[Chem. Pharm. Bull. 26(9)2914—2919(1978)]

UDC 547.564.3.03.04 : 547.495.9.04

Mechanism of the Color Reaction of Active Methylene Compounds with 1,3,5-Trinitrobenzene Derivatives. X.[1] Three Isomers of 1:2 Complex of Picric Acid and Creatinine

KAZUYA KOHASHI, YASUTO TSURUTA, and YOSUKE OHKURA

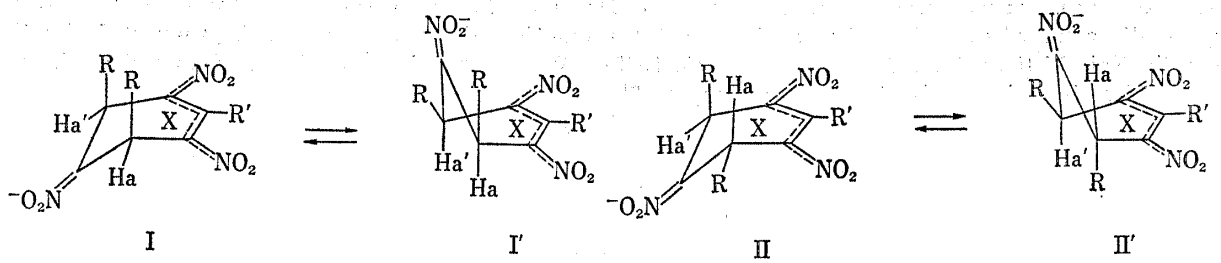
Faculty of Pharmaceutical Sciences, Kyushu University[2]

(Received May 13, 1978)

Three isomers of 1:2 complex of picric acid and creatinine were isolated in crystalline forms from the alkaline reaction mixture of picric acid and excess creatinine. The structures were discussed on the basis of the nuclear magnetic resonance (NMR) and high performance liquid chromatography studies of the three isomers. NMR, infrared and ultraviolet spectra of them were presented.

Keywords——picric acid; creatinine; Jaffé reaction; 1:2-complex of picric acid and creatinine; three isomers of 1:2 complex; comparison of NMR, IR and UV spectra

It has been known that 1:2 complexes of 1,3,5-trinitrobenzene (TNB) and nucleophiles can exist in isomeric *cis* and *trans* forms[3] (I and II, respectively) as shown in Chart 1.



1:2 Complex of TNB and nucleophile: R=nucleophile, R'=H, X=—
1:2 Complex of picric acid and creatinine: R=creatinine residue, R'=O, X=2–

Chart 1

In the previous paper of this series,[1] we isolated a 1:2 complex of picric acid and creatinine in crystalline forms from the alkaline reaction mixture of creatinine with excess picric acid. However, the *cis* or *trans* configuration of two creatinine residues remained unresolved. Recently, we found that three isomers of 1:2 complex formed in the alkaline reaction mixture of picric acid and excess creatinine. This paper describes the isolation of the three isomers and discusses their structures, which may contribute to a mechanistic study of the Jaffé reaction.[4]

Results and Discussion

The alkaline reaction mixture of picric acid and excess creatinine showed color change from red to orange yellow. The resulting orange yellow mixture separated into three main

1) Part IX: K. Kohashi, Y. Tsuruta and Y. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **25**, 2127 (1977).
2) Location: *Maidashi, Higashi-ku, Fukuoka, 812, Japan.*
3) M.R. Crampton, *Adv. Phys. Org. Chem.*, **7**, 211 (1969); M.J. Strauss, *Chem. Rev.*, **70**, 667 (1970); M.J. Strauss and S.P.B. Taylor, *J. Am. Chem. Soc.*, **95**, 3813 (1973); M.R. Crampton and M.J. Willison, *J. C. S. Chem. Comm.*, 215 (1973).
4) M. Jaffé, *Z. Physiol. Chem.*, **10**, 391 (1886). For reviews, see T. Nambara, *Bunseki Kagaku*, **13**, 184 (1964); K. Kohashi and Y. Ohkura, *ibid.*, **23**, 91 (1974).

colored spots on thin-layer chromatoplate (*Rf* values: 0.45, 0.25 and 0.15). When ethanol was added to the mixture, orange yellow precipitate separated out. The precipitate also showed the same three spots as those of the reaction mixture on thin-layer chromatoplate, indicating that the precipitate was a mixture of three colored species. Repeated recrystallizations of the precipitate from water gave pale yellow crystals (complex a). The analytical data[5] of complex a agreed with those of the 1:2 complex of picric acid and creatinine described in the previous paper.[1] The overall filtrate after separation of complex a was treated as described in Experimental and orange yellow plates were obtained. Recrystallization of the crystals from water–ethanol mixture gave pale yellow plates (complex b). The addition of small amounts of ethanol to the residual filtrate after separation of complex b gave pale yellow prisms (complex c). *Rf* values of complex a, b and c were 0.45, 0.25 and 0.15, respectively, which agreed with those of three colored spots observed on the thin-layer chromatograms of the alkaline reaction mixture and the orange yellow precipitate described above. The data of elemental analyses of complex b and c described in Experimental agreed with those of complex a,[1] indicating that the three complexes were stereo isomers derived from one molecule of picric acid and two molecules of creatinine, respectively. Complex a, b and c were soluble in water but insoluble in other solvents.[6] The UV spectra of these complexes in water were shown in Fig. 1. Complex a, b and c showed absorption maxima at 390, 394 and 396 nm, with shoulder around 460 nm, respectively. The IR spectra of complex a, b and c were shown in Fig. 2. Absorption at 1700 cm^{-1} in complex a and b and that at 1680 cm^{-1} in complex c were assigned to the amide group[7] of two creatinine residues. Although the amide band of creatinine picrate[8] was also observed at 1700 cm^{-1}, creatinine itself showed unresolved broad band in a range of 1650—1700 cm^{-1}. Unambiguous assignment of the other absorptions in Fig. 2 was difficult to make. The NMR spectra of complex a, b and c were shown in Fig. 3. NMR signals of complex b and c were assigned to protons of 1:2 complex (III) (Chart 2) in the same way as those of complex a described in the previous paper.[1] A singlet at δ (ppm) 3.10 (6H) in complex c (Fig. 3, c) and two singlets at δ 2.76 (3H) and 3.10 (3H) in complex b (Fig. 3, b) were assigned to methyl protons of creatinine residues

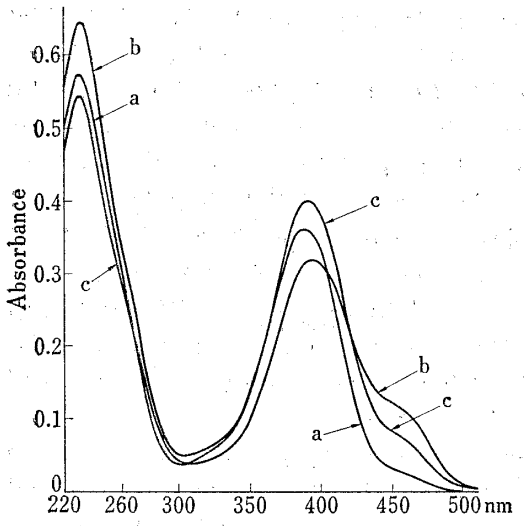


Fig. 1. Absorption Spectra of Complex a, b and c (each 0.25×10^{-4} M)

a) complex a, b) complex b, c) complex c.
The spectra were measured against H_2O immediately after dissolution of the samples in H_2O.

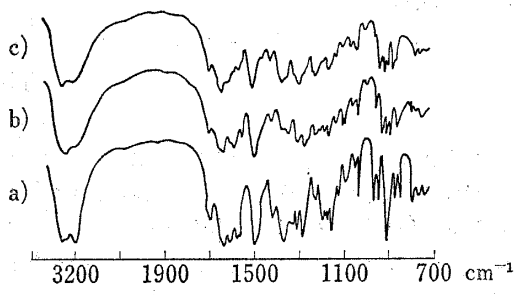


Fig. 2. IR Spectra of Complex a, b and c

a) complex a, b) complex b, c) complex c.

5) Elemental analyses and ultraviolet and visible (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectra.
6) Methanol, ethanol, dimethylformamide, dimethylsulfoxide and acetone were examined.
7) K. Nakanishi, "IR Absorption Spectroscopy," Nankodo, Tokyo, 1960, p. 51.
8) Creatinine picrate was prepared by mixing aqueous solution of picric acid and that of creatinine. Pale yellow needles from H_2O, mp 220—221°.

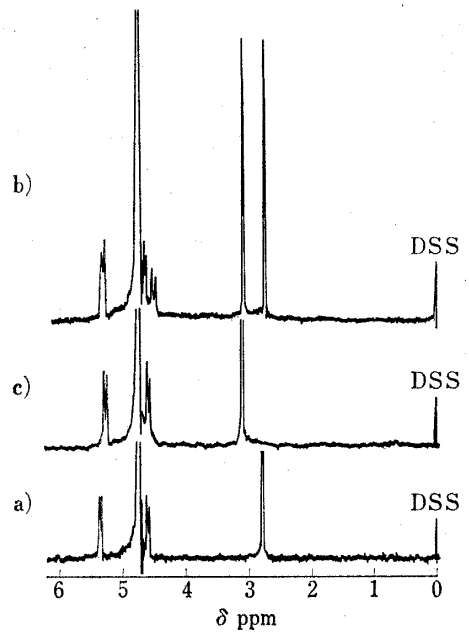
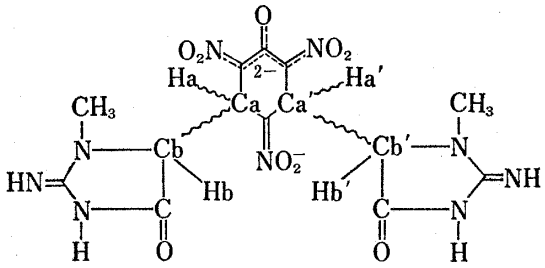


Fig. 3. NMR Spectra of Complex a, b and c in D_2O

a) complex a, b) complex b, c) complex c.



III

Chart 2

bonded to the picrate ring, respectively. A doublet at δ 4.58 (J=3.0 Hz, 2H) in complex c and two doublets at δ 4.61 (J=2.0 Hz, 1H) and 4.50 (J=4.0 Hz, 1H) in complex b were assigned to the methine protons (Hb and Hb') of two creatinine residues, respectively. Another doublet at δ 5.26 (J=3.0 Hz, 2H) in complex c and overlapped two doublets at δ 5.29 (J=2.0 Hz, 1H and J=4.0 Hz, 1H) in complex b were assigned to the protons (Ha and Ha') attached to the ring carbon atoms, respectively. The doublets at lower field and higher field coupled to each other (*cf.*, Fig. 5).

The NMR spectra of complex a, b and c in deuterium oxide (D_2O) measured at various temperatures (3, 29, 40 and 80°) remained unchanged except for the shift of the solvent signal from δ 5.20 (at 3°) to 4.80 (at 80°). However, when each solution was heated up to 95°, the signals due to complex a, b and c gradually decreased in intensities without broadening, respectively and new signals, which were assigned to the others, picric acid and creatinine, appeared with increasing intensities.[9] These facts indicated that complex a, b and c changed partly into the others and partly decomposed to picric acid and creatinine at about 95°, respectively. In the alkaline medium, the conversion of each of complex a, b and c to the others occurred without decomposition at ambient temperature, which were proved by high performance liquid chromatography (HPLC). Chromatograms of complex a, b and c were shown in Fig. 4 (1). When the aqueous solution of complex a was made alkaline and allowed to stand for more than 10 min, the peak of complex a at the retention time of 9 min decreased in intensity and two peaks due to complex b and c appeared at the retention time of 8 and 6.4 min, respectively. Finally, the peak heights became constant. Chromatogram of the resulting solution of complex a was shown in Fig. 4 (2). If decomposition occurred during the conversion of complex a to the others, the peak of creatinine would appear at the retention time of 4 min.[10] Similar results were also obtained in the case of complex b and c. Alkaline solutions of complex a, b and c (dissolved in 10% NaOH or NaOD) showed the same NMR spectra with three singlets at δ 2.90, 2.60 and 2.59. Their solutions, which were neutralized by sodium dihydrogen phosphate solution, separated into three peaks by

9) Creatinine in H_2O showed two singlets at δ 3.03 and 4.04 due to methyl and methylene protons. Their intensities and chemical shifts did not change even on addition of various concentrated NaOH solutions. In D_2O, the signal of methylene protons at δ 4.04 gradually decreased in intensity and disappeared at temperature raised up to 90°, indicating that exchange of the protons with deuterium occurred. When sodium deuterium oxide (NaOD) solution was added, the exchange occurred rapidly at ambient temperature. Picric acid showed a singlet at δ 8.94 in the media.

10) Picric acid was adsorbed on the column packing and the peak was not observed on the chromatogram of the present HPLC.

HPLC (*cf.*, Fig. 4) and three spots by thin-layer chromatography. These facts indicated that each of complex a, b and c partly changed into the others without decomposition in the alkaline medium.

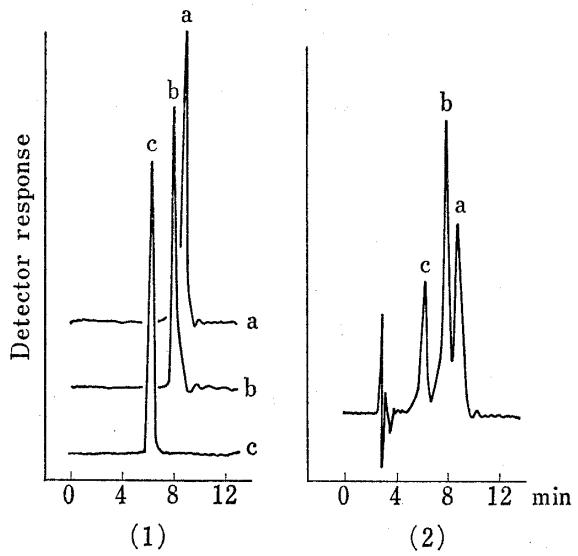


Fig. 4. Chromatograms of Complex a, b and c

(1) a: complex a, b: complex b, c: complx c.
(2) Change of complex a to complex b and c.
To 1.0 ml of aqueous solution of complex a (4 mg/5 ml), 1.0 ml of 5% NaOH was added and the mixture was allowed to stand for 60 min at 25°, then neutralized by 1.0 ml of 10% NaH_2PO_4. The resulting solution (20 μl) was injected. HPLC Conditions: eluent; MeOH–H_2O (20: 80) containing 5% K_2HPO_4 and 0.5% NaCl, flow rate; 0.5 ml/min at 100 kg/cm², temperature; ambient, detector; absorbance at 254 nm (0.16 AUFS), chart speed of recorder; 2.5 cm/min.

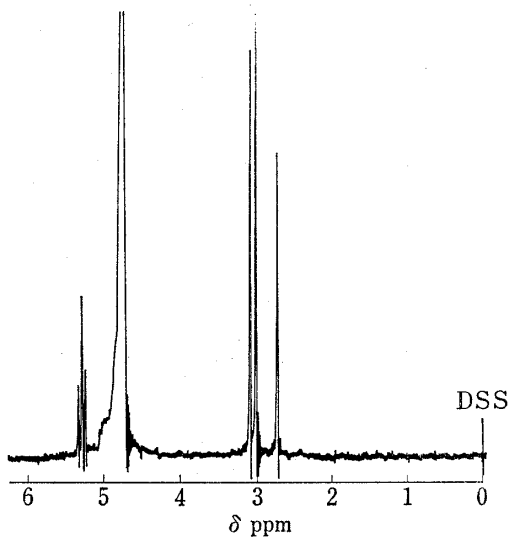


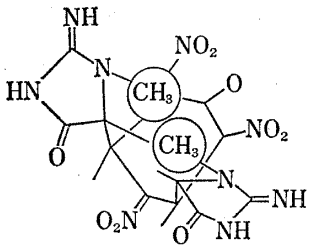
Fig. 5. NMR Spectrum of Reaction Mixture in D_2O

To a mixture of 0.5 ml of creatinine (4.52 mg/ml) and 2.0 ml of picric acid (1.14 mg/ml), 0.5 ml of NaOD (2%) was added and the mixture was allowed to stand for 30 min at 29°.

The NMR spectrum of the reaction mixture of picric acid and excess creatinine in the pressence of NaOD was shown in Fig. 5. Signal of methylene protons of excess creatinine was not observed[9] though that of methyl protons appeared at δ 3.03. The mixture showed three colored spots on the thin-layer chromatoplate in the same way as the aqueous reaction mixture described above, indicating the presence of three 1: 2 complexes which were derived from picric acid and creatinine with methylene group replaced by deuterium.[9] The chemical shifts of three singlets (δ 5.35, 5.29 and 5.26) agreed with those of the ring protons (Ha and Ha′) which appeared as a doublet in complex a, b and c, respectively (*cf.*, Fig. 3). Two singlets at δ 2.76 and 3.10 were assigned to methyl protons of the three 1: 2 complexes.

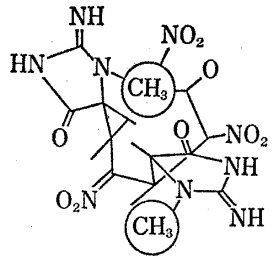
Simplicity of the NMR spectra shown in Fig. 3 and 5 suggested that two creatinine residues of the three complexes attached to two ring carbon atoms in *cis* configuration like I or I′. If the two creatinine residues attached in *trans* configuration like II and II′, ring protons (Ha and Ha′) were magnetically nonequivalent, and would be expected to exhibit different chemical shifts and to appear as more complex signals than observed in Fig. 3 and 5. The agreement of chemical shifts of methyl protons of complex b with those of complex a and c also suggested that one of two methyl groups of complex b was situated in a magnetically equivalent position to methyl groups of complex a, and the other was to those of complex c, respectively.

From the above results and considerations, it would be possible to interpret the relative configurations of the three isomers in the following manner: *cis* and pseudo-axial form of two creatinine residues like I gave four isomers because of two groups of adjacent asymmetric carbon atoms (Ca, Ca′, Cb and Cb′ in III). They were schematically represented by IV, V, V′ and VI (Chart 3). However, none of them could be assigned to complex a, b and c because



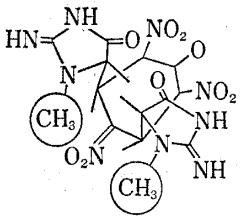
IV

Two methyl groups of two creatinine residues are directed towards dinitro oxo propenide group of picrate ring.



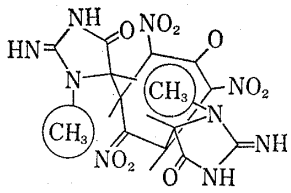
V

One of two methyl groups is directed towards dinitro oxo propenide group, the other is towards nitronate group.



VI

Two methyl groups are directed towards nitronate group.

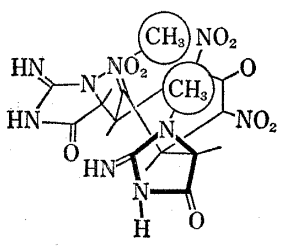


V'

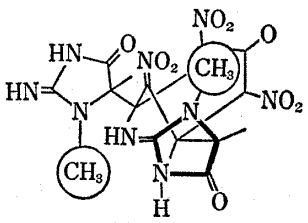
enantiomer of V

Chart 3

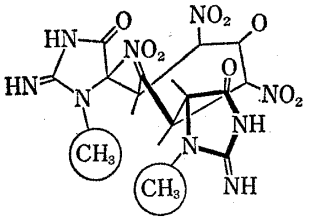
none of them changed to the others unless the bond cleavage and another type of bond formation occurred successively. On the other hand, *cis* and pseudo-equatorial form like I′ gave sixteen isomers (four equatorial forms for each of the four axial forms shown in Chart 3), which were constructed by models, because large steric hindrance among two creatinine residues and nitro and nitronate groups restricted the rotation of two creatinine residues about the bond axes. Eight equatorial forms derived from V and V′ were ruled out because of their unsymmetrical configurations. The equatorial forms derived from VI were schematically shown in Chart 4, which were indistinguishable from those derived from IV from the NMR spectral point of view (*cf.*, Fig. 3). The behavior of complex a, b and c described above was well explained by both equatorial forms. Therefore, whether the structures of complex a, b and c were equatorial forms of IV or those of VI could not be determined with certainty. In the case of equatorial forms of VI shown in Chart 4, if complex a was tentatively assigned to VIa, complex b and c would be assigned to VIb (VIb′) and VIc, respectively.



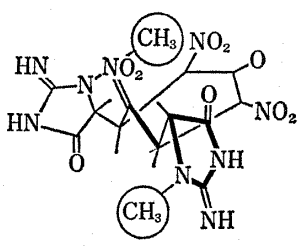
VIa



VIb



VIc



VIb′
(enantiomer of VIb)

Chart 4

Experimental[11]

I) Complex a——To a mixture of creatinine (1 g) and picric acid (1 g) in H_2O (20 ml), 10 ml of NaOH (20%) was added and stirred. After the mixture was allowed to stand for 60 min at 60°, 10 ml of EtOH was added. Orange yellow precipitates were filtered off and dried *in vacuo*. The precipitates were recrystallized from H_2O two times. Pale yellow plates were obtained. Yield, 0.5 g. The analytical data[5] of the crystals were the same as those described in the previous paper.[1]

II) Complex b——After the separation of the orange yellow precipitates in I), 50 ml of EtOH was added and the mixture was allowed to stand in refrigerator (2°) overnight. Pale yellow crystals separated out. Recrystallization from H_2O–EtOH (80: 20) gave pale yellow plates. Yield, 1.0 g. mp 152° (dec.). *Anal.* Calcd. for $C_{14}H_{14}Na_3N_9O_9 \cdot 6H_2O$: C, 26.72; H, 4.16; N, 20.03; Na, 10.96. Found: C, 26.90; H, 4.40; N, 19.82; Na, 11.02. $\lambda_{max}^{H_2O}$ nm (ε): 394 (17000).

III) Complex c——After the separation of complex b, the filtrate was concentrated to about 20 ml *in vacuo*. 10 ml of EtOH was added dropwise to the filtrate and the mixture was allowed to stand overnight in refrigerator (2°). Pale yellow prisms were obtained. Yield, 0.5 g. mp 152° (dec.). *Anal.* Calcd. for $C_{14}H_{14}Na_3N_9O_9 \cdot 6H_2O$: C, 26.72; H, 4.16; N, 20.03; Na, 10.96. Found: C, 27.00; H, 4.38; N, 20.35; Na, 11.10. $\lambda_{max}^{H_2O}$ nm (ε): 396 (16200).

Thin-layer chromatographic conditions: adsorbent; Wakogel B-5, 0.25 mm thickness, dried by being allowed to stand for 15 hr at ambient temperature. Solvent system; EtOH: H_2O: 28% NH_3 (10: 12: 1). The samples were dissolved in 2% NaOH and spotted on the plate.

Acknowledgement The authors express their gratitudes to Mr. H. Matsui for IR spectral measurements, to Mr. Y. Tanaka and Miss Y. Soeda for NMR spectral measurements and to Mr. M. Abe for elemental analyses. Thanks are given to Mr. S. Hashimoto for his skillful assistance in the experimental work of HPLC.

This study was aided in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, which is gratefully acknowledged.

11) UV spectra were measured by a Shimazu UV 200S Double Beam Spectrometer in a cell of 10 mm optical path length. NMR spectra were measured by a JEOL 100H Spectrometer using about 10% D_2O solution of sample containing sodium dimethylsilapentansulfonate (DSS) as an internal standard. IR spectra were measured by a Nihonbunko DS 701G Spectrophotometer in KBr pellets. HPLC was performed by a Hitachi 635 Liquid Chromatograph, equipped with a Hitachi Wavelength Tunable Effluent Monitor and a Hitachi 056 Recorder. Stainless steel column (250 × 4 mm i.d.) packed with Hitachi gel # 3011-N was used. Specific HPLC conditions were given in the legend of Fig. 4.