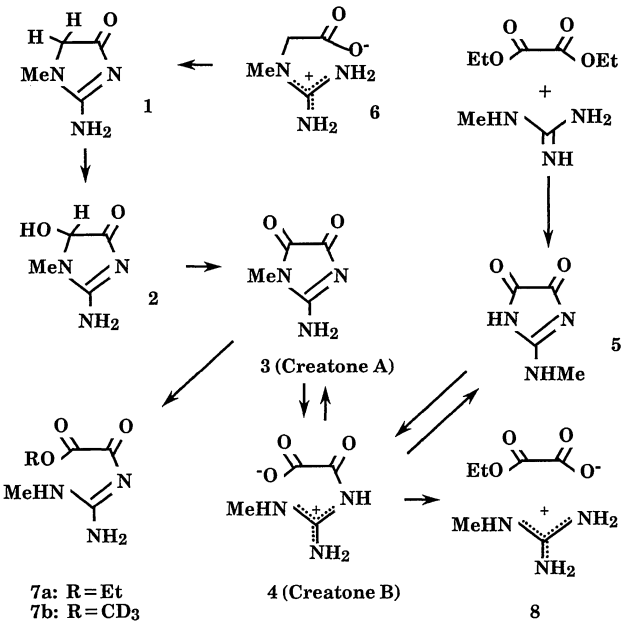
Creatones A and B. Revision of the Structure for the Product of Oxidation of Creatinine and Creatine

Ko NAKAMURA, Chikara OHIRA,† Hiroshi YAMAMOTO,†
Wolfgang PFLEIDERER,†† and Kazuharu IENAGA*
Institute of Bio-Active Science (IBAS), Nippon Zoki Pharmaceutical Co. Ltd.,
Yashiro-cho, Kato-gun, Hyogo 673-14
†Faculty of Science, Okayama University, Tsushima, Okayama 700
††Fakultät für Chemie, Universität Konstanz,
D-7750 Konstanz, West Germany
(Received September 30, 1989)

Synopsis. The structure for a product of the oxidation of creatinine is revised to be *N*-(*N'*-methylamidino)oxamic acid (**4**). Acyclic product **4** and its cyclic product, 2-amino-1-methyl-4,5-imidazoledione, are coined as creatones B and A, respectively. The first synthesis of 2-methylamino-4,5-imidazoledione is also described.

Although creatinine (**1**) was previously believed to be an end-metabolite in mammals, our discovery of two hydantoins, 1-methyl-2,4-imidazolidinedione and its 5-hydroxy derivative in inflamed mammalian skin tissues,[1] and the first isolation of creatol, 2-amino-5-hydroxy-1-methyl-4(5*H*)-imidazolone (**2**),[2] have led to proposals of two analogous oxidative pathways[2–4] for the catabolism of **1** in mammals. The first pathway involves the conversion of **1** into 1-methylurea via methylhydantoins and the second into the uremic toxin 1-methylguanidine via **2**, oxo-compound **3** and its ring-opened derivative **4**, successively.[4] During the preparation of authentic specimens to obtain evidence for intermediates **3** and **4** in vivo, we have found that structure **5**, previously assigned[5–7] to the oxidation product (creatone) of creatine (**6**) or **1** with aqueous mercury(II) acetate, should be revised on the basis of newly obtained physicochemical evidence. We now wish to redefine the term "creatone" as a cyclic or acyclic compound that is obtainable by the oxidation of **1** and contains a carbonyl group in place of the original 5-methylene group of **1**: we use two names, creatones A and B, in order to avoid confusion like that which has existed in the literatures.[5–7]



Scheme 1. Formation and reactions of creatones A and B.

Creatone A (2-Amino-1-methyl-4,5-imidazoledione) (3). This cyclic compound was prepared from its *t*-Boc-derivative, obtained by the oxidation of *t*-Boc-creatinine.[7] The structure **3** has been unambigously confirmed by newly available B/E linked scan SIMS[8,9] and 400-MHz ^{1}H NMR spectra (see Fig. 1 and Exptl part). Compound **3** was also obtained as a minor product in the condensation of 1-methylguanidine with diethyl oxalate.

Creatone B [*N*-(*N'*-Methylamidino)oxamic Acid] (4). Product **4** from a mercury(II) acetate oxidation of **6** in water (pH ca. 3) was first assigned the structure *N*-methyl-*N*-amidinooxamic acid.[10,11] Later, the same compound was isolated by the oxidation of **1** and formulated as 2-methylamino-4,5-imidazoledione (**5**), supposedly formed via **3** and **4**.[5] However, structure **4** (in Zwitterion form) is now found to be more appropriate for the product (creatone B) on the basis of 400-MHz ^{1}H NMR and B/E linked-scan SIMS analyses (see Fig. 1 and Exptl part). Because **6** readily cyclizes[12] to **1** and creatone B (**4**) is easily formed by the hydrolysis of creatone A (**3**),[7] the oxidation of **6** (or **1**) is likely to first give **3**, followed by hydrolysis to yield **4** during the reaction and/or work-up using an aqueous media. It has now been confirmed by an HPLC study that any drying procedure at above ca. 100 °C in vacuo (and EI mass spectral measurement) for creatone B (**4**) caused dehydrative cyclization to a mixture of creatone A (**3**) and its isomer (**5**); such a facile dehydration was the main reason for the incorrect structural assignment.[5] While the alcoholysis of **3** gave esters **7a**, **b** at 20 °C, the ethanolysis of **4** yielded 1-methylguanidium ethyl hydrogen oxalate (**8**) upon refluxing for 24 h. The material **4** was also obtained as a minor product from the condensation of 1-methylguanidine with diethyl oxalate, obviously after hydrolysis by moisture.

Isomer of Creatone A (2-Methylamino-4,5-imidazoledione) (5). The condensation of 1-methylguanidine and diethyl oxalate in absolute ethanol gave, together with minor products **3** and **4**, the main product (70%) to which we assign structure **5** based on IR, UV, 400-MHz ^{1}H NMR, high-resolution MS, and B/E linked-scan SIMS measurements. The isolated main

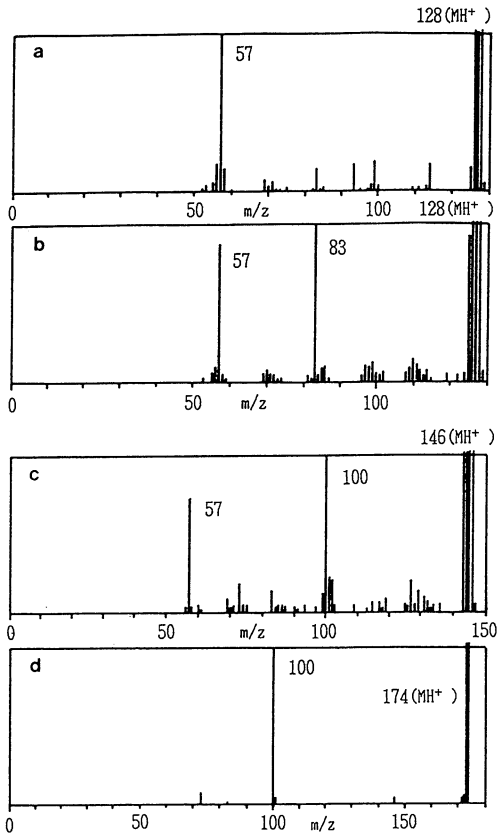


Fig. 1. B/E linked scan SIMS of creatones and their derivatives. a. Creatone A (**3**). b. Isomer (**5**) of creatone A. c. Creatone B (**4**). d. Ethyl ester (**7a**) of creatone B.

product from the same condensation reaction was previously thought to have a structure of either the 2-imino-form of **3**[13] or **4** (after aqueous work-up).[5] Compound **5** is readily hydrolyzed to give **4**, as in the case of **3**.

As a consequence of these findings, it is now possible for us to investigate the provisional roles of creatones A and B in the toxin-producing pathway operating in creatininemic mammals.

Experimental

Melting points are uncorrected. ^{1}H NMR spectra were obtained in D_2O (*t*-butyl alcohol, δ 1.23, as standard), in $(CD_3)_2SO$ (TMS standard) or acetone-d_6 (TMS standard) using a Bruker AM-400 spectrometer. EI-MS, SIMS and B/E linked-scan SIMS[8,9] spectra were taken on a Hitachi-M80-B.

(2-Amino-1-methyl-4,5-imidazoledione)[7] (3: Creatone A). (a) The structure **3** of the dioxo-material, prepared from **1** according to a method described in the literature,[7] was confirmed by instrumental analyses: EI-MS *m/z* 127 (M^+; 61), 99 (89), 71 (72), 56 (92), 55 (84), 42 (100); SIMS *m/z* 128 (MH^+); ^{1}H NMR (D_2O) δ=3.21 (3H, s), [$(CD_3)_2SO$] δ=3.03 (3H, s), 9.15 (2H, brs).

(b) After filtration of crystalline **5** from the reaction mixture of 1-methylguanidine and diethyl oxalate (vide infra), the ^{1}H NMR spectrum of the residue showed that **3** was produced in ca. 10% yield.

***N*-(*N'*-Methylamidino)oxamic Acid (4: Creatone B).** (a) The oxidation of **1** (10 mmol) with mercury(II) acetate (50 mmol) in water (80 ml) for 4 d, followed by removal of mercury as mercury(II) sulfide gave an acidic supernatant from which crude crystals were isolated. Recrystallization from an acidic aqueous solution and drying at 60 °C over P_2O_5 in vacuo gave pure **4** in 74% yield: mp 180 °C decomp (lit,[5] 38%, mp 197—199 °C decomp); EI-MS *m/z* 127 (M^+-H_2O; 61), 99 (69), 71 (45), 56 (100), 55 (92), 42 (88); SIMS *m/z* 146 (MH^+); ^{1}H NMR (D_2O) δ=3.00 (3H, s), [$(CD_3)_2SO$] δ=2.86 (3H, d, *J*=5 Hz), 8.48 (2H, brs), 9.03 (1H, brq, *J*=5 Hz), 11.25 (1H, brs). Found: C, 33.35; H, 5.14; N, 28.81%. Calcd for $C_4H_7N_3O_3$: C, 33.11; H, 4.86; N, 28.96%.

(b) Creatone A (100 mg) was quantitatively hydrolyzed in 1 M acetic acid (5 ml) [1 M=1 mol dm^{-3}] at 25 °C for 1 d to give a crystalline product, which was identical with creatone B.

(c) After filtration of the crystalline **5** from the reaction mixture of 1-methylguanidine and diethyl oxalate (vide infra), the ^{1}H NMR spectrum of the residue showed that creatone B was produced in ca. 5% yield.

2-Methylamino-4,5-imidazoledione (5). To an ethanolic solution (2 ml) of salt-free 1-methylguanidine (2.0 mmol), prepared from 1-methylguanidine hydrochloride and sodium ethoxide and filtered under nitrogen) was added diethyl oxalate (1.3 mmol) at 5 °C. After the reaction mixture had been stirred at 25 °C for 30 min and then allowed to stand for 2 d under nitrogen, the resulting crystalline product was collected by filtration and washed with dry chloroform to give **5** in 75% yield as a colorless crystalline powder: mp 209—212 °C decomp (from dry CF_3CO_2H-AcOEt); EI-MS *m/z* 127 (M^+; 82), 99 (28), 71 (17), 56 (93), 55 (100); SIMS *m/z* 128 (MH^+); ^{1}H NMR (D_2O) δ=3.21 (3H, s); [$(CD_3)_2SO$] δ=2.92 (3H, brs); IR (KBr) 3020, 2920, 2680, 1787, 1747, 1670 cm^{-1}; UV (MeOH) λ_{max} 240 (log ε 3.89), 287 nm (sh 3.35). Found: *m/z* 127.0415. Calcd for $C_4H_5N_3O_2$: M, 127.0382.

Ethyl *N*-(*N'*-Methylamidino)oxamate (7a). Creatone A (**3**) (10 mmol) was dissolved in a minimal amount of anhydrous trifluoroacetic acid, evaporated in vacuo, and the residual syrup dissolved in absolute ethanol (10 ml). After being stirred for 1 d at room temperature, the resulting crystals were collected by filtration to give chromatographically pure **7a** in 43% yield: mp 92—95 °C; EI-MS *m/z* 127 (M—C_2H_5OH; 75), 99 (100), 71 (50), 69 (67), 56 (65), 42 (65); SIMS *m/z* 174 (MH^+); ^{1}H NMR of CF_3CO_2H salt [$(CD_3)_2SO$] δ=1.29 (3H, t, *J*=7 Hz), 2.87 (3H, d, *J*=5 Hz), 4.29 (2H, q, *J*=7 Hz), 8.44 (1H, brs), 8.65 (1H, brs), 8.91 (1H, brq, *J*=5 Hz); IR (KBr) 3300, 3020, 1763, 1724, 1694 cm^{-1}; UV (MeOH) λ_{max} 232 (log ε 3.56), 292 nm (sh 2.84).

Methanolysis of Creatone A. The ^{1}H NMR spectra for creatone A (**3**) (1 mg) in 0.6 ml of methanol-d_4 (99.5% *d*), measured at intervals, showed that the $t_{1/2}$ value from creatone A to methyl-d_3 *N*-(*N'*-methylamidino)oxamate (**7b**) was about 80 min. **7b**: ^{1}H NMR (MeOH-d_4) δ=2.86 (s).

Ethanolysis of Creatone B. A suspension of creatone B (**4**) (10 mmol) in 10 ml of absolute ethanol was heated under reflux in an argon atmosphere for 24 h. The reaction mixture was concentrated. After the addition of acetone, the crystals were collected by filtration. This material was identical with the salt derived from an authentic 1:1 mixture (**8**) of 1-methylguanidine and ethyl hydrogen oxalate: mp 123—124 °C; SIMS *m/z* 264 ($MH+MG^+$), 147 ($2MG+H^+$), 74 ($MG+H^+$); ^{1}H NMR [$(CD_3)_2SO$] δ=1.17 (3H, t, *J*=7 Hz), 2.70 (3H, d, *J*=5 Hz), 3.98 (2H, q, *J*=7 Hz), 7.29 (4H, brs), 8.15 (1H, brq, *J*=5 Hz). Found: C, 38.05; H, 7.13; N, 22.25%. Calcd for $C_6H_{13}N_3O_4$; C, 37.69; H, 6.85; N, 21.96%

We thank Dr. Desmond. J. Brown (the Australian National Univ.) for some advice and Miss H. Morino

(IBAS) for measurement of some physical data.

References

1) K. Ienaga, K. Nakamura, T. Goto, and J. Konishi, *Tetrahedron Lett.*, **28**, 4587 (1987).
2) K. Nakamura and K. Ienaga, *Experientia*, **46**, 470 (1990).
3) K. Ienaga, K. Nakamura, F. Naka, and T. Goto, *Biochim Biophys. Acta*, **967**, 441 (1988).
4) K. Ienaga, K. Nakamura, A. Ishii, T. Taga, Y. Miwa, and F. Yoneda, *J. Chem. Soc., Perkin Trans. 1.*, **1989**, 1153.
5) H. Yamamoto and W. Pfleiderer, *Bull. Chem. Soc. Jpn.*, **55**, 1912 (1982).
6) H. Yamamoto, T. Takahashi, and S. Inokawa, *Heterocycles*, **20**, 1067 (1983).
7) H. Yamamoto, C. Ohira, T. Aso, and W. Pfleiderer, *Bull. Chem. Soc. Jpn.*, **60**, 4115 (1987).
8) K. Ienaga, K. Nakamura, K. Higashiura, Y. Toyomaki, and H. Kimura, *Chem. Pharm. Bull.*, **36**, 2796 (1988).
9) K. Nakamura, K. Higashiura, and K. Ienaga, *Chem. Pharm. Bull.*, **37**, 73 (1989).
10) L. Baumann and T. Ingvaldsen, *J. Biol. Chem.*, **35**, 277 (1918).
11) I. Greenwald, *J. Am. Chem. Soc.*, **41**, 1109 (1919).
12) G. Edgar and W. S. Hinegardner, *Org. Synth.*, Coll. Vol. I, 172 (1932) and references cited therein.
13) W. Traube and K. Gorniak, *Z. Angew. Chem.*, **42**, 379 (1929).