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Although the reaction of caffeine with hydrogen peroxide/hydrochloric acid or nitric acid and then with ammonia has been known to give a purple coloration (Murexide reaction), the use of hydrazine instead of ammonia is found to provide no purple coloration. The reaction of caffeine with hydrogen peroxide/hydrochloric acid and then with hydrazine hydrate afforded a yellow reaction mixture, from which 4-methyl-6-(*N*-methylcarbamoyl)-3,5-dioxo-2,3,4,5-tetrahydrotriazine **9**, oxalyl hydrazide **10** and hydroxylamine hydrochloride were isolated. The reaction of caffeine with nitric acid and then with hydrazine hydrate furnished a yellow reaction mixture, from which 8-amino-1,3,7-trimethyl-2,6-dioxo-1*H*,3*H*,7*H*-xanthine **11**, **9** and hydroxylamine nitrate were isolated. Compound **9** was clarified to be produced from 3-hydroxy-4,6-dimethyl-oxazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione **3** and 1,3-dimethylalloxan **7** by the ring transformation with hydrazine.

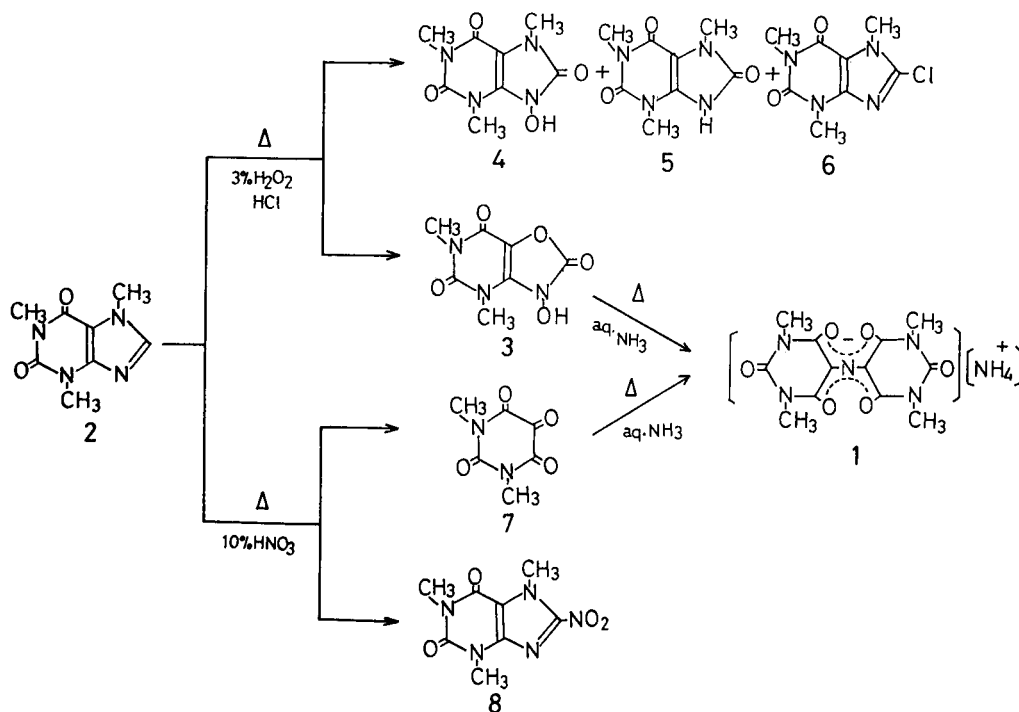
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### Introduction.

The murexide reaction has been used to detect uric acid and related purines, wherein the purine compounds are oxidized first with hydrogen peroxide/hydrochloric acid or nitric acid and then treated with ammonia to give a purple coloration. This purple coloration has been found to be due to the formation of murexoin **1** [1]. In previous papers, we clarified that the oxidation of caffeine **2** with hydrogen

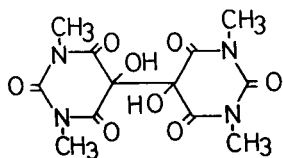
peroxide/hydrochloric acid provided 3-hydroxy-4,6-dimethyl-oxazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione **3**, 9-hydroxy-1,3,7-trimethyl-2,6,8-trioxo-1*H*,3*H*,7*H*-xanthine **4**, 1,3,7-trimethyl-2,6,8-trioxo-1*H*,3*H*,7*H*-xanthine **5** and 8-chloro-1,3,7-trimethyl-2,6-dioxo-1*H*,3*H*,7*H*-xanthine **6** [2], and the oxidation of caffeine with nitric acid furnished 1,3-dimethylalloxan **7** and 1,3,7-trimethyl-8-nitro-2,6-dioxo-1*H*,3*H*,7*H*-xanthine **8** [3]. Treatment of **3** or **7** with am-

Scheme 1



monia produced the purple colored substance murexoin **1** (Scheme 1). Concerning the purple coloration, amalic acid **A** (Chart 1) has been reported to be an intermediate to murexoin **1** [4], but we rejected a contribution of amalic acid **A** as an intermediate to murexoin **1** [5].

Chart 1



A

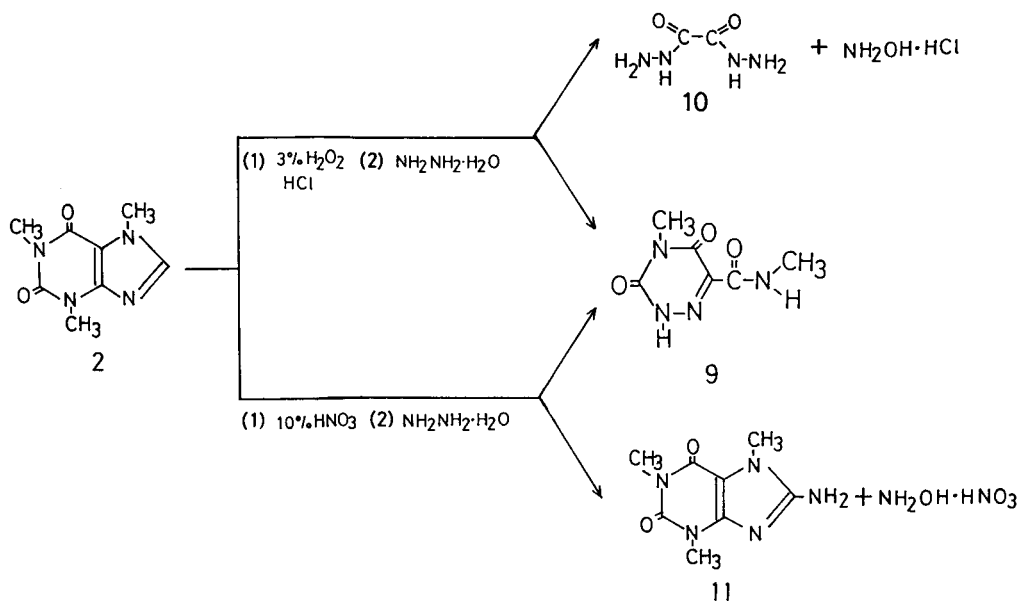
On the other hand, we examined the use of various amines such as aliphatic or aromatic amines in place of ammonia as a color reagent in the murexide reaction, but our examination confirmed that ammonia was superior to other amines. However, hydrazine has not been employed yet as a color reagent in the murexide reaction. Accordingly, we studied the reactions of foregoing oxidized intermediates **3** and **7** with hydrazine, since such reactions were postulated to form the dimer **B** (Chart 2) which would provide a specific coloration. In contrast, these reactions

neither produced the dimer **B** nor provided a specific coloration, but we found an interesting ring transformation of compounds **3** and **7** into the 1,2,4-triazine **9**. This paper describes the above ring transformation and all products in the reaction of caffeine with hydrogen peroxide/hydrochloric acid or nitric acid and then with hydrazine.

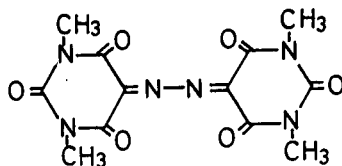
### Results and Discussion.

A mixture of caffeine, hydrogen peroxide and hydrochloric acid was heated to dryness on a boiling water bath to give a yellowish red oily substance [2], whose reaction with hydrazine hydrate afforded a yellow reaction product yielding three compounds, 4-methyl-6-(*N*-methylcarbamoyl)-3,5-dioxo-2,3,4,5-tetrahydrotriazine **9**, oxalyl hydrazide **10** and hydroxylamine hydrochloride, although compounds **3**, **4**, **5** and **6** were not isolated. On the other hand, the oxidation of caffeine with nitric acid under similar conditions to the above also provided a yellow oily substance [3], whose reaction with hydrazine hydrate furnished a yellow reaction product yielding three compounds, compound **9**, 8-amino-1,3,7-trimethyl-2,6-dioxo-1*H*,3*H*,7*H*-xanthine **11** and hydroxylamine nitrate, although compounds **7** and **8** were not obtained. These results are shown in Scheme 2. The triazine **9** was assumed to be produced by the ring transformation of pyrimidine

Scheme 2

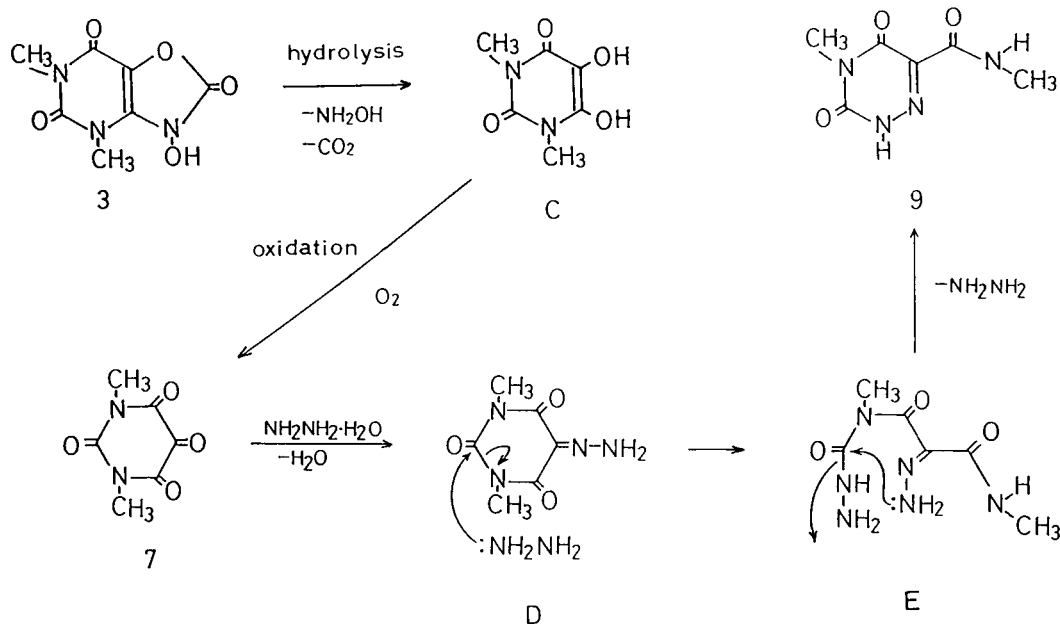


(Chart 2)



B

Scheme 3



or pyrimidine moiety, and hence an experiment was carried out to determine intermediates to the triazine **9**. As the results, compounds **3** and **7** were found to be intermediates to the triazine **9** in two oxidation routes using 3% hydrogen peroxide/hydrochloric acid and nitric acid, respectively. Namely, the reaction of **3** and **7** with hydrazine hydrate resulted in ring transformation to provide the triazine **9** in good yields, respectively, presumably *via* intermediates **C**~**E** (Scheme 3). Recently, we found that **3** spontaneously changed into **7** presumably *via* an intermediary reductone **C** by allowing it to stand at room temperature for about one week in the air. Accordingly, the sequence of **3** to **9** may be supported in the above ring transformations.

In conclusion, the use of hydrazine hydrate instead of ammonia in the murexide reaction did not give a specific coloration, but afforded only a yellow coloration. However, we found that the reaction of the oxidation product **3** or **7** with hydrazine hydrate resulted in ring transformation to provide the triazine **9**.

### EXPERIMENTAL

Absorption spectra were measured with a Hitachi 124 spectrophotometer in a cell of 10 mm optical length, ir spectra with a JASCO IR-G spectrophotometer,  $^1\text{H}$ -nmr spectra with a JEOL EC100 spectrometer at 100 MHz using TMS as an internal standard, and mass spectra (ms) with a JMS-D100 mass spectrometer. Melting points were determined with a Yamato Scientific stirred liquid apparatus and are uncorrected. Elemental analyses were carried out with a Perkin-Elmer 240 B elemental analyzer.

### Reaction of Caffeine with 3% Hydrogen Peroxide/Hydrochloric Acid and Hydrazine Hydrate.

A mixture of caffeine (1 g, 5 mmoles), 3% hydrogen peroxide (40 ml) and concentrated hydrochloric acid (5~6 drops) in a crucible or dish was heated on a boiling water bath, and subsequent heating until dry up gave a yellowish red oily reaction mixture [2]. Subsequent addition of hydrazine hydrate (1 ml) to this oily reaction mixture brought about a yellow coloration. This reaction mixture was dissolved in methanol/water with heating and then filtered. Cooling of the filtrate to room temperature precipitated yellow crystals **10**, which were collected by suction filtration. Evaporation of the filtrate *in vacuo* provided yellow crystals, whose column chromatography on silica gel with chloroform/methanol (20:1,v/v) yielded 4-methyl-6-(*N*-methyl-carbamoyl)-3,5-dioxo-2,3,4,5-tetrahydrotriazine **9** as colorless crystals ( $R_f$  value = 0.50). Subsequent elution with methanol gave hydroxylamine hydrochloride.

### Reaction of Caffeine with 10% Nitric Acid and Hydrazine Hydrate.

A mixture of caffeine (1 g, 5 mmoles) and 10% nitric acid (40 ml) in a crucible or dish was heated on a boiling water bath and subsequent heating until dry gave a yellow oily reaction mixture. Subsequent addition of hydrazine hydrate (1 ml) to this mixture brought about a yellow coloration. This reaction mixture was dissolved in water. Caffeine was extracted with chloroform from the aqueous solution, and, at the same time, 8-amine-1,3,7-trimethyl-2,6-dioxo-1*H*,3*H*,7*H*-xanthine **11** precipitated in the aqueous layer. After the chloroform layer was separated, compound **11** was collected by suction filtration. The filtrate was concentrated to a small volume by evaporation *in vacuo* until crystals began to precipitate. Subsequent addition of methanol to the aqueous solution precipitated hydroxylamine nitrate as colorless crystals, which were collected by suction filtration. Evaporation

of the filtrate *in vacuo* afforded yellow crystals, whose column chromatography on silica gel with chloroform/methanol (20:1, v/v) furnished **9**.

#### Compound **9**.

Recrystallization from methanol gave a colorless powder (20 mg), mp 255 ~ 256°; ms: m/z 184 (M<sup>+</sup>); <sup>1</sup>H-nmr (DMSO-d<sub>6</sub>): δ 2.73 (d, J = 5 Hz, 3H, N-CH<sub>3</sub>), 3.13 (s, 3H, N-CH<sub>3</sub>), 8.44 (d, J = 5 Hz, 1H, NH), 12.82 (s, 1H, NH); ir: ν cm<sup>-1</sup> 3330 (NH), 3100 (NH), 1740 (C=O), 1660 (C=O), 1640 (C=N).

*Anal.* Calcd. for C<sub>6</sub>H<sub>8</sub>N<sub>4</sub>O<sub>3</sub>: C, 39.13; H, 4.38; N, 30.42. Found: C, 39.28; H, 4.25; N, 30.37.

#### Compound **10**.

Recrystallization from methanol/water gave a colorless scaly crystals (60 mg). The ir spectrum and melting point of this sample were identical with those of a sample obtained by the reaction of diethyl oxalate with hydrazine hydrate.

#### Compound **11**.

Recrystallization from methanol gave a colorless powder (60 mg), mp > 300°; ms: m/z 209 (M<sup>+</sup>); <sup>1</sup>H-nmr (DMSO-d<sub>6</sub>): δ 3.13 (s, 3H, N-CH<sub>3</sub>), 3.52 (s, 3H, N-CH<sub>3</sub>), 6.70 (s, 2H, NH<sub>2</sub>); ir: ν cm<sup>-1</sup> 3400 ~ 3200 (NH<sub>2</sub>), 1690 (C=O), 1640 (C=O).

*Anal.* Calcd. for C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>2</sub>: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.73; H, 5.11; N, 33.75.

Hydroxylamine hydrochloride was obtained in a yield of 10 mg. Hydroxylamine nitrate was obtained in a yield of 40 mg. The

ir spectra of these compounds were identical with those of the samples obtained by the reaction of hydrazine hydrate with hydrochloric acid or nitric acid.

#### Reaction of Compounds **3** and **7** with Hydrazine Hydrate.

Compound **3** (50 mg) was dissolved in methanol (10 ml) in a crucible under heating on a boiling water bath. Subsequent addition of hydrazine hydrate (1 ~ 2 drops) to the above residue left colorless crystals of **9**. Recrystallization from methanol gave a colorless powder (40 mg).

The reaction of compound **7** (30 mg) with hydrazine hydrate (1 ~ 2 drops) under similar conditions to that above also provided a colorless substance **9**. Recrystallization from methanol gave a colorless powder (20 mg).

#### Acknowledgement.

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