the table. Cadmium chloride was also given in drinking water and the metals in metallothionein fractions were analyzed in the same way. Copper was again one of the major metals (VII—VIII in the table), but none of the three metals was found in metallothionein fractions in the case of control animals (IX in the table). Figurelis a typical Sephadex G-75 elution pattern of the kidney supernatant from the sample IV in the table. Thus, the content of copper in kidney metallothionein was found extremely high regardless of the methods of cadmium exposure. This is the marked difference from the result of equine kidney metallothionein,[1] an example of environmental cadmium exposure. The metal contents of liver metallothionein fractions of experiments I to VIII in the table were also analyzed in the same way. Although zinc and cadmium were found as major metals in every experiment, copper was a minor metal (less than 2—3% of cadmium content by weight per cent) in liver metallothionein fractions. As a typical example, the metal contents of liver metallothionein in experiment IV were shown as following; zinc 2.08×10^{-7}, cadmium 1.63×10^{-7}, and copper 0.02×10^{-7} mol/g wet tissue, respectively.

Our results can be summarized as follows. i) The cupric ion has stronger affinity to metallothionein than zinc and cadmium ions. ii) The copper content in cadmium-exposed rat kidney metallothionein fractions is very high. iii) On the other hand, the copper content in cadmium-exposed rat liver metallothionein is very low.

These results suggest that much attention should be focused on the toxicity of metallothionein in kidney in relation to copper, especially the possible demasking effects of copper to the masked cadmium toxicity as metallothionein.

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Syntheses of Heterocycles *via* Intramolecular Cycloaddition of Azahexatrienes. Photochemical Cyclization of 5-Arylazo-6-dimethylaminomethyleneamino-1,3-dimethyluracils

Photochemical cyclization of 5-arylazo-6-dimethylaminomethyleneamino-1,3-dimethyluracils (I), 1,2,5-triazahexatriene-type precursors, under aerobic condition resulted in the formation of 6-aryl-1,3-dimethyl-6,7-dihydro-6-azalumazine-7-ones. On the other hand, the photolysis of I under anaerobic condition gave 8-arylaminotheophyllines.

Keywords——Intramolecular cycloaddition; azahexatriene; photolysis; 6-azalumazine-7-one; 8-arylaminotheophylline; 5-arylazo-6-dimethylaminomethyleneamino-1,3-dimethyluracil

Recent investigations in this laboratory have established the novel synthetic method for preparation of heterocycles such as purines,[1] pteridines[2,3] and pyrazolo[3,4-*d*]pyrimidines[1,4] by intramolecular cycloaddition of aza analogs of hexatriene. This paper describes

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the photochemical cycloaddition of 5-arylazo-6-dimethylaminomethyleneamino-1,3-dimethyluracils, which are attractive candidates for the 1,2,5-triazahexatriene-type precursors, under both aerobic and anaerobic conditions.

A solution of 5-arylazo-6-dimethylaminomethyleneamino-1,3-dimethyluracils (Ia and Ib)[1] (0.5 g) in benzene (500 ml) in a Pyrex flask was exposed to the direct sunlight (Kumamoto, June, unclouded) at ambient temperature for 10 hr or irradiated with a 100 W high-pressure mercury lamp at room temperature for 40 hr under aerobic condition. Removal of the solvent and recrystallization of the residue from ethanol gave 6-phenyl- (IIa, mp 219°) and 6-*m*-tolyl-1,3-dimethyl-6,7-dihydro-6-azalumazine-7-one (IIb, mp 221°) in 48 and 53% yields, respectively. The structures of IIa and IIb were confirmed by comparison of the products with authentic samples prepared by the condensation of the corresponding 6-amino-5-arylazo-1,3-dimethyluracils (IIIa and IIIb) with urea at 180° for 1 hr.

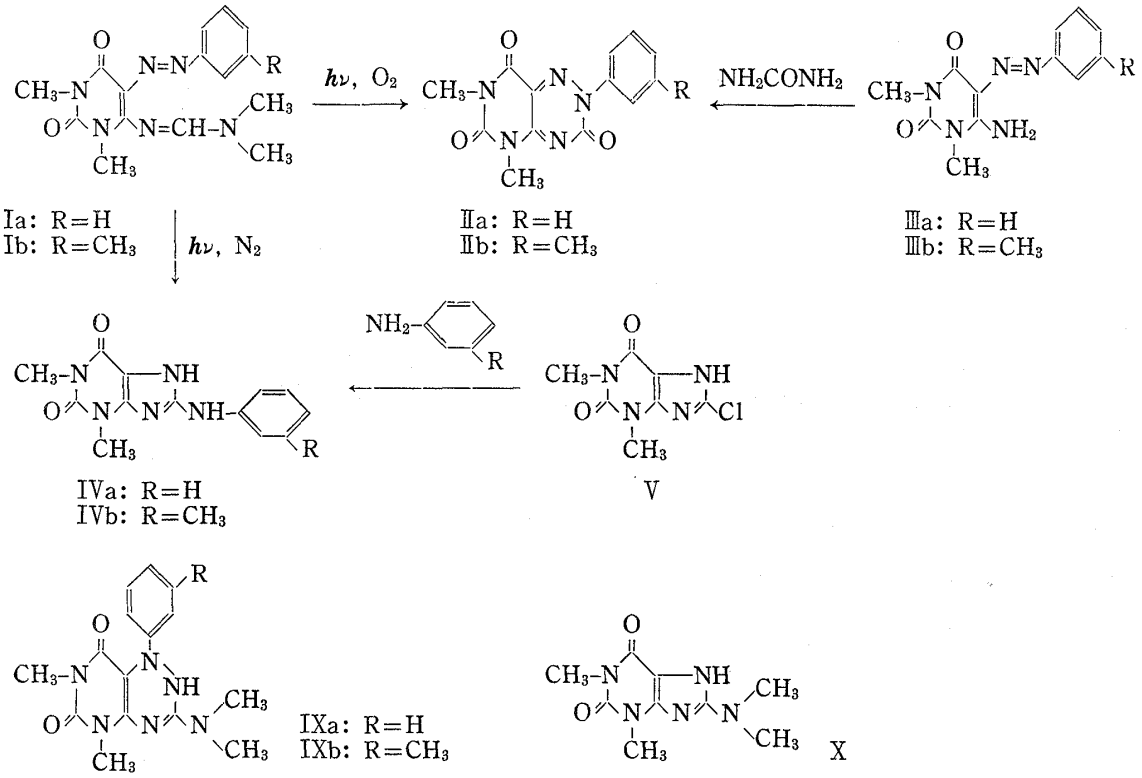

Chart 1

The photolysis of Ia and Ib under the same conditions except anaerobic circumstance resulted in the exclusive formation of 8-anilino- (IVa, mp >300°)[5] and 8-*m*-toluidino-theophylline (IVb, mp >300°) in 45 and 40% yields, respectively. These were identical in all respects with authentic samples prepared by the reaction of 8-chlorotheophylline (V) with aniline and *m*-toluidine.

The above reactions probably involve the initial formation of the intermediary 6-aryl-7-dimethylamino-1,3-dimethyl-5,6-dihydro-6-azalumazine (VI) by intramolecular cyclization and subsequent hydrogen transfer. The formation of II is well rationalized by a pathway involving photooxygenation of VI to a hydroperoxide intermediate (VII), followed by elimination of an oxygen atom and then dimethylamine. Under anaerobic condition, the key intermediate (VI) could undergo photo-induced rearrangement to 8,8-disubstituted theophyllines (VIII) followed by photohydrogenation and elimination of dimethylamine to give the corresponding 8-arylaminotheophyllines (IV).

5) K. Senga, M. Ichiba, H. Kanazawa, S. Nishigaki, M. Higuchi, and F. Yoneda, *Synthesis*, **1977**, 264.

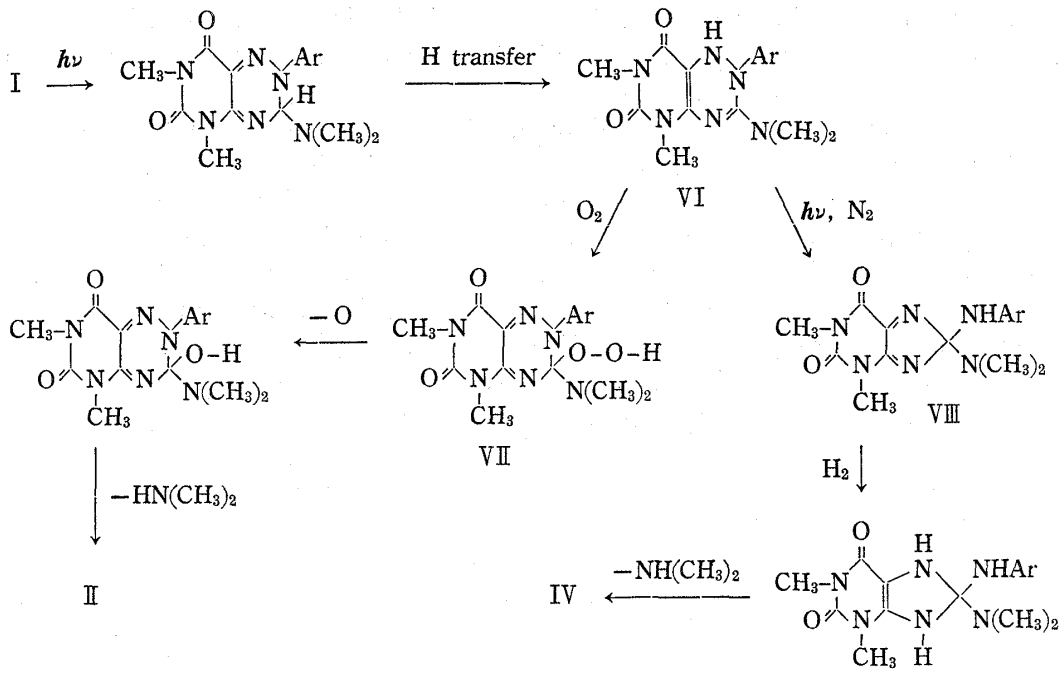

Chart 2

It is interesting to note that the thermal cyclization of Ia and Ib gave the corresponding 5-aryl-7-dimethylamino-1,3-dimethyl-5,6-dihydro-6-azalumazines (IXa and IXb) and 8-dimethylaminotheophylline (X).[1)]

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Quantitative Determination of Hydrazines derived from Isoniazid in Man

Hydrazine formation took place in man after the oral administration of isoniazid. The excreted amount of free hydrazine in human urine was determined quantitatively by the new method, *i.e.* mass fragmentography using ^{15}N-hydrazine as an internal standard. The unchanged isoniazid and the other metabolites, acetylisoniazid, monoacetylhydrazine and diacetylhydrazine were simultaneously analyzed.

Keywords——isoniazid–antituberculosis drug; drug metabolism; determination of free hydrazine; human urine; mass fragmentography; internal standard–^{15}N-hydrazine

Because of toxicity, mutagenicity and carcinogenicity of hydrazine, the fate of hydrazine moiety of isoniazid (INH) *in vivo* has drawn attention of some researchers since INH