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Mechanism of the Color Reaction of Active Methylene Compounds with 1,3,5-Trinitrobenzene Derivatives. VII.[1] Color Reactions of Acetophenone with 1,3,5-Trinitrobenzene in Methanol and Methanol-Water Mixtures

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Color reactions of 1,3,5-trinitrobenzene and acetophenone in methanol and methanol-water mixtures under the Janovsky conditions were investigated by use of the stopped flow rapid scan spectrophotometric technics. The observations that the color changes in methanol were quite different from those in methanol-water mixtures of high water content were discussed in comparison with the absorption spectral features of the coloring matters (I-Na and II-Na). The color reaction pathways presented in these media were summarized.

Keywords——Janovsky reaction; acetophenone; 1,3,5-trinitrobenzene; stopped flow rapid scan spectrometric technic; color reaction mechanism

It has been known that 1,3,5-trinitrobenzene (TNB) and acetophenone reacts in the presence of sodium hydroxide to yield a coloring matter, sodium salt of anion I (I–Na) (Chart 1), which shows a characteristic absorption band having two maxima at 465 and 555 nm in methanol.[3] In the previous paper of this series,[4] it was reported that another coloring matter, sodium salt of anion II (II–Na) (Chart 2) showing a maximum around 500 nm was formed along with I–Na in aqueous dimethylformamide solution.

During the course of study on absorption spectral behavior of I–Na, II–Na and the reaction mixtures of TNB and acetophenone under the Janovsky conditions,[5] it was found that alkaline reaction mixture of TNB and acetophenone in methanol showed quite different color change from that observed in methanol-water mixture of high water content. And also a very rapid color change in the earlier time of the reaction, which could not be followed by conventional spectrophotometer, was observed to occur in preference to the appearance of the color due to the anion I in the reactions in both methanol and methanol-water mixtures. This paper describes the spectral changes of I–Na and II–Na in alkaline media and the reaction mixtures of TNB and acetophenone under the Janovsky conditions in both methanol and methanol-water mixtures by use of the stopped flow and rapid scan spectrometric technics and discussed the mechanisms of the color reactions to account for the above observations.

Results and Discussion

Spectral Features of I-Na and II-Na

The absorption band of I–Na in methanol–water (40: 60) mixture showed two maxima at 472 and 545 nm (Fig. 1, a). Immediately after the addition of sodium hydroxide solution it was rapidly transformed into a new band with a maximum at 500 nm showing two isosbestic points at 485 and 523 nm (Fig. 1, b_{1-3}). In methanol, the band of I–Na (Fig. 2, a) slightly

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[1] Part VI: T. Kabeya, K. Kohashi, and Y. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **22**, 711 (1974).

[2] Location: *Maidashi, Higashi-ku, Fukuoka, 812, Japan.*

[3] M. Kimura, *Yakugaku Zasshi*, **77**, 1219 (1953).

[4] K. Kohashi, T. Kabeya, Y. Ohkura, and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **21**, 2187 (1973).

[5] K. Kohashi and Y. Ohkura, *Kagaku No Ryoiki*, **27**, 303 (1973).

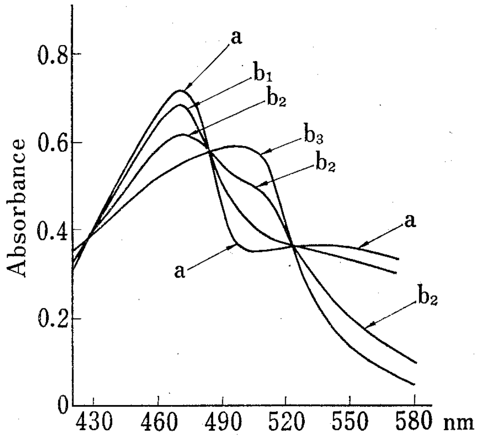


Fig. 1. Absorption Spectral Change of I–Na in Methanol–Water (40:60) Mixture

a) Equal volumes of a solution of I-Na (1×10^{-5}M) dissolved in methanol–water (40:60) mixture and a methanol–water (40:60) mixture were mixed.

b) Equal volumes of the solution of I-Na in a) and a solution of NaOH (2.0%) dissolved in methanol–water (40:60) mixture were mixed. The spectra were recorded after the periods (1; 0.2, 2; 1.6, 3; 40 sec) against H_2O.

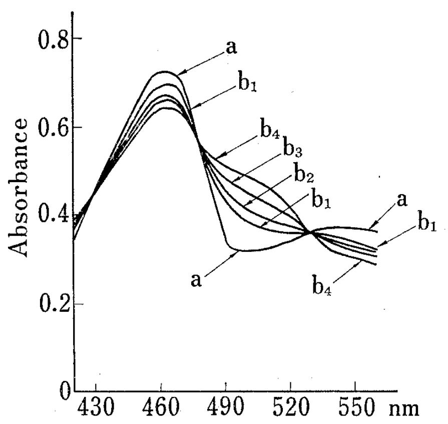
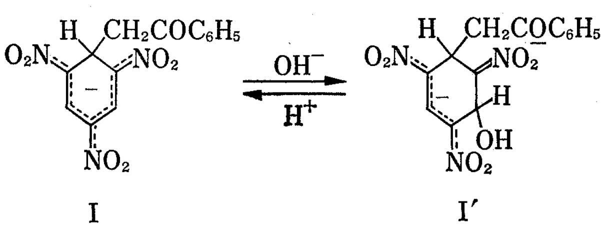


Fig. 2. Absorption Spectral Change of I–Na in Methanol

a) Equal volumes of a methanolic solution of I-Na (1×10^{-5}M) and methanol were mixed.

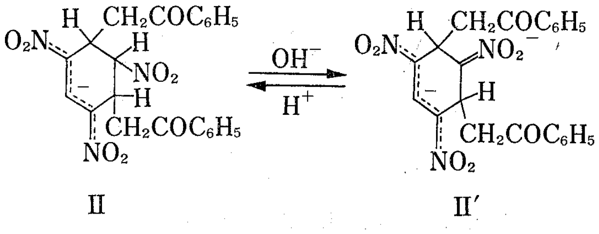
b) Equal volumes of the methanolic solution of I-Na in a) and a methanolic NaOH (2.0%) solution were mixed. The spectra were recorded after the preiods (1; 10 msec, 2; 10 sec, 3; 40 sec, 4; 160 sec) against H_2O.

changed on adding sodium hydroxide solution to form a shoulder around 500 nm showing two isosbestic points at 478 and 531 nm (Fig. 2, b_{1-4}). By neutralization of the resulting alkaline solutions, the new band observed in the methanol-water mixture returned into the original band due to the anion I[6] and the shoulder observed in methanol disappeared. These observations indicate the presence of a mobile equilibrium between the anion I and I′[4] (Chart 1). It is considered that the equilibrium lies so far to the right in the methanol-water mixture and to the left in methanol.



(1)

Chart 1



(2)

Chart 2

On the other hand, when an excess of acetophenone was added to I–Na freshly dissolved in the methanolic aqueous sodium hydroxide and the methanolic sodium hydroxide solutions, respectively and the mixtures were kept standing for a longer period (30—60 min) at 25°, spectra with the maximum at 500 nm in the methanolic aqueous medium and with the shoulder around 500 nm in the methanolic medium were observed, which were similar to the spectra shown in Fig. 1, b_3 and Fig. 2, b_4, respectively, but remained unchanged even if the mixtures were neutralized. II–Na was isolated from the neutralized mixtures which showed a spectrum

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6) The regenerated absorption bands were lower than the original one in intensity since the alkaline solutions of I-Na were unstable, especially in aqueous medium.[4]

7) Other spectral features of II-Na in these media were omitted here because they were almost the same as those in aqueous dimethylformamide solution described in the previous paper.[4]

with the maximum around 500 nm in both alkaline and neutralized solutions,4) indicating that the unchanged absorption around 500 nm was due to the anion II. The anion II changed into a species thought to be II′ (Chart 2) on the addition of sodium hydroxide solution and the equilibrium between II and II′ was reached in the alkaline medium.7)

Color Reactions of TNB and Acetophnone in Methanol

On mixing a sodium hydroxide solution with a TNB solution containing excess acetophenone in the stopped flow apparatus, an absorption band having two maxima at 423 and 500 nm began to appear at about 0.6 msec (Fig. 3, 1). The intensity increased to reach maximum at about 6 msec and was kept constant up to about 100 msec (Fig. 3, 2), then decreased with the appearance of the band due to the anion I, showing an isosbestic point at 440 nm. Finally, the mixture showed a band due to the anion I (Fig. 3, 7). In the absence of acetophenone, the absorption band with two maxima at 423 and 500 nm also appeared and did not change in shape and intensity for more than 30 min at 25°. Therefore, the initially appeared band shown in Fig. 3, 1—2 was attributed to the anion III8) (Chart 3) formed from TNB and sodium hydroxide.

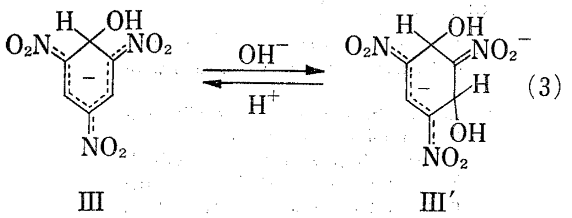

Chart 3

When a higher concentrated sodium hydroxide solution (4%) was used, the anion III′ (Chart 3) was expected to be formed by further interaction of the anion III with

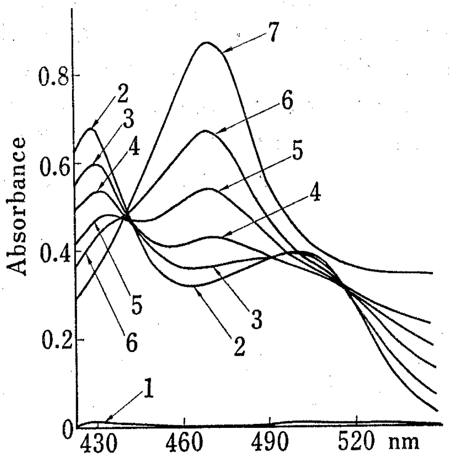

Fig. 3. Absorption Spectral Change of Color Reaction Mixture of TNB and Acetophenone in Methanol (sodium hydroxide concentration, 0.5% in the reaction mixture)

Equal volumes of a solution of TNB (2×10^{-4}M) and acetophenone (4×10^{-2}M) dissolved in methanol and a methanolic NaOH (1%) solution were mixed. The spectra were recorded after the periods (1; 0.6 msec, 2; 6—100 msec, 3; 6.4 sec, 4; 16 sec, 5; 32 sec, 6; 64 sec, 7; 5 min) against H_2O.

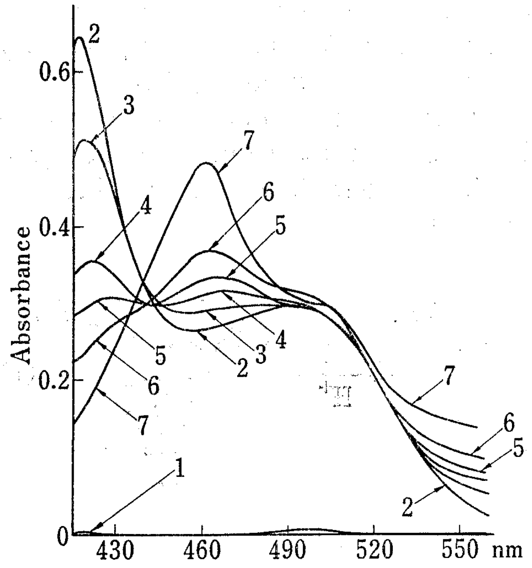

Fig. 4. Absorption Spectral Change of Color Reaction Mixture of TNB and Acetophenone in Methanol (sodium hydroxide concentration, 2% in the reaction mixture)

The spectra were recorded in the same way as described in Fig. 3, but using 4% NaOH solution after the periods (1; 0.6 msec, 2; 6—100 msec, 3; 6.4 sec, 4; 16 sec, 5; 32 sec, 6; 64 sec, 7; 5 min) against H_2O.

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8) The formations of III and III′ by the action of hydroxide ion on TNB have been investigated by several workers. For reviews, see M.R. Crampton, *Adv. Phys. Org. Chem.*, **7**, 211 (1969); M.J. Strauss, *Chem. Rev.*, **70**, 667 (1970); C.A. Fyfe, "The Chemistry of the Hydroxyl Group," Part 1, ed. by S. Patai, Interscience, John Wiley & Sons, New York, 1971, p. 51.

hydroxide ion.[8] However, hardly any band due to the anion III′, which had a maximum around 500 nm, was observed, being covered with highly intensive band of the anion III under the present conditions of the color reaction (Fig. 4, 2). The same spectral change as that shown in Fig. 3 was also observed in the higher alkaline concentration, except that the band of the anion I, which appeared with an decrease in the band intensity of the anion III, had a shoulder around 505 nm (Fig. 4, 7). The shoulder disappeared by the neutralization of the mixture in an earlier time, but remained unchanged when the mixture was neutralized after being allowed to stand for more than 30 min at 25°. The spectral behavior of the mixtures was the same as that of the reaction mixture of I–Na and acetophenone described above.

From the above results, the color reaction in methanol is explained in terms of the reaction schema shown in Chart 4.

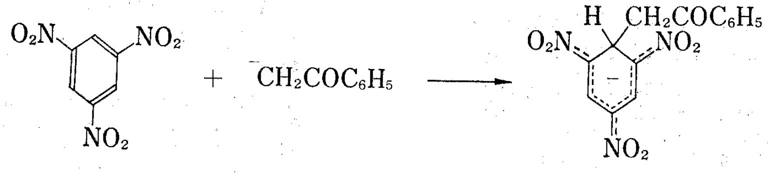
$$C_6H_5COCH_3 + OH^- \rightleftharpoons C_6H_5COCH_2^- + H_2O \qquad (4)$$

$$\mathrm{TNB} + OH^- \rightleftharpoons \mathrm{III} \qquad (3')$$

$$\mathrm{III} + C_6H_5COCH_2^- \rightleftharpoons \mathrm{I} + OH^- \qquad (5)$$

Chart 4

The formation of phenacyl anion (Chart 4, (4)) might be very fast, which was undetectable by means of the present experimental device. The reaction of TNB with hydroxide ion takes place very rapidly to attain an equilibrium (Chart 4, (3′)). The anion I is produced by way of a nucleophilic substitution of hydroxyl group of the anion III by phenacyl group (Chart 4, (5)). The expression (5) is well consistent with the spectral change shown in Fig. 3, though the formation of σ-complex like the anion I has been conventionally represented by the scheme (6) (Chart 5), where a nucleophilic attack of a carbanion intermediate on the TNB dissociated from III.[8]



(6)

I

Chart 5

In addition to the reaction schema shown in Chart 4, two reaction pathways (7) and (8) shown in Chart 6 must be considered in the higher alkaline concentration to explain the spectral behavior of the shoulder around 500 nm described above.

$$(\mathrm{TNB} \overset{OH^-}{\rightleftharpoons} \mathrm{III} \xrightarrow{C_6H_5COCH_2^-}) \mathrm{I} \overset{OH^-}{\rightleftharpoons} \mathrm{I}' \xrightarrow{C_6H_5COCH_2^-} \mathrm{II}' \qquad (7)$$

$$(\mathrm{TNB} \overset{OH^-}{\rightleftharpoons}) \mathrm{III} \overset{OH^-}{\rightleftharpoons} \mathrm{III}' \xrightarrow{C_6H_5COCH_2^-} \mathrm{I}' \xrightarrow{C_6H_5COCH_2^-} \mathrm{II}' \qquad (8)$$

Chart 6

In (7), the conversion of I into I′ occurs very rapidly to attain an equilibrium (1) shown in Chart 1, which lies to the left in methanol as shown in Fig. 2, and then the anion I′ reacts with an excess of acetophenone to yield the anion II′.

In (8), the reaction of TNB and hydroxide ion occurs very rapidly to produce the anions III and III′, and the reaction proceeds with the successive formation of the colored species I′ from III′, and II′ from I′ in the presence of an excess of acetophenone.

Color Reactions of TNB and Acetophenone in Methanol-Water Mixtures

In the case of the reaction in methanol–water (40: 60) mixture, the absorption band due to the anion III showing two maxima at 431 and around 500 nm was observed immediately after mixing of the reactants (Fig. 5, 1—2). The intensity at 431 nm rapidly decreased with an increase in the intensity around 470 nm (Fig. 5, 2—4). The spectral change so far observed (Fig. 5, 1—4) was identical with that of the mixture of TNB and sodium hydroxide in water,[9] indicating the conversion of the anion III into III′. Finally, the mixture showed a band with the maximum around 500 nm (Fig. 5, 9). In the course of the spectral changes under the present aqueous conditions, the band due to the anion I was not observed. However, if the alkaline reaction mixture was neutralized in a time earlier than 30 min, the band of the anion I was generated, which indicated that the equilibrium between I and I′ (1) shown in Chart 1 lay so far to the right in the alkaline medium.

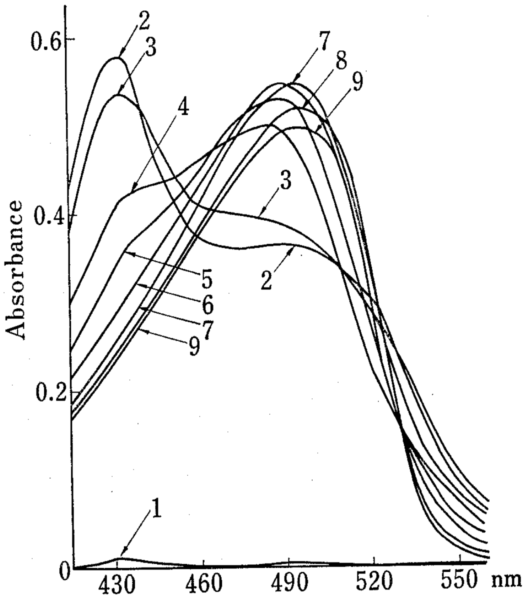


Fig. 5. Absorption Spectral Change of Color Reaction Mixture of TNB and Acetophenone in Methanol–Water (40: 60) Mixture

Equal volumes of a solution of TNB (2×10^{-4}M) and acetophenone (4×10^{-2}M) dissolved in methanol–water (40: 60) mixture and a solution of NaOH (1%) dissolved in methanol–water (40: 60) mixture were mixed. The spectra were recorded after the periods (1; 0.6 msec, 2; 6—100 msec, 3; 400 msec, 4; 6.4 sec, 5; 16 sec, 6; 32 sec, 7; 64 sec, 8; 25 min, 9; 30 min) against H_2O.

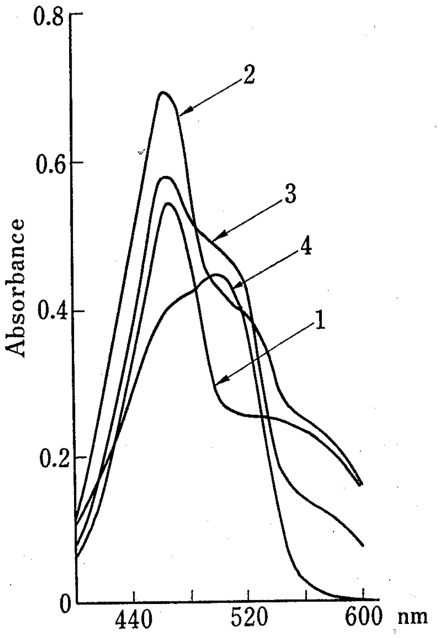


Fig. 6. Absorption Spectra of Neutralized Color Reaction Mixture of TNB and Acetophenone in Methanol–Water (40: 60) Mixture

A mixture of 2 ml each of a solution of TNB (1×10^{-4}M) and acetophenone (2×10^{-2}M) dissolved in methanol–water (40: 60) mixture and a solution of NaOH (1%) dissolved in methanol–water (40: 60) mixture was kept at 25° for the periods (1; 30 sec, 2; 5 min, 3; 15 min, 4; 30 min) and neutralized with 6 ml of $NaH_2PO_4\cdot2H_2O$ (10%) dissolved in methanol–water (40: 60) mixture. The spectra were measured against H_2O.

The spectra of the mixtures neutralized after being allowed to stand for each period are shown in Fig. 6. The band of the anion I (Fig. 6, 1) changed gradually into that of another species, thought to be II, with a maximum around 500 nm (Fig. 6, 4). From the neutralized mixture was isolated II–Na as described in Experimental, which showed a maximum around 500 nm in the same solvent composition as that of the reaction mixture.

The color reaction in this aqueous medium was successfully explained in terms of the reaction pathway (8) shown in Chart 6.

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[9] R. Gaboriaud and R. Schaal, *Bull. Soc. Chim. France*, **1969**, 2683.

When the methanol content of the reaction mixture was increased, for example, in methanol–water (60:40) mixture, the band of the anion I was observed even in alkaline solution but gradually decreased in intensity with time (Fig. 7, 1—5). In the cases of the reactions in aqueous mixtures of higher methanol content than 80%, the spectral changes in the earlier time were almost the same as those in methanol in higher alkaline concentrations shown in Fig. 4. Then, the band due to the anion I gradually decreased in intensity with time and a new band with a maximum around 500 nm was formed. The spectral change was similar to that observed in Fig. 7, 2—6. In these cases, the absorptions around 500 nm were attributed to the anion I′, being in an equilibrium with the anion I (Chart 1), and to the anion II′ derived from I′ and excess of acetophenone. The color changes in earlier time in these aqueous media (Fig. 7, 1—2) are explained in terms of the reaction pathways (7) and (8). The slower change observed in Fig. 7, 2—6 are explained in terms of the pathway (7).

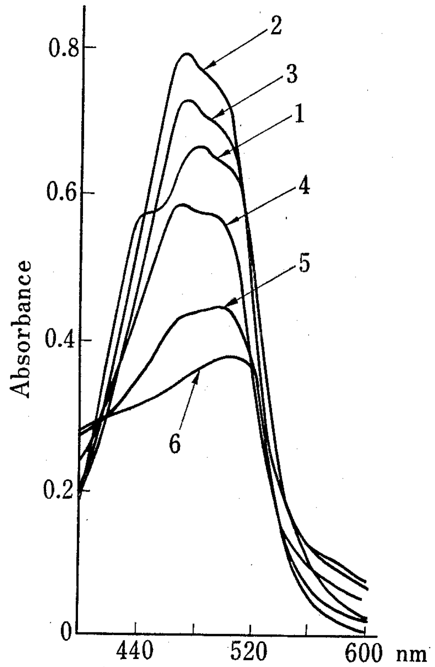

Fig. 7. Absorption Spectral Change of Color Reaction Mixture of TNB and Acetophenone in Methanol–Water (60:40) Mixture

Equal volumes of a solution of TNB (1×10^{-4}M) and acetophenone (2×10^{-2}M) dissolved in methanol–water (60:40) mixture and a solution of NaOH (1%) dissolved in methanol–water (60:40) mixture were mixed. The spectra were measured after the periods (1; 15 sec, 2; 1 min, 3; 7 min, 4; 15 min, 5; 30 min, 6; 60 min) against H_2O.

Overall Reaction Pathways

On the basis of the above results, the color reaction pathways of TNB and acetophenone under the Janovsky conditions described above could be summerized in Chart 7.

The overall reaction scheme was also consistent with the facts that II–Na was isolated from the reaction mixtures of TNB or I–Na, acetophenone and sodium hydroxide in both methanol and methanol–water mixtures and not from the reaction mixture of I–Na and acetophenone in the absence of the alkali.

$$\begin{tikzcd}
\text{TNB} \arrow[r, shift left, "OH^-"] & \text{III} \arrow[l, shift left] \arrow[r, shift left, "OH^-"] \arrow[d, "C_6H_5COCH_2^-"] & \text{III}' \arrow[l, shift left] \arrow[d, "C_6H_5COCH_2^-"] \\
& \text{I} \arrow[r, shift left] & \text{I}' \arrow[l, shift left, "OH^-"] \arrow[d, "C_6H_5COCH_2^-"] \\
& \text{II} \arrow[r, shift left] & \text{II}' \arrow[l, shift left, "OH^-"]
\end{tikzcd}$$

Chart 7

The color change in methanol was mainly due to the formation of I from III and that in methanol–water mixture of high water content was due to the successive formation of III, III′, I′ and II′.

Experimental[10]

TNB was purified as previously described.[11] The other chemicals used were reagent grade.

I-Na——To a solution of TNB (0.5 g) and acetophenone (1.5 g) in MeOH (20 ml), 20 ml methanolic or aqueous NaOH (2%) solution was added. After the mixture was kept standing for 30 min at 25°, 20 ml of

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[10] Infrared spectra were measured by a Nihonbunko DS 701G Infrared Spectrophotometer in KBr pellets. Nuclear magnetic resonance spectra were measured by a JEOL 100H Spectrometer using about 10% acetone-d_6 solution of the sample containing tetramethylsilane as an internal standard.

[11] T. Momose, Y. Ohkura, K. Kohashi, T. Tanaka, Y. Yano, and N. Itakura, *Rinsho Kensa*, **10**, 747 (1966).

aqueous solution of $NaH_2PO_4 \cdot 2H_2O$ (20%) was added and stirred for 20 min. The precipitate was filtered off. The filtrate was evaporated to dryness *in vacuo*. The residue was washed with dry C_6H_6 several times and dissolved in a small amount of dry $(CH_3)_2CO$ and filtered. When an appropriate amount of ether was added to the filtrate, dark purple crystals were obtained. Yield, 0.5 g. Analytical data (elemental analyses, infrared (IR), nuclear magnetic resonance (NMR), ultraviolet and visible (UV) spectra) of the compound were consistent with those of I-Na, which was prepared in aqueous dimethylformamide solution as described in the previous paper.$^{4)}$

II-Na——a) From TNB, Acetophenone and NaOH: To a solution of TNB (0.5 g) and acetophenone (2.5 g) in MeOH (20 ml), 20 ml of methanolic or aqueous NaOH (2%) solution was added. After the mixture was kept standing for 2.5 hr at 25°, 20 ml of aqueous solution of $NaH_2PO_4 \cdot 2H_2O$ (20%) and 2 g of $NaH_2PO_4 \cdot 2H_2O$ were added and stirred for 30 min. The precipitate was filtered off. The filtrate was evaporated to dryness *in vacuo*. The residue, dissolved in MeOH (20 ml) and filtered, was chromatographed on a column of neutral alumina (Merck, Brockmann, Activity II—III) and eluted with MeOH. Two main fractions were separated. From the first eluate, fraction of dark purple color, I-Na, was obtained. After the first eluate was removed, the orange fraction adsorbed over the whole column was eluted with MeOH. The eluate was evaporated to dryness *in vacuo*. The residue was dissolved in a small amount of dry acetone and filtered. When an appropriate amount of dry ether was added to the filtrate, bright orange plates were obtained. Yield, 0.5g. Analytical data (elemental analyses and IR, NMR, and UV spectra) of the compound were identical with those of II-Na, which was obtained from aqueous dimethylformamide medium described in the previous paper.$^{4)}$

b) From I-Na, Acetophenone and NaOH: A solution of I-Na (0.5 g) and acetophenone (2.0 g) in MeOH (20 ml) and 20 ml of methanolic or aqueous NaOH (2%) solution were mixed. After the mixture was kept standing for 2 hr at 25°, 20 ml of $NaH_2PO_4 \cdot 2H_2O$ (20%) and 2 g of $NaH_2PO_4 \cdot 2H_2O$ were added and stirred for 30 min. The succeeding procedures were carried out in the same manner as described in a). 0.3 g of bright orange plates was obtained. Analytical data were identical with those of II-Na in a).

Rapid visible absorption spectral changes shown in Fig. 1, 2, 3, 4, and 5 were measured by a Union RA 1300 Stopped-Flow Rapid Scan Analyser. After mechanical injection of equal volume of the reactant solutions into the reaction cell (optical path length of 1 cm) of the stopped flow apparatus thermostated at 25°, the absorption spectra in a range from 420 to 570 nm were recorded on the digital memory at appropreate time intervals and regenerated on the X—Y recorder.

Other absorption spectra shown in Fig. 6 and 7 were measured by a Shimadzu UV 200S Double Beam Spectrophotometer in a cell of 1 cm optical path length.

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