[Chem. Pharm. Bull. 20(5)1026—1032(1972)]

UDC 547.495.9.04 : 547.655.1.04

Studies on the Voges-Proskauer Reaction. VII.[1] Pigments obtained from the Reaction with 2- and 4-Chloro-, and 2,4-Dichloro-1-naphthols and Reactivity of Some Phenols and Naphthols[2]

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(Received November 22, 1971)

The structure of pigments derived from 2-chloro- (Pigment III) and 4-chloro- (Pigment I), and 2,4-dichloro-1-naphthol (Pigment II), and the reactivity of some phenols and naphthols were investigated in the reaction mixture of diacetyl and 1,1-pentamethyleneguanidine in alkaline medium.

The structures of Pigments I and II were identical with 4-chloro derivative of Pigment A obtained from 1-naphthol as reported previously, and Pigment III was a *para*-isomer relative to the structure of Pigment I and II. It was found that Pigment III was also identical with 2-chloro derivative of Pigment B which could not be separated from 1-naphthol because of its instability.

For the reactivity of phenols and naphthols, 1,3-dihydroxy type such as 2-methyl- and 5-methyl resorcinol, 3,5-dihydroxy benzoic acid, and 1,3-dihydroxynaphthalene gave striking coloration in comparison with 1-naphthol, while 1,2- or 1,4-dihydroxy type, which is immediately oxidized to a quinone form, did not give positive coloration.

Diacetyl or acetoin, which are products of the growth of certain organisms in glucose-pepton cultures, give eosin-like coloration with creatine in the presence of 40% potassium hydroxide solution (O'Meara's method[4]), which is known as ordinary Voges–Proskauer reaction.[5] This reaction was improved by Barritt[6] to a strikingly sensitive coloration by the addition of 1-naphthol. Eggleton, *et al.*[7] applied Barritt's modification to the colorimetric determination of diacetyl, acetoin, and creatine in the field of biochemical analysis (Eggleton's method). Although they also obtained a crude pigment from the colored solution of diacetyl, creatine, and 1-naphthol, the pigment could not be isolated in a pure crystalline form and its structure has not yet been determined.

In a previous work of this series,[8–10] we demonstrated that three kinds of pigments, A,B, and C are produced from diacetyl, guanidines, and 1-naphthol, in which pigments A and B were the main products and the other pigment C was a by-product which was also obtained from diacetyl-guanidines in alkaline medium (O'Meara's method). The pigment A and C were obtained in a pure crystalline form and their structures were determined (Chart 1), while pigment B could not be isolated because of its high instability. It was expected that pigment B is a *para*-isomer relative to the structure of pigment A as shown in Chart 1.

1) Part VI: T. Sakaguchi, S. Tanabe, U. Nonaka, M. Takeuchi, Y. Onuki, and M. Ishizaki, *Yakugaku Zasshi*, **91**, 695 (1971).
2) Presented at 19th Annual Meeting of the Japan Society for Analytical Chemistry, Nagoya, October 1970.
3) Location: *1-33, Yayoi-cho, Chiba, 280, Japan.*
4) R.A.Q. O'Meara, *J. Pathol. Bacteriol.*, **34**, 401 (1931).
5) O. Voges and B. Proskauer, *Z. Hyg. Infektionkrankh.*, **28**, 20 (1898).
6) M.M. Barritt, *J. Pathol. Bacteriol.*, **42**, 441 (1936).
7) P. Eggleton, S.R. Elsden, and N. Gaugh, *Biochem. J.*, **37**, 526 (1943).
8) T. Sakaguchi, S. Tanabe, I. Koyahara, and T. Inoue, *Yakugaku Zasshi*, **90**, 1418 (1970).
9) T. Sakaguchi, K. Kijima, S. Tanabe, T. Inoue, and I. Koyahara, *Yakugaku Zasshi*, submitted. T. Nishimura, C. Yamazaki, S. Tanabe, T. Ueno, S. Kitashima, T. Inoue, I. Koyahara, K. Kijima, and T. Sakaguchi, *Tetrahedron Letters*, **55**, 4815 (1970).
10) K. Kijima, I. Koyahara, S. Tanabe, and T. Sakaguchi, *Yakugaku Zasshi*, **91**, 115, (1971).

In the present work, the coloring matters derived from 2- and 4-chloro-, and 2,4-dichloro-1-naphthols were obtained in crystalline form and the relation of their structures and pigment B produced from 1-naphthol were investigated, and further discussion was made on the reactivity of phenols and naphthols.

Reactivity of Phenols and Naphthols

Roos and Siest[11] reported that a few of phenols and naphthols besides 1-naphthol underwent coloration in Voges–Proskauer reaction. However, the relationship between the structure of the compounds and their reactivity was not discussed. In order to clarify this relation, we tested the reactivity of 23 kinds of phenols and naphthols by the use of diacetyl and 1,1-pentamethyleneguanidine under the Eggleton condition, and the results of color reaction are summarized in Table I.

Table I. Color Reaction of Phenols and Naphthols under Eggleton Condition

Compd. No.	Naphthols	λmax (nm)	Color intensity[a]	Compd. No.	Phenols	λmax (nm)	Color intensity[a]
1	1-naphthol	535	1.0	14	phenol	—	—
2	2-naphthol	495	0.32	15	1,2-dihydroxybenzene (catechol)	—	—
3	1-naphthol-2-sulfonic acid sodium salt	535	0.12	16	1,3-dihydroxybenzene (resorcinol)	525	0.97
4	1-naphthol-4-sulfonic acid potassium salt	505	0.04	17	2-methylresorcinol	530	1.30
5	2-chloro-1-naphthol	535	0.53	18	4-hexylresorcinol	540	0.76
6	4-chloro-1-naphthol	540	0.27	19	5-methylresorcinol	525	1.20
7	2,4-dichloro-1-naphthol	540	0.01	20	3,5-dihydroxybenzoic acid	525	1.30
8	1,2-dihydroxynaphthalene[b]	—	—	21	1,4-dihydroxybenzene[b] (hydroquinone)	—	—
9	2,3-dihydroxynaphthalene	515	0.32	22	1,2,3-trihydroxybenzene (pyrogallol)	—	—
10	1,3-dihydroxynaphthalene[b]	515	1.04	23	1,3,5-trihydroxybenzene (phloroglucinol)	480	0.17
11	1,4-dihydroxynaphthalene[b]	—	—				
12	1,5-dihydroxynaphthalene[c]	580	0.35				
13	1,7-dihydroxynaphthalene[c]	525	0.39				

a) Color intensity was calculated as absorbance ratio of each derivative *vs.* absorbance of 1-naphthol.
b) Sample immediately changed to dark brown in alkaline medium.
c) Sample gradually developed pink or reddish violet color in alkaline medium.

From the results in Table I, it would be concluded that polyhydroxy-benzenes and naphthalenes with 1,3-dihydroxy group (Compd. No. 10 and 16—20 in Table I), which are able to change easily to a ketomethylene, exhibit a stronger color reaction than 1-naphthol, but those with 1,2- or 1,4-dihydroxy group (No. 8, 11, 15, 21, and 22), which are immediately oxidized to a quinone form in alkaline medium, did not give positive results.

Of all the naphthols which gave positive results in Voges–Proskauer reaction other than 1,3-dihydroxy type, 1-naphthols substituted in *ortho-* or *para*-position (No. 3—7), had less color intensity than 1-naphthol, and especially, 1-natphols substituted with an electronegative SO_3^{2-} group in *ortho-* or *para*-position strongly exhibited this tendency, and 1,5- and 1,7-dihydroxynaphthalene (No. 12 and 13), whose balnk colors gradually developed to pink or reddish violet and color of the reaction mixtures faded in a short time, did not give an intense coloration.

Relation of the Coloring Matters obtained from 1-Naphthol and Its Chloro Derivatives.

Reults shown in Table I and absorption spectra in Fig. 1 indicated that the color intensity of 2- and 4-chloro-, and 2,4-dichloro-1-naphthol was reduced to less than half of that of 1-

11) F. Roos and G. Siest, *Bull. Soc. Pharm. Nancy*, **70**, 40 (1966).

naphthol, and that the reactivity of 2,4-dichloro-1-naphthol diminished when compared with that found in other chloro derivatives.

This result clearly indicated that *ortho*- and *para*-positions of 1-naphthol are an important active site in this reaction. Therefore, crude pigments were prepared from each of the reaction mixtures of 1-naphthol and its chloro derivatives under the condition described in Experimental, and separated by thin-layer chromatography (TLC, silica gel), developed with benzene–ethyl acetate–ethanol. Only one fraction (Pigments I, II, and III) besides fraction C, which was a common species, was separated from the crude pigments of chloro derivatives (b, c, and d in Fig. 2), while three fractions, A, B, and C, were separated from that of 1-naphthol (a in Fig. 2), and *Rf* value of fraction I was identical with that of II, as shown in Fig. 2.

These chromatographic data indicate that both fractions I (or II) and III were intimately connected with the structure of pigments A and B obtained from diacetyl, 1,1-pentamethyleneguanidine, and 1-naphthol.

Structure of Pigment I (Fraction I)

Pigment I was obtained as dark red needles from the reaction mixture with 4-chloro-1-naphthol. This pigment was identical with the main colored fraction I (Fig. 2), and its absorption spectrum in alkaline solution was similar to that of the reaction mixture (Fig. 1). Shape of the spectrum of this pigment, which gave a characteristic curve having five absorption maxima in the visible region in EtOH solution, was identical with that of pigment A (Fig. 3).

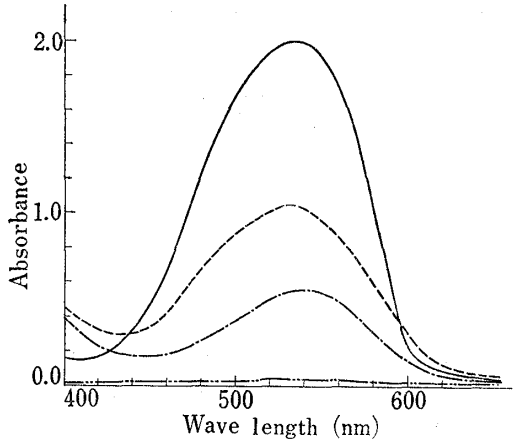


Fig. 1. Visible Absorption Spectra of Colored Solution in Alkaline Medium

———: 1-naphthol
- - - - -: 2-chloro-1-naphthol
—··—··: 2,4-dichloro-1-naphthol
—·—·: 4-chloro-1-naphthol

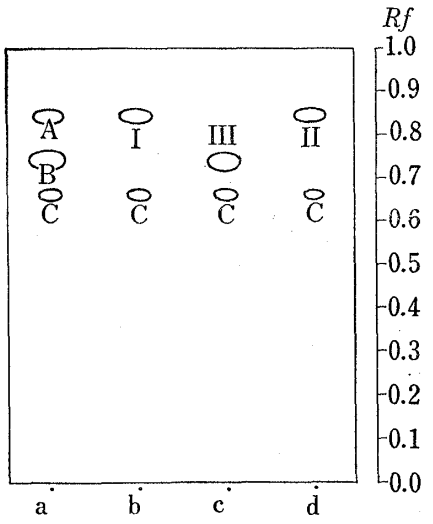


Fig. 2. Thin-Layer Chromatography of the Crude Pigments obtained under Eggleton Condition

absorbent: Silica gel B-5 (Wako)
developing solvent: bezene–AcOEt–EtOH (3:1:1)
a: 1-naphthol b: 4-chloro-1-naphthol
c: 2-chloro-1-naphthol d: 2,4-dichloro-1-naphthol
A: Pigment A B: Pigment B C: Pigment C

The molecular formula of pigment I was established from the molecular ion (M^+) in mass spectrum and elemental analysis as $C_{20}H_{20}ON_3Cl$. The infrared (IR) spectrum of pigment I showed the presence of a quinoid-carbonyl at 1630 cm^{-1} due to the intramolecular hydrogen bonding between the N–H and carbonyl oxygen.[9] Furthermore, the absorption bands at 3270—2700 (aromatic hydrogen vibration and C–H streching vibration of methyl and pentamethylene ring), 1600—1580 (C=N streching and aromatic ring vibration), and 800—740 cm^{-1} (ring proton out-of-plane vibration) were also observed similar to those of pigment A shown in a previous paper[9] (Fig. 4).

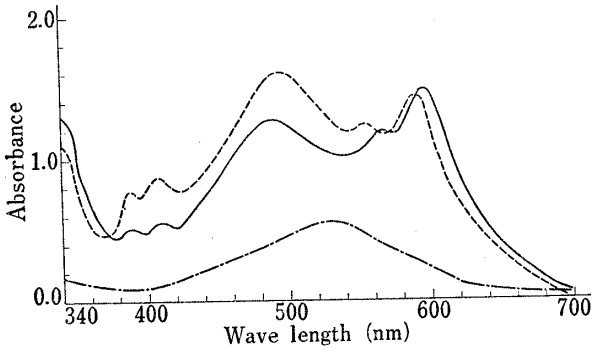


Fig. 3. Visible Absorption Spectra of Pigments A, I, II, and III in EtOH Solution

———: pigment A —·—·: pigment III
- - - - -: pigment I and II

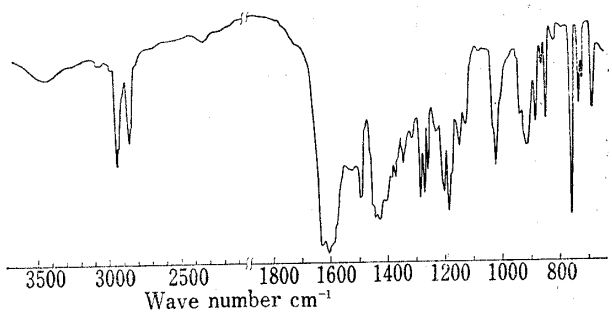


Fig. 4. Infrared Spectrum of Pigment I in KBr Disk

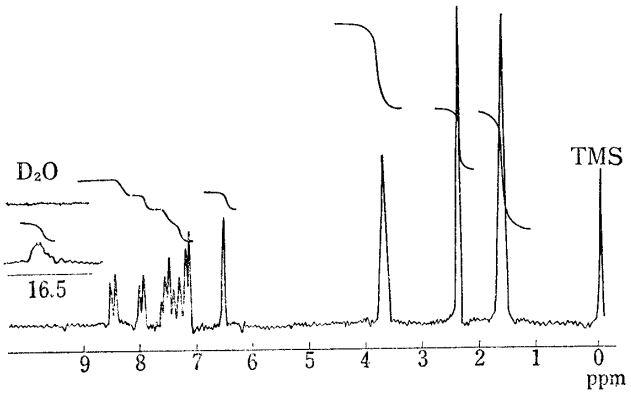
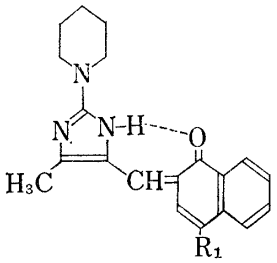
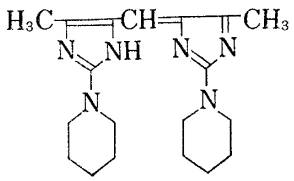


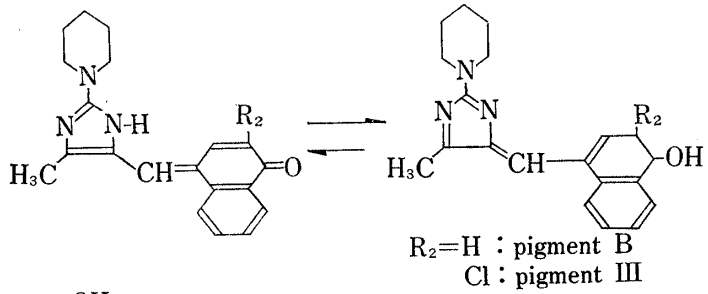
Fig. 5. Nuclear Magnetic Resonance Spectrum of Pigment I in $CDCl_3$ at 100 MHz



R_1=H : pigment A
Cl : pigment I



pigment C



R_2=H : pigment B
Cl : pigment III

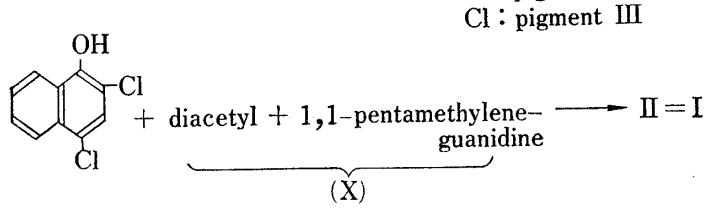


Chart 1

The mass spectrum indicated the presence of a characteristic isotope peak at m/e: 355 (M^++2) due to one chlorine atom substituted in pigment A (molecular weight 319) and of fragment ions of M^+-CH_3, M^+-OH, M^+-CO+H, M^+-$C_5H_{10}N$, and $C_5H_{10}N$.[9]

The nuclear magnetic resonance (NMR) spectrum of pigment I is shown in Fig. 5. The three singlet signals at 1.5, 2.7, and 3.6 ppm and double-doublet at 8.5 ppm were assigned to pentamethylene ring (6H), methyl (3H), pentamethylene ring (4H), and 8-position of 1-naphthol ring (1H, $J_{7,8}$=10 cps), as in the previously described spectrum of pigment A.[9] Three signals at 7.18 (singlet, 1H), 8.0 (double-doublet, 1H, $J_{5,6}$=10 cps), and 7.5 ppm (multiplet, 2H) are observed in pigment I. The signal at 7.18 ppm comes from the 3-position which is adjacent to the 4-chlorine atom in the 4-chloro-1-naphthol ring, and other two signals at 8.0 and 7.5 ppm can be assigned to the 5-position coupled with 6-position and 6,7-positions of 4-chloro-1-naphthol, respectively. The broad signal at 16.5 ppm, which disappeared immediately on addition of deuterium oxide, would be assigned to the N–H proton which shifted to a lower magnetic field by the intramolecular hydrogen bonding between N–H and carbonyl oxygen.[9]

From these spectral evidences, the structure of pigment I obtained from 4-chloro-1-naphthol was identified as the 4-chloro derivative of pigment A as shown in Chart 1.

Structure of Pigment II (Fraction II)

As the reactivity of 2,4-dichloro-1-naphthol was less than that of 4- and 2-chloro-1-naphthols (Table I), its pigment was extracted with chloroform from reaction mixture, and pigment II was isolated as dark red needles. The molecular weight of pigment II was estanlisbed from the molecular ion (M^+) in mass spectrum as 353. The mass spectrum of pigment II showed the characteristic isotope peak of $M^+ + 2$ to support the presence of one chlorine atom, and its fragment pattern was the same as that of pigment I (Fig. 4).

From these mass and infrared (IR) spectra, pigment II obtained from 2,4-dichloro-1-naphthol was identified with the structure of pigment I (Chart 1).

The reaction mechanism of pigment II can be tentatively assumed as follows: The condensation reaction of diacetyl and 1,1-pentamethyleneguanidine in alkaline medium takes place in the first stage, and then this product (X) would the selectively attack the 2-position of 2,4-dichloro-1-naphthol. Finally, the chlorine atom in 2-position is eliminated (Chart 1). Substitution of 4-position in 2,4-dichloro-1-naphthol with X is difficult to occur in this reaction.

Structure of Pigment III (Fraction III)

The coloring matter produced from 2-chloro-1-naphthol gave one spot (Rf=0.74) other than pigment C in TLC, as shown in Fig. 2. Synthesis of the crude pigment was carried out in the same way as pigment I and a purple precipitate was obtained by neutralization. This pigment was unstable as pigment B derived form 1-naphthol in the process of separation, and it was difficult to obtain it in pure crystalline form.

However, a small quantity of the pure pigment III (dark red plates) was obtained by four repeated colum chromatography. From the results of mass spectrum and elemental analysis, the molecular formula of pigment III agreed with the same formula of $C_{20}H_{20}ON_3Cl$ as pigment I (or II). In its mass spectrum, the characteristic peak depending on chlorine atom and the fragment ions of M^+-CH_3, M^+-CO+H, M^+-$C_5H_{10}N$, and $C_5H_{10}N$ were observed at *m/e* 355, 338, 324, 269, and 84, respectively, similar to that of pigment I, but the peak at *m/e* 336 (M^+-OH) was not observed in the spectrum of pigment III. Relative abundance due to *m/e* 324 (M^+-CO+H) was increased by about 20% compared to that of pigment I. On the other hand, pigment B previously obtained from 1-naphthol,[5] which was separated on the TLC, gave the molecular ion (M^+) at *m/e* 319 the same as that in pigment A.

In the IR spectrum of pigment III, as shown in Fig. 6, the quinoid-carbonyl absorption band, which was observed in the spectra of pigment I (or II) and pigment A[9] at 1630 cm^{-1}, disappeared, and a strong absorption due to the hydroxyl group was observed at 3250 cm^{-1}. From the presence of the fragment ion of M^+-CO+H in the mass spectrum, this absorption was known to be the OH streching band due to the aromatic hydroxyl group. This conclusion was further supported by the ultraviolet (UV) spectrum of pigment III at various pH as shown in Fig. 7. This UV spectra shows that the absorption spectrum of pigment III at pH 13 appears similar to that of pigment A, but this pigment has the characteristic absorption maxima due to the naphthol structure in the region of 280—355 nm at pH 7 and 1. The absorption spectrum of pigment III in the visible region (EtOH solution) did not exhibit the characteristic absorption like pigments I and A as shown in Fig. 3.

The IR spectrum of pigment III further showed the presence of the C–H out-of-plane bending bands due to substituted naphthalene at around 860—820 and 755 cm^{-1}; these bands correspond to an isolated hydrogen atom on the one ring and 4 adjacent hydrogen atoms on the other ring, and the absorption bands due to aromatic C=C or C=N and C–O vibrations at 1600—1535 and 1250 cm^{-1}, respectively.

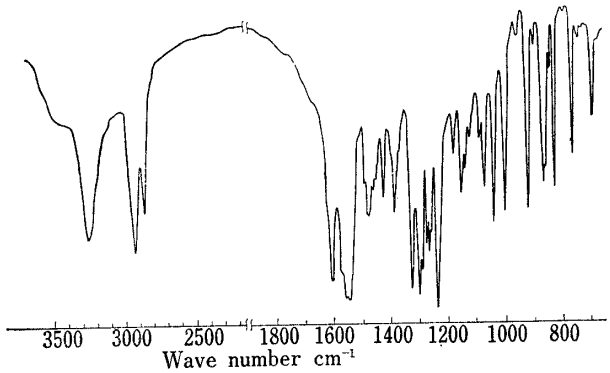


Fig. 6. Infrared Spectrum of Pigment III in KBr Disk

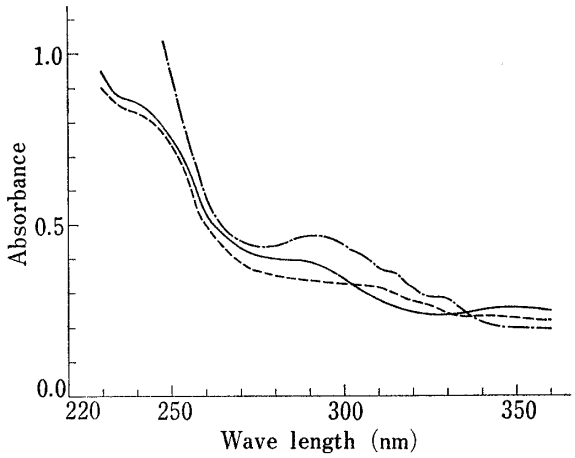


Fig. 7. Ultraviolet Spectra of Pigment III at Various pH

———: pH 13 - - - - -: pH 7 —·—·: pH 1

These results and afore-mentioned discussion suggested that pigment III is a *para*-isomer of pigment I, taking an enol structure, as shown in Chart 1, and that it had the same skeleton as the structure of pigment B, as pigment I has against pigment A.

Fraction C was obtained as a by-product from 2- and 4-chloro-, and 2,4-dichloro-1-naphthol under the Eggleton condition, as shown in Fig. 2. It was confirmed from IR and NMR spectra, and from mixed melting point determination that this pigment was completely identical with the authentic pigment C[10] (Chart 1).

Finally, the condensation product (X) of α-diketone and guanidines was isolated from the reaction mixture recently and its identity will be published in the near future.

Experimental[12]

Material——All the reagents used were of analytical grade. The following materials were prepared according to the methods described in the literature: 1,1-Pentamethyleneguanidine sulfate (PMG),[13] mp 286° (decomp); 2-chloro-1-naphthol,[14] mp 60—61.5°; 4-chloro-1-naphthol,[14] mp 123°; 1,2- and 1,4-dihydroxynaphthalene.[15]

Color Reactio nof Phenols and Naphthols under Eggleton Condition——One ml of 0.05% diacetyl solution and 1 ml of 0.5 μmole/ml PMG solution were place in a 10 ml test tube with a stopper, 2 ml of 1% alkaline solution of the sample (prepared by dissolving 12 g of NaOH and 32 g of Na_2CO_3 in distilled water and diluting to 200 ml) was added to this solution, and this mixture was diluted to 10 ml with distilled water. The reaction mixtures were stored in the dark after shaking thoroughly. After 15 min, visible absorption spectra were determined to know absorption maxima and color intensity of the reaction mixtures employing a Hitachi automatic recording spectrophotometer EPS-3.

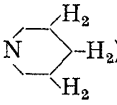
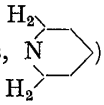
A solution prepared by diluting 2 ml of 1% alkaline solution of the sample to 10 ml with distilled water was used as a blank.

12) All melting points are uncorrected. The NMR spectra were taken on JMN 4H-100 spectrometer at 100 Mc with tetramethylsilane as an internal standard, UV spectra were measured by a Hitachi EPS-3T automatic recording spectrophotometer, IR spectra by a Hitachi-215T spectrometer in KBr disk, and mass spectra were measured by a Hitachi RMU-6E spectrometer.
Thin-layer chromatography was carried out on silica gel (500 μ thickness, Wako-gel B-5). Column chromatography was carried out on alumina (300 mesh, Wako) and silica gel (100 mesh, Mallinchrodt).

13) W.F. Cockburn and R.A.B. Bannard, *Can. J. Chem.*, **35**, 1285 (1957).

14) R. Lesser and G. Gad, *Ber.*, **56**, 963 (1923).

15) L.F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, **56**, 1565 (1934), **61**, 596 (1939); Y. Nagase and U. Matsumoto, *Yakugaku Zasshi*, **74**, 9 (1954).

Isolation of Pigment I obtained from 4-Chloro-1-naphthol——To a solution of 0.86 g of diacetyl and 1.78 g of PMG in 300 ml of H_2O, 1.72 g of 4-chloro-1-naphthol dissolved in 100 ml of 4% NaOH solution was added and the mixture was stirred for 60 min at room temperature. The reaction mixture was neutralized with 1N HCl solution till the reddish violet precipitation appeared. The precipitate was collected by filtration, washed with water, and dried *in vacuo*. The precipitate was purified by alumina column chromatography (developing solvent, benzene: EtOH=19:1) as a violet fraction, which was chromatographed over silica gel column and eluted with benzene–AcOEt (6:1). Dark red needles, mp 193—195° (decomp), were obtained. *Anal.* Calcd. for $C_{20}H_{20}ON_3Cl$: C, 67.89; H, 5.70; N, 11.87. Found: C, 66.42; H, 5.58; N, 11.93. IR ν_{max}^{KBr} cm^{-1}: 3270, 2980, 2870 (aromatic C=C and C–H streching vibration), 1630 (quinoid carbonyl), 1600—1580 (aromatic C=C or C=N vibration), 800—740 (ring proton out-of-plane vibration). UV and visible λ_{max}^{EtOH} nm: 235, 260 (shoulder), 280, 320, 333, 345, 386, 408, 490, 550, 590. Mass Spectrum *m/e*: 355 (M^++2), 353 (M^+), 338 (M^+-CH_3), 336 (M^+-OH), 269 ($M^+-C_5H_{10}N$). NMR (in $CDCl_3$) ppm: 1.5 (6H, s, ), 2.7 (3H, s, =C-CH_3), 3.6 (4H, s, ), 6.67 (1H, s, =CH–), 7.18 (1H, s, aromatic C_3-H), 7.50 (2H, m, aromatic $C_{6,7}$-H), 8.0 (1H, d-d, $J_{5,6}$=10 cps, aromatic C_5-H), 8.5 (1H, d-d, $J_{7,8}$=10 cps, aromatic C_8-H), 16.5 (1H, s, N–H).

Isolation of Pigment II obtained from 2,4-Dichloro-1-naphthol——The reaction was carried out in the same way as in the case of pigment I. The reddish violet or reddish orange reaction mixture was extracted with $CHCl_3$. The extract was dried over Na_2SO_4, $CHCl_3$ was evaporated, and the residue was purified by silica gel column chromatography employing benzene–EtOH (6:1) as the developing solvent. II was obtained as dark red needles, mp 193—195° (decomp). The IR spectrum in KBr disk, NMR, and mass spectra of II were quite identical with those of pigment I.

Isolation of Pigment III obtained from 2-Chloro-1-naphthol——The reaction was carried out in the same way as in the case of pigment I. The reaction mixture was neutralized with 1N HCl solution till the purple red precipitation appeared. The precipitate was purified by silica gel column chromatography employing benzene–AcOEt–EtOH (3:1:1) as the developing solvent, and dark red plates, mp above 300°, were obtained. *Anal.* Calcd. for $C_{20}H_{20}ON_3Cl$: C, 67.89; H, 5.70; N, 11.87. Found: C, 64.27; H, 5.84; N, 11.45. IR ν_{max}^{KBr} cm^{-1}: 3250 (aromatic OH), 1600, 1560, 1550, 1535 (aromatic C=C or C=N), 860—820, 755 (ring proton out-of-plane). UV and visible λ_{max}^{EtOH} nm: 307, 330 (shoulder), 535. Mass Spectrum *m/e*: 355 (M^++2), 353 (M^+), 338 (M^+-CH_3), 324 (M^+-CO+H), 269 ($M^+-C_5H_{10}N$).

Isolation of Colored Matter obtained from 1-Naphthol——To a solution of 100 μmoles of PMG and 75 μmoles of diacetyl in 50 ml of H_2O, 100 μmoles of 1-naphthol dissolved in 20 ml of 4% NaOH solution was added and the mixture was stirred for 60 min at room temperature. The reaction mixture was neutralized with 1N HCl solution till the reddish violet precipitation appeared. The precipitate was collected by filtration, washed with water, and dried *in vacuo*. The precipitate was dissolved in a small amount of EtOH and this EtOH solution was spotted on a silica gel plate (500 μ thickness, Wako-gel B-5) for TLC which was developed with benzene–AcOEt–EtOH (3:1:1). After the chromatogram was dried in air at room temperature, three fractions (*Rf*=0.85, 0.74, and 0.67) were scraped and extracted with EtOH. These procedures were repeated several times to obtain a single fraction.

I, Pigment at *Rf*=0.85——Pigment A, dark red needles, mp 167° (decomp.).[9]

II, Pigment at *Rf*=0.74——Pigment B, dark red with golden luster, mp above 300°. Mass Spectrum *m/e*: 319 (M^+). Visible absorption spectrum in EtOH, λ_{max} 535 nm.

III, Pigment at *Rf*=0.67——Pigment C, reddish orange needles, mp 159—161° (decomp.)[10]

From the coloring matter isolated from 2- and 4-chloro-, and 2,4-dichloro-1-naphthol, fraction C was located at *Rf*=0.67 similar to that from 1-naphthol. *Anal.* Calcd. for $C_{19}H_{28}N_6$: C, 67.05; H, 8.24; N, 24.71. Found: C, 66.83; H, 8.34; N, 23.97. The IR, NMR, and mass spectra were identical with those of an authentic pigment C.[10]

Acknowledgement We thank Miss F. Kuriyagawa and Miss Y. Tomura for NMR and mass spectra measurement. We are also grateful to Mr. A. Nara of the Kowa Reserch Laboratories, Tokyo, for the elemental analysis.