water or in other organic solvents.

To avoid this, a new route is described in this communication. First, adenosine was acetylated to 2',3',5'-tri-O-acetyladenosine (IV),[3] which could be extracted with chloroform, crystallized well from ethanol, and was more easily soluble in acetic acid-water mixture (solvent of next step) than tribenzoate. (IV) was dissolved in above mixture and deaminohydroxylated[*2] in 6-position with sodium nitrite. 2',3',5'-Tri-O-acetylinosine (V) produced was easily extracted and recrystallized from ethanol after evaporation of the solvent.

Well dried (V) was then thiolated by treatment with phosphorus pentasulfide in pyridine and afforded a new compound, 2',3',5'-tri-O-acetylthioinosine (VI), m.p. 198~200°. When (VI) was deacetylated with methanolic ammonia, thioinosine (I) and tri-O-acetyladenosine were obtained. (I) was obtained in a good yield by deacetylation with sodium methoxide in anhydrous methanol. When the synthesis of 6-substituted purine-nucleoside is required, acetylated methylthioinosine can be reacted directly with various nucleophilic reagents. The synthesis of several nucleosides along this line will be reported later.




*1 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (池原森男).

*2 $NH_2 \rightarrow OH$ conversion has been incorrectly termed "deamination." J. F. Bunnett's nomenclature was used (J. Chem. Soc., **1954**, 4717).

1) P. A. Levene: J. Biol. Chem., **111**, 313(1935); J. M. Gulland, E. R. Holiday: J. Chem. Soc., **1936**, 765; J. Davoll: J. Chem. Soc., **1948**, 1685.
2) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr: J. Am. Chem. Soc., **80**, 1669(1958).
3) H. Bredereck: Ber., **80**, 401(1947).

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## Experimental

**2′,3′,5′-Tri-O-acetyladenosine (IV)**—Five g. of adenosine[4] (dried over $P_2O_5$ for 5 hr. at 3 mm./Hg) was added to 70 cc. of pyridine under vigorous stirring. When 5 g. of $Ac_2O$ was added at room temperature, suspended adenosine dissolved gradually to form a yellow solution. After 1.5 hr. of stirring, the insoluble amorphous solid was removed by filtration (recovered as adenosine) and the filtrate was evaporated below 50° *in vacuo*. Residual syrup was taken up in $CHCl_3$, dried ($Na_2SO_4$), evaporated *in vacuo*, and recrystallized from EtOH to 4.1 g. of (IV), m.p. 166～167°. $\lambda_{max}^{EtOH}$ 258.0 mμ ($\lambda_{max}$ of 6-acetamino compound, 283～284 mμ).

**2′,3′,5′-Tri-O-acetylinosine (V)**—A clear solution was obtained from 1.5 g. of (IV) dissolved in AcOH (4 cc.)-$H_2O$ (15 cc.) mixture, 0.5 g. of $NaNO_2$ was dissolved into this and the mixture was set aside at room temperature for 48 hr. Gentle bubbling of the yellow reaction mixture almost ceased at the end of this period. UV absorption spectrum after 20 hr. showed $\lambda_{max}$ at 244.9 mμ. The solvent was removed *in vacuo* and the residue was extracted with hot dehyd. EtOH. Small, white needles, m.p. 236～238°, were obtained (800 mg.), which was identical with a sample derived from inosine. When recrystallization was difficult, $CHCl_3$-extraction was employed for total removal of AcOH and pyridine.

**2′,3′,5′-Tri-O-acetylthioinosine (VI)**—300 mg. of (IV) was dissolved in dry pyridine and 800 mg. of $P_2S_5$ was added. The whole was refluxed for 4 hr. until two layers, upper yellow and lower orange, separated. Both were poured into boiling water in order to destroy $P_2S_5$. Granular precipitate was collected by decantation, triturated with EtOH-$H_2O$, and filtered. Recrystallization from EtOH-$Me_2CO$ gave 250 mg. of (VI), m.p. 214～216°. Mixed fusion with (V) melted at 205～208°. *Anal.* Calcd. for $C_{16}H_{18}O_7N_4S$: C, 46.88; H, 4.64; N, 13.67. Found: C, 47.26; H, 4.65; N, 13.21. $\lambda_{max}^{EtOH}$ 315 mμ, $\lambda_{min}^{EtOH}$ 250 mμ.

**Deacetylation of (VI) with MeOH-$NH_3$**—A solution of 100 mg. of (VI) dissolved in 20 cc. of MeOH was saturated with dry $NH_3$ at 0°. The whole was kept standing for 24 hr. at room temperature. Evaporation of the solvent under reduced pressure afforded a syrup containing colorless crystals (i) (10 mg.). When the syrup was triturated with MeOH, 35 mg. of (VI) was recovered. From the mother liquor, 20 mg. of thioinosine (VII) was obtained, which was confirmed by direct comparison with an authentic sample. (i) was found to be triacetyladenosine, m.p. 166°, $\lambda_{max}$ 259 mμ.

**Deacetylation of (VI) with NaOMe**—2.5 g. of (VI) was dissolved in 185 cc. of anhyd. MeOH and 4 cc. of *N* NaOMe was added. On refluxing for 4.5 hr., a slightly yellow solution formed. The solvent was removed by evaporation *in vacuo*, 15 cc. of $H_2O$ was added, and adjusted to pH 8.5 with AcOH. This solution was extracted with $CHCl_3$ and pH was adjusted to 4～5 (at this stage ca. 15 cc.). Brownish white precipitate which appeared was collected and dried. Recrystallization from EtOH gave cream-colored crystals, m.p. 208～210° (800 mg.), which was identified as thioinosine by direct comparison with an authentic sample.

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4) Y. Mizuno, K. Nakamura, T. Ueda: Yakugaku Zasshi, **77**, 683(1957).

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## Hiroshi Igeta: Syntheses of Pyridazine Derivatives. IV.[1] Reaction of 3,6-Dimethoxypyridazine 1-Oxide with Phosphoryl Chloride.

(*National Hygienic Laboratory*[*1])

As reported in Part III[1] of this series, the reaction of 3-methoxypyridazine 1-oxide, in which the neighboring position of the N-oxide group is unsubstituted, with phosphoryl chloride yielded 3-methoxy-6-chloropyridazine. The present paper is a report on the reaction of 3,6-dimethoxypyridazine 1-oxide, in which *ortho* position of the N-oxide group is substituted with methoxyl group, with phosphoryl chloride.

When 3,6-dimethoxypyridazine 1-oxide[2] (I) was treated with phosphoryl chloride at room temperature, a chloro-dimethoxyl compound (II) was obtained. This chloro-dimethoxypyridazine was reacted with sodium methoxide in methanol and produced a trimeth-

*1 Tamagawa-Yogamachi, Setagaya-ku, Tokyo (井下田 浩).

1) Part III: This Bulletin, **7**, 938(1959).

2) Part II. T. Itai, H. Igeta: Yakugaku Zasshi, **75**, 996(1955).

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