Studies on the Voges-Proskauer Reaction. II. The Structure of a Pigment from the Diacetyl Reaction of 1-Benzyl-1-methylguanidine[1]

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A pigment formed in the diacetyl reaction of 1-benzyl-1-methylguanidine was isolated as reddish purple prisms. The reduced pigment was colorless and rapidly converted back to the original pigment on exposure to the air. On the basis of IR, NMR, and mass spectral evidence, the structures of the pigment and the reduced one were established to be 2-(*N*-benzyl-*N*-methylamino)-4-methyl-5-(1-oxo-1,2-dihydro-2-naphthylidenemethyl)-imidazole and 2-(*N*-benzyl-*N*-methylamino)-4-methyl-5-(1-hydroxy-2-naphthylmethyl)imidazole, respectively.

Voges and Proskauer[2] found that glucose peptone cultures of a certain microorganism produced a pink color on addition of aqueous potassium hydroxide solution. It was shown that the coloration occurs as a result of the reaction between acetoin and creatine or certain similar substances, and the former is converted into diacetyl during the course of reaction.[3,4] Barritt[5] modified the reaction by the addition of 1-naphthol, which resulted in a great intensification of the color. This reaction has been called the diacetyl reaction. It has been demonstrated by several investigators that the color reaction is characteristic of 1,1-disubstituted, mono-, and unsubstituted guanidines and the intensity of coloration decreased in the order mentioned.[5-10] It is also obvious from literature[5,11,12] that the ketonic component should be α-diketone having at least one methyl group attached to the carbonyl carbon atom. Although Eggleton *et al.*[6] obtained a crude coloring matter in the diacetyl reaction of creatine, the pigment has not yet been isolated in a pure crystalline form and consequently its structure has remained unknown. The present work deals with the structure determination of a pigment produced in the diacetyl reaction of 1-benzyl-1-methylguanidine as the first step for elucidation of the mechanism of this color reaction.

Results and Discussion

The crude pigment from creatine according to Eggleton's method gave numerous coloring bands on column chromatography, which made it impossible to obtain a pure pigment. Thus we selected 1-benzyl-1-methylguanidine in expectation of ease in isolation of the aimed pigment, because of its expected good solubility in non-polar solvents in addition to good stability of coloration.[10]

Preliminary experiments revealed that the yield of pigment was best, when the guanidine, diacetyl, and 1-naphthol were allowed to react in water in a molar ratio of 1 : 2 : 5. The pigment was formed by the addition of 1-naphthol in 2.5 N sodium hydroxide to a mixture of diacetyl and the guanidine hydrochloride in water, followed by agitation of the mixture at room temperature. The crude pigment was obtained either by adjusting the reaction mixture to pH 10 with hydrochloric acid, or by extracting the sodium chloride-saturated mixture with benzene. The pigment was purified by column chromatography on silica gel with benzene, giving dark reddish purple prisms with mp 149—149.5°C in about 2% yields on the basis of the guanidine employed. It was also obtained in about 5% yields by conducting the reaction in chloroform with an equimolar proportion of reactants. The pigment thus isolated gave a visible spectrum having the same maximum absorption as and a similar shape to that obtained under the conditions of quantitative determination.[10]

Elemental analysis and mass spectrometry of the pigment gave the molecular formula $C_{23}H_{21}ON_3$. NMR data showed the presence of *N*-methyl (3.21

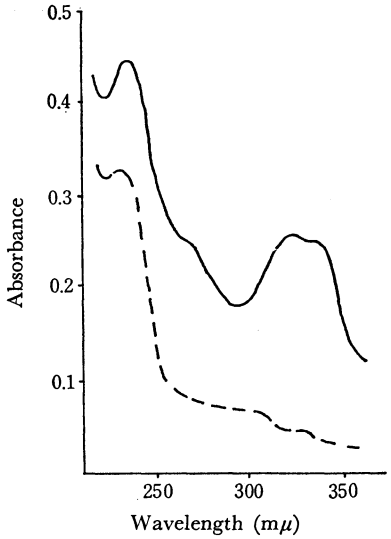

Fig. 1. UV spectra of pigment and reduced pigment (MeOH).
(——): Pigment, (----): Reduced

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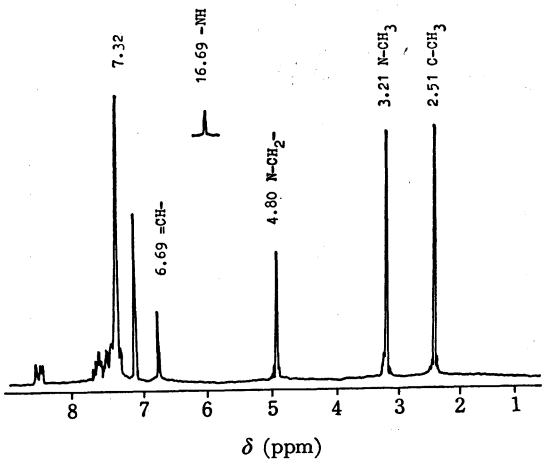


Fig. 2a. NMR spectrum of pigment (CCl_4).

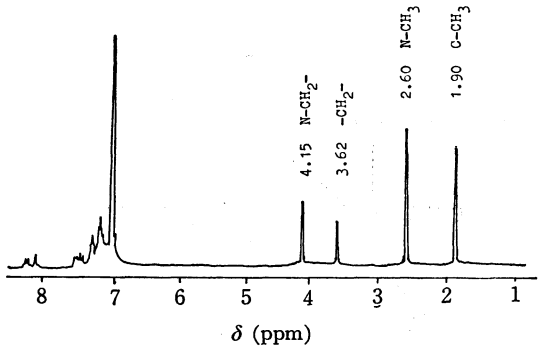


Fig. 2b. NMR spectrum of reduced pigment (CCl_4).

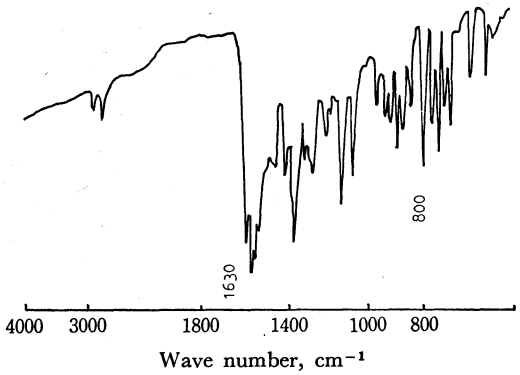


Fig. 3a. IR spectrum of pigment (KBr).

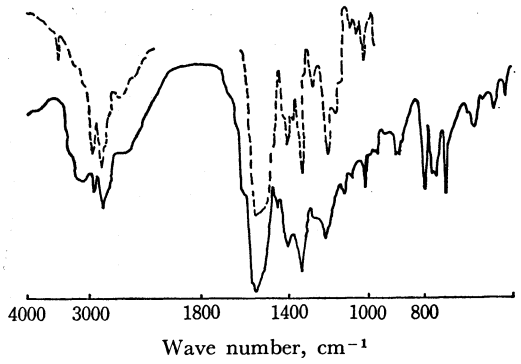
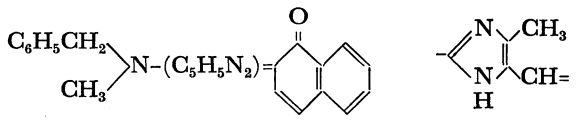


Fig. 3b. IR spectrum of reduced pigment.
(——): KBr, (----): CCl_4

s), *N*-methylene (4.80, s), phenyl (7.32, s), and naphthalene nucleus (7.0—8.4, m) as shown in Fig. 2a.

The IR spectrum of the pigment showed strong peaks at 1630 cm^{-1} (C=O) and 800 cm^{-1} (ring hydrogen out-of-plane vibration) as shown in Fig. 3a. The 800 cm^{-1} band was observed in 1,2-naphthoquinone but not in 1,4-naphthoquinone suggesting the presence of 1,2-naphthoquinoidal system. The pigment absorbed hydrogen on catalytic hydrogenation and yielded a colorless solution, which rapidly restored the original reddish color upon exposure to the air. The recovered pigment gave the same IR spectrum as that of the original pigment. The reduced pigment showed an associated O–H stretching band at 3150—3270 cm^{-1} in KBr disk and a sharp unassociated band at 3460 cm^{-1} in carbon tetrachloride, with simultaneous disappearance of the carbonyl band (Fig. 3b). It also showed a C–O stretching band at 1250 cm^{-1} (in CCl_4) or 1260 cm^{-1} (in KBr). The UV spectrum of the reduced pigment (Fig. 1) was similar to that of 1-naphthol. The hydrogenated pigment should therefore have a naphthol structure. The fact that it has no signal in the neighborhood of 6.7 ppm (Fig. 2b) at which 2-proton of 1-naphthols[13] usually resonates, further suggests that the reduced pigment is 2-substituted 1-naphthol. The absence of this signal in 2-substituted 1-naphthol was confirmed by comparing the NMR spectrum of 2-chloro-1-naphthol with that of 4-chloro-1-naphthol. On the basis of these findings, it was inferred that the pigment has the structure I.

In the NMR spectrum of the reduced pigment (Fig. 2b), the =CH– signal (6.69 ppm) disappeared and a new signal (–CH_2–) was observed at 3.62 ppm. In consideration of the presence of an aromatic C-methyl (2.51 ppm, s) and of the highly probable preservation of the guanidine structure in the pigment, it might be reasonable to conclude that the unknown moiety $C_5H_5N_2$ in I is II.



I II

An NH chemical shift in imidazole has been reported to be in 11.10—13.50 ppm,[14] whereas the NH signal of the pigment was found in a markedly lower field (16.69 ppm, disappearing on addition of CH_3OD). The exceptional down-field shift and the unusual low frequencies of both N–H (3040—3090 cm^{-1}, independent of concentrations in CCl_4 and the intensity reduced on treatment with CH_3OD) and C=O stretching vibrations in the IR spectrum might be interpreted as the result of intramolecular hydrogen bonding between the NH and carbonyl oxygen atom and might provide an additional evidence supporting the presence of 1,2-quinoid structure. Thus the fol-

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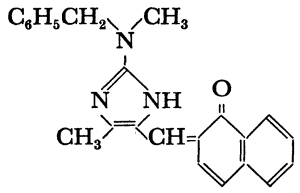
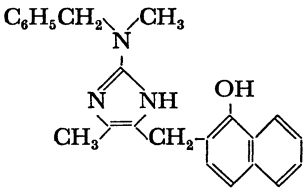
lowing structures III and IV can be derived for the pigment and the reduced pigment, respectively.



III



IV

These structures were further confirmed on the basis of mass spectrometric evidence. The molecular and major fragment ions of the pigment and the reduced one are listed in Tables 1 and 2.

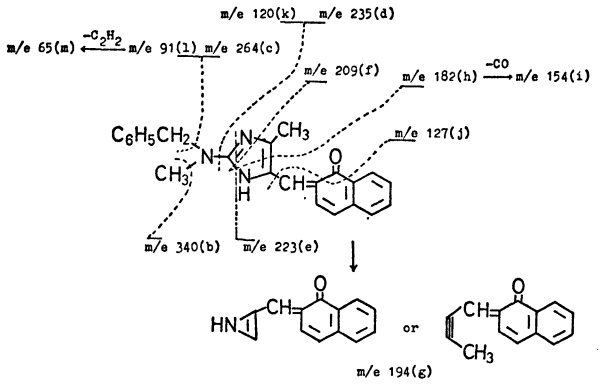
TABLE 1. MASS SPECTRUM OF THE PIGMENT

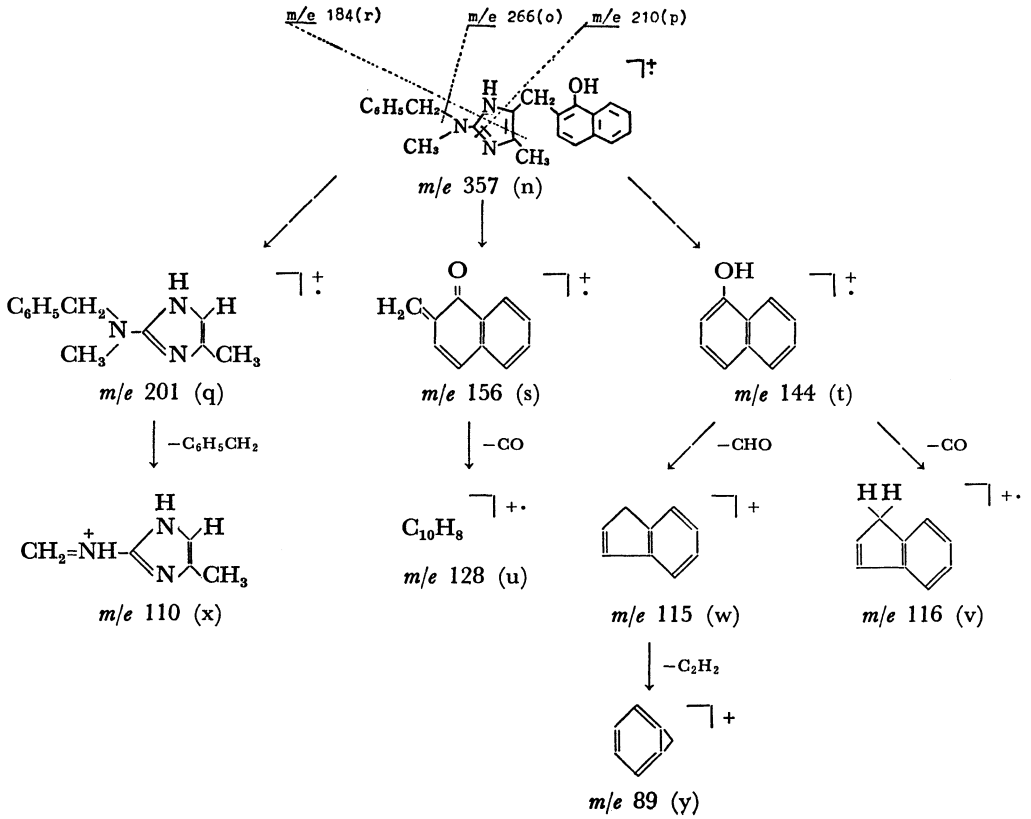
	m/e	Intensity	Composition	Calcd	Found
a	355 (M)	100	$C_{23}H_{21}ON_3$	355.168	355.169
b	340	17	$C_{22}H_{18}ON_3$	340.145	340.142
c	264	8	$C_{16}H_{14}ON_3$	264.114	264.113
d	235	3	$C_{15}H_{11}ON_2$	235.087	235.088
e	223	21	$C_{14}H_{11}ON_2$	223.100	223.097
f	209	6	$C_{14}H_{11}ON$	209.084	209.086
g	194	4	$C_{14}H_{10}O$	194.073	194.068
			$C_{13}H_8ON$	194.065	
h	182	91	$C_{12}H_8ON$	182.059	182.063
i	154	7	$C_{11}H_8N$	154.066	154.072
j	127	31	$C_{10}H_7$	127.055	127.052
k	120	6	$C_8H_{10}N$	120.051	120.053
l	91	38	C_7H_7	91.055	91.057
m	65	11	C_5H_5	65.021	65.039

TABLE 2. MASS SPECTRUM OF THE REDUCED PIGMENT

	m/e	Intensity	Composition	Calcd	Found
n	357 (M)	23	$C_{23}H_{23}ON_3$	357.184	357.184
o	266	9	$C_{16}H_{16}ON_3$	266.129	266.129
p	210	12	$C_{14}H_{12}ON$	210.092	210.092
q	201	74	$C_{12}H_{15}N_3$	201.127	201.125
r	184	5	$C_{12}H_{10}ON$	184.076	184.082
s	156	4	$C_{11}H_8O$	156.058	156.058
t	144	67	$C_{10}H_8O$	144.058	144.058
u	128	6	$C_{10}H_8$	128.063	128.062
v	116	25	C_9H_8	116.063	116.060
w	115	44	C_9H_7	115.055	115.055
x	110	100	$C_5H_8N_3$	110.072	110.072
y	89	7	C_7H_5	89.039	89.040

The mass spectrum of the pigment was consistent with the structure III as shown in the following fragmentation scheme:





Treatment of the pigment with CH_3OD resulted in the significant increase in M+1 and *m/e* 183 ion peaks, indicating that deuteration occurred at position 1. Thus the methyl group can be located at position 4 in the imidazole ring from the latter peak.

The mass spectrum of the reduced pigment can be explained by the following fragmentation scheme: The ions q, s, and t may arise from the molecular ion n by the hydrogen rearrangement-dissociation reaction by analogy with the fragmentation of diarylmethanes containing *o*-methyl or *o*-hydroxyl substituent,[15] and the presence of the former two ions substantiates the reduced pigment to be 2-substituted-1-naphthol.

From the foregoing discussions it is concluded that the pigment produced in the diacetyl reaction of 1-benzyl-1-methylguanidine is 2-(*N*-benzyl-*N*-methylamino)-4-methyl-5-(1-oxo-1,2-dihydro-2-naphthylidenemethyl)imidazole (III), and the reduced form should be 2-(*N*-benzyl-*N*-methylamino)-4-methyl-5-(1-hydroxy-2-naphthylmethyl)imidazole (IV). Thus it becomes evident that the pigment produced by Barritt's or Eggleton's method differs from that formed by the original Voges-Proskauer reaction carried out in the absence of 1-naphthol.

Recently, Sakaguchi *et al.*[16] reported that the Voges-Proskauer reaction of diacetyl, 1,1-dimethyl guanidine and 1-naphthol by Eggleton's method gives two spots A (reddish purple, R_f 0.76) and B (red, R_f 0.62) on tlc (silica gel, benzene–AcOEt–EtOH (3 : 1 : 1)), and the major product is pigment B. Our pigment corresponds to Sakaguchi's pigment A in view of the color and the R_f value. Isolation of the pigment corresponding to Sakaguchi's pigment B was not successful, because of its instability.

Experimental

Melting points are uncorrected. NMR spectra were obtained on a Japan Electron Optics JNM-100 or Hitachi 3H-60 spectrometer. Chemical shifts are reported as δ values using tetramethylsilane as an internal standard. Abbreviations used in NMR data are: s, singlet; m, complex multiplet. The mass spectra were determined on a Japan Electron Optics JMS-01S high resolution mass spectrometer (Mattauch-Herzog type) operating with an ionizing energy of 70 eV by the direct inlet procedure. The source temperatures were 220°C for the pigment and 170°C for the reduced one. Exact mass measurement was carried out with perfluorokerosene to provide reference masses. Infrared spectra were obtained with a Hitachi recording spectrophotometer EPI G_2. Visible and ultraviolet spectra were obtained with a Hitachi spectrophotometer 101 or EPS-2.

Materials. Commercially available 1-naphthol and 1,4-naphthoquinone were purified by steam distillation and column chromatography on silica gel with benzene, respectively. 2-Chloro-1-naphthol,[17] 4-chloro-1-naphthol,[18] 1,2-naphthoquinone,[19] and 1-benzyl-1-methylguanidine hydrochloride[10] (mp 134—135°C)[20] were prepared according to the known procedures.

Preparation of Pigment. (a) To a solution of 1-benzyl-1-methylguanidine (1.63 g, 0.01 mol) in chloroform (25 m*l*) were added successively diacetyl (0.86 g, 0.01 mol) in chloroform (3 m*l*) and 1-naphthol (1.44 g, 0.01 mol) in chloroform (10 m*l*). The mixture was stirred at 24°C for 6 hr and then allowed to stand at room temperature for 16 hr. The resulting deep red solution was evaporated at about 40°C and the residue, after being dissolved in a small amount of benzene, was charged onto a column (made from 100 g of about 300 mesh Wako-gel, 60 mm diameter and 68 mm depth) and then eluted with benzene. After the first 850 m*l* fraction containing 1-naphthol and unidentified substances had been removed, the main fraction (about 2,800 m*l*) corresponding to a deep reddish purple band of the chromatogram was collected and evaporated at 37°C to give crude pigment (532 mg), which was found to consist of the desired pigment and three other minor components by means of tlc (silica gel, benzene). A reddish purple amorphous product showing a single spot on tlc was obtained by repeated chromatographic purification. The product dissolved in a small amount of benzene gave 182 mg of dark reddish purple prisms, mp 149—149.5°C, on addition of several drops of *n*-hexane and refrigeration. The pigment was readily soluble in ethyl acetate, carbon disulfide, benzene, and carbon tetrachloride; moderately in methanol, ethanol, and dilute aqueous alkali solution; insoluble in water. Found: C, 77.59; H, 6.54; N, 11.62%. Calcd for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82%. UV: λ_{max} 535 mμ (ε=37200) in aqueous NaOH+Na_2CO_3.[10]

(b) To a mixture of 1-benzyl-1-methylguanidine hydrochloride (1.50 g, 7.5 mmol) in water (300 m*l*) and diacetyl (1.31 g, 15 mmol) in water (350 m*l*) was added a solution of 1-naphthol (5.41 g, 37.5 mmol) in 2.5 N NaOH (300 m*l*) under stirring at room temperature. The resulting reddish purple solution was stirred at room temperature for 10 min and then saturated with sodium chloride. After 20 min, the solution was separated by decantation from a dark purple gummy precipitate which did not contain the desired pigment. The solution was extracted several times with benzene, and the combined extracts (3500 m*l*) were dried over anhydrous sodium sulfate for 12 hr and then concentrated at 42—45°C to a small volume. The concentrate was purified in the same manner as above to give the pure pigment (131 mg).

Catalytic Hydrogenation of Pigment. A solution of pigment (50 mg) in benzene (50 m*l*) was hydrogenated in the presence of platinic oxide (20 mg) at ambient pressure and temperature. The red color of the pigment disappeared within 100 min, after which the solution was evaporated under reduced pressure to give a glass. Similar results were obtained in combinations of methanol–Pd/C and methanol–PtO_2.

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20) The lower value reported[10] can be ascribed to insufficient drying.