A SIMPLE DETERMINATION OF VITAMIN A IN MARGARINE

II. ON THE REACTION BETWEEN YELLOW AB OR OB AND ALDEHYDES

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In the preceding paper (1), a simple method has been reported to remove the artificial dyes, yellow AB and OB, from the unsaponifiable fraction of margarine by treating the petroleum ether phase with glacial acetic acid containing a small amount of acetaldehyde. The present paper deals with the study to elucidate the reaction mechanism between the dyes and acetaldehyde. Furthermore, the condensation products of the dyes with various kinds of aldehyde were synthesized and described.

Yellow AB, *i. e.*, phenylazo- β -naphthylamine and OB, *i. e.*, *o*-tolylazo- β -naphthylamine differ from each other only by a methyl group in their chemical structure as described below and their chemical properties are very similar. The reaction with acetaldehyde was assumed to occur in the common β -naphthylamine radical of both dyes, since they behaved similarly to acetaldehyde contained in glacial acetic acid. There have been many investigations on the reaction between β -naphthylamine and aromatic aldehydes, whereby aldehyde- β -naphthylimides, condensed products at amino group, have been obtained. Therefore, the reaction in our case was supposed also to be the condensation of the amine with aldehyde in a similar manner, yielding phenylazo-aldehyde- β -naphthylimide (I) or *o*-tolylazo-aldehyde- β -naphthylimide (II), respectively, as follows:

As the compounds (I) and (II) have never been described so far in the literature, the condensation products of both pigments with various aldehydes were prepared and their chemical properties were investigated.

EXPERIMENTAL

1. The Synthesis and Identification of Phenylazo-acetaldehyde- β -naphthylimide.

FIG. 1 Ultraviolet Absorption Spectrum of Phenylazoacetaldehyde- β -naphthylimide

An adequate quantity of acetaldehyde (10 ml) was added to an alcoholic solution of phenylazo- β -naphthylamine (5 g) and the mixture was boiled for 30 minutes on a water bath. A red product appeared in a viscous solution when the reaction mixture was left standing overnight. The precipitate (5 g) was separated from viscous mother liquor by filtration and recrystallized from hot alcohol several times. The red color of the crystal faded gradually. Then the crystal was treated with animal charcoal in alcohol. Finally colorless hexagonal

plate crystals were obtained, mp 236° (decomp. uncorr.). The ultraviolet absorption spectrum of this crystal in alcohol is shown in Fig. 1. Absorption maximum lies at 244–246 m μ ; $E_{1\%}^{1\text{cm}}$ 245 m μ = 2,032.

		C	H	N	m. w.
$\text{C}_{18}\text{H}_{15}\text{N}_3$	Calc.	79.12	5.49	15.38	271.3
	Found	78.23	5.41	14.39	286

The molecular weight was measured by ebullioscopy using glacial acetic acid or carbon tetrachloride as solvent.

The infrared spectra of this compound and of phenylazo β naphthylamine are shown in Fig. 2. The spectrum of the latter revealed the absorption maxima at 2.90 and 6.14 μ , indicating the presence of NH_2 -radical, but these absorption maxima were not observed in that of the former and a characteristic absorption of $-\text{N}=\text{C}$ linkage at 6.10 μ was clearly observed. This is a powerful evidence for the formation of phenylazo-acetaldehyde- β -naphthylimide (III).

The crystal is easily soluble in chloroform, carbon tetrachloride, carbon disulfide, ethyl formate, and glacial acetic acid, soluble in ether, benzene, and

alcohol, but insoluble in petroleum ether, benzene, ligroin, and water. This solubility coincides well with the principle adopted in the standard procedure. The crystal assumed a light pink color under the sunlight.

2. *o*-Tolylazo-acetaldehyde β naphthylimide.

This compound was prepared from *o*-tolylazo- β -naphthylamine and acetaldehyde in the same manner as described above. It crystallized in colorless hexagonal plates from alcohol, mp 176.5° (decomp. uncorr.). The ultraviolet absorption spectrum of this crystal in alcohol is about the same as that of (III). Absorption maximum lies at 245–246 m μ ; $E_{1\%}^{1\text{cm}}$ 245 m μ = 1,920.

		C	H	N	m. w.
$\text{C}_{19}\text{H}_{17}\text{N}_3$	Calc.	79.40	5.92	14.63	287.5
	Found	79.43	6.31	13.83	280

As the result of the infrared analysis of this compound, a characteristic absorption of $\text{N}=\text{C}$ linkage was observed at 6.10 μ as shown in Fig. 2.

From these data, the crystal was identified as *o*-tolylazo-acetaldehyde- β -naphthylimide (IV).

The solubility of this crystal is just the same as that of (III). This compound differs from (III) in the resistance to the sunlight.

3. The Yield of Phenylazo-acetaldehyde- β -naphthylimide in the Standard Method.

In the standard method described in the previous paper (1), the pigments

FIG. 2 Infrared Spectra of Yellow AB, Phenylazo- and *o*-Tolylazo-acetaldehyde- β -naphthylimide

were observed to transfer themselves into the acetic acid layer, assuming a reddish-violet color, when glacial acetic acid and acetaldehyde solution were added to petroleum ether solution of the dyes. But, as mentioned above, the main condensation product (III), was obtained in colorless crystals and it was necessary to estimate of the yield of (III) in the standard method. The acid layer obtained according to the standard method was neutralized with 10 per cent sodium hydroxide solution, and the red viscous products were separated from the solution. The red products were extracted with ether and the ether fraction was washed with water several times. After complete removal of ether, the viscous red residue was obtained, but the crystallization was not attained even by using several different solvents. The concentration of (III) in the residue was determined spectrophotometrically.

The red viscous product obtained from yellow AB in the same manner, was dissolved in alcohol and its absorbancy was measured at 245 $m\mu$. Since the yield of (III) corresponded to 78 per cent of the theoretical value, (III) was considered to be the main product.

Thus, it was assumed that about 80 per cent of phenylazo- β -naphthylamine condensed with acetaldehyde to (III) in this procedure and that the substance assuming a reddish-violet color was small in quantity and should be considered to be an unknown byproduct accompanying the reaction, although seemingly dominant in appearance.

4. The Condensation of Pigments with Various Aldehydes.

The acetic acid solution

FIG. 3 Ultraviolet Absorption Spectra of Some Phenylazo- and *o*-Tolylazo-aldehyde- β -naphthylimides

similar absorption spectra.

The solubility of all compounds obtained was just the same as that of (III); phenylazo compounds assumed a light pink color under the sunlight, but *o*-tolylazo compounds failed to show any color.

The infrared spectra of a few representative compounds are represented in

of yellow AB or OB produced a red or reddish violet color on addition of aldehydes except polymer such as paraformaldehyde. The condensation products of pigments with various aldehydes were synthesized and their chemical properties investigated.

Compounds were prepared from yellow AB or OB and aldehydes in a similar procedure as described above and their properties, *e. g.*, melting point, crystal form, $\lambda_{\max.}$, are listed in the following Tables. Most of the compounds were obtained in colorless crystals from alcohol, but only cinnamic aldehyde compound in a light yellow color from the same solvent. The ultraviolet absorption spectra of each saturated aliphatic aldehyde compound was about the same as that of (III), and revealed a single absorption maximum at about 245 $m\mu$. In the case of unsaturated aliphatic and aromatic aldehydes, the absorption spectra of condensation products revealed characteristic two or three principal absorption maxima as shown in Fig. 3. Phenylazo- and *o*-tolylazo compounds of the same aldehyde excluding the citronellal compounds showed very

TABLE I *Phenylazo-aldehyde-β-naphthylimide*

Aldehyde	R	Formula	Crystal form	mp °C (decomp., uncorr.)	λ max, mμ (alcohol)	E 1% at λ max. (alcohol)	Elementary analyses				m. w.
							C	H	N		
<i>Saturated aliphatic aldehydes</i>											
Propylaldehyde	—CH ₂ •CH ₃	C ₁₉ H ₁₇ N ₃	tetragonal plates	227—228	244—246	2,083	Calcd. Found	79.44 78.17	5.92 5.67	14.63 14.92	287.5 279
Butylaldehyde	—(CH ₂) ₂ •CH ₃	C ₂₀ H ₁₉ N ₃	hexagonal plates	246	244—246	2,083	Calcd. Found	79.73 79.82	6.31 5.94	13.95 13.79	301.7 305
Isovaleraldehyde	—CH ₂ •CH:(CH ₃) ₂	C ₂₁ H ₂₁ N ₃	tetragonal plates	240	244—246	2,078	Calcd. Found	80.00 80.19	6.66 6.51	13.33 12.65	315.4 317.3
Oenantaldehyde	—(CH ₂) ₅ •CH ₃	C ₂₃ H ₂₃ N ₃	needles	183	242—244	1,790	Calcd. Found	80.46 80.41	7.28 7.96	12.24 12.35	343.4 349
<i>Unsaturated aliphatic aldehyde</i>											
Citronellal	—CH ₂ •(CH ₃)•CH•CH ₂ CH ₂ :(CH ₃)C•CH ₂ •CH ₂	C ₂₆ H ₂₉ N ₃	tetragonal plates	207	246—247 329	1,599 115	Calcd. Found	81.46 81.37	7.57 7.89	10.96 11.01	— —
<i>Aromatic aldehydes</i>											
Benzaldehyde	—C ₆ H ₅	C ₂₃ H ₁₇ N ₃	plates	192	232—235 270 335—336	1,286 970 289	Calcd. Found	82.38 81.81	5.07 5.10	12.53 12.13	— —
Anisaldehyde	—C ₆ H ₄ •OCH ₃	C ₂₄ H ₁₉ N ₃ O	”	227—228	231 332	1,315 196	Calcd. Found	78.90 78.87	5.21 5.27	11.51 11.74	— —

TABLE II *o*-Tolylazo-aldehyde- β -naphthylimide

Aldehyde	R	Formula	Crystal form	mp °C (decomp., uncorr.)	λ max. mμ (alcohol)	$E_{1\text{ cm}}$ at λ max.	Elementary analyses				m. w.
							C	H	N		
<i>Saturated aliphatic aldehydes</i>											
Propylaldehyde	$-\text{CH}_2 \cdot \text{CH}_3$	$\text{C}_{20}\text{H}_{19}\text{N}_3$	rectangular plates	188	245—246	1,930	Calcd. 79.73 Found 78.03	6.31 6.14	13.95 13.80	301.7 290	
Butylaldehyde	$-(\text{CH}_2)_2 \cdot \text{CH}_3$	$\text{C}_{21}\text{H}_{21}\text{N}_3$	"	180.5	244—248	1,983	Calcd. 80.00 Found 79.38	6.66 6.36	13.33 13.26	315.4 307	
Isovaleraldehyde	$-\text{CH}_2 \cdot \text{CH}(\text{CH}_3)_2$	$\text{C}_{22}\text{H}_{23}\text{N}_3$	tetragonal plates	207—208	244—248	1,821	Calcd. 80.24 Found 79.87	6.99 6.66	12.76 12.70	329.4 335	
Oenantaldehyde	$-(\text{CH}_2)_5 \cdot \text{CH}_3$	$\text{C}_{24}\text{H}_{27}\text{N}_3$	needles	166	245—248	1,703	Calcd. 80.67 Found 79.98	7.56 7.00	11.76 10.28	358.1 363	
<i>Unsaturated aliphatic aldehydes</i>											
Citronellal	$-\text{CH}_2 \cdot (\text{CH}_3) \cdot \text{CH} \cdot \text{CH}_2$ $\text{CH}_2 : (\text{CH}_3) \text{C} \cdot \text{CH}_2 \cdot \text{CH}_2$	$\text{C}_{27}\text{H}_{31}\text{N}_3$	tetragonal plates	170	227—228 244—248	1,100 1,400	Calcd. 81.61 Found 81.71	7.81 7.13	10.58 10.51	408.5 383	
Citral	$-\text{CH} : (\text{CH}_3) \text{C} \cdot \text{CH}_2$ $(\text{CH}_3)_2 \text{C} : \text{CH} \cdot \text{CH}_2$	$\text{C}_{27}\text{H}_{29}\text{N}_3$	needles	150	233—235 268 333	814 868 300	Calcd. 82.03 Found 81.23	7.34 6.80	10.63 10.14	395.5 392	
<i>Aromatic aldehydes</i>											
Benzaldehyde	$-\text{C}_6\text{H}_5$	$\text{C}_{24}\text{H}_{29}\text{N}_3$	needles	209	233—235 270 338	1,223 958 279	Calcd. 82.57 Found 82.54	5.44 5.26	12.03 11.02	— —	
Anisaldehyde	$-\text{C}_6\text{H}_4 \cdot \text{OCH}_3$	$\text{C}_{25}\text{H}_{21}\text{N}_3\text{O}$	hexagonal plates	217	232 333	1,413 225	Calcd. 79.15 Found 78.76	5.54 5.43	10.52 10.13	— —	
Cinnamic aldehyde	$-\text{CH} : \text{CH} \cdot \text{C}_6\text{H}_5$	$\text{C}_{26}\text{H}_{21}\text{N}_3$	hexagonal pillars	210	238 288 363	987 589 724	Calcd. 83.20 Found 83.19	5.60 5.36	11.20 10.49	— —

FIG. 4 Infrared Spectra of Phenylazo- and o-Tolylazo-citronellal- β -naphthylimide, and o-Tolylazo-benzaldehyde- β -naphthylimide

Fig. 4. In all the cases, the absorption maxima indicating the presence of $-N=C<$ linkage, naphthalene and benzene rings were observed at 6.10, about 12.4 and 13.4 μ , respectively.

SUMMARY

In the simple assay method of vitamin A in margarine which has been devised by the authors, yellow AB and OB were found by condensing with acetaldehyde to form phenylazo-acetaldehyde- β naphthylimide or *o*-tolylazo-acetaldehyde- β -naphthylimide which can be removed easily by acetic acid.

Various phenylazo-aldehyde- β -naphthylimides and *o*-tolylazo-aldehyde- β -naphthylimides were prepared from yellow AB or OB and their chemical properties were described.

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