Murexide Reaction of Caffeine with Hydrogen Peroxide and Hydrochloric Acid. II

Matajiro Koyama*

Pharmaceutical Department, Cancer Treatment Center of Saitama,
Komuro, Inamachi, Kitaadachigun, Saitama 362, Japan

Hiroshi Kozuka

Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University,
Sugitani, Toyama 930-01, Japan

Received August 25, 1989

In continuation of the study on the murexide reaction of caffeine with 3% hydrogen peroxide/hydrochloric acid and then with ammonia giving a purple coloration, we investigated the oxidation reaction of caffeine with 6% hydrogen peroxide/hydrochloric acid to isolate ten reaction products, 3-hydroxy-4,6-dimethyloxazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione **1**, 1,3-dimethylalloxan **2**, murexoin **3**, 1,3,7-trimethyl-2,6,8-trioxo-9-hydroxy-1*H*,3*H*,7*H*-xanthine **5**, 1,3,7-trimethyl-2,6,8-trioxo-1*H*,3*H*,7*H*-xanthine **6**, 1,3,7-trimethyl-2,6-dioxo-8-chloro-1*H*,3*H*,7*H*-xanthine **7**, 5-(1,3-dimethyl-1,2,3,4,5,6-hexahydro-2,4,6-trioxopyrimidin-5-yl)-aminomethylene-1,3-dimethyl-1,2,3,4,5,6-hexahydro-2,4,6-trioxopyrimidine ammonium salt **9**, 1,3-dimethylparabanic acid **10**, 1-methyl-2,4,5-trioxoimidazole **11**, 3-hydroxy-5,7-dimethyloxazolo[5,4-*d*]pyrimidine-2,4,6(3*H*,5*H*,7*H*)-trione **12** and 4,6,8-trimethyl-1,2,4-dioxazino[6,5-*d*]pyrimidine-3,5,7(4*H*,6*H*,8*H*)-trione **13**.

The oxidation reaction using 6% hydrogen peroxide/hydrochloric acid was found to produce a similar purple coloration to that of the murexide reaction despite no subsequent addition of ammonia, indicating the liberation of ammonia by the oxidation of caffeine.

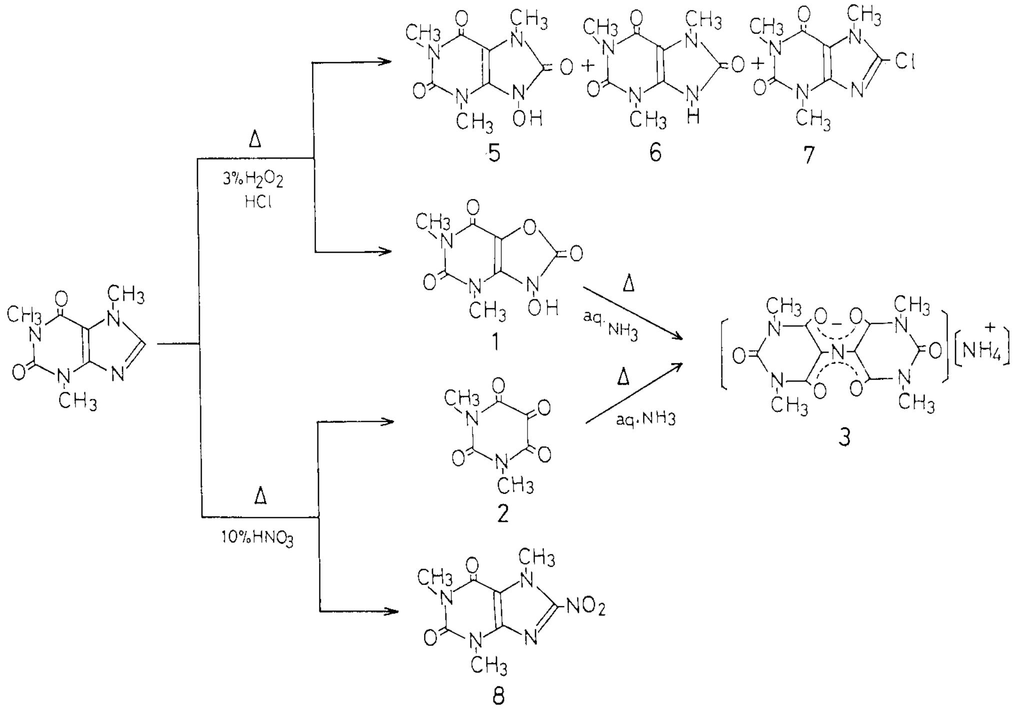
Among the above compounds, the purple colored substance murexoin **3** and the yellow colored compound **9** were both ammonium salts, and compound **5** was the red colored substance. In the present investigation, these three compounds were found to contribute to the coloration.

J. Heterocyclic Chem., **27**, 667 (1990).

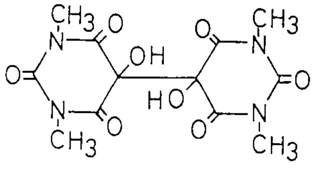
Introduction.

The murexide reaction has been known as the specific color reaction for uric acid and related purine compounds [1]. In this reaction, an oxidizing agent such as hydrogen peroxide/hydrochloric acid [2] or nitric acid [3] is employed at the first stage, and then an addition of con-

Scheme 1



centrated ammonia gives rise to a purple coloration (525 nm). In previous reports, we have investigated the murexide reactions of caffeine with 3% hydrogen peroxide/hydrochloric acid [2] and with nitric acid [3] to clarify the mechanisms of the coloration, whose results are summarized in Scheme 1. Thus, we isolated two intermediates 3-hydroxy-4,6-dimethyloxazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione **1** and 1,3-dimethylalloxan **2**, which could be transformed into the purple colored substance murexoin **3** (525 nm). Moreover, we indicated that amalic acid (tetramethylalloxanthine) **4** [4] (Chart 1) was not an intermediate to murexoin [5]. Besides the above intermediates **1** and **2**, four reaction products 1,3,7-trimethyl-2,6,8-trioxo-9-hydroxy-1*H*,3*H*,7*H*-xanthine **5**, 1,3,7-trimethyl-2,6,8-trioxo-1*H*,3*H*,7*H*-xanthine **6**, 1,3,7-trimethyl-2,6-dioxo-8-chloro-1*H*,3*H*,7*H*-xanthine **7** and 1,3,7-trimethyl-2,6-dioxo-8-nitro-1*H*,3*H*,7*H*-xanthine **8** were obtained from the murexide reaction mixture of caffeine. Compound **5** was the red colored substance (450 nm) [2], but this compound was not an intermediate to murexoin **4**.



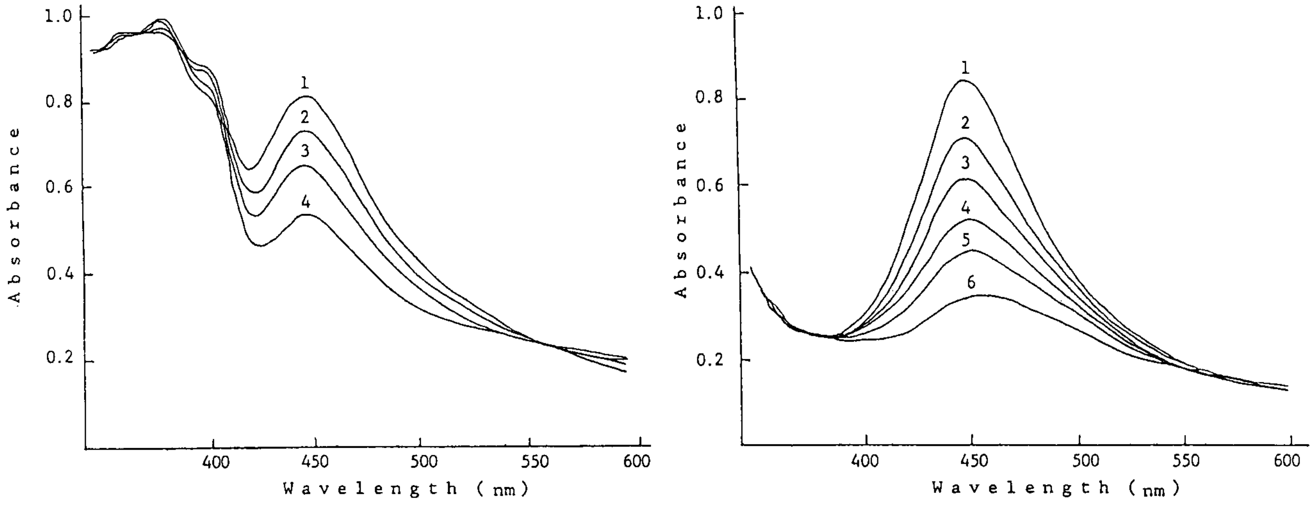
4

Chart 1

In continuation of the above work, we examined the conditions to intensify the sensitivity of the murexide reaction. Namely, caffeine was oxidized with 6% hydrogen peroxide(2-fold concentration)/hydrochloric acid in order to increase the yield of an intermediate **1**, which would lead to the improvement of the sensitivity. As a result, this reaction was found to afford a purple colored reaction mixture in spite of no subsequent addition of ammonia, and the absorption maxima of this reaction mixture were observed at 360, 380, 400 and 445 nm [Figure 1 (A)]. The absorption maxima at 360, 380 and 400 nm were specific in this reaction, since these maxima did not appear in the reaction mixture of caffeine with 3% hydrogen peroxide/-hydrochloric acid [Figure 1 (B)]. Thus, the oxidation reaction of caffeine with 6% hydrogen peroxide/hydrochloric acid provided unexpected results, and it was interesting for us to clarify this reaction mechanism. In fact, this reaction gave new products as well as the purple colored ammonium salt murexoin **3**. The paper describes the elucidation of this color reaction mechanism.

Results and Discussion.

A mixture of caffeine with 6% hydrogen peroxide/hydrochloric acid was heated on a boiling water bath, and a continued heating of this mixture until evaporation to dryness provided a purple reaction mixture with generation of white fumes, which was presumably due to ammonium chloride (Method 1). This purple reaction mixture was triturated with methanol to precipitate yellow insoluble 5-(1,3-dimethyl-1,2,3,4,5,6-hexahydro-2,4,6-trioxopyrimidin-5-yl)aminomethylene-1,3-dimethyl-1,2,3,4,5,6-hexahydro-2,4,6-trioxopyrimidine ammonium salt **9** (Scheme 2), whose absorption maxima were observed at 380 and 400



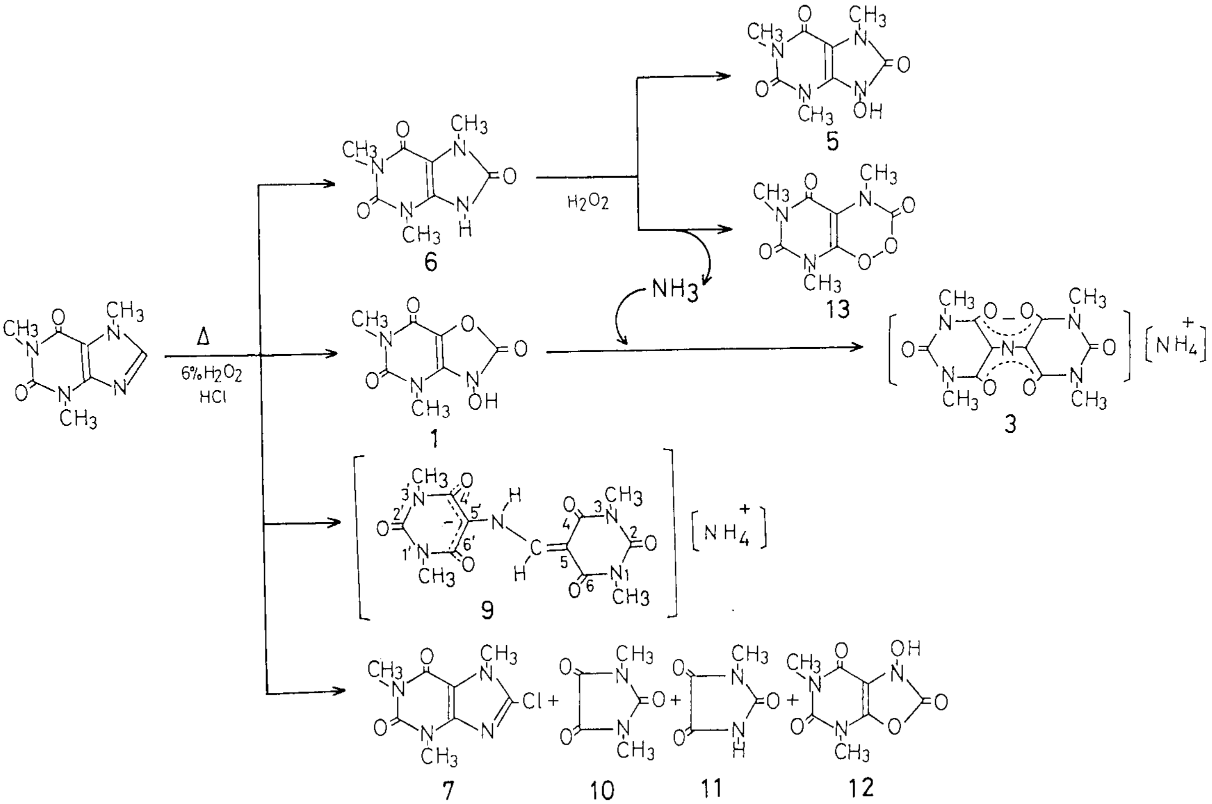
(A) (B)

Figure 1. Absorption spectra of the reaction mixture of caffeine with 6% (**A**) and 3% (**B**) hydrogen peroxide/hydrochloric acid in methanol.

A purple colored residue (**A**) and a yellowish-red oily residue (**B**) were obtained by concentration of the reaction mixture of caffeine (0.5 mmole) with 6% and 3% hydrogen peroxide (5 ml) and hydrochloric acid (1 ~ 2 drops), respectively.

These residues were dissolved in 5 ml of methanol and kept for various times (1 ~ 5 and 1 ~ 6) at room temperature (15°). The times were as follows: 1-immediately; 2-5 minutes; 3-10 minutes; 4-15 minutes; 5-20 minutes; 6-25 minutes.

Scheme 2



nm. Column chromatography of the above residual methanol solution on Sephadex LH-20 provided the purple colored substance murexoin **3**. The other components were eluted and then separated by rechromatography on silica gel to give eight compounds **1**, **5**, **6**, 1,3-dimethylparabanic acid **10**, 3-methyl-2,4,6-trioxoimidazole **11**, 3-hydroxy-5,7-dimethyloxazolo[5,4-*d*]pyrimidine-2,4,6(3*H*,5*H*,7*H*)-trione **12** and 4,6,8-trimethyl-1,2,4-dioxazino[6,5-*d*]pyrimidine-3,5,7(4*H*,6*H*,8*H*)-trione **13**. Among the above compounds, **1**, **5**, **6** and **7** have already been isolated from the reaction mixture of caffeine with 3% hydrogen peroxide/hydrochloric acid, and **10** and **11** are known in literature [6,7]. The structural assignments of **9**, **12** and **13** were based on the analytical and spectral data.

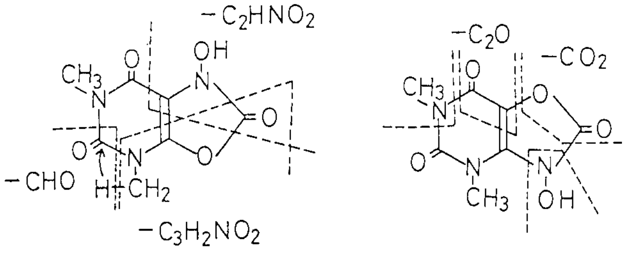
The nmr spectrum of **9** showed the vinylic proton signal (δ 9.55) coupling with the NH proton (δ 12.30) (J = 17 Hz). The ^{13}C-^{1}H COSY spectrum exhibited the correlation of the vinylic proton (δ 9.55) to the $C_6=O$, $C_4=O$, C_5 and $C_{5'}$ carbons [δ 163.52 (or 161.87), 161.87 (or 163.52), 87.79 and 93.09]. The chemical shift due to the $C_{5'}$ carbon (δ 93.09) was distinguished from that due to the C_5 carbon (δ 87.79) by radiation at the NH proton signal (δ 12.38) in the selective NOE ^{13}C-[^{1}H] spectrum. The C_2 (δ 151.81) and $C_{2'}$ (δ 151.05) carbon signals changed into siglet signals by radiation at the N-Me proton signal (δ 3.14). The vinylic carbon signal appeared at δ 146.87 ppm, and the ^{1}J (vinylic ^{1}H-^{13}C) and ^{2}J (NH-vinylic C) were 176.5 and 3.5 Hz, respectively. The ^{3}J [vinylic H-$C_6=O$ (or $C_4=O$)] and ^{3}J [vinylic H-$C_4=O$ (or $C_6=O$)] were found to be 8.6 and 4.0 Hz, respectively, by radiation at the N-Me proton signal (δ 3.14), and the ^{3}J (methyl H-$C_6=O$) were shown to be both 3.5 Hz by radiation at the vinylic proton signal (δ 9.57). The ^{3}J (NH-$C_{4'}=O$) and ^{3}J (NH-$C_{6'}=O$) were clarified to be both 5.0 Hz by radiation at the N-Me proton signal (δ 3.14). Thus, the nmr spectral data supported the structure of **9** shown in Scheme 2. The carbon signal assignment is represent in Table 1.

Table 1

The ^{13}C-NMR Chemical Shift (δ) for Compound **9**.

163.52 (C_6 or C_4)	168.87 (C_4 or C_6)	157.10 (C_6 and C_4)	151.87 (C_2)
151.05 ($C_{2'}$)	146.87 (vinylic)	93.09 ($C_{5'}$)	87.79 (C_5)
27.33 (N_3- or N_1-Me)	26.87 ($N_{1'}$- and $N_{3'}$-Me		26.67 (N_1- or N_3-Me)

Compound **12** was found to be an isomer of **1**. Namely, the N-Me proton signals of **12** were observed at δ 3.10 and 2.83 ppm, whose values were similar to those of **1** (δ 3.08 and 2.82) [2]. The lactone C = O absorption band of **12** was observed at 1850 cm^{-1}, while that of **1** appeared at 1840 cm^{-1} [2]. However, the mass spectral fragmentation pattern of **12** was eminently different from that of **1** as shown in Chart 2 and Table 2. Accordingly, compound **12** was assumed to have the oxazolo[5,4-*d*]pyrimidine nucleus.



12 1

Chart 2

Table 2

Mass Spectral Data for **12** and **1**

Compound		m/z	Calcd.	Found	Formula
12	213	(M^+)	213.039	213.039	$C_7H_7N_3O_5$
	142	213-C_2HNO_2	142.038	142.034	$C_5C_6N_2O_3$
	129	213-$C_3H_2NO_2$	129.030	129.029	$C_4H_5N_2O_3$
	100	129-CHO	100.027	100.028	$C_3H_4N_2O_2$
1 [2]	213	(M^+)	213.039	213.038	$C_7H_7N_3O_5$
	169	213-CO_2	169.052	169.048	$C_6C_7N_3O_3$
	129	169-CCO	129.055	129.053	$C_4H_7N_3O_2$
	100	129-NCH_3	100.028	100.027	$C_3H_4N_2O_2$
	69	100-NOH	69.021	69.021	C_3H_3NO

[2] Reference No. 2.

The nmr spectral data of **13** supported the structure of 1,2,4-dioxazino[6,5-*d*]pyrimidine (Table 3). Namely, the quartet signals due to the C_3 (δ 164.86) and C_{4a} (δ 152.71) carbons changed into the singlet signals by radiation at the N_4-Me proton signal (δ 3.11), and the quartet signals due to the C_5 (δ 164.16) and C_{8a} (δ 90.10) carbons turned to the singlet signals by radiation at the N_6-Me (δ 2.99) and N_8-Me (δ 2.91) proton signals, respectively. Moreover, the sevenfold signal of the C_7 carbon (δ 155.13) became the quartet signal by either radiation at the N_6-Me or N_8-Me proton signal. All the ^3J (^1H-^{13}C) coupling constants were 7.0 Hz.

Table 3

NMR Spectral Data for Compound **13**

Proton	Chemical Shift (δ)	Carbon	Chemical Shift (δ)	Coupling Constant $^3J(^1H-^{13}C)$ (Hz)
N_4-CH_3	3.11	C_3	164.86	7.0 (q)
N_6-CH_3	2.99	C_{4a}	152.71	7.0 (q)
N_8-CH_3	2.91	C_5	164.16	7.0 (q)
		C_7	155.13	7.0 (q,q)
		C_{8a}	90.10	7.0 (q)

When a mixture of caffeine with 6% hydrogen peroxide/hydrochloric acid was heated on a boiling water bath without evaporation to dryness (Method 2), **1**, **5**, **6**, **7**, **10**, **11**, **12** and **13** were isolated. In the method 2, the ammonium salts **3** and **7** were not obtained, indicating that the mild oxidation of caffeine with 6% hydrogen peroxide/hydrochloric acid did not liberate ammonia.

As described above, murexoin **3** (ammonium salt), **5** and **9** (ammonium salt) are the colored substances and their absorption maxima agree with those of the original purple colored reaction mixture [Figure 1 (A)]. Accordingly, these three compounds were found to contribute the coloration.

In conclusion, we clarified the mechanism of the murexide reaction of caffeine with 6% hydrogen peroxide/hydrochloric acid. Caffeine was oxidized or chlorinated at first in the 8-position, and further oxidation would liberate ammonia, which led to the formation of the ammonium salts **3** and **9**. Compounds **9**, **12** and **13** were unknown in literature.

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, ^1H-nmr spectra with a JEOL EC100 spectrometer at 100 MHz using TMS as an internal standard, ^{13}C-nmr spectra with a VXR-30 spectrometer at 300 MHz, 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 analyses were carried out with a Perkin-Elmer 240B elemental analyzer. All Rf values were obtained with silica gel plates using chloroform:methanol (20:1, v/v) as developing solvent.

Reaction of Caffeine using 6% Hydrogen Peroxide/Hydrochloric Acid.

Method 1.

A mixture of caffeine (1 g, 5 mmoles), 6% hydrogen peroxide (40 ml) and concentrated hydrochloric acid (5 ~ 6 drops) in a crucible or dish was heated on a boiling water bath and evaporated to dryness. A purple colored mixture was obtained with white fuming. This purple colored mixture was dissolved in methanol (50 ml) with heating, and the insoluble yellow substance **9** was collected by suction filtration. The filtrate was evaporated *in vacuo* to give crystals, which were dissolved in methanol and then subjected to column chromatography on sephdex LH-20. Elution with methanol gave purple colored fraction, and evaporation of the solvents provided murexoin **3** (10 mg). Eluate of the other components was combined, and the solvent was evaporated *in vacuo* to afford crystals, which were dissolved in methanol and then submitted to column chromatography on silica gel. Elution with chloroform/methanol (20:1, v/v) gave fraction of **13** (Rf 0.80), **7** (Rf 0.76), caffeine (Rf 0.60), **12** (Rf 0.55), **11** (Rf 0.52), **10** (Rf 0.45), **6** (Rf 0.30) and **5** (Rf 0.15).

Method 2.

A mixture of caffeine (1 g, 5 mmoles), 6% hydrogen peroxide (40 ml) and concentrated hydrochloric acid (5 ~ 6 drops) in a crucible or dish was heated on a boiling water bath without evaporation to dryness. A yellowish red oily reaction mixture was obtained. This oily reaction mixture was dissolved in methanol, and evaporation of the solvent provided an oily residue. Column chromatography of the oily residue on silica gel in a similar manner to the method A gave fractions of **1** (400 mg, Rf 0.66) and **11**, **7**, caffeine, **12**, **11**, **10**, **6** and **5**.

Compound **9**.

Ten mg of **9** was obtained. Recrystallization from methanol/water gave a yellow powder (5 mg), mp >300°; ms: m/z 337 (M$^+$); ^{1}H-nmr (DMSO-d_6): δ 3.12 (s, 6H, N-CH_3), 3.13 (s, 3H, N-CH_3), 3.16 (s, 3H, N-CH_3), 7.09 (br s, 4H, NH_4^+), 9.55 (d, J = 17 Hz, 1H, C-H), 12.30 (d, J = 17 Hz, 1H, NH); ir: ν cm^{-1} 3150 (NH_4^+), 1480 (NH_4^+), 1710 (C=O), 1690 (C=O), 1620 (C=N); vis: λ max nm (log ϵ) 380 (3.65).

Anal. Calcd. for $C_{13}H_{18}N_6O_6$: C, 44.06; H, 5.12; N, 23.72. Found: C, 44.17; H, 5.03; N, 23.42.

Compound **10**.

Thirty-five mg of **10** was obtained. Recrystallization from methanol gave a colorless powder, mp 145 ~ 146°; ms: m/z 142 (M$^+$); ^{1}H-nmr (DMSO-d_6): δ 2.97 (s, 6H, N-CH_3); ir: ν cm^{-1} 1760 (C=O), 1730 (C=O), 1710 (C=O).

Anal. Calcd. for $C_5H_6N_2O_3$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.00; H, 4.23; N, 19.55.

Compound **11**.

Forty mg of **11** was obtained. Recrystallization from methanol gave a colorless powder, mp 153 ~ 154°; ms: m/z 128 (M$^+$); ^{1}H-nmr (DMSO-d_6): δ 2.98 (s, 3H, N-CH_3), 11.97 (br s, 1H, NH); ir: ν cm^{-1} *ca.* 3200 (NH), 1800 (C=O), 1750 (C=O), 1720 (C=O).

Anal. Calcd. for $C_6H_4N_2O_3$: C, 37.51; H, 3.15; N, 21.87. Found: C, 37.41; H, 3.12; N, 22.15.

Compound **12**.

Two hundred mg of **12** was obtained. Repurification by column chromatography on silica gel gave a yellow oil (40 mg); ^{1}H-nmr (DMSO-d_6): δ 2.83 (s, 3H, N_5-CH_3), 3.10 (s, 3H, N_7-CH_3), 9.30 (br s, 1H, OH); ir: ν cm^{-1} 1850 (lactone); ms: m/z 213.0390 (M$^+$). Calcd. for $C_7H_7N_3O_5$: M, 213.0386.

Compound **13**.

Four hundred mg of **13** was obtained. Recrystallization from methanol gave a colorless powder, mp 199 ~ 201°; ms: m/z 227 (M$^+$); ^{1}H-nmr (DMSO-d_6): δ 2.88 (s, 3H, N_6-CH_3), 2.98 (s, 3H, N_4-CH_3), 3.10 (s, 3H, N_8-CH_3); ir: ν cm^{-1} 1840 (lactone), 1795 (C=O), 1735 (C=O).

Anal. Calcd. for $C_8H_9N_3O_5$: C, 42.29; H, 3.99; N, 18.50. Found: C, 42.10; H, 3.98; N, 18.51.

Acknowledgement.

The authors wish to thank Dr. Yoshihisa Kurasawa and Dr. Katsuhiko Nagahara, School of Pharmaceutical Sciences, Kitasato University, for their helpful suggestions.

REFERENCES AND NOTES

[1] F. Wohler and J. Liebig, *Ann. Chem.*, **26**, 241 (1838).

[2] H. Kozuka, M. Koyama and T. Okitsu, *Chem. Pharm. Bull.*, **29**, 433 (1981).

[3] H. Kozuka, M. Koyama and T. Okitsu, *Chem. Pharm. Bull.*, **30**, 941 (1982).

[4] "Interpretation of the Japanese Pharmacopoeia" VIII ed, Vol **1**, Hirokawa Publishing Co., Tokyo, 1971, p C-555.

[5] *Beilstein*, **25**, II, 397.

[6] T. L. Patton, *J. Org. Chem.*, **32**, 383 (1967).

[7] K. J. Kolonko, R. H. Shapiro, R. M. Barkley and R. E. Sievers, *J. Org. Chem.*, **44**, 3769 (1979).