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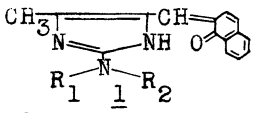
MECHANISM OF VOGES-PROSKAUER REACTION

Tamio NISHIMURA, Hiroshige TOKU, Tetsuro UENO,
Shinji SHIBAMOTO, and Toshio IMAI
Department of Chemistry, School of Hygienic Sciences,
Kitasato University, Minato-ku, Tokyo

Presumable intermediates of pigments formed by the Voges-Proskauer reaction were synthesized from 1-methyl-1-benzylguanidine and acetylbenzoyl and the mechanism of this color reaction was proposed.

Voges and Proskauer[1] reported the production of an eosin-like coloration in glucose peptone cultures of certain organisms to which aqueous KOH was added. This color reaction has generally been referred to as the Voges-Proskauer (V-P) reaction and has been shown due to the reaction of diacetyl (DA) with guanidine derivatives.[2,3] On the basis of these findings, O'Meara[4] devised a sensitive method for detecting acetoin formed by bacteria. Barritt[5] found that color intensity is remarkably increased by addition of α-naphthol and this method was elaborated and applied to the determination of guanidines, DA or acetoin by Eggleton et al.[6] Throughout this paper, the reaction in the absence of α-naphthol will be referred to as the Original V-P reaction and that in it's presence as the Barritt reaction.

We already proposed[7] the structure of pigments (1) that were produced from DA and 1,1-disubstituted guanidines by the Barritt reaction. On the other hand, the structure of an Original V-P pigment (2) which was formed from DA and N-amidinomorpholine was reported by Kijima et al.[8]



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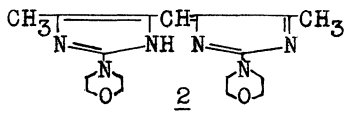

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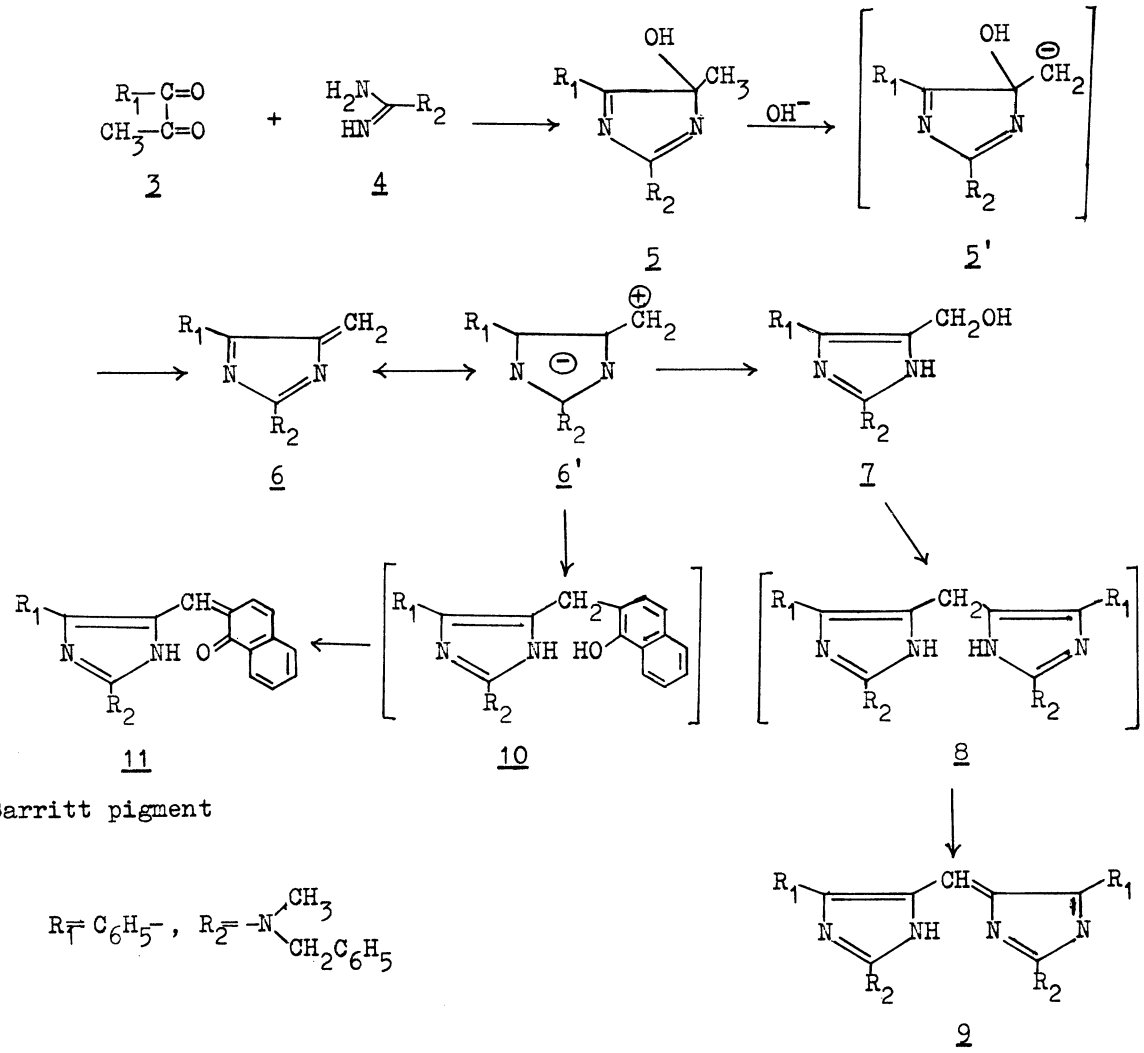
In order to elucidate the mechanism of the Original V-P and Barritt reactions, we attempted without success to obtain the intermediates of the pigments from DA and 1-methyl-1-benzylguanidine (4). However, by replacing acetylbenzoyl (3) for DA, we succeeded to synthesize the intermediates (5,6,7) and could propose the reaction mechanism as shown in Chart 1.



Barritt pigment

R_1= C_6H_5-, R_2= $-N(CH_3)CH_2C_6H_5$

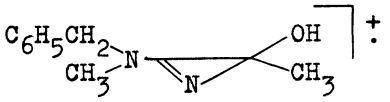
Original V-P pigment

Chart 1

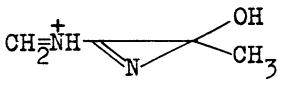
Column chromatography (Wako-gel C-200, 10% CH_3OH in $CHCl_3$) of the crude pigment obtained by the reaction of 3 and 4 according to O'Meara's method[4] gave an Original V-P pigment (9), violet needles, mp 152-153°C; $C_{35}H_{32}N_6$ (Calcd. 536.2688, Found 536.2699); MS (m/e): 416 (M^+-$C_6H_5CH_2NCH_3$), 354 (M^+-2 $C_6H_5CH_2$); UV: λmax 570mμ (ε, 3.78×10^4) in C_6H_6; λmax 560mμ (ε, 3.19×10^4) in EtOH; NMR in $CDCl_3$ (δ): 3.07 (2 N-CH_3), 4.80 (2 N-CH_2), 7.27-8.30 (-CH=, -NH-, 4 C_6H_5); IR (KBr, cm^{-1}): 820 (-CH=).

The reaction of 3 and 4 in water under N_2 produced 2-(N-methyl-N-benzylamino)-4-hydroxy-4-methyl-5-phenyl-4H-imidazole (5), yellow prisms, mp 141.5-142°C; $C_{18}H_{19}ON_3$ (Calcd. 293.1528, Found 293.1533); MS (m/e): 278 (M^+-CH_3), 276 (M^+-OH), 190 (M^+-C_6H_5CN), 120 ($C_6H_5CH_2\overset{+}{N}H=CH_2$), 99 ($M^+$-$C_6H_5CN$ and $C_6H_5CH_2$); NMR in $CDCl_3$ (δ): 1.63 (C-CH_3), 3.10 (N-CH_3), 4.86 (N-CH_2), 5.86 (OH), 7.28-8.40 (2 C_6H_5); IR (KBr, cm^{-1}): 1150 (tert. OH). The attachment of C_6H_5 to the conjugated system is suggested by UV spectrum (λmax 274.5mμ) and NMR signal of C_6H_5, which shifted to relatively lower magnetic field. The position of C_6H_5 on the 4H-imidazole ring

was further substantiated by the presence of m/e 190 and m/e 99 on the mass spectrum of 5.



m/e 190



m/e 99

A dilute HCl solution of 5 was evaporated to dryness under reduced pressure to yield dihydrochloride of 2-(N-methyl-N-benzylamino)-4(5)-phenyl-5(4)-hydroxymethyl-imidazole (7), orange powder, mp 168°C (d.); $C_{18}H_{19}ON_3 \cdot 2\,HCl$; MS (m/e): 293 ($M^+$), 276 ($M^+$-OH), 275 ($M^+$-$H_2O$), 262 ($M^+$-$CH_2OH$); NMR in $CDCl_3$ (δ): 3.05 (N-C$\underline{H}_3$), 4.65 (C-C$\underline{H}_2$), 4.75 (N-C$\underline{H}_2$, O$\underline{H}$), 7.23-7.32 (2 C$_6\underline{H}_5$), 12.60-12.95 (2 N$\underline{H}$); IR (KBr, cm^{-1}): 1035 (prim. OH).

Addition of conc. HCl to 5 or 7 yielded dihydrochloride of 2-(N-methyl-N-benzylamino)-4- methylene-5-phenyl-4H-imidazole (6), white needles, mp 118-119°C (d.); $C_{18}H_{17}N_3 \cdot 2\,HCl$; MS (m/e): 275 ($M^+$), 249 ($M^+$-CH=CH), 155 ($M^+$-$C_6H_5CH_2NCH_3$); NMR in $CDCl_3$ (δ): 3.01 (N-C$\underline{H}_3$), 4.61 (C=C$\underline{H}_2$), 4.80 (N-C$\underline{H}_2$), 7.24-7.80 (2 C$_6\underline{H}_5$), 12.20-12.58 (2 N$\underline{H}$). 6 was quantitatively converted to 7 in the presence of water.

In order to test whether 5 and 7 are the intermediates of the Barritt pigment or not, solutions of 3+4, 5, and 7 in ethanol were treated by Eggleton's method[6] and their absorbance was measured at 575mμ. 5 gave the same coloration rate and maximum color intensity as 3+4, while 7 showed about 1/3 as small intensity as 5 or 3+4, with relatively lower coloration rate. From the above result, 5 was considered to be the intermediate of the Barritt pigment, but not 7. Coloration of 7 might be ascribable to the production of the Original V-P pigment.

Separation and purification by column chromatography (Wako-gel C-200, C_6H_6) of the reaction products from 5 gave an Barritt pigment (11), violet needles, mp 155-155.5°C; $C_{28}H_{23}ON_3$ (Calcd. 417.1841, Found 417.1848); UV: λmax 525mμ (ε, 1.64×10^4), 565mμ (sh., ε, 1.41×10^4), 610mμ (ε, 1.30×10^4) in C_6H_6; λmax 525mμ (sh., ε, 1.56×10^4), 565mμ (ε, 1.60×10^4), 610mμ (ε, 1.75×10^4) in EtOH; IR (KBr, cm^{-1}): 1630 (C=O), 810 (-CH=). Main fragment ions of MS corresponded with those of the Barritt pigment[7] from DA and 4. However, the presence of pigment 11 in the reaction mixture from 7 could not be confirmed.

In order to know, whether 5 and 7 are the intermediates of the Original V-P pigment or not, they were treated by O'Meara's method[4] and the change in absorbance was followed at 570mμ. Color intensity and coloration rate increased in the order 7>5>3+4. The pigments isolated in cases of 5 and 7 were found to have the same structure as that of 9. Therefore, 5 and 7 are considered to be the intermediates

of the Original V-P pigment.

Because of high reactivity of 6 with water or ethanol, its possibility of being the intermediate was tested by treating a solution of 6 in $CHCl_3$ with Eggleton's method.[6] After 30 min, produced pigments were isolated by TLC, dissolved in benzene and their absorbance was measured. For comparison, 5 and 7 were treated under the same conditions. The ratio of amounts of the Barritt pigment produced was 6:5:7=4:1:0, and that of the Original V-P pigment was 7:6:5=4:1:1/35. Considerably smaller amount of the Original V-P pigment from 5 is due to the side reactions occurred at lower alkaline concentration than that employed in O'Meara's[4] conditions. This was confirmed by independent spectroscopic study. Therefore, 6 is the intermediate of both pigments and located between 5 and 7 in the reaction pathway.

On the basis of the above results, most plausible mechanism is as follows (Chart 1). Abstraction of a proton from 4-methyl group of 4H-imidazole 5 by OH^- produces carbanion 5', which then loses OH^- to form 4-methylene-4H-imidazole 6. Methylene carbon of 6 may have electrophilic character because of considerable contribution of a resonance structure 6'. This property of 6 reasonably accounts for the formation of primary alcohol 7 by nucleophilic attack of OH^- and the formation of reduced Barritt pigment 11 by attack of naphtholate ion. It is also highly probable that these processes may be effected by protonated 6, which has stronger electrophilic methylene carbon. This mechanism successfully explaines the reason why Eggleton's method[6] needs very large excess of naphthol, since naphtholate ion must compete with OH^-. It is evident from previous paper[7] that Original V-P pigment 9 and Barritt pigment 11 are easily produced by air-oxidation of respective reduced pigments 8 and 10. The mechanism by which 8 is formed from 7 is under investigation.

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