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Studies on the Reaction between Polynitrobenzene Compounds and Active Methylene Groups. VII.[1] On the Colored Substances of the Janovsky and the Zimmermann Reactions

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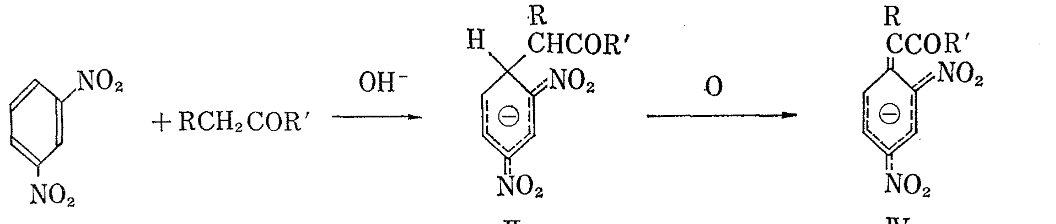
The color–substance, $C_9H_9O_5N_2K$, (III) was successfully isolated from the Janovsky reaction–mixture of acetone, *m*–dinitrobenzene (I), and potassium hydroxide. The spectral and chemical properties were studied in comparison with the potassium salt of 2,4–dinitrobenzyl methyl ketone (V), the Meisenheimer–like complex structure of III being thus confirmed: Absorption spectra of III and V in acetone were quite identical with those of the Janovsky and the Zimmermann reaction–mixture, respectively (Fig. 1); λ_{max} of III varied highly in different solvents, in contrast to that of V (Table I). NMR spectrum gave the sextet structure of the proton (Hδ) attached to the ring sp^3 hybridized carbon atom (Table II). When the solution of III was kept at pH 1, diacetonyl–dinitroazoxybenzene, V, and I were isolated. Strong alkali decomposed III to 2,4–dinitrophenol and unknown substances; a remarkable difference from V was shown as for the elimination of nitrite ion. Ultra–violet irradiation on III gave the unknown substance, free of carbonyl absorption in IR spectrum.

Since the color reaction of *m*–dinitrobenzene with active methylene compounds in the presence of alkali was first described by Janovsky,[3] it has been widely employed in the detection of aldehydes and ketones having an α–hydrogen atom. The Zimmermann reaction, on the other hand, has been used for the quantitative estimation of steroidal ketones and these two reactions have been considered to be quite identical in the mechanism.[4] Corker, *et al.*,[5] however, suggested that the products from these two reactions are different from each other, because the Janovsky reaction is carrried out with a large excess of the ketone (Janovsky condition) and the Zimmermann reaction is carried out with a large excess of *m*–dinitrobenzene (Zimmermann condition). Foster, *et al.*[6] examined also these reactions in details and drew the same conclusion that the color observed in the Janovsky reaction is due to the species (II) formed by the attack of the conjugated base of the ketone at the 4–position of *m*–dinitrobenzene, whereas that of the Zimmermann reaction is due to the species (IV) formed by further oxidation of II, probably with nitro compound as shown below:



I II IV

(III : R=H ; R'=CH_3) (V : R=H ; R'=CH_3)

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[1] Part VI: *Yakugaku Zasshi*, **78**, 1401 (1958).

[2] Location: *Nishi-6-chome, Kita-12-jo, Sapporo.*

[3] J.V. Janovsky, *Ber.*, **19**, 2158 (1886); **24**, 971 (1891).

[4] W. Zimmermann, *Z. Physiol. Chem.*, **233**, 257 (1935).

[5] C.S. Corker, J.K. Norymberski, and R. Thow, *Biochem. J.*, **83**, 583 (1962).

[6] R. Foster and R.K. Mackie, *Tetrahedron*, **18**, 1131 (1962).

The colored products of the Zimmermann condition were isolated from the alkaline reaction–mixture of *m*–dinitrobenzene with each of acetophenone,7) dehydroepiandrosterone5) as well as androsterone.8) In spite of some trials, however, the intermediate (II) of the Zimmermann reaction, that is, the colored substance of the Janovsky reaction, has never been isolated from the reaction–mixture using *m*–dinitrobenzene (I) itself, though the corresponding compounds were isolated in cases using some derivatives of I.9,10) If this would be successful, the reaction mechanism might become more evident. The present paper describes on the isolation11) of III as well as V and the discussion of the relation between them.

Although the pigment formed under the Janovsky conditions is generally too unstable to be isolated, it became evident from our studies on the reaction that the color is fairly stable in some aprotic solvents such as acetone, dimethylformamide and dimethylsulfoxide, in contrast to that in alcohol and water. It was also observed that the coloration is highly stable under anhydrous conditions. When *m*–dinitrobenzene was thus treated with a large excess of acetone dried in advance with pellets of potassium hydroxide which acted simultaneously as alkali, the dark–violet needles $C_9H_9O_5N_2K$, were obtained. The absorption spectrum of this pigment in acetone was quite identical with that of the Janovsky reaction–mixture (Fig. 1). Thin–layer chromatography (TLC) on silica–gel of this pigment showed only one spot. It might thus be considered that *m*–dinitrobenzene reacted with hydroxyl ion to form an addition compound of the type described by Meisenheimer12,13) which in turn reacted with acetone to form the colored derivative (III) having an ionized *aci*–nitro group; Foster, *et al.*14) derived the Janovsky complex of acetone–trinitrobenzene (VI)9) from the Meisenheimer–like complex (VII)15) in acetone. On the other hand, it may be alternatively assumed that acetonyl anion is formed at the first step followed by the reaction with I to produce III as pointed by Canbäck16) and this is clearly supported by the fact that the more anhydrous condition was carried out, the more amount of pigment was produced in our experiments.

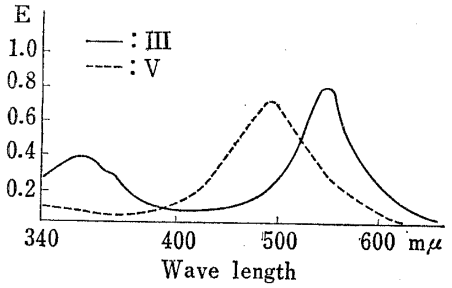
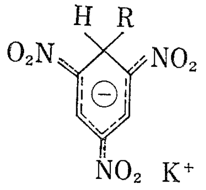


Fig. 1. Absorption Spectra of Janovsky Complex (III) and Zimmermann Compound (V)



(Ⅵ: $R=CH_2COCH_3$)9)
(Ⅶ: $R=OCH_3$)15)

Spectral Observation on the Pigments

The Janovsky complex (III) which was obtained as pure crystals, dissolved readily in acetone, dimethylformamide and dimethylsulfoxide to give violet solutions; it was also soluble in waterand alcohol but insoluble in benzene and chloroform. The solubility of the Zimmermann

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7) T.J. King and C.E. Newall, *J. Chem. Soc.*, **1962**, 367.
8) O. Neunhoeffer, K. Thewlt, and W. Zimmermann, *Z. Physiol. Chem.*, **323**, 116 (1961).
9) M. Kimura, *Yakugaku Zasshi*, **73**, 1219 (1953).
10) M. Akatsuka, *Yakugaku Zasshi*, **80**, 378, 384 (1960).
11) M. Kimura, M. Kawata, and M. Nakadate, *Chem. Ind.* (London), **1965**, 2065.
12) J. Meisenheimer, *Ann.*, **323**, 205 (1902).
13) V. Gold and C.H. Rochester, *J. Chem. Soc.*, **1964**, 1710.
14) R. Foster and C.A. Fyfe, *Tetrahedron*, **21**, 3372 (1965).
15) R. Foster and R.K. Mackie, *J. Chem. Soc.*, **1963**, 3796.
16) T. Canbäck, *Farm. Revy*, **48**, 153 (1949).

compound (V, K–salt) prepared by the known method[17] was quite similar to that of III. The absorption spectra of III and V in acetone were identical with those observed on the Janovsky and the Zimmermann reaction–mixtures, respectively, whereas their absorption maxima were apparently different from each other (579 and 493 mμ, respectively) as shown in Fig. 1; Pollitt, *et al.*[18] reported the former to be at 576 mμ. Foster, *et al.*[6] observed that the nature of active methylene compounds did not appreciably affect λ_{max} under the Janovsky conditions (570 mμ in acetone, for example) while it caused considerable shifts of the maximum in the Zimmermann reaction (490 mμ in acetone, for example). King, *et al.*[7] described that λ_{max} was similarly sensitive to the substituent R in V because of its attachment to a carbon atom conjugated with dinitrophenyl– and carbonyl groups. In the course of our studies the absorption spectra of the Janovsky reaction in several different solvents were shown to be not identical, the solvent effect on these pigments being noticed. The absorption maximum of III was found to highly vary in a range from 480 (water)to 587 mμ (dimethylsulfoxide), whereas V showing almost identical maxima in these solvents as summarized in Table I; this might suggest that acetonyl group in III can be replaced by the conjugated base of the solvent. In contrast to the higher stability in aprotic solvents, these two pigments were rather unstable in water as well as in alcohol and the solvent effect remains unexplained.

Infra–red spectrum of *m*–dinitrobenzene shows the symmetric and asymmetric N–O stretching vibration bands at 1347 and 1542 cm^{-1} (in $CHCl_3$), respectively.[19] The Meisenheimer structure (III) may lead to a decreased N–O bond order of the nitro groups, and thus to lowering of the asymmetric and symmetric N–O stretching frequencies,[20] as has been reported on the quinoidal form of nitrobenzene derivatives.[21] The strong bands at 1290 cm^{-1} in III and 1310 cm^{-1} in V may, therefore, be assigned to the symmetric N–O stretching. The asymmetric ones were observed at 1470 and 1453 cm^{-1} in III and V, respectively. The transfer of negative charge to the nitro group was thus indicated in agreement with the proposed structures. The carbonyl stretching vibration band of III appeared at 1719 cm^{-1}, being assigned to the aliphatic C=O band. That of V, on the contrary, was at the frequencies of 1610 cm^{-1}, lower than those of ordinary ketones, suggesting the abnormally higher conjugation with double bond.

TABLE I. The Absorption Maximum of Janovsky Complex (Ⅲ) and Zimmermann Compound (Ⅴ) in Different Solvents

Solvent	Janovsky complex		Zimmermann compound	
	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε
Water	480	6700	487	580
Methanol	536	7700	485	420
Ethanol	555	10800	490	1600
Methylcellosolve	563	9800	494	15000
Tetrahydrofuran	555	11900	493	1700
Acetone	579	19200	493	5600
Dimethylsulfoxide	587	19400	498	20000
Dimethylformamide	582	18100	495	28000
Pyridine	580	5600	500	4000

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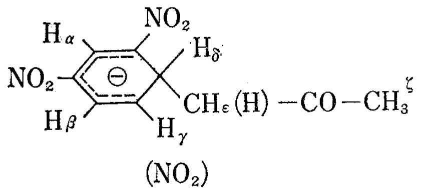
17) W. Borche, *Ber.*, **42**, **611** (**1909**).
18) R.J. Pollitt and B.C. Saunder, *J. Chem. Soc.*, **1965**, 4618.
19) C.D. Conduit, *J. Chem. Soc.*, **1959**, 3273.
20) L.K. Dyall, *J. Chem. Soc.*, **1960**, 5166.
21) R.R. Randle and D.H. Whiffen, *J. Chem. Soc.*, **1952**, 4153; R. Foster and D.L. Hammick, *ibid.*, **1954**, 2153.

Table Ⅱ. NMR Chemical Shifts (τ) of Meisenheimer–like Complexes and Related Compounds in Dimethyl Sulfoxide

	H_α	H_β	H_γ	H_δ	H_ε	H_ζ
Complex (Ⅲ) from acetone–*m*–dinitrobenzene	1.74	3.43	4.64	5.83[a]	6.88	7.95
Complex (Ⅵ) from acetone–trinitrobenzene	1.75	1.75	—	5.04[b]	[c]	7.91
2,4,6–Trinitrobenzyl methyl ketone	0.93	0.93	—	—	5.62	7.75
2,4–Dinitrobenzyl methyl ketone	1.25	1.52	2.29	—	5.66	7.80
Potassium salt (V) of dinitrobenzyl methyl ketone	0.88	2.43	0.66	—	3.32	7.98



a) sextet, $J_{\gamma\delta}$ 4.5 cps, $J_{\delta\varepsilon}$ 7 cps
b) triplet, $J_{\delta\varepsilon}$ 4.8 cps
c) covered by the solvent absorption

Nuclear magnetic resonance spectral properties of III and V as the potassium salts are summarized in Table II, together with those of 2,4–dinitro–, 2,4,6–trinitrobenzyl methyl ketones and the Janovsky complex (VI) as the reference substances. Foster, *et al.*[22] observed NMR spectrum of the anion (VI) in dimethylsulfoxide, the signals of the protons H_α and H_δ being at 8.35 and 5.08 ppm, respectively. According to them, the low solubility of the σ–complex (III) and the presence of small concentrations of free radical which are in rapid exchange with other nitro–compound species present have made the measurement in this system impracticable to date.[22] On the contrary, the expected sextet structure of the proton (H_δ) attached to the ring sp^3 hybridized carbon atom could be observed on the potassium salt (III)[11] prepared in this laboratory, as is shown in Table II.

Chemical Properties of the Pigments

Effect of Changing pH: It has already been pointed out that these color reactions mentioned above are affected by the basicity of media. When the solution of III was acidified to pH 2 or less, the absorption band of visible region disappeared immediately. Although the coloration is rather stable at pH 7, hypochromic change occurred at pH 8—9 and hypsochromic shift was accompanied further at pH 10 or more; these changes ceased at pH 13 showing the absorption maximum at 398 mμ as summarized in Table III. When the

Table Ⅲ. The Absorption Maximum of Janovsky Complex (Ⅲ) and Zimmermann Compound (Ⅴ) at Various pH

pH	Janovsky complex				Zimmermann compound			
	5 min		10 min		5 min		10 min	
	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε
13	398	6000	396	5960	487	10100	487	6500
12	416	7300	408	6500	487	9200	487	8600
11	475	6200	465	5100	487	1700	487	1700
9	480	6400	480	6300	*	*	*	*
7	480	6700	480	6700	*	*	*	*
3	480	5000	480	3700	*	*	*	*
2	*	*	*	*	*	*	*	*

* No maximum was observed in visible region.

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[22] R. Foster and C.A. Fyfe, *Rev. Pure and Appl. Chem.*, **16**, 75 (1966).

solution of V was also kept at pH 13 for long time, the maximum appeared at the similar wave length (396 mμ). It seemed, therefore, that III and V might be decomposed into the similar substances under strongly basic media as described later.

In Acidic Media: When the alcoholic solution of V was kept at about pH 1, 2,4-dinitrobenzyl methyl ketone was obtained quantitatively as expected. When the methanol solution of III was treated with the same buffer solution, on the other hand, *m*-dinitrobenzene, 2,4-dinitrobenzyl methyl ketone and diacetonyl-dinitroazoxybenzene were isolated in 12, 25 and 24% yields, respectively. It may, therefore, be reasonably assumed that the bond between benzene nucleus and acetone is of rather sensitive C–C bond. From the fact that dinitrobenzyl methyl ketone was obtained in 25% yield, the intramolecular and/or intermolecular oxidoreduction was shown to be concerned on this treatment with acid. The polarographic reduction of III was studied in aqueous solution at different pH, giving the results summarized in Table IV; this may support the consideration of Corker, *et al.*5) that V can be formed in the Zimmermann condition under the oxidation by I.

TABLE Ⅳ. Hlaf-wave Reduction Potentials*a)*

	pH 3	pH 7	pH 9	pH 13
m-Dinitrobenzene	*b)*	0.41	0.54	0.62
Janovsky complex (Ⅲ)	0.33	0.54	0.61	0.68
Zimmermann compound (Ⅴ)	0.35	0.41	0.52	0.63

a) against the saturated calomel elctrode at 25° *b)* not measured

In Basic Media: The solution of III and V were kept in a strongly basic media and the decomposition products in turn were examined by means of TLC during the elapse of time. When ethanol solution of III was treated with 10% potassium hydroxide, the spot of dinitrobenzyl methyl ketone were slightly increased at a time, then turned to decrease and finally disappeared and two or three spots of unknown substances were observed instead. Since almost same phenomena on TLC were observed also with respect to V, it seemed that these two pigments might be decomposed into the similar substances. Chromatography of the reaction products from III gave three substances and the first fraction eluted with benzene gave 2,4-dinitrophenol as yellow scales in 0.9% yield. The phenol might be formed by the direct replacement of acetonyl ion with hydroxide ion, so that the contribution of ionic characters in the bond between carbon atom of benzene uncleus and that of acetone moiety may present in some extent. Other two substances which seemed to be main products failed to be crystallized and were not identified in this time.

A remarkable difference between III and V was shown as for the formation of nitrite ion. Gold, *et al.*13) pointed out the elimination of nitro group from the trinitrobenzene derivatives on treatment with alkali. Nitrite ion originated thus from nitro group was clearly detected also in the alkaline solution of III, whereas it failed on V where the contribution of acetonyl group into the quinoidal structure may be assumed to decrease the electropositivity of the carbon atom adjacent to the nitro group that can otherwise be eliminated as nitrite anion under the attack of hydroxyl ion.

As shown in Fig. 2 the absorption maximum of 550 mμ shifted toward 490 mμ during the course of the Janovsky reaction. In the early stage of the reaction, the initial spot of III disappeared on TLC giving that of V instead, which was then afterwards disappeared and finally two spots of *Rf* 0.1—0.2 were observed as shown in Fig. 3. It may, therfore, be evident that the reaction would proceed as follows:

$$\text{I}+\text{Acetone} \xrightarrow{\text{OH}^-} \text{III} \xrightarrow{\text{oxidation}} \text{V} \xrightarrow{\text{OH}^-} \text{unknown substances}$$

and that in the case of the Zimmermann reaction the pigment (III) is the intermediate which is then oxidized by itself or probably by *m*–dinitrobenzene (I) into the pigment (V).

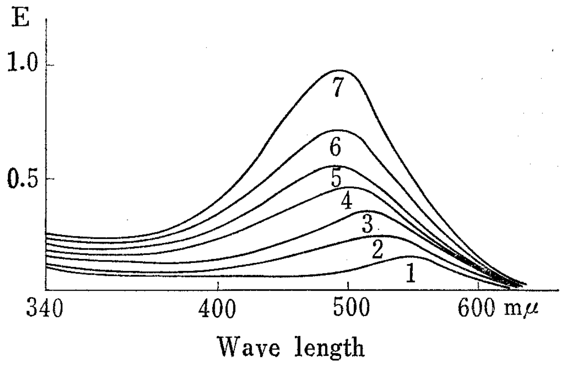

Fig. 2. Shift of $\lambda_{\max}$ in the Janovsky Reaction–mixture[a]

[a] Ethanolic solution of acetone, *m*–dinitrobenzene, and potassium hydroxide under the Canbäck condition[7] was allowed to stand at room temperature and the absorption spectra were measured at every hour.

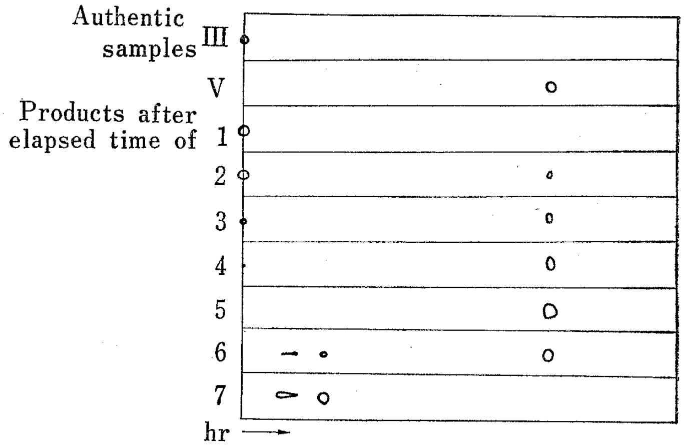

Fig. 3. Thin–layer Chromatogram of Products by the Janovsky Reaction

Developing solvent: hexane–benzene–ethanol (4:4:1)

Sensitivity for Light: It has been pointed out that the Janovsky reaction, particularly when 3,5–dinitrobenzoic acid and picric acid are used instead of I, is sensitive to light and that the analytical routine works for the biological samples such as 17–ketosteroids and creatinine should be carried out in the dark at the stage of colorimetry. The acetone solution of III was observed to fade away when it was irradiated with the ultraviolet ray. The ethanol and aqueous solutions of the product thus obtained showed the absorption maxima at 272 and 266 mμ, respectively. Almost the same results including the thin–layer chromatographical observations were given also in the pigment (V) and in the solvents such as water and ethanol which were used for the irradiation instead of acetone. IR absorption spectra of the product revealed the presence of nitro group and aromatic ring, whereas the carbonyl absorption disappeared. Details on the chemistry of this compound and the above–mentioned degradation products by alkali are in progress.

Experimental[23]

Isolation of Janovsky Complex (III)——A mixture of *m*–dinitrobenzene (1 g) and acetone (10 ml) dried with KOH pellets, was vigorously shaken for 15 min. The reaction mixture of deep bluish–purple color was filtered off with glass filter and KOH pellets were washed with acetone. When benzene (20 ml) was added to the combined acetone solution, black–violet precipitates were produced. Recrystallization from acetone–benzene gave balck–violet needles which were then washed with benzene and recrystallized repeatedly. Decomp. >250°. Yield: 10%. *Anal.* Calcd. for $C_9H_9O_5N_2K$: C, 40.91; H, 3.41; N, 10.61. Found: C. 40.92; H, 3.39; N, 10.47. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1719 (C=O), 1470 and 1290 (NO_2). NMR τ (in DMSO): 1.74 (H_α, doublet), 3.43 (H_β, quartet), 4.64 (H_γ, quartet), 5.83 (H_δ, sextet), 6.88 (H_ε, doublet), 7.95 (H_ζ, singlet).

Preparation of Zimmermann Compound (V)——A solution of 2,4–dinitrobenzyl methyl ketone (1 g) prepared by known method[17] in EtOH (10 ml) was vigorously shaken with KOH pellets (2 g) for 5 min. The mixture was filtered off with glass filter and KOH pellets were washed with acetone. The combined solution was similarly treated as in the case of III and black–violet needles, decomp. >250°, were obtained in almost quantitative yield. *Anal.* Calcd. for $C_9H_7O_5N_2K$: C, 41.18; H, 2.67; N, 10.67. Found: C, 41.10;

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[23] All melting points were uncorrected. Thin–layer chromatography was carried out on Wakogel B–5 (Wako Pure Chemical Industries, Ltd.). UV and V spectra were taken with a Hitachi EPS–2U recording spectrophotometer. IR spectra were obtained using a Koken D101 spectrophotometer. NMR spectra were recorded on a JNM 3H–60 (Japan Electron Optics Laboratory Co., Ltd.) spectrometer operating at 60 Mcps.

H, 2.60; N, 10.60. IR ν_{max}^{KBr} cm^{-1}: 1610 (conjugated C=O), 1453 and 1310 (NO_2). NMR τ (in DMSO): 0.66 (H_γ, doublet), 0.88 (H_α, doublet), 2.43 (H_β, quartet), 3.32 (H_ϵ, singlet), 7.98 (H_ζ, singlet).

Decomposition of Pigments——The pigments III (2.890 mg) and V (2.322 mg) were dissolved in each 10 ml of water, and each 0.5—1 ml of these solutions was diluted successively with different buffer solutions. The light absorptions and thin-layer chromatograms of the solution were then compared progressively with each other.

To a solution of III (9 g) in MeOH (300 ml) was added under stirring 300 ml of buffer solution of pH 1.0. After standing for 30 min the solution was neutralized with 2 N KOH and extracted with benzene. The benzene extracts were dried over Na_2SO_4 and chromatographed over alumina. From the first fraction eluted with benzene, the pale yellow crystals were obtained. Recrystallization from EtOH gave *m*-dinitrobenzene (I) as colorless needles, mp 87°, in 12.3% yield. On admixture of the needles with an authentic specimen, no depression of melting point was observed and their IR spectra were also identical. From the second fraction eluted with benzene, the yellow crystals were obtained. Recrystallization from EtOH–H_2O gave 2,4-dinitrobenzyl methyl ketone as pale yellow needles, mp 75°, in 25.3% yield. On admixture of this ketone and its *p*-nitrophenylhydrazone with the respective specimen, no depression of melting point was observed. From the third fraction eluted with benzene–MeOH (1:1), the brown solid was obtained. Recrystallization from $CHCl_3$ gave yellow plates, mp 205—205.5°. Yield: 24.3%. It may be assumed that this product is diacetonyldinitroazoxybenzene from the data described below. The molecular weight measured by Rast's method revealed 391.8. *Anal.* Calcd. for $C_{18}H_{16}O_7N_4$: C, 54.00; H, 4.03; N, 14.50. Found: C, 53.36; H, 4.18; N, 14.08. UV λ_{max}^{EtOH} mμ: 326 (in alkaline solution: 320 and 520). IR ν_{max}^{KBr} cm^{-1}: 1713 (C=O), 1612 (aromatic C=C or N=N), 1520 (NO_2), 1340 (NO_2).

To a solution of III (8 g) in 50% EtOH–H_2O (100 ml) was added under stirring 2 N KOH. After standing for 1 hr at room temperature, the solution was neutralized with 2 N HCl and extracted with benzene. The extracts were dried over Na_2SO_4 and chromatographed over alumina. From the first fraction eluted with benzene, the yellow solid was obtained. Recrystallization from MeOH gave 2,4-dinitrophenol as yellow scales, mp 111—112°. Yield: 0.9%. On admixture of the product with an authentic specimen, no depression of melting point was observed and their IR spectra were identical. The second fraction eluted with $CHCl_3$ gave reddish brown powder (1.5 g) showing one spot of *Rf* 0.2 on TLC developed by hexane–benzene–EtOH (4:4:1). UV λ_{max}^{EtOH} mμ: 240 and 350 (in alkaline solution: 240, 282, 330 and 490). IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O), 1615 (aromatic C=C), 1532 and 1349 (NO_2). From the third fraction eluted with $CHCl_3$–MeOH (1:1), a brown solid (0.5 g) was obtained, which was purified on silica-gel chromatography and gave one spot on TLC: *Rf*=0.1 (developed by hexane–benzene–EtOH=4:4:1), 0.5 (by EtOH). UV λ_{max}^{EtOH} mμ: 240 and 310 (in alkaline solution: 240 and 402). IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1740 (C=O), 1610 (aromatic C=C), 1540 and 1350 (NO_2).

Polarography of Pigments——Reduction of the pigment (*ca.* 10^{-4} M) in an aqueous ethanol solution was carried out at 25±1° under nitrogen atmosphere using a Yanagimoto recording polarograph Model P-B4. The pHs of buffer solutions were checked with a glass electrode and the half-wave potentials were measured against the saturated calomel electrode. The values summarized in Table IV are the means of three to five determinations.

Decomposition by Irradiation——Acetone solution (1 liter) of V (246 mg) was divided into thirty-six test tubes, 2.7 mm in diameter and *ca.* 1 mm thick, and irradiated by the ordinary UV lamp from a distance of *ca.* 5 cm for *ca.* 1 hr. The faded solution was concentrated under reduced pressure and chromatographed on alumina (7.5 g). From the fraction eluted with $CHCl_3$, a yellow and sublimable residue (10 mg) was obtained, which decomposed at 240—248° and gave one spot of *Rf* 0.65 on TLC developed by CH_2Cl_2–MeOH–DMF (80:15:15). UV λ_{max} mμ: 273 (in EtOH), 266 (in H_2O). IR ν_{max}^{KBr} cm^{-1}: 1608 (aromatic C=C), 1568 (C=C), 1530 and 1350 (NO_2).

Elimination of Nitro Group——On the alkaline solution of III described above, nitrite analyses were carried out spectrophotometrically by Rider and Mellon's method.[24] No nitrite ion was detected in the case of V. No effect of light was also observed on the elimination.

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24) B.F. Rier and M.G. Mellon, *Anal. Chem.*, **18**, 96 (1946).