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Mechanism of the Color Reaction of Active Methylene Compounds with 1,3,5-Trinitrobenzene Derivatives. II. The Color Reaction of Acetone with 1,3,5-Trinitrobenzene[1]

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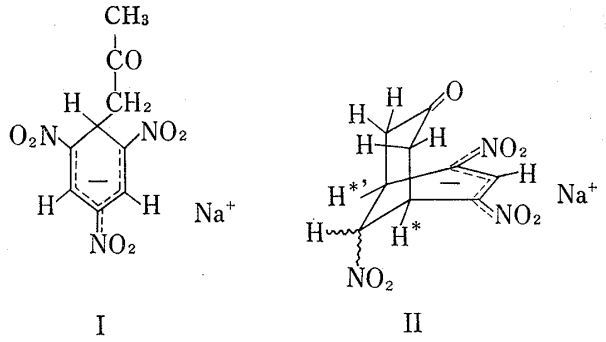
The spectral behaviors of two coloring matters (I and II) were investigated, which were formed in the color reaction of acetone with TNB. An equilibrium reaction system related with I and II was proposed as shown in Chart 4. We had found that the colorations of acetone with TNB in sodium hydroxide solutions were intensified by neutralizing the reaction mixtures with sodium dihydrogen phosphate. Now, the phenomenon was successfully explained by the scheme. On the other hand, another coloring matter (III) was isolated from the reaction mixture, which clarified the cause why the final absorption intensity at 480 mμ much increased when the conversion of I to II was carried out in the presence of excessive TNB. The structure of III, a tetracyclic compound, was determined as III' as shown in Chart 5. The correlations among the three coloring matters and their roles in the color reaction were discussed.

In the previous paper of this series,[3] it was shown that the Meisenheimer type compound[4] (I) and a bicyclic type compound[5] (II) were formed as the main coloring matters of the reaction of acetone with 1,3,5-trinitrobenzene (TNB) under the conditions of the improved spot test procedure.[6] However, the following phenomena still remained with unsatisfactory explanation in the previous paper: The coloration of acetone with TNB in a sodium hydroxide solution was intensified by neutralizing it with sodium dihydrogen phosphate, and the absorption intensity at 480 mμ much increased when I was converted into II in the presence of TNB.



I II

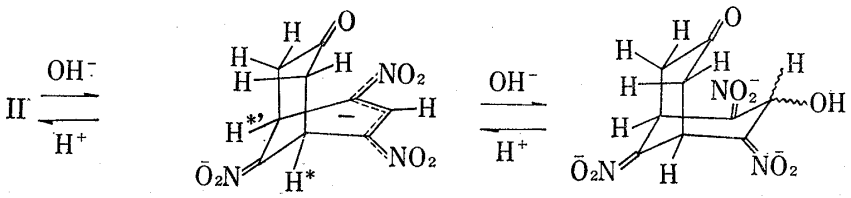
Chart 1

In the present paper, in order to clarify the mechanisms of the above facts, we isolated another new coloring matter (III), and discussed the correlation among the three coloring matters (I, II and III), and their roles in the color reaction mainly in relation to the spot test procedure.

1) This forms "Organic Analysis LXXVIII." A part of this work was presented at "Communications to the Editor" in *Chem. Pharm. Bull.* (Tokyo), **19**, 213 (1971). Part LXXVII: M. Kageura, Y. Ohkura and T. Momose, *Chem. Pharm. Bull.* (Tokyo), in press.
2) Location: *Katakasu, Fukuoka.*
3) K. Kohashi, Y. Ohkura and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **18**, 2151 (1970).
4) M. Kimura, *Yakugaku Zasshi*, **73**, 1219 (1953); For reviews, see R. Foster and C.A. Fyfe, *Rev. Pure and Appl. Chem.*, **16**, 61 (1966); M.J. Strauss, *Chem. Rev.*, **70**, 667 (1970).
5) T. Momose, Y. Ohkura and K. Kohashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 858 (1969); the analogous compounds had been isolated from the reaction mixture of TNB and phloroglucinol in potassium hydroxide solution by T. Severin, *Ber.*, **90**, 2898 (1957); T. Severin, *ibid.*, **92**, 1517 (1959), and also from the reaction mixtures of TNB, dibenzyl ketone or acetylacetone and triethylamine by R. Foster, M.I. Foreman and M.J. Strauss, *Tetrahedron Letters*, **1968**, 4949; M.I. Foreman, R. Foster and M.J. Strauss, *J. Chem. Soc.* (*C*), **1969**, 2112.
6) T. Momose, Y. Ohkura and K. Kohashi, *Chem. Pharm. Bull.* (Tokyo), **11**, 301 (1963).

Equilibrium System of II

The absorption spectra of an aqueous solution of II were changed on adding various concentrations of sodium hydroxide and turned into the original one on neutralizing the resulting alkaline solutions with sodium dihydrogen phosphate, as described in the previous paper.[3] These facts suggested the presence of an equilibrium as shown in Chart 2.



IIa IIb

Chart 2

The structures of the anions (IIa and IIb) were confirmed by the nuclear magnetic resonance (NMR) spectra of II in the sodium hydroxide solutions (Fig. 1). When an equivalent sodium hydroxide to II was added to an aqueous solution of II, a triplet newly appeared at δ (ppm) 6.3 and the intensity of a triplet at δ 5.7, caused by H-C-NO_2 proton, remarkably decreased (Fig. 1,b). The new triplet was assigned to H* and H*' in IIa, which coupled with geminal protons adjacent to the carbonyl group. The signals of the protons, H* and H*' would be expected to appear at a lower field than those of the corresponding protons of II, H* and H*', because they attached to the sp^3 ring carbon atoms between nitronate anion and nitro group on the delocalized dinitropropenide anionic group in the TNB ring system.[7] The triplet at δ 5.7 disappeared and a singlet and a doublet newly appeared at δ 8.3 and 2.0 respectively with more increasing concentration of the alkali (Fig. 1,c). A singlet at δ 8.5 due to the proton on the dinitropropenide group and a multiplet at δ 3.3—2.4 due to the geminal protons adjacent to the carbonyl group in II disappeared when the concentration of the alkali further increased (Fig. 1,d). The new doublet was assigned to methylene protons adjacent to the carbonyl group in bicyclo(3,3,1)nonan-3-one system (IIb), in which the flattening of the ring might occur and this caused the distance between the chemical shifts of the geminal protons to be smaller.[8] The new singlet was assigned to H-C-OH proton between two nitronate anions in IIb. Support for these assignments came from the observations that the intensity of the singlet at δ 8.5 decreased with the appearance of the new singlet at δ 8.3. The signal of hydroxyl proton of IIb could not be observed because of its exchange with water.

These successive NMR and the absorption spectral changes of II as described in the previous paper[3] were satisfactorily explained by the above equilibrium. Thus, the absorption curves, which had two maxima at 262 and 480 mμ and a maximum at 282 mμ,[3] were ascribed to IIa and IIb respectively. The visible absorption spectrum of IIa resembled that of II. IIb had no conjugated chromogenic group but gave an absorption band closely similar to II on neutralizing with sodium dihydrogen phosphate, the dinitropropenide group being recovered.

7) The values of chemical shifts of the similar ring protons formed in other TNB derivative ring systems had been reported by M.R. Crampton and V. Gold, *Chem. Commun.*, **1965**, 256; R. Foster and C.A. Fyfe, *Tetrahedron*, **21**, 3365 (1965); K.L. Servis, *J. Am. Chem. Soc.*, **89**, 1508 (1967); M.R. Crampton, *J. Chem. Soc.* (*B*), **1967**, 1341; M.R. Crampton, *ibid.*, **1968**, 1208; M.R. Crampton and E. El-Ghariani, *J. Chem. Soc.* (*B*), **1969**, 330.

8) M. Fisch, S. Smallcombe, J.C. Gramain, M.A. McKervey and J.E. Anderson, *J. Org. Chem.*, **35**, 1886 (1970).

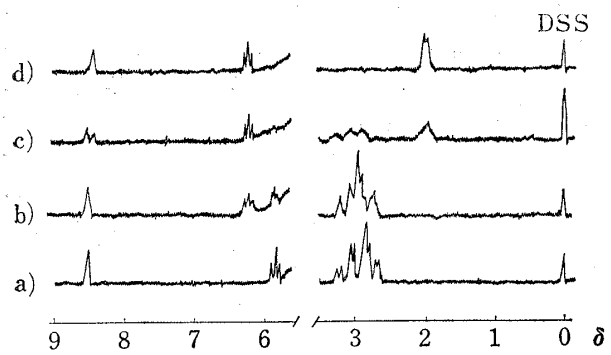


Fig. 1. The NMR Spectral Changes of Aqueous Solution of II in Sodium Hydroxide Solution

a) 29.3 mg of II was dissolved in 0.5 ml of H_2O.
b) To 29.3 mg of II dissolved in 0.4 ml of H_2O, 0.1 ml of aq. 4% NaOH was added (molar ratio of II to NaOH, 1:1).
c) To 0.4 ml of aq. solution of II in b, 0.1 ml of aq. 6% NaOH was added (molar ratio, 1:1.5).
d) To 0.4 ml of aq. solution of II in b, 0.1 ml of aq. 8% NaOH was added (molar ratio, 1:2).

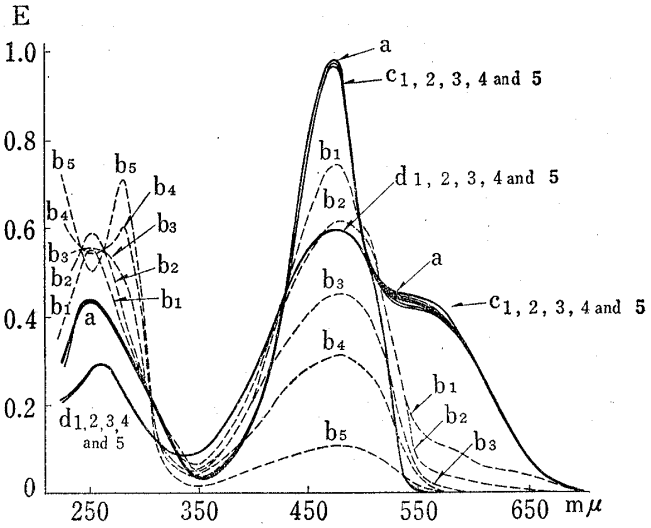


Fig. 2. Absorption Spectral Changes of I in Neutral and Alkaline Media

a: 2.0 ml of aq. solution of I (1.0×10^{-4}M) was diluted with 3.0 ml of H_2O.
b: To 2.0 ml of aq. solution of I in a, 1.0 ml each of H_2O and variously concentrated NaOH (1—5) were successively added. Immediately after mixing well, the mixtures were diluted with 1.0 ml of H_2O. No correction was applied to compensate for the finite time of scanning (3 min).
NaOH concentration is 1, 0.4; 2, 0.8; 3, 1.6; 4, 2.4; 5, 4.0%.
c: 2.0 ml of aq. solution of I in a was treated in the same way as b. After mixing well, each alkaline mixture was neutralized with 1.0 ml of variously concentrated $NaH_2PO_4\cdot2H_2O$ (1—5).
$NaH_2PO_4\cdot2H_2O$ concentration is 1,2; 2,4; 3,8; 4, 12; 5,20%.
d: 2.0 ml of aq. solution of I in a was treated in the same way as c. After adding NaOH, each mixture was kept at 37° for 60 min, then neutralized with $NaH_2PO_4\cdot2H_2O$.
The spectra were measured against H_2O.

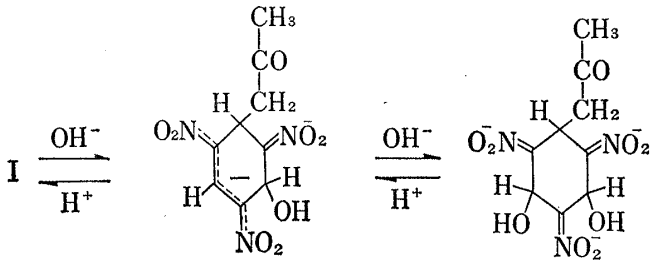


Chart 3

Spectral Behaviors of I and Its Equilibrium System

The absorption spectral changes of I in neutral and alkaline media are shown in Fig. 2. In ultraviolet (UV) region, the aqueous solution of I showed a maximum at 248 mμ (Fig. 2,a). When sodium hydroxide was added to the solution, the maximum shifted to 252 mμ with increasing intensity (Fig. 2,$b_{1,2}$) and finally transferred to 282 mμ (Fig. 2,b_5) with increasing concentration of the alkali. These spectra showed isosbestic points at 242, 264 and 306 mμ. In the visible region, the Meisenheimer type band[4] (Fig. 2,a) changed to new one with a single maximum at about 480 mμ (Fig. 2,b_3). The intensity of the new band was decreased with more increasing concentration of the alkali (Fig. 2,$b_{4,5}$). When the aqueous solution of I was made alkaline with sodium hydroxide solutions described above and then neutralized with sodium dihydrogen phosphate, the initial spectrum was observed unchanged (Fig. 2,c_{1-5}), indicating that the spectral changes were reversible.

The above spectral behaviors of I closely resembled those of II,[3] and indicated that alkaline solutions of I had the similar chromophores to those of IIa and IIb and there existed such equilibrium as shown in Chart 3. Therefore, the absorption curves, which had two maxima at 252 and 480 mμ (Fig. 2,b_2) and a maximum at 282 mμ (Fig. 2,b_5), were assigned to Ia and Ib respectively.[9]

9) The NMR spectra of I in variously concentrated sodium hydroxide solutions could not be measured successfully on account of the bulky absorptions of solvents and the complicated signals caused by the mixtures of I, Ia and Ib.

On the other hand, irreversible spectral changes were observed when the alkaline solutions were allowed to stand for a long time. For example, when the solutions were neutralized after standing at 37° for 60 min, they all showed a spectrum ascribable to II[3] (Fig. 2,d_{1-5}), though the shapes and intensities of the UV absorption curves ascribed to Ia and Ib were similar to those of IIa and IIb respectively and remained unchanged until they were neutralized. These facts suggested that the conversion of Ia to IIa and of Ib to IIb might occur. On the other hand, when each alkaline solution of I was neutralized at a time earlier than 60 min, each reaction mixture showed various two component bands, which were ascribed to the spectra of I and II, respectively (*cf.* Fig. 4,b_{1-4}). Above results might be explained by the scheme as shown in Chart 4.

I is changed to Ia and Ib, which are gradually transformed into IIa and IIb, respectively, in the presence of alkali. When neutralized with sodium dihydrogen phosphate, both Ia and Ib return to I, and both IIa and IIb to II, respectively.[10]

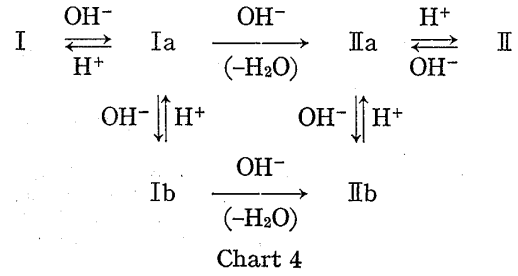


Chart 4

The fact that the coloration of acetone with TNB in the alkaline solution was intensified by neutralizing it with sodium dihydrogen phosphate was successfully explained by the above scheme.

The scheme was also applicable well to explain the Janovsky like reaction,[11] which corresponded to the reaction of TNB and a large amount of acetone in sodium hydroxide solution. In this case, we observed various transient spectra, which were ascribed to I in the early stage of the reaction (Fig. 3,1), then to I and Ia (Fig. 3, 2—4) and finally to IIa (Fig. 3,7).

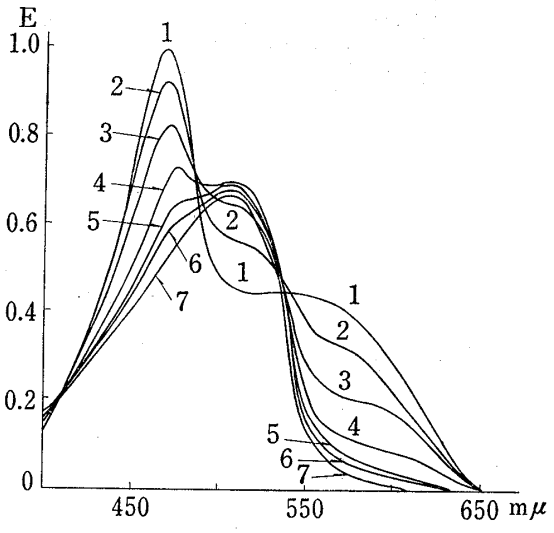


Fig. 3. The Spectral Changes of the Janovsky Like Reaction Mixture

To 2.0 ml of acetone solution of TNB (2.5×10^{-5}M), 2.0 ml of H_2O and 1.0 ml of aq. 0.25% NaOH were successively added and mixed well. The spectra were measured after the periods, 1,2; 2,5; 3,15; 4,30; 5,45; 6,60; 7,120 min against H_2O. No correction was applied to compensate for the finite time of scanning (2 min).

A New Coloring Matter (III) in the Color Reaction

In the course of study on the spectral behaviors of I and II, we found that their absorption intensities much increased when the aqueous solution of I or II was treated with excess TNB in the presence of sodium hydroxide.

The mode of spectrum of I gradually changed to that of II (Fig. 4,b_{1-4}), then the absorption intensities at 480 mμ of the new band increased with time (Fig. 4,b_{5-9}) under the conditions described in the legend of Fig. 4. In the reaction of II with TNB, the absorption

10) Another reaction path leading from the Meisenheimer type anion to the bicyclic anion in the reaction systems of aromatic nitro compounds, diethyl- or triethylamine and ketone or keto ester had been proposed by M.J. Strauss and H. Schran, *J. Am. Chem. Soc.*, **91**, 3974 (1969); M.J. Strauss, T.C. Jensen, H. Schran and K. O'Conner, *J. Org. Chem.*, **35**, 383 (1970).

11) J.V. Janovsky and L. Erb, *Ber.*, **19**, 2155 (1886); J.V. Janovsky, *ibid.*, **24**, 971 (1891). For a review, see E. Buncel, A.R. Norris and K.E. Russell, *Quart. Rev.* (London), **22**, 123 (1968).

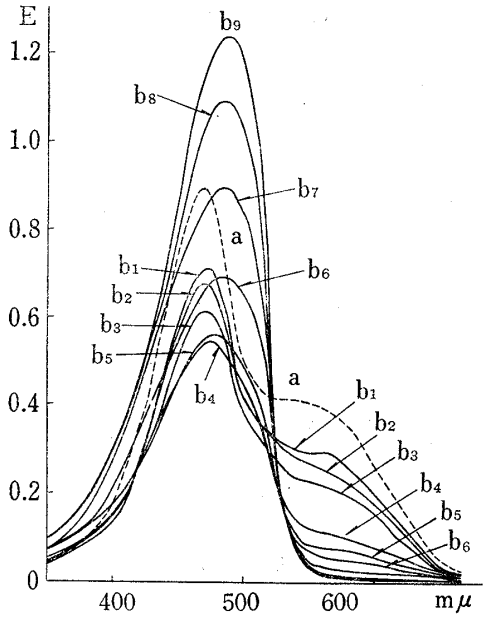


Fig. 4. Absorption Spectral Changes of I in the Presence of TNB

a: 2.0 ml of aq. solution of I (1.0×10^{-4}M), 1.0 ml of aq. 40% dimethylformamide (DMF) and 2.0 ml of H_2O were mixed. The spectrum was measured against H_2O.

b: To 2.0 ml of aq. solution of I in a, 1.0 ml each of 0.1% TNB (100 mg of TNB was dissolved in aq. 40% DMF) and aq. 1.8% NaOH were successively added and kept at room temperature (23°) for several periods (1—9), then neutralized with 1.0 ml of aq. 18% $NaH_2PO_4\cdot2H_2O$. The time is 1,2; 2,3; 4,7; 5,9; 6,13; 7,15; 8,30; 9,60 min.
The spectra were measured against the reagent blank.

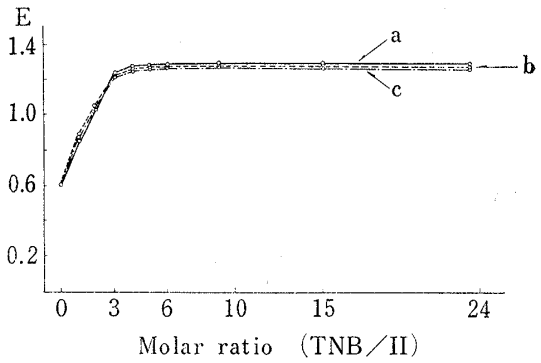


Fig. 5. Effect of TNB on the Absorption Intensity of II in Various Concentrations of Sodium Hydroxide

To 2.0 ml of aq. solution of II (1×10^{-4}M), 1.0 ml each of various concentrations of TNB (dissolved in aq. 40% DMF) and NaOH (a, b and c) were successively added and kept at 37° for 60 min. Each alkaline solution was neutralized with 1.0 ml of $NaH_2PO_4\cdot2H_2O$ (a, b and c).
NaOH concentration is a, 4.0; b,1.6; c, 0.4%. $NaH_2PO_4\cdot2H_2O$ concentration is a, 20; b, 8; c, 2%.
The absorption intensities at 480 mμ were measured against the reagent blanks.

intensities at 480 mμ increased twice as much as the initial intensity of II at the molar ratio of TNB more than 3:1 as shown in Fig. 5.

Above results suggested that another type of coloring matter might also be formed. Thus, a new compound (III) was separated in a crystalline form from the reaction mixture of acetone with TNB by thin-layer chromatographic technics as described in Experimental.

III showed no melting point and exploded when ignited or heated rapidly. Its aqueous solution gave a similar visible absorption curve (Fig. 6,a) to those of the color reaction mixture of acetone with a large amount of TNB at a prolonged reaction time and to the reaction mixture of I or II with TNB (*cf.* Fig. 4,b_9). These facts indicated that III was also a main coloring matter. The data of elemental analyses and infrared (IR) spectrum of III were consistent with a structure containing four sodium, two moieties of TNB and one carbonyl group, and those led us to postulate two structural isomeric forms (III' and III'').

The increase of color intensities in the reaction of I or II with TNB could be well explained by introducing another dinitropropenide group into II. Then, the structure of III was determined as III' from the following NMR assignments (Fig. 7).

Two singlets at δ 8.50 (1H) and 8.55 (1H) were assigned to a proton (Ha) on the dinitropropenide group of A ring, and a proton (Hb) on that of B ring, respectively. The value of chemical shift of Ha was observed at slightly higher field than that of Hb, probably due to the effect of the nitronate anion on B ring, which occupied a position directly above Ha. The signals of two protons (Hc and Hc') on B ring would be expected to appear at higher fields than the corresponding protons (Hd and Hd' on A ring or H* and H*' of II) by the

shielding effect of the delocalized anionic group of A ring, which occupied the position directly above Hc and Hc'. Then, a doublet at δ 5.00 (2H, J=2.9 cps) was assigned to Hd and Hd' and another doublet at δ 4.25 (2H, J=3.0 cps) to Hc and Hc'. By the irradiation of a poorly resolved triplet at δ 3.30 (2H), which was assigned to two protons (He and He') adjacent to the carbonyl group, above two doublets turned into two singlets, respectively. These absorptions were consistent with the structure of III'.

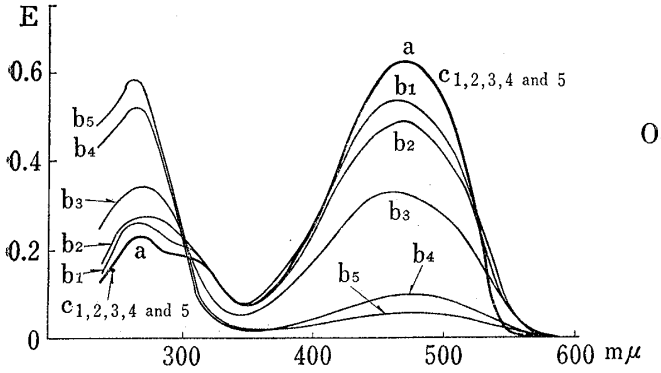


Fig. 6. Absorption Spectral Changes of III in Neutral and Alkaline Media

a: 2.0 ml of aq. solution of III (0.5×10^{-4}M) was diluted with 3.0 ml of H_2O.
b: To 2.0 ml of aq. solution of III in a, 1.0 ml each of H_2O and variously concentrated NaOH (1—5) were successively added, kept at 37° for 60 min and diluted with 1.0 ml of H_2O. NaOH concentration is 1, 0.4; 2, 1.6; 3, 4.0; 4, 20; 5, 40%.
c: 2.0 ml of aq. solution of III in a was treated in the same way as b, and after keeping at 37° for 60 min, each alkaline reaction mixture was neutralized with 1.0 ml of variously concentrated $NaH_2PO_4\cdot2H_2O$ (1—3) and H_3PO_4 (4, 5). $NaH_2PO_4\cdot2H_2O$ concentration is 1, 2; 2, 8; 3, 20% and H_3PO_4 concentration is 4, 20; 5, 40%.
The spectra were measured against H_2O.

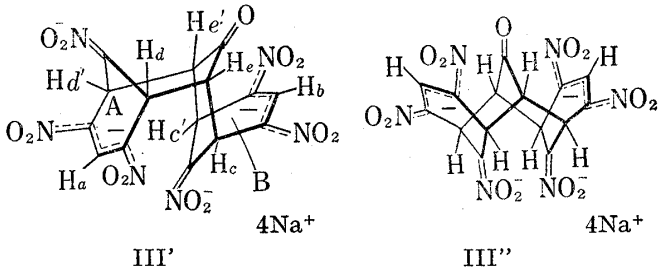


Chart 5

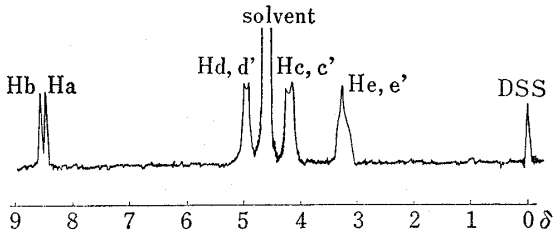
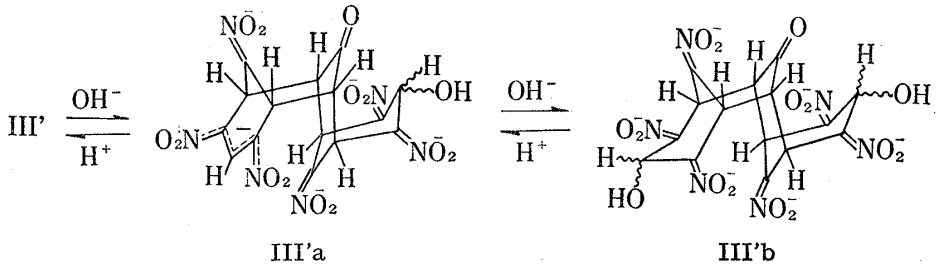


Fig. 7. The NMR Spectrum of III in D_2O

The addition of sodium hydroxide to the aqueous solution of III' caused the decrease of absorption intensity in the visible region (Fig. 6,b). The solutions were almost decolorized in higher concentrations of the alkali (Fig. 6,$b_{4,5}$), and the reversible color change was also observed when the alkaline solutions were neutralized (Fig. 6,c). These facts also indicated the presence of equilibrium between III' and other structural anions, which were assumed as shown in Chart 6.



III'a III'b

Chart 6

The above decrease of absorption intensity with the alkali might be attributed to the formations of III'a and III'b. The latter structure (III'b), which had no conjugated chromogenic group, was thought to be formed only in a higher concentration of the alkali (Fig. 6,$b_{4,5}$). In the procedure for the color reaction in which about 2% sodium hydroxide solution was used,[6] it was assumed that equilibrium between III' and III'a was mainly formed.

When the solution was neutralized, III'a turned into III'. This caused the increase of absorption intensity at 480 mμ.

Above results and the fact that III might be derived from the reactions between II and TNB in each alkali concentration (*cf.* Fig. 5) were successfully explained by the scheme as shown in Chart 7.[12]

On the basis of the spectral changes of I in the presence of TNB (Fig. 4) and the equilibrium systems related with three coloring matters (Chart 4 and 7), the colorations of acetone with TNB under the improved spot test procedure could be successfully explained as follows: When a large amount of acetone was used, the developed color showed the spectrum ascribable to I, but with a prolonged reaction time, the spectrum gradually changed to that of II. In this case, the colors were mainly ascribed to those of I or II. On the other hand, in the usual spot test in which a low concentration of sample was used in the presence of excessive TNB, we observed mainly the final color ascribed to II and III, because the color changes might proceed rapidly from I to II and III.

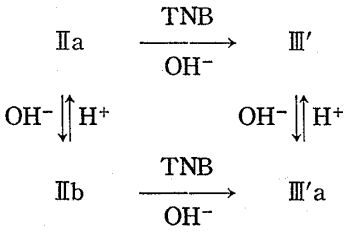


Chart 7

The color reaction mechanisms of other active methylene compounds with TNB under the conditions of the improved spot test procedure[6] will be published in the near future.

Experimental[13]

Preparation of I and II——They were prepared as previously described.[3]

Isolation of III——The procedure was carried out in the same way as in the case of I and II using 0.5 g of acetone in 150 ml of H_2O, a solution of 3.5 g of TNB in 150 ml of DMF, 100 ml of aq. 1.8% NaOH and 100 ml of 18% $NaH_2PO_4 \cdot 2H_2O$ for the reaction time of 100 min. The concentrated reaction mixture was spotted on silica gel layers (about 250 μ thickness, Wakogel B-5) and developed by *n*-BuOH saturated with aq. 3% NH_4OH. After drying the chromatogram in the air at room temperature, the main colored fraction ($Rf=0.25$) was scraped and extracted with MeOH. After evaporating MeOH, the purple red residue was dissolved in a small amount of acetone and rechromatographed to give finally a single fraction on the chromatogram. The last refined residue was dissolved in acetone and filtered. When an appropriate amount of dry ether was added to the acetone filtrate, dark crimson crystals began to separate gradually, which were recrystallized from acetone and benzene (1:3). *Anal.* Calcd. for $C_{15}H_8O_{13}N_6Na_4$: C, 31.48; H, 1.39; N, 14.68; Na, 16.07. Found: C, 31.67; H, 1.49; N, 14.56; Na, 16.00. UV and visible $\lambda_{max}^{H_2O}$ mμ (log ε): 267 (4.07), 470 (4.45). IR ν_{max}^{KBr} cm^{-1}: 1730 (CO), 1550, 1350—1320 (NO_2). It was soluble in H_2O, acetone, MeOH, DMF and dimethylsulfoxide and insoluble in absolute EtOH, ethyl acetate, benzene, ethyl ether, chloroform and petroleum ether.

Acknowledgement The authors express their gratitude to Mr. H. Matsui and Miss Y. Soeda for the IR spectral measurements, Messrs. K. Ishimura and M. Shido for the elemental analyses and Mr. Y. Tanaka for the NMR spectral measurements.

12) Three moles of TNB might be essentially required to form III from the reaction of II and TNB as shown in Fig. 5. One molecule of the reagent was consumed directly to form III and others might be used as oxidizing agent.

13) Absorption spectra were measured by a Shimadzu Recording Spectrophotometer SV-50A and Hitachi 139 Spectrophotometer in a cell of 10 mm optical length; the latter was used for the accurate measurement of absorbance. IR spectrum was measured by a Koken DS-301 Infrared Spectrophotometer in KBr pellet. NMR spectra were measured by JEOL C-60H NMR Spectrometer. Solutions were prepared immediately prior to use. All chemical shifts expressed as δ values were measured in ppm from sodium dimethylsilapentanesulfonate as internal standard.