[Chem. Pharm. Bull. 20(1) 35—40 (1972)]

UDC 547.281.1.04 : 547.551.52.04 : 547.581.2.04

Mechanism of the Color Reaction between Aldehyde and Diazotized Aromatic Amine. I. Color Reaction Products of Formaldehyde with Diazotized Sulfanilamide and with Diazotized *p*-Aminobenzoic Acid

Masaru Nakamura, Kunihide Mihashi, Keiko Egami
and Toshiko Arita

Faculty of Pharmaceutical Sciences, Fukuoka University[1]

(Received April 7, 1971)

Two main coloring matters were isolated in crystalline forms in the color reaction of formaldehyde and diazotized sulfanilamide. Their structures were determined as 1,3,5-tri-(*p*-sulfamoyl)phenylformazan and 1,5-di-(*p*-sulfamoyl)phenylformazan.

Similarly, two main coloring matters of the reaction of formaldehyde with diazotized *p*-aminobenzoic acid were obtained as their methyl esters. They were identified as 1,3,5-tri-(*p*-carbomethoxy)phenylformazan and 1,5-di-(*p*-carbomethoxy)phenylformazan.

The color reaction of aldehyde with diazotized aromatic amine in an alkaline medium in the presence of sodium amalgam has been utilized for its detection,[2] but the structure of the coloring matter has remained unknown.

This paper describes the isolation of some coloring maters produced by the reaction of formaldehyde with diazotized sulfanilamide and with diazotized *p*-aminobenzoic acid, and discusses their chemical structures.

Isolation of the Color Reaction Products

Diazobenzenesulfonic acid has been commonly used as a diazotized amine and was shown not to require sodium amalgam in its reaction with some aliphatic aldehydes.[2] And so, we first attempted the reaction of formaldehyde with this reagent in the absence of the amalgam, but the reaction product could not be successfully separated in crystalline form. Thus, diazotized sulfanilamide and diazotized *p*-aminobenzoic acid were selected as the reagents, which gave the similar color reactions to that of diazobenzenesulfonic acid.

An aqueous solution of formaldehyde gave a red brown color with an excess of diazotized sulfanilamide in a sodium hydroxide solution at room temperature. But, when a large amount of formaldehyde was used in the reaction, the coloration appeared to be red violet and its intensity greatly increased.[3] And the maximum intensity was observed in the molar ratio of the aldehyde to the diazotized amine, about 5.5: 1. Therefore, this ratio was adopted to isolate the reaction products. The reaction mixture was neutralized, and the precipitate thus separated was extracted with acetone. The extract was chromatographed on an alumina column to give two large fractions which were divided by their adsorption colors on alumina. The resulting first and second eluates left red needles of mp 285° (I) and dark brown prisms of mp 264° (II), respectively. Several other small fractions were observed in the chromatogram, but they were difficult to separate successfully.

The absorption spectra of I and II showed their maxima at 528 and 560 mμ, respectively, when dissolved in a 2N sodium hydroxide solution (Fig. 1). On the other hand, the reaction mixture had an absorption maximum at 540 mμ (Fig. 1), indicating that I contributed mainly to the coloration and II partially.

---

[1] Location: *Nanakuma, Fukuoka.*

[2] F. Penzolt and E. Fischer, *Ber.*, **16**, 657 (1883).

[3] The excessive aldehyde might be considered to act as a reducing agent in the reaction.[2]

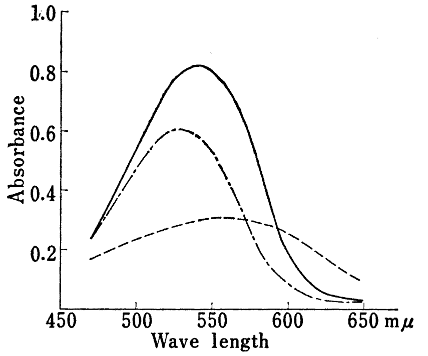

Fig. 1. Absorption Spectra of I, II and the Reaction Mixture of Formaldehyde and Diazotized Sulfanilamide

———: the reaction mixture of formaldehyde and diazotized sulfanilamide[a]
—·—·—: I[b]
--------: II[c]

a) The reaction mixture of formaldehyde and diazotized sulfanilamide, described in Experimental, was diluted 40 times with aqueous 2N NaOH.
b) 0.537 mg of I was dissolved in 100 ml of aqueous 2N NaOH.
c) 0.382 mg of II was dissolved in 100 ml of aqueous 2N NaOH.

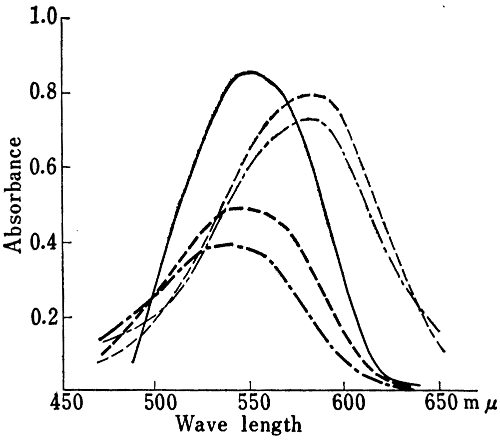

Fig. 2. Absorption Spectra of III, IV and the Reaction Mixture of Formaldehyde and Diazotized *p*-Aminobenzoic Acid

———: the reaction mixture of formaldehyde and diazotized *p*-aminobenzoic acid[a]
—·—·—: III[b]
—·—·—: III, three hours later after dissolving in an alkaline medium[c]
-------: IV[d]
------: IV, three hours later after dissolving in an alkaline medium[e]

a) 1 ml of the reaction mixture, described in Experimental, was dissolved in a mixture of 18 ml of acetone, 8.5 ml of aqueous 1 N NaOH and 32.5 ml of H_2O (*Eventual concentrations of the alkali and acetone are* 0.2N and 30%, respectively).
b) 0.474 mg of III was dissolved in a mixture of 80 ml of acetone and 20 ml of 0.5N NaOH. III and IV were sparingly soluble in aqueous alkali solution, and developed colors were unstable, so aqueous 80% acetone was utilized for a solvent to dissolve the coloring matters, and to stabilize the colors.
c) 1 mg of III was dissolved in a mixture of 67.5 ml of acetone, 67.5 ml of 0.4N NaOH, and 90 ml of 0.2N NaOH (NaOH: 0.2N, acetone: 30%). The spectrum was measured after standing for three hours.
d) 0.340 mg of IV was dissolved in a mixture of 80 ml of acetone, and 20 ml of 0.5N NaOH.
e) 1 mg of IV was dissolved in a mixture of 150 ml of acetone, 150 ml of 0.4N NaOH and 200 ml of 0.2N NaOH (NaOH: 0.2N, acetone: 30%). The spectrum was measured after standing for three hours.

Similarly, formaldehyde showed a red violet color with diazotized *p*-aminobenzoic acid in a sodium hydroxide solution. In the reaction, the molar ratio of the aldehyde to the diazotized amine, about 6:1, gave the maximum color intensity. The reaction mixture thus obtained was neutralized, and the separated precipitate was collected, which could not be divided directly by a column chromatographic technic. Hence, the dried precipitate was extracted with acetone and methylated with diazomethane and then chromatographed on a silica gel column to afford two major fractions which were divided by their adsorption colors. The first and second eluates left red needles of mp 232° (III) and orange needles of mp 182° (IV), respectively. Several other fractions were seen on the chromatogram, but they were so small and could not be separated successfully. III and IV showed absorption maxima at 585 and 588 mμ, respectively, when dissolved in a mixture of aqueous sodium hydroxide solution and acetone. The maxima were located at a longer wave length region than that of the reaction mixture, 550 mμ (Fig. 2). But, the maxima of III and IV shifted to 537 and 545 mμ, respectively, when their alkaline solutions were left standing at room temperature for three hours (Fig. 2). These data suggested that III and IV might be the methyl esters of coloring matters of the reaction and were hydrolyzed to the original ones by the alkali.

Structures of the Reaction Products

The data of elemental analysis of I agreed with the formula of $C_{19}H_{19}O_6N_7S_3$, suggesting that I might be formed from one mole of formaldehyde and three moles of diazotized sulfanilamide. The infrared (IR) spectrum of I had a characteristic absorption band at 1598 cm^{-1}, which might be caused by formazan moiety $(\text{-N=N-}\underset{|}{\text{C}}\text{=N-}\underset{\substack{|\\ \text{H}}}{\text{N}}\text{-})$ of the molecule.[4] The data of nuclear magnetic resonance (NMR) spectrum of the compound are shown in Table I.

Table I. NMR Spectral Data of I, II, III and IV

Product	Solvent	Signal[a] δ (ppm)	Assignment
I	$(CD_3)_2SO$	7.43 s 6H	amide H
		8.02 vs 12H	phenyl H
		13.5 bs 1H	imino H (hydrogen bonding)
II	$(CD_3)_2SO$	7.45 s 4H	amide H
		about 8 bvs 9H	phenyl H and methine H
		13.4 bs 1H	imino H (hydrogen bonding)
III	$CDCl_3$	3.90 s 9H	methoxy H
		8.05 vs 4H	C-phenyl H
		7.65 d 4H, $J=9.5$	N-phenyl H (adjacent two hydrogens)
		8.09 d 4H, $J=9.5$	
		15.3 bs 1H	imino H (hydrogen bonding)
IV	$(CD_3)_2SO$	3.90 s 6H	methoxy H
		8.80 s 1H	methine H
		7.65 d 4H, $J=9.5$	phenyl H (adjacent two hydrogens)
		8.08 d 4H, $J=9.5$	
		12.2 bs 1H	imino (hydrogen bonding)

a) abbreviation: s, singlet; d, doublet; b, broad; v, virtual

Signals ascribable to aromatic protons on benzene rings were observed as a virtual singlet. The presence of a hydrogen bonding imino proton was suggested by a broad signal, which was located at a down field and disappeared on adding heavy water to the sample solution.[5] But it could not be confirmed from the IR spectrum because a broad band due to amino of sulfonamide around 3300 cm^{-1} might veil the imino absorption band, which might originally be broad and weak.[6]

The above data indicated that I might have a structure of triphenylformazan (red form) written in Chart 1.

The data of elemental analysis of II accorded with the formula of $C_{13}H_{14}O_4N_6S_2$, and indicated that II might be resulted by the reaction of one mole of formaldehyde with two moles of diazotized sulfanilamide. The IR spectrum of the compound also showed a characteristic band due to formazan moiety at 1598 cm^{-1} as in I. A broad signal at an extremely down field in the NMR spectrum of II (Table I) was as-

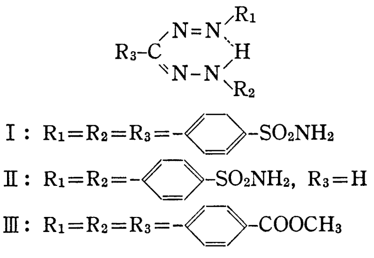

Chart 1. Structures of I, II, and III

---

4) This characteristic band was also observed in the IR spectrum of authentic triphenylformazan.

5) Triphenylformazan dissolved in dimethylsulfoxide-d_6 also gave a broad signal due to imino proton at δ 14.3 in the NMR spectrum.

6) G.N. Lipunova, K.V. Aglitskaya and N.P. Bednyagina, *Izv. Sib. Otd. Akad. Nauk SSSR*, *Ser. Khim. Nauk*, **4**, 50 (1967) [*C. A.*, **69**, 101315m (1968)].

cribed to a hydrogen bonding imino proton from the result of deuterium exchange. But, the existence of the imino group could not be clarified from the IR spectrum as in I.

A broad virtual singlet at δ about 8 in the NMR spectrum of II dissolved in dimethylsulfoxide-d_6 (Table I) might be assigned to aromatic protons on benzene rings and methine proton from the integral intensity. The assumption was confirmed by the fact that two doublets at δ 8.05 (4H, J=9.0 cps) and 8.42 (4H, J=9.0 cps) (four aromatic adjacent two protons), and a singlet at δ 9.85 (1H) (methine proton) were observed in a pyridine-d_5 solution of II. These data indicated that II might be a diphenylformazan (red form) described in Chart 1.

The molecular formula of III was established from elemental analysis and the molecular ion (M^+) in the mass spectrum (MS) as $C_{25}H_{22}O_6N_4$. This suggested that III might be a trimethyl ester of the compound derived from the reaction between one mole of formaldehyde and three moles of diazotized *p*-aminobenzoic acid. The IR spectrum of the compound had characteristic bands caused by a formazan moiety and ester carbonyls at 1608 and 1725 cm^{-1}, respectively. The NMR spectrum of III (Table I) proved the existence of hydrogen bonding imino group by the signal of a strongly deshielded proton, which disappeared on adding a small amount of heavy water, though it could not be clarified from the IR spectrum.[6] The signals of aromatic protons on benzene rings were observed as a virtual singlet and two doublets, which were ascribed as described in Table I, respectively.

From the data described above, the structure of III might be the red form of a triphenylformazan, as shown in Chart 1.

The data of elemental analysis of IV and its M^+ in the MS agreed well with the formula of $C_{17}H_{16}O_4N_4$, which corresponded to the composition of a dimethyl ester of a diphenylformazandicarboxylic acid. The IR spectrum of IV showed the presence of the formazan moiety and the imino group[7] by their absorption bands at 1605 and 3263 cm^{-1}, respectively. The NMR spectrum confirmed the imino group by the broad signal of deshielded proton which disappeared on adding heavy water, and also indicated the presence of methine proton and aromatic protons as shown in Table I.

Above data might support that IV was di-(carbomethoxy)phenylformazan. Furthermore, the characteristic absorption band of ester carbonyls was observed as two bands at 1696 and 1715 cm^{-1} in the IR spectrum of the compound, indicating that the environments around the carbonyls were different each other. Therefore, IV might be the yellow form of the formazan in the solid state (Chart 2).

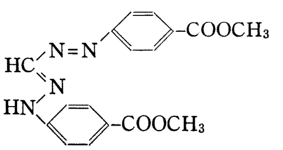

Chart 2. Structure of IV

In order to confirm the assumed structures of I, II, III and IV, the authentic samples of 1,3,5-tri-(*p*-sulfamoyl)phenylformazan, 1,5-di-(*p*-sulfamoyl)phenylformazan, 1,3,5-tri-(*p*-carbomethoxy)phenylformazan and 1,5-di-(*p*-carbomethoxy)phenylformazan were newly prepared by treating malonic acid[8] with diazotized sulfanilamide and with diazotized *p*-aminobenzoic acid, as described in Experimental. The physical properties (melting point, IR and NMR spectra) of the samples were entirely identical with those of I, II, III and IV, respectively.

Now, it may be said that formazan derivatives are main coloring matters of the reaction between formaldehyde and diazotized aromatic amine.

---

[7] In general, the IR spectrum of the yellow form of formazan has the characteristic band due to the stretching vibration of the free NH group at 3200—3500 cm^{-1} in the solid state, but that of the red from hasn't the corresponding band in this region: W. Otting and F.A. Neugebauer, *Z. Naturforsch.*, **23b**, 1064 (1968).

[8] This acid has been commonly utilized with diazotized aromatic amine to synthesize formazan derivatives: W. Otting and F.A. Neugebauer, *Chem. Ber.*, **102**, 2520 (1969).

Experimental[9]

Isolation of I and II——To a solution of 1.7 g of sulfanilamide in 6 ml of concentrated HCl and 30 ml of H_2O, 1 g of $NaNO_2$ freshly dissolved in a small amount of H_2O was added with stirring under ice-water cooling at about 8°. To the resulting diazotized sulfanilamide solution, 5 ml of 37% formaldehyde and 9 g of NaOH dissolved in 40 ml of H_2O were successively added with shaking. The reaction mixture was allowed to stand overnight and then neutralized with dilute HCl. The precipitation of brown product occurred. The collected brown product was washed thoroughly with H_2O, air-dried and dissolved in acetone.

The above procedure was repeated ten times. The acetone solutions were combined, dried over Na_2SO_4, concentrated *in vacuo* and poured onto a column packed with about 200 g of neutralized Al_2O_3[10] and eluted with acetone to afford two main fractions, from which the crystals (I and II) were separated when the each eluate was concentrated.

I——Red needles, mp 285° (from acetone), yield 30 mg. *Anal.* Calcd. for $C_{19}H_{19}O_6N_7S_3$: C, 42.45; H, 3.56; N, 18.24. Found: C, 42.72; H, 3.67; N, 18.94. No depression was observed on admixture with an authentic sample of 1,3,5-tri-(*p*-sulfamoyl) phenylformazan. Its IR and NMR spectra were identical with those of authentic sample. UV $\lambda_{max}^{2N\ NaOH}$ mμ (log ε): 528 (4.79).

II——Dark brown prisms, mp 264° (from acetone), yield 100 mg. *Anal.* Calcd. for $C_{13}H_{14}O_4N_6S_2$: C, 40.83; H, 3.69; N, 21.98. Found: C, 40.73; H, 3.67; N, 21.67. No depression was observed on admixture with an authentic sample of 1,5-di-(*p*-sulfamoyl)phenylformazan. Its IR and NMR spectra were the same as those of authentic sample. UV $\lambda_{max}^{2N\ NaOH}$ mμ (log ε): 560 (4.49).

Isolation of III and IV——To a solution of 1.3 g of *p*-aminobenzoic acid dissolved in 5 ml of concentrated HCl and 40 ml of H_2O, 0.7 g of $NaNO_2$ dissolved in a small amount of H_2O was added with stirring under ice-water cooling at about 8°. To the resulting diazotized *p*-aminobenzoic acid solution, a mixture of 5 ml of 37% formaldehyde and 16 g of NaOH dissolved in 50 ml of H_2O was added with shaking. The reaction mixture was then treated as in the isolation of I and II. The above procedure was repeated five times, and the combined acetone solution of the product thus obtained was concentrated to dryness *in vacuo*. The residue was methylated by dispersing it in the etherial diazomethane prepared from 10 g of nitrosomethylurea. After the removal of the solvent, the product was dissolved in a small amount of a mixture of benzene and acetone (50:1) and poured onto a column packed with about 200 g of SiO_2-gel (Mallinckrodt) and developed with the mixture of benzene and acetone to show two main fractions, from which the crystals (III and IV) were separated when the eluates were separately concentrated.

III——Red needles, mp 232° (from benzene), yield 25 mg. *Anal.* Calcd. for $C_{25}H_{22}O_6N_4$: C, 63.29; H, 4.67; N, 11.81; mol. wt., 474. Found: C, 63.30; H, 4.76; N, 11.79; mol. wt. (mass spectrum), 474. No depression was observed on admixture with an authentic sample of 1,3,5-tri-(*p*-carbomethoxy)phenylformazan. Its IR and NMR spectra were the same as those of authentic sample. UV λ_{max} (solvent: 0.1N NaOH in aqueous 80% acetone) mμ (log ε): 585 (4.90).

IV——Orange needles, mp 182° (from benzene), yield 50 mg. *Anal.* Calcd. for $C_{17}H_{16}O_4N_4$: C, 60.00; H, 4.74; N, 16.46; mol. wt., 340. Found: C, 60.27; H, 4.48; N, 16.39; mol. wt. (mass spectrum), 340. No depression was observed on admixture with an authentic sample of 1,5-di-(*p*-carbomethoxy)phenylformazan. Its IR and NMR spectra were identical with those of authentic sample. UV λ_{max} (0.1 N NaOH in aqueous 80% acetone) mμ (log ε): 588 (4.87).

1,3,5-Tri-(*p*-sulfamoyl)phenylformazan and 1,5-Di-(*p*-sulfamoyl)phenylformazan——Diazotized sulfanilamide solution was prepared from 7.2 g of sulfanilamide, 8.75 ml of concentrated HCl and 2.85 g of $NaNO_2$ in the usual manner. This was treated with 1.3 g of malonic acid in the presence of 62.5 ml of dimethylformamide and 12.5 ml of pyridine under the conditions for the preparation of triphenylformazan.[8] The chromatographic separation of the resulting product was performed as described in the isolation of I and II, and gave two fractions. The first and second eluates left 40 mg of 1,3,5-tri-(*p*-sulfamoyl)phenylformazan, red needles of mp 285° (from acetone), and 50 mg of 1,5-di-(*p*-sulfamoyl)phenylformazan, dark brown prisms of mp 264° (from acetone), respectively. The data of elemental analyses of them well agreed with the calculated values, respectively.

1,3,5-Tri-(*p*-carbomethoxy)phenylformazan and 1,5-Di-(*p*-carbomethoxy)phenylformazan——Diazotized *p*-aminobenzoic acid solution obtained by treating 6.20 g of *p*-aminobenzoic acid, 10.5 ml of concentrated HCl and 3.42 g of $NaNO_2$ was treated with 1.56 g of malonic acid in the presence of 75 ml of dimethylformamide and 15 ml of pyridine, in the same way as described above. The product was methylated with diazo-

---

[9] UV spectra were measured by a Shimadzu Double-40 Multiconvertible Spectrophotometer in a cell of 10 mm optical length, IR spectra by a Hitachi EPI-G3 Infrared Spectrophotometer in KBr pellets otherwise noted, NMR spectra by a JEOL-60H NMR Spectrometer at 60 Mc with tetramethylsilane as an internal standard, and MS by a JEOL JMS 01-SG Mass Spectrometer. All melting points are uncorrected.

[10] Commercial activatied alumina (Merck, 100 mech) was dispersed in H_2O, neutralized with 10% HCl and filtered. After washing with H_2O, it was air-dried and activated at 120° for 10 hr.

methane and chromatographed to be separated into two fractions in the same way as described in the isolation of III and IV. The first and second fractions gave 20 mg of 1,3,5-tri-(*p*-carbomethoxy)phenylformazan, red needles of mp 232° (from benzene), and 10 mg of 1,5-di-(*p*-carbomethoxy)phenylformazan, orange needles of mp 182°, respectively. Mass Spectra *m*/*e*: 474 (M^+) and 340 (M^+), respectively. The data of elemental analyses of them well agreed with the calculated values, respectively.

Acknowledgement The authors are greatly indebted to Professor T. Momose and Assistant Professor Y. Ohkura of Kyushu University for their valuable advices. They are also grateful to Miss Y. Inatsu and Mr. M. Shido of Kyushu University for mass spectral measurement and elementary analyses, respectively.