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

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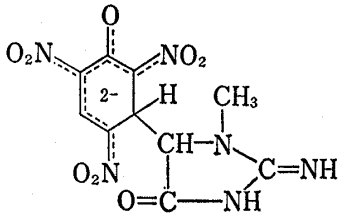
1:2 Complex of picric acid and creatinine was isolated in crystalline forms from the alkaline reaction mixture of creatinine and excess picric acid. Structural characterization of the complex and its absorption spectral behavior were discussed.

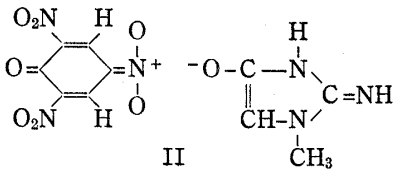
Keywords——Jaffé reaction; isolation of crystalline 1:2 complex; visible absorption spectral behavior; characterization of the structure by NMR; preparation of 1:2 complex

Studies on the structure of the main coloring matter formed in the Jaffé reaction have been made by several workers. Kimura proposed a structure of 1:1 complex of picric acid and creatinine like I (Chart 1) from visible absorption spectroscopic and paper chromatographic studies.[4] Butler, on the basis of kinetic studies, also proposed a similar structure of 1:1 complex.[5] On the other hand, Vasiliades[6] confirmed the structure of 1:1 complex like II (Chart 2), proposed formerly by Anslow and King[7] and recently by Seelig and Wüst,[8] by carbon nuclear magnetic resonance (NMR) spectroscopy. Recently, Butler[9] and Vasiliades[6] postulated the formation of 1:2 complex, which was first described by Greenwald,[10] in the reaction of picric acid with excess creatinine from data of kinetic studies.



I

Chart 1



II

Chart 2

During the course of study on mechanism of the Jaffé reaction, we found that a 1:2 complex like III (Chart 3) was formed even in the reaction of creatinine with excess picric acid. This paper describes the isolation and structural characterization of the complex and its absorption spectral behavior.

1) Part VIII: K. Kohashi, Y. Tsuruta, M. Yamaguchi, and Y. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **25**, 1103 (1977).
2) A part of this paper was presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, Apr., 1976.
3) Location: *Maidashi, Higashi-ku, Fukuoka, 812, Japan.*
4) M. Kimura, *Pharm. Bull.* (Tokyo), **3**, 81 (1955).
5) A.R. Butler, *Clin. Chim. Acta*, **59**, 227 (1975).
6) J. Vasiliades, *Clin. Chem.*, **22**, 1664 (1976).
7) W.K. Anslow and H. King, *J. Chem. Soc.*, **1929**, 1210.
8) H.P. Seelig and H. Wüst, *Arztl. Lab.*, **15**, 34 (1969).
9) A.R. Butler, *J. Chem. Soc. Perkin Trans.*, **2**, 853 (1975).
10) I. Greenwald, *J. Biol. Chem.*, **86**, 333 (1930).

Results and Discussion

When methanol was added to an aqueous alkaline solution of creatinine and excess picric acid, red precipitate was obtained as described in Experimental. The precipitate was hygroscopic and soluble in water but insoluble in other solvents.[11] The absorption spectrum of the aqueous solution showed two maxima at 406 and 480 nm (Fig. 1, 1), which gradually changed to the other band (Fig. 1, 4) when the solution was allowed to stand for 1 hr at 25°. The NMR spectrum of the precipitate in deuterium oxide (D_2O) showed complicated absorptions which were difficult to assign to the protons on the structure I or II and also changed with time[12] (Fig. 2). However, the spectrum of the solution obtained after being allowed

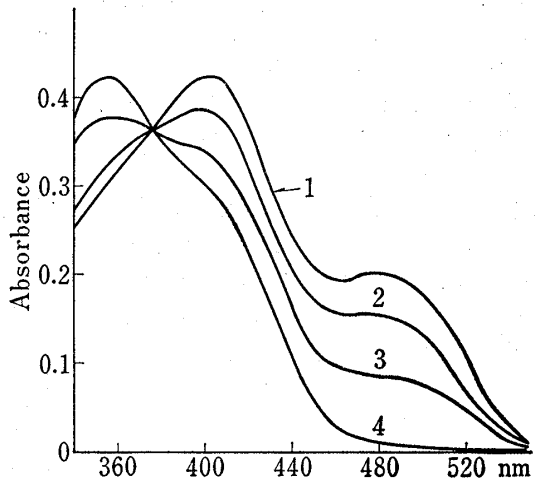


Fig. 1. Absorption Spectral Change of Red Precipitate with Time

1.0 ml of aq. solution (4.0 mg/dl) of red precipitate obtained as described in Experimental was diluted with 3.0 ml of H_2O. The spectra were measured (1, immediately after dissolution; 2, after 10 min; 3, 30 min; 4, 60 min) against H_2O.

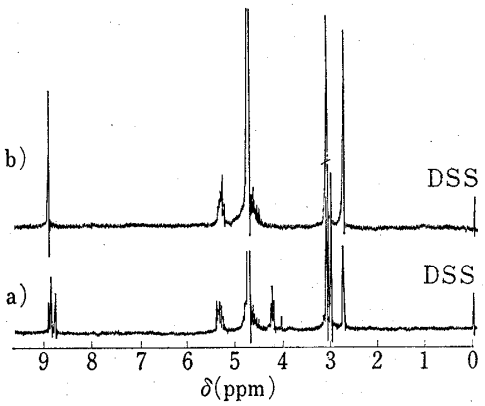
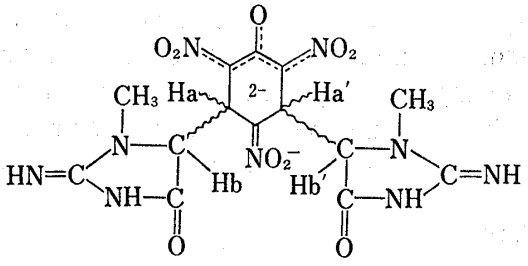


Fig. 2. NMR Spectral Change of Red Precipitate in D_2O with Time

a) immediately after dissolution.
b) after 40 hr.

to stand for more than 40 hr at 25° did not change any longer (Fig. 2, b). When methanol was added to the final solution, orange yellow solid separated out. From the methanol filtrate, sodium picrate was isolated, which showed a signal in the NMR spectrum at δ (ppm) 8.92 due to aromatic ring protons(*cf.*, Fig. 2, b). The same orange yellow solid was also obtained from the alkaline reaction mixture of picric acid and excess creatinine described in Experimental or according to Greenwald's method.[10] The NMR spectrum of the solid in D_2O was almost identical with that shown in Fig. 2,b in the absence of the absorption at δ 8.92 due to the picrate. Repeated recrystallizations of the solid from water gave pale yellow plates. The data of elemental analyses were consistent with the formula of $C_{14}H_{14}N_9Na_3O_9 \cdot 6H_2O$, suggesting that the compound was derived from one molecule of picric acid and two molecules of creatinine. A structure of the compound was determined as trisodium salt of the anion III shown in Chart 3 (III-Na), in which two creatinine residues were considered to attach to sp_3 ring carbon atoms.[13] The NMR spectrum was shown in Fig. 3. A singlet

11) Methanol, ethanol, dimethylformamide, dimethylsulfoxide, acetone, ethyl acetate, benzene, ether and acetic acid were examined.
12) Freshly prepared aqueous solution of the red precipitate separated four colored fractions on thin-layer chromatoplate (adsorbent: Wakogel B-5; solvent: methanol–water–28% ammonia (10: 5: 1)), indicating the precipitate was a mixture of more than four species.
13) The configuration of two creatinine residues with respect to the trinitrocyclohexenide ring plane remains unknown.



III

Chart 3

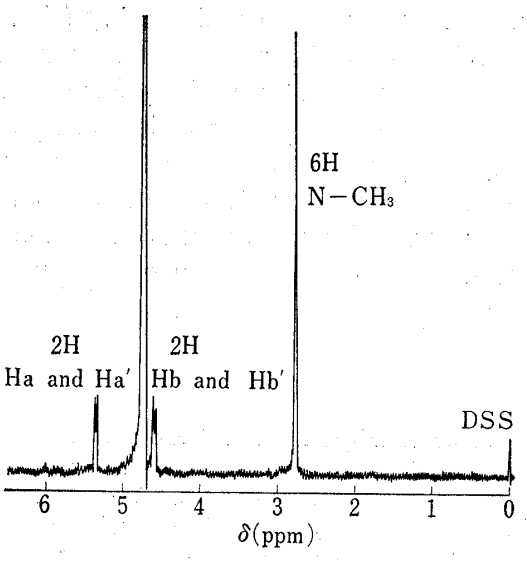


Fig. 3. NMR Spectrum of III-Na in D_2O

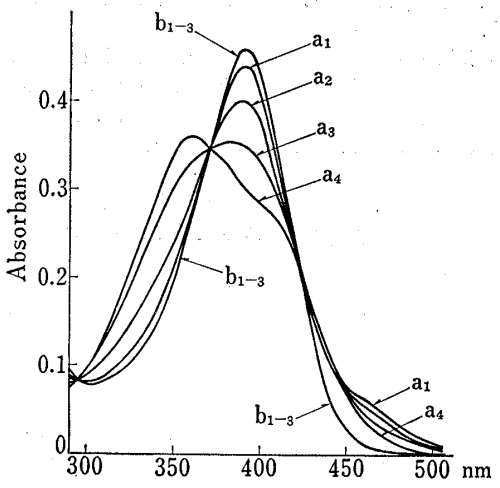


Fig. 4. Absorption Spectral Changes of III-Na with Time in Neutral and Alkaline Media

a) 1.0 ml of aq. solution of III-Na (1.0×10^{-4} M) was diluted with 3.0 ml of H_2O. The spectra were measured after (1, 1 min; 2, 30min; 3, 60 min; 4, 180 min) against H_2O.
b) To 1.0 ml of aq. solution of III-Na (1.0×10^{-4} M), 1.0 ml of NaOH (1, 1%; 2, 4%; 3, 8%) was added. The spectra were measured after 10 and 60 min against H_2O.

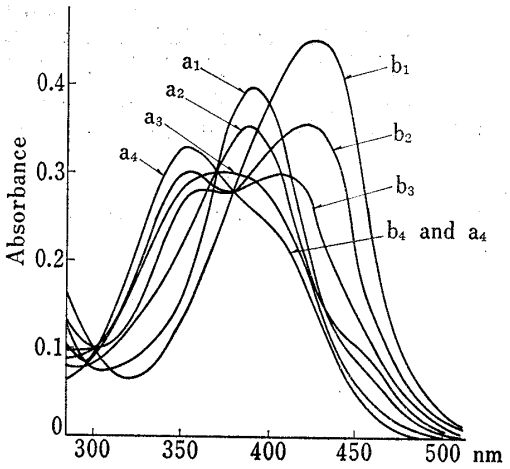


Fig. 5. Absorption Spectral Changes of III-Na with Time in Acidic Media

a) to 1.0 ml of aq. solution of III-Na (1.0×10^{-4} M), 1.0 ml of 0.2% $NaH_2PO_4\cdot2H_2O$ and 2.0 ml of H_2O were added. The spectra were measured after (1, 1 min; 2, 10 min; 3, 30 min; 4, 60 min) against H_2O.
b) instead of $NaH_2PO_4\cdot2H_2O$ in a), 1.0 ml of 10% CH_3COOH was added. The spectra were measured after (1, 1 min; 2, 10 min; 3, 30 min; 4, 60 min) against H_2O.

at δ 2.76 (6H) was assigned to N–CH_3 protons of two creatinine residues. A doublet at δ 5.35 (J=3.0 Hz, 2H) was assigned to the protons Ha and Ha′ on the trinitrocyclohexenide ring carbon atoms, which coupled with the absorption at δ 4.59 (doublet, J=3.0 Hz, 2H) due to the methine protons Hb and Hb′ in the two creatinine residues, respectively.[14] An aqueous solution of III-Na (1.0×10^{-4} M) had a pH value of 8.0 at 20° and showed absorption band with a maximum at 390 nm and a small shoulder around 460 nm, which gradually changed with time (Fig. 4, a_{1-4}). When the pH value of the solution was made 5.2, the

14) Details on the absorptions other than those of III-Na and sodium picrate observed in Fig. 2 will be published in the near future.

change of spectra occurred rapidly (Fig. 5, a_{1-4}). However, when a higher concentrated acidic solution, which made the pH value of the final solution 2.5, was added,[15] the maximum at 390 nm rapidly decreased to form a new maximum at 427 nm and then the intensity gradually decreased with time (Fig. 5, b_{1-4}). The final spectra of these mixtures (Fig. 4, a_4 and Fig. 5, a_4 and b_4) were similar to that of an aqueous solution of creatinine picrate, which showed a maximum at 352 nm and a shoulder around 400 nm. On the other hand, in various concentrated sodium hydroxide solutions, the changes of spectra with time were not observed (Fig. 4, b_{1-3}). However, when the alkaline mixtures were neutralized or acidified, similar changes of spectra to those observed in Fig. 4,a and Fig. 5 occurred.

Experimental[16]

Isolation of III-Na from Red Precipitate——To a solution of creatinine (0.5 g) and picric acid (2.3 g) in H_2O (100 ml), 50 ml of NaOH (2.5%) solution was added. After the mixture was allowed to stand for 30 min at 25°, 300 ml of MeOH was added. Red precipitate was filtered off, washed with MeOH several times and dried *in vacuo*. Yield, 2.5 g. The solution of the precipitate (2.0 g) in H_2O (100 ml) was allowed to stand for 40 hr at 25°. Orange yellow solid separated out by adding MeOH (200 ml). The solid was washed with MeOH and dried *in vacuo*. Yield, 1.0 g. When the orange yellow solid was recrystallized from H_2O three times, pale yellow plates were obtained. Yield, 0.5 g. mp 152° (decomp.). *Anal.* Calcd. for $C_{14}H_{14}N_9Na_3O_9 \cdot 6H_2O$: C, 26.72; H, 4.16; N, 20.03; Na, 10.96. Found: C, 26.90; H, 4.18; N, 20.11; Na, 10.88. $\lambda_{max}^{H_2O}$ nm (ε): 390 (16500). IR ν_{max}^{KBr} cm^{-1}: 1640 (CONH), 1580, 1320 (NO_2).

Preparation of III-Na from Picric Acid and Excess Creatinine——To a solution of creatinine (1 g) and picric acid (1 g) in H_2O (50 ml), 5 ml of aq. NaOH (10%) was added. After warming on a water-bath (80°) for 20 min, 50 ml of MeOH was added. Yellow precipitate was recrystallized from H_2O two times. Pale yellow plates. Yield, 1.5 g. Analytical data agreed with those of III-Na described above.

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15) In this case, brown precipitate separated out when the concentration of III-Na was more than 1%. The precipitate was insoluble in water and organic solvents[11] but soluble only in alkaline solution. Although III-Na was reprecipitated by adding methanol to the alkaline solution, the chemical structure of the brown precipitate remained unresolved.

16) Absorption spectra were measured by a Shimazu UV 200S Double Beam Spectrophotometer in a cell of 1 or 10 mm optical path length. NMR spectra were measured by a JEOL 100H Spectrometer using about 10% D_2O solution of the sample containing sodium dimethylsilapentansulfonate (DSS) as an internal standard. Infrared spectra (IR) were measured by a Nihonbunko DS 701G Infrared Spectrophotometer in KBr pellet. PH was measured by a Hitachi-Horiba pH Meter M-7. Samples were dissolved in distilled water free from carbon dioxide.