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STUDIES ON THE VOGES-PROSKAUER REACTION. II.

STRUCTURE OF PIGMENTS FORMED IN THE DIACETYL REACTION OF 1,1-DISUBSTITUTED GUANIDINES.

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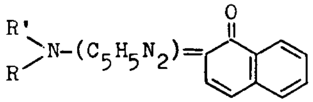
Voges and Proskauer1) reported that glucose pepton cultures of certain organisms produced an eosin-like coloration on addition of 10% KOH. The coloration was shown to occur as a result of the reaction between diacetyl and creatine or certain similar substances2,3). Addition of 1-naphthol greatly intensified the coloration4) and this reaction has been called the diacetyl reaction. Although Eggleton et al.5) obtained a crude coloring matter in the diacetyl reaction with creatine, the pigment has not yet been isolated in a pure crystalline form and consequently its structure has remained unknown. The first paper6) of this series has shown that among substituted guanidines, 1,1-disubstituted ones gave especially strong coloration. The authors have established the structure of pigments obtained by using 1,1-disubstituted guanidines6), $RR'NC(=NH)NH_2$ (I) where RR'N- denotes benzylmethylamino, dimethylamino, piperidino or morpholino group, instead of creatine and found these pigments to have an interesting structure of imidazolylnaphthoquinomethane. (The four pigments will be designated as A, B, C and D in the above order.)

The pigments were formed by addition of 1-naphthol in 2.5N NaOH to a mixture of diacetyl and a guanidine hydrochloride in water, followed by agitation of the mixture at room temperature. The crude pigments were precipitated by neutralizing the reaction mixture with HCl to pH 7. Crude pigment A was also obtained by extracting the NaCl-saturated mixture with benzene. They were purified by column chromatography on alumina with benzene + methanol and then on silica gel with n-hexane + chloroform except pigment A, which was separated on silica gel with benzene. Thus, dark reddish purple prisms with mp 149.0-149.5°(A), 164° (B), 167°(C), and 182-3°(D)(uncorr.) were obtained in about 2% yields. Pigment A was also obtained in about 5% yield by conducting the reaction in chloroform. Each pigment thus isolated gave a visible spectrum having the same maximum absorption (λ_{max} 535mμ) as and a similar shape to that obtained under the conditions of quantitative determination[6].

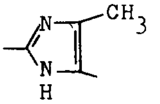
The elemental analyses and mass spectrometry of these pigments gave the molecular formulas of $C_{23}H_{21}ON_3$(A), $C_{17}H_{17}ON_3$(B), $C_{20}H_{21}ON_3$(C), and $C_{19}H_{19}O_2N_3$(D). The NMR data on pigment A (ppm in δ-value, in CCl_4) showed the presence of N-methyl(3.21, s), N-methylene(4.80, s), phenyl(7.32, s), and naphthalene nucleus (about 7.0-8.4, m). Other pigments also showed the signals corresponding to the respective substituents of guanidines used. These pigments are therefore composed of a common structural unit of $C_{15}H_{11}ON_2$ and a disubstituted amino moiety.

The IR spectra of these pigments showed strong peaks at 1630 cm^{-1}(C=O), 1595 and 1580 cm^{-1}(aromatic ring vibration), and 800 and 740 cm^{-1}(ring proton out-of-plane vibration). The 800 cm^{-1} band is observed in 1,2-naphthoquinone but not in the 1,4-isomer, suggesting the presence of a 1,2-naphthoquinoidal system. Pigment A absorbed one molar equivalent of hydrogen on catalytic hydrogenation in methanol in the presence of Pd/C or Pt and yielded a colorless solution, which rapidly restored the original reddish color upon exposure to 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 3270-3150 cm^{-1} in KBr disk and a sharp unassociated band at 3460 cm^{-1} in CCl_4 solution, with simultaneous disappearance of the carbonyl band. It has therefore a naphthol structure. The fact that it has no signal in the neighborhood of 6.68 ppm at which 2-proton of 1-naphthols$^{7)}$ usually resonates further suggests the presence of a 1,2-quinoidal system. On the basis of these findings, it can be inferred that the pigments have the structure II.

In the NMR spectrum of the reduced pigment, the =CH- signal (6.69 ppm) disappeared and a new signal ($-CH_2-$) at 3.62 ppm was observed. 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 II should be III.

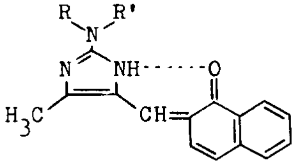


(II)

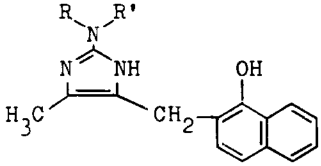


(III)

An NH chemical shift in imidazole is reported to be in 11.10-13.50 ppm depending upon the concentration$^{8)}$, whereas the NH signal of the pigments was found in a markedly lower field (16.67 ppm; relatively broad, disappeared on addition of CH_3OD). The exceptional downfield shift and the unusual low frequencies of both N-H (3090-3060 cm^{-1}, independent of concentrations in CCl_4) and C=O stretching vibrations in the IR spectrum may be interpreted as a result of intramolecular hydrogen bonding between the NH and the carbonyl oxygen atom and provide an additional evidence supporting the presence of 1,2-quinoid structure. Thus the following structures IV and V can be derived for the pigments and the reduced pigments, respectively:



(IV)

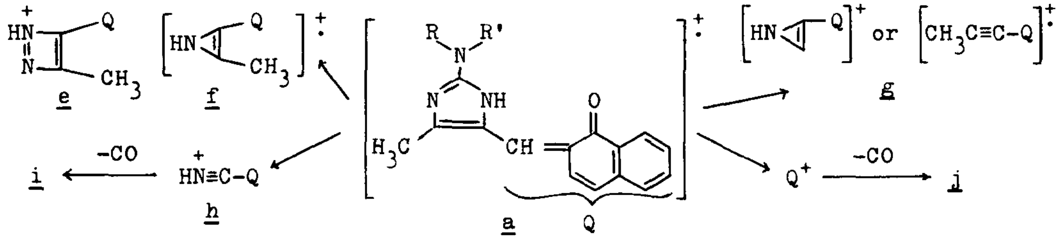


(V)

The structure IV was further confirmed on the basis of mass spectrometric evidence. The molecular and major fragment ions of pigment A are listed below:

	m	l	k	j	i	h	g	f	e	d	c	b	a
m/e	65	91	120	127	154	182	194	209	223	235	264	340	355(M)
I(%)	11	38	6	31	7	91	4	6	21	3	8	17	100

Exact mass measurements established these ions, a - m, in pigment A to have the following composition: $C_{23}H_{21}ON_3$(a), $C_{22}H_{18}ON_3$(b), $C_{16}H_{14}ON_3$(c), $C_{15}H_{11}ON_2$(d), $C_{14}H_{11}ON_2$(e), $C_{14}H_{11}ON$(f), $C_{14}H_{10}O$ or $C_{13}H_8ON$(g), $C_{12}H_8ON$(h), $C_{11}H_8N$(i), $C_{10}H_7$ (j), $C_8H_{10}N$(k), C_7H_7(l), C_5H_5(m). Other pigments showed the ion peaks corresponding to these ions. The fragment ions, b and d, correspond to the loss of CH_3 and R-N-R' from the molecular ion a and the latter was observed as k ion. *The* c *ion in pigment A may be formed by loss of* $CH_2C_6H_5$ *from* a *and this was ob*served as l ion, which may then lose an acetylene molecule to give m. The formation of the e to j ions could plausibly be interpreted from the following fragmentation scheme:



Treatment of pigment A with CH_3OD resulted in the significant increase in M+1 and m/e 183 ion peaks. This indicates that deuteration occurred at position 1 and that the methyl group is located at position 4 in the imidazole ring.

From the foregoing discussions, it is concluded that the pigments formed in the diacetyl reaction of I are 2-N,N-disubstituted amino-4-methyl-5-(1-oxo-1,2-dihydro-2-naphthylmethylene)imidazole IV and the reduced form should be (2-N,N-disubstituted amino-4-methyl-5-imidazolyl)(1-hydroxy-2-naphthyl)methane V.

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