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Murexide Reaction of Caffeine using Nitric Acid

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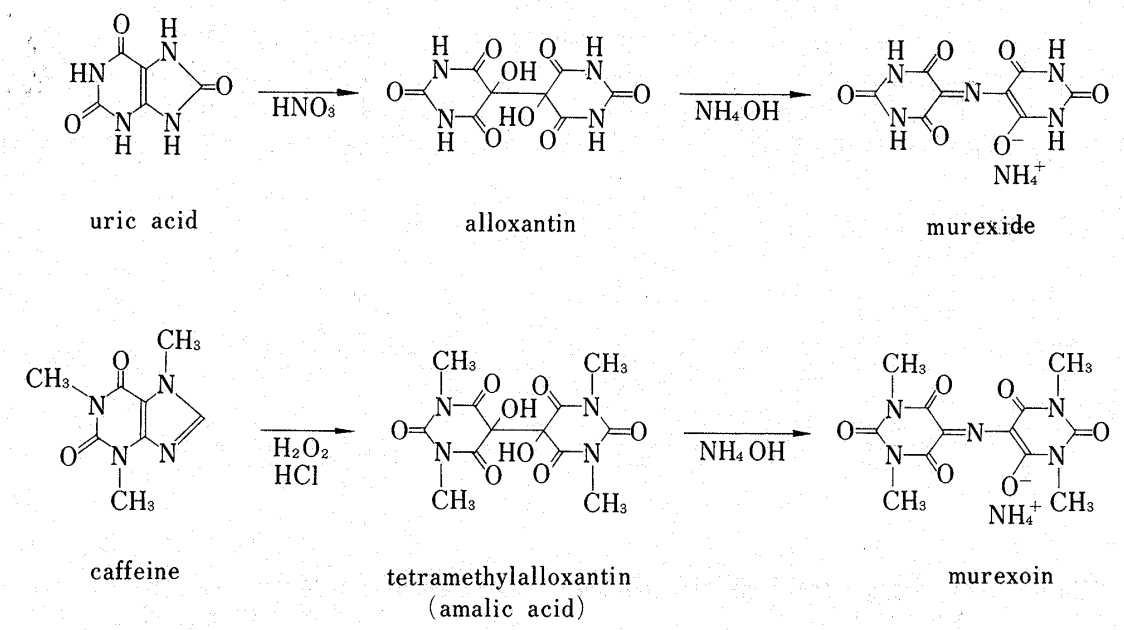
The murexide reaction of caffeine was investigated to clarify the pathway of the coloration. From the reaction mixture of caffeine with nitric acid, 1,3-dimethylalloxan (II) and 1,3,7-trimethyl-2,6-dioxo-8-nitro-1*H*,3*H*,7*H*-xanthine (IV) were isolated. Compound II was found to be the key intermediate, since it was converted to a purple-red-colored substance, murexoin (III), by treatment with conc. ammonia. It was found that 1-hydroxy-5,7-dimethyl-2,4,6-trioxo-1*H*,5*H*,7*H*-oxazolo[4,5-*d*]pyrimidine (I), previously obtained by the oxidation of caffeine with hydrogen peroxide and hydrochloric acid, was also transformed to III with conc. ammonia. Consequently, the murexide reaction of caffeine was shown to have two pathways of coloration depending on the oxidizing agent employed. From the spectral data, a symmetrical structure (III) was assigned to murexoin in solution. Amalic acid, which has been reported as an intermediate of the murexide reaction of caffeine, can be ruled out on the basis of our experimental results.

Keywords——murexide reaction; coloration; caffeine; amalic acid; oxidizing agent; nitric acid; hydrogen peroxide and hydrochloric acid; 1,3-dimethylalloxan; 1-hydroxy-5,7-dimethyl-2,4,6-trioxo-1*H*,5*H*,7*H*-oxazolo[4,5-*d*]pyrimidine; 1,3,7-trimethyl-2,6-dioxo-8-nitro-1*H*,3*H*,7*H*-xanthine; murexoin

The murexide reaction which is used to detect uric acid and related purines has been studied by many workers,[1-3] and the reaction mechanisms for uric acid and caffeine have been assumed to be as shown in Chart 1.[4] In these reactions, two kinds of oxidizing agent have been used; one is nitric acid for uric acid and the other is a mixture of hydrogen peroxide and hydrochloric acid for caffeine. These oxidation reactions have been reported to produce alloxantin and amalic acid (tetramethylalloxantin) (Chart 1), respectively, as intermediates, and these are converted to murexide[5] and murexoin,[6] which give rise to the purple colorations

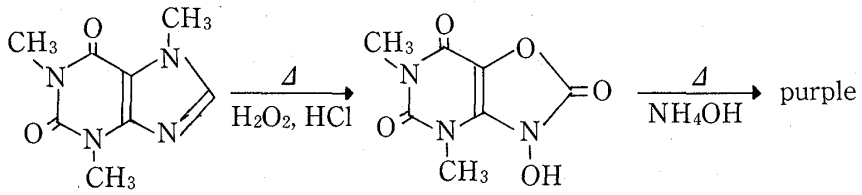


uric acid — alloxantin — murexide

caffeine — tetramethylalloxantin (amalic acid) — murexoin

Chart 1

on subsequent treatment with conc. ammonia. However, these reaction pathways are not fully understood. As described in a previous report,[7] it was clarified that the murexide reaction of caffeine using hydrogen peroxide and hydrochloric acid afforded 1-hydroxy-5,7-dimethyl-2,4,6-trioxo-1*H*,5*H*,7*H*-oxazolo[4,5-*d*]pyrimidine (I), which was regarded as an intermediate for the purple-red-colored substance ($\lambda_{max}^{H_2O}$ 525 nm), as shown in Chart 2.



I

Chart 2

In these experiments, however, we could not obtain amalic acid (Chart 1) which had been reported as an intermediate for the murexide reaction of caffeine using hydrogen peroxide and hydrochloric acid.[6] On the other hand, the reaction of uric acid with nitric acid was reported to give alloxantin as an intermediate (Chart 1). Therefore, it may be assumed that the intermediate of the murexide reaction depends upon the oxidizing agent employed in the reaction. Thus, we expected that amalic acid, which was not obtained in the reaction of caffeine with hydrogen peroxide and hydrochloric acid, might be formed as an intermediate in the reaction of caffeine with nitric acid. This paper describes the elucidation of the pathway in the murexide reaction of caffeine using nitric acid.

The reaction of caffeine with nitric acid gave a yellow mixture. Subsequent addition of conc. ammonia to the yellow mixture brought about a purple-red coloration ($\lambda_{max}^{H_2O}$ 525 nm). From the yellow reaction mixture, three compounds were isolated; the starting material (*Rf* 0.63) and two compounds, II (*Rf* 0.68) and IV (*Rf* 0.75) (silica gel, $CHCl_3$: CH_3OH=20: 1, v/v).

Compound II was a colorless substance assigned as 1,3-dimethylalloxan from the spectral data. Its molecular formula was established as $C_6H_6N_2O_4$ by high resolution mass spectrometry, and the infrared (IR) spectrum (Fig. 1) showed a C=O absorption band at 1680 cm^{-1}. The nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited N–CH_3 protons at δ 3.31 ppm.

Compound IV was a yellow compound assigned as 1,3,7-trimethyl-2,6-dioxo-8-nitro-1*H*,-3*H*,7*H*-xanthine[8] (Chart 3) based on the spectral and elemental analytical data. Its ^{1}H-NMR spectrum showed three signals due to N–CH_3 protons at δ 3.42, 3.57, and 4.45 ppm, and the IR spectrum exhibited two C=O absorption bands at 1710 and 1670 cm^{-1}, and NO_2 bands

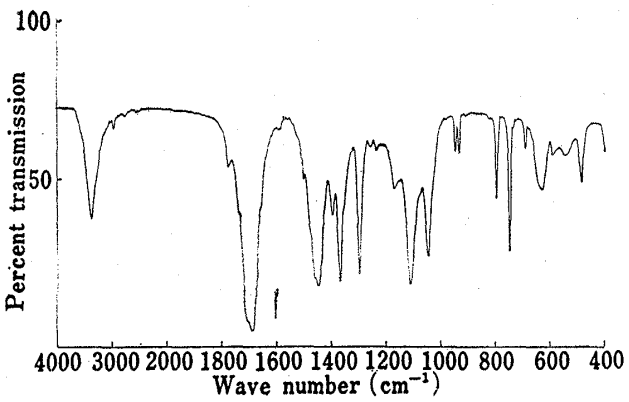


Fig. 1. IR Spectrum of II (in KBr)

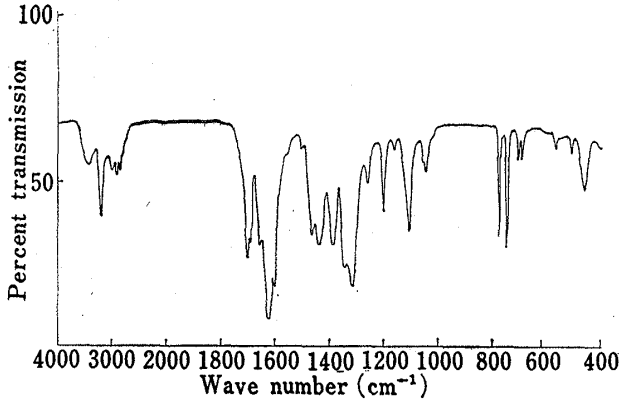


Fig. 2. IR Spectrum of III (in KBr)

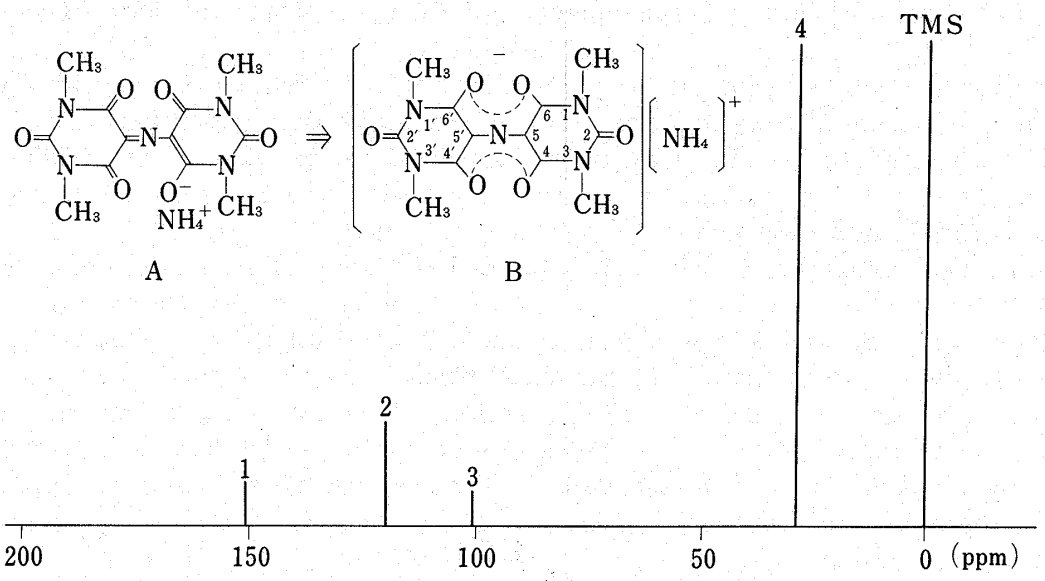


Fig. 3. ^{13}C-NMR Spectrum of III in DMSO-d_6

A: Commonly reported structure for murexoin.
B: Revised structure for murexoin.

No	ppm	
1:	150.97	------ C(2,2').
2:	119.40	------ C(4,4', 6,6').
3:	100.91	------ C(5,5').
4:	27.81	------ N-$\underline{C}H_3$.

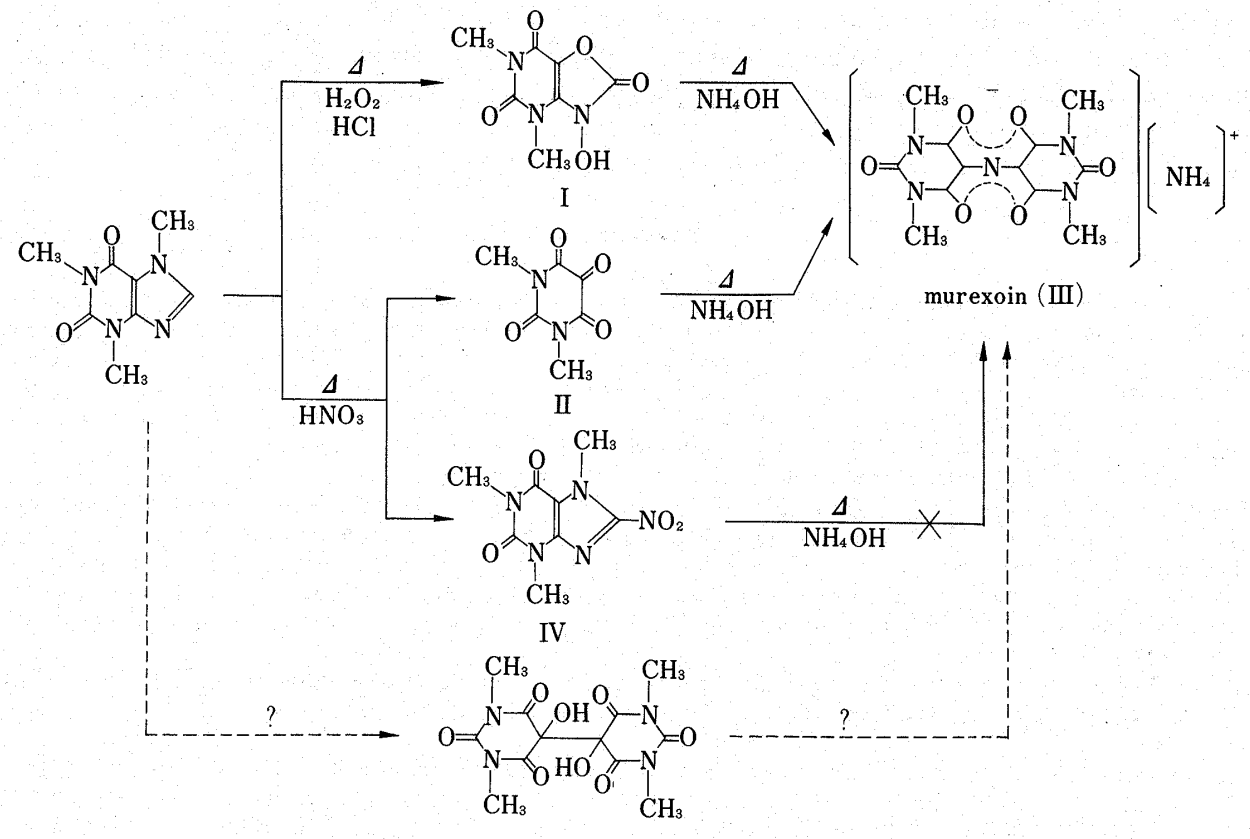


Chart 3

at 1530, 1340, and 1320 cm^{-1}. This compound was the main product of the reaction in terms of yield.

Compound II turned purple-red on treatment with conc. ammonia,[9] and its absorption maximum ($\lambda_{max}^{H_2O}$ 525 nm) coincided with that of the murexide reaction product of caffeine obtained with nitric acid. Therefore, II was concluded to be the key intermediate in the formation of the purple-red-colored substance. However, IV was concluded not to be involved in this coloration, since it was unaffected by conc. ammonia.

The purple-red-colored substance (III) was isolated from the purple-red-colored reaction mixtures of caffeine, II, and I with conc. ammonia in order to clarify its structure. On the basis of the analytical and spectral data, III was assigned as murexoin. Moreover, III was assumed to adopt a symmetrical structure in solution (Chart 3). Its IR spectrum (Fig. 2) showed NH_4^+ absorption bands at *ca.* 3200, 1470, and 1440 cm^{-1}, a C=O band at 1700 cm^{-1}, and a C=N band at 1620 cm^{-1}. The ^{1}H-NMR spectrum showed the N–CH_3 protons signal at δ 3.17 ppm (singlet, 12H). The signal of NH_4^+ protons was observed at δ 7.30 ppm (broad, 4H). Furthermore, the ^{13}C-NMR spectrum (Fig. 3) exhibited the N–CH_3 carbons signal at 27.81 ppm. The signals at 100.91, 119.40, and 150.97 ppm were assigned as C–N–C (5,5′), C=O (4,4′, 6,6′), and C=O (2, 2′) carbons, respectively. The above results are summarized in Chart 3.

In conclusion, it was found that the murexide reaction of caffeine using nitric acid gave 1,3-dimethylalloxan (II) and 1,3,7-trimethyl-2,6-dioxo-8-nitro-1*H*,3*H*,7*H*-xanthine (IV). Compound II turned purple-red on treatment with conc. ammonia, and hence II was regarded as the key intermediate in the murexide reaction of caffeine using nitric acid. It was clear that the murexide reaction of caffeine afforded two different intermediates, I and II, when hydrogen peroxide and hydrochloric acid, and nitric acid, respectively, were used as oxidizing agents. Both I and II were converted to murexoin (III) with conc. ammonia. Consequently, the murexide reaction of caffeine was shown to have two pathways of coloration depending upon the kind of oxidizing agent, as shown in Chart 3. Furthermore, the molecular anion of murexoin was proposed to have a symmetrical structure in solution. Amalic acid can be ruled out as an intermediate on the basis of our experimental results.

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}H-NMR spectra with a JEOL EC100 spectrometer at 100 MHz, ^{13}C-NMR spectra with a JEOL PET-100 at 25 MHz with TMS as an internal standard, mass spectra (MS) with a JMS-D100 mass spectrometer, and high resolution mass spectra with a JMS-01S spectrometer. Melting points were determined with a Yamato Scientific stirred liquid apparatus and are uncorrected. Elemental analysis was carried out with a Perkin-Elmer 240B elemental analyzer.

Reaction of Caffeine with Nitric Acid——A mixture of caffeine (1 g, 5 mmol) and 10% HNO_3 (40 ml) in a crucible was heated on a boiling water bath and evaporated to dryness. The yellow residue obtained was dissolved in methanol (100 ml) with heating, then the solution was cooled to room temperature. The yellow substance (IV) that precipitated was collected by suction filtration. The filtrate was evaporated to dryness *in vacuo* to give a yellow residue, which was dissolved in H_2O (100 ml). Caffeine and a trace amount of IV were extracted with $CHCl_3$ (50 ml × 3) from the H_2O layer. Removal of H_2O by evaporation gave colorless crystals (II).

Compound II——Recrystallization from methanol gave colorless prisms (20 mg), mp 51—52°C. MS *m/e*: M^+, 170.0327. Calcd for $C_6H_6N_2O_4$: M, 170.0325. ^{1}H-NMR δ ($CDCl_3$): 3.31 (6H, s, N–CH_3). IR ν_{max}^{KBr} cm^{-1}: 1680 (C=O).

Compound IV——Recrystallization from methanol gave yellow scaly crystals (130 mg), mp 171—172°C. MS *m/e*: 239 (M^+). *Anal.* Calcd for $C_8H_9N_5O_4$: C, 40.17; H, 3.79; N, 29.28. Found: C, 40.17; H, 3.69; N, 29.16. ^{1}H-NMR δ ($CDCl_3$): 3.42 (3H, s, N–CH_3), 3.57 (3H, s, N–CH_3), 4.45 (3H, s, N–CH_3). IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O), 1670 (C=O), 1530 (NO_2), 1340 (NO_2), 1320 (NO_2).

Murexoin (III)——The reaction of caffeine (1 g, 5 mmol) with 10% HNO_3 (40 ml) gave a yellow residue, as described in the case of the above reaction. The yellow residue was then treated with conc. ammonia (2—3 drops) to afford a purple-red reaction mixture. The mixture was dissolved in methanol (50 ml) with heating, and the insoluble purple-red-colored substance (III) that precipitated was collected by suction.

Recrystallization from CH_3OH–H_2O gave a purple-red powder (5 mg), mp 258—260°C (dec.). MS. *m/e*: M^+, 323.0861. Calcd for $C_{12}H_{13}N_5O_6$: M, 323.0866. *Anal.* Calcd for $C_{12}H_{16}N_6O_6$: C, 42.35; H, 4.74; N, 24.70. Found: C, 41.92; H, 4.80; N, 24.51. ^{1}H-NMR δ (DMSO-d_6): 3.17 (12H, s, N–CH_3), 7.30 (4H, brs, NH_4^+). ^{13}C-NMR (DMSO-d_6) ppm: 27.81 (4C, t, N–CH_3), 100.91 (2C, s, C–N–C, C: 5,5′), 119.40 (4C, s, C=O, C: 4,4′, 6,6′), 150.97 (2C, s, C=O, C: 2,2′). VIS $\lambda_{max}^{H_2O}$ nm (log ε): 525 (3.54). IR ν_{max}^{KBr} cm^{-1}: 3200 (NH_4^+), 1470 (NH_4^+), 1440 (NH_4^+), 1700 (C=O), 1620 (C=N).

The reactions of I (15 mg) and II (25 mg) with conc. ammonia (2—3 drops) also gave III (5 mg from I and 10 mg from II), whose identity was confirmed by IR and absorption spectral measurements.

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9) To describe the coloration of the murexide reaction, the expression "purple-red" is now preferred to "purple," which was used in our previous paper.[7] Moreover, the use of conc. ammonia (28%) in the present investigation afforded a more intense purple-red coloration than that of dil. ammonia (10%) in the previous papers.[4,7] Therefore, we recommend the employment of conc. ammonia and the description of the coloration as "purple-red."