Reaction of Thiobarbituric Acid with Saturated Aldehydes

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The reaction of thiobarbituric acid (TBA) with saturated aldehydes, i.e., 1-butanal, 1-hexanal and 1-heptanal, produced a 455-nm yellow and a 532-nm red pigment. Formation of the pigments depended on the reaction conditions. The yellow pigment was unstable in the presence of excess amounts of the saturated aldehydes. The red pigment was formed only when the reaction was performed at a TBA/aldehyde ratio of 1:1 in aqueous acetic acid. Formation of the yellow and red pigments required molecular oxygen. The colorless adducts, intermediates for the yellow and the red pigments, were isolated from the reaction mixtures. Aldol condensation and dehydration of 2 mol of the saturated aldehydes initially gave the α,β-unsaturated aldehydes, which in turn reacted with TBA to form the colorless adducts, pyranopyrimidine derivatives. The adducts were then converted into the yellow and red pigments under aerobic conditions.

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The thiobarbituric acid (TBA) test is commonly used for measurement of peroxidation of unsaturated fatty acids or lipids (1). The peroxidation of lipids has been shown to give malonaldehyde (2) and monofunctional aldehydes (3). TBA produces a red pigment with a maximum absorption at 532 nm due to malonaldehyde (4,5). TBA also reacts with the saturated and unsaturated monofunctional aldehydes (6-11), giving, respectively, an unstable yellow pigment with a maximum absorption at 452 nm and a red pigment with a maximum absorption at 532 nm.

The reaction of 2 mol of TBA with 1 mol of malonaldehyde gives the red crystalline 2:1 adduct whose structure has been unambiguously established (12,13). The mechanisms of the reaction of TBA with the saturated and unsaturated monofunctional aldehydes, however, remain obscure. We investigated the reaction of TBA with the saturated aldehydes and found that a yellow and a red pigment were formed from an intermediate colorless adduct in the presence of molecular oxygen. We describe here the structure of the intermediate colorless adducts and their relevance to the formation of the yellow and red pigments.

MATERIALS AND METHODS

Materials. TBA, 1-butanal and 1-hexanal were the products of Wako Pure Chemical Industries Ltd. (Osaka, Japan). 1-Heptanal, 2-ethyl-2-hexenal and malonaldehyde bis(dimethylacetal) were the products of Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Thin layer chromatography (TLC) was performed with Wakogel B-5F (Wako Pure Chemical Industries Ltd.). Silica gel column chromatography was performed by use of silica gel for column chromatography (above 100 mesh) from Kanto Chemical Co. Ltd. (Tokyo, Japan).

Analysis. Absorption spectra were measured with a Shimadzu UV-240 UV-visible recording spectrophotometer. Mass spectra were obtained with a Hitachi M-80 double focusing mass spectrometer. ^{1}H-Nuclear magnetic resonance (NMR) spectra were taken in d_6-dimethylsulfoxide on a Bruker AM-400 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were taken in d_6-dimethylsulfoxide on a JEOL JNM-FX-100 Fourier-Transform NMR spectrometer with TMS as an internal standard, using both noise and off-resonance decoupling techniques. High performance liquid chromatography (HPLC) was carried out with a Shimadzu LC-5A liquid chromatograph equipped with a stainless steel column (4.6 mm × 25 cm) of Zorbax ODS. The chromatograph was operated by elution with a solvent mixture of MeOH/H_2O (8:2, v/v) or MeOH/0.04 M acetate buffer (pH 5.5) (4:6, v/v) at a flow rate of 0.8 ml/min. The peak was detected by use of a Shimadzu SPD-2A spectrophotometer.

Formation of a yellow and a red pigment. TBA and each aldehyde at the indicated concentration were dissolved in 5.0 ml of glacial acetic acid or 15% acetic acid in a test tube with a screw cap. The mixture was kept or heated either in air or under nitrogen gas. A solution of each colorless adduct at the indicated concentration in 5.0 ml of 15% acetic acid was similarly treated. After cooling, the absorption spectrum of the mixture was measured. HPLC of the reaction mixture was performed.

Colorless adducts of TBA and the saturated aldehydes. (i) Adduct W_B from 1-butanal. A mixture of 1.44 g (10 mmol) of TBA and 1.16 g (16 mmol) of 1-butanal in 170 ml of 15% acetic acid was heated for 15 min under reflux. The pale yellow mixture was extracted with chloroform several times. The chloroform extracts were washed with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue was crystallized from ethyl alcohol to afford colorless needles of W_B in a yield of 15% (384 mg). Recrystallization from ethyl alcohol gave pure sample of W_B, mp 179-182 C (dec). UV: λ_{max}^{EtOH} 293 nm (ε: 13600), 336 nm (16800). ^{13}C-NMR and ^{1}H-NMR spectra are shown in Tables 1 and 2, respectively. Mass spectrum: m/e (rel int); M^+ 252 (62), M^+-15 ($CH_3\cdot$) 237 (6), M^+-29 ($C_2H_5\cdot$) 223 (22), M^+-43 ($C_3H_7\cdot$) 209 (100). TLC: Rf 0.50 (solvent: $CHCl_3$/MeOH, 9:1, v/v). Anal: calcd for $C_{12}H_{16}N_2O_2S$: C, 57.11; H, 6.40; N, 11.10. Found: C, 57.06; H, 6.36; N, 11.06.

(ii) Adduct W_B from 2-ethyl-2-hexenal. A mixture of 5.0 g (35 mmol) of TBA and 5.0 g (40 mmol) of 2-ethyl-2-hexenal in 170 ml of 15% acetic acid was heated for 15 min under reflux. The pale yellow reaction mixture was extracted with chloroform as described. White needles of W_B were obtained from ethyl alcohol in a yield of 42% (3.70 g). Recrystallization from ethyl alcohol gave pure specimen, mp 181-184 C (dec). UV: λ_{max}^{EtOH} 293 nm (ε: 13700), 337 nm (17100). ^{1}H-NMR spectrum taken in d_6-dimethylsulfoxide was identical to that of the adduct obtained from 1-butanal. Mass spectrum: m/e (rel int); 252 (40), 237 (5), 223 (10), 209 (100). Mixed fusion test of this specimen and the adduct obtained from 1-butanal showed that they were identical. Anal: calcd for $C_{12}H_{16}N_2O_2S$: C, 57.11; H, 6.40;

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N, 11.10. Found: C, 57.14; H, 6.32; N, 11.14.

(iii) Adduct W_{Hx}. A mixture of 5.0 g (35 mmol) of TBA and 5.0 g (50 mmol) of 1-hexanal in 170 ml of 15% acetic acid was heated for 45 min under reflux. To a pale yellow reaction mixture was added an equal amount of chloroform. Insoluble yellow precipitate was removed by filtration. The chloroform layer was separated from the aqueous layer and dried over anhydrous sodium sulfate. It was evaporated to dryness, and the residue was applied to a column of silica gel (3 × 21 cm). Compound W_{Hx} was eluted with chloroform, and it was crystallized from ethyl alcohol-water as colorless granules in a yield of 1.9% (198 mg), mp 132–138 C (dec). Recrystallization from the same solvent gave pure sample, mp 142–146 C. UV: λ_{max}^{EtOH} 293 nm (ε: 12600), 340 nm (16000). ^{1}H-NMR (d_6-dimethylsulfoxide): ppm; 13.08 (1H, s, NH), 12.21 (1H, s, NH), 5.99 (1H, s, =CH-), 4.98 (1H, d, =CH-), 2.01 (2H, m, $-CH_2-$), 1.71 (1H, m), 1.2-1.6 (11H, bm), 0.89 (3H, t, $-CH_3$), 0.87 (3H, t, $-CH_3$). Mass spectrum: m/e (rel int); M^+ 308 (40), M^+-43 ($C_3H_7\cdot$) 265 (10), M^+-57 ($C_4H_9\cdot$) 251 (12), M^+-71 ($C_5H_{11}\cdot$) 237 (100). TLC: Rf 0.60 (solvent: $CHCl_3$/MeOH, 9:1, v/v). Anal: calcd for $C_{16}H_{24}N_2O_2S$: C, 62.29; H, 7.86; N, 9.08. Found: C, 62.15; H, 7.88; N, 9.12.

(iv) Adduct W_{Hp}. A mixture of 5.0 g (35 mmol) of TBA and 5.0 g (44 mmol) of 1-heptanal was treated and purified as in the case of the reaction of TBA and 1-hexanal. A small amount of colorless needles of W_{Hp} was obtained (7 mg) from ethyl acetate-*n*-hexane, mp 122–124 C (dec). UV: λ_{max}^{EtOH} 290 and 340 nm. Mass spectrum: m/e (rel int); M^+ 336 (80), M^+-43 ($C_3H_7\cdot$) 293 (10), M^+-57 ($C_4H_9\cdot$) 279 (20), M^+-71 ($C_5H_{11}\cdot$) 265 (95), M^+-85 ($C_6H_{13}\cdot$) 251 (100). TLC: Rf 0.62 (solvent: $CHCl_3$/MeOH, 9:1,v/v).

RESULTS

Formation of a yellow and a red pigment in the reaction of TBA with saturated aldehydes. Formation of a yellow and a red pigment in the reaction of TBA with 1-butanal, 1-hexanal and 1-heptanal was investigated. TBA was reacted with the 0.2–8 equivalents of 1-butanal. When 0.5 mM TBA was reacted with an equivalent amount of 1-butanal in glacial acetic acid at 100 C for 15 min, absorbance at 455 nm of the reaction mixture increased from 0.05 to 2.4 during standing at room temperature for 21 hr (Fig. 1). When the reaction mixture was heated for a longer period (6 hr), the absorbance decreased to 0.5. Thus, the yellow pigment formed in the reaction mixture was degraded. This instability of the yellow pigment may be due to the amount of 1-butanal. This possibility was supported by the fact that treatment of TBA with 4 and 8 equivalents of 1-butanal produced less yellow pigment after 15-min heating followed by 21-hr standing and also after 6-hr heating. In these reactions in glacial acetic acid, no significant amount of red pigment was produced.

TBA (0.5 mM) was reacted with an equivalent amount of 1-butanal in 15% acetic acid at 25 C. A yellow pigment with a maximum at 455 nm was produced gradually, but it subsequently degraded due to its instability in the aqueous solution (6–9,11) (Fig. 2). When the same reaction mixture was heated at 100 C for 6 hr, a yellow pigment was completely degraded and a

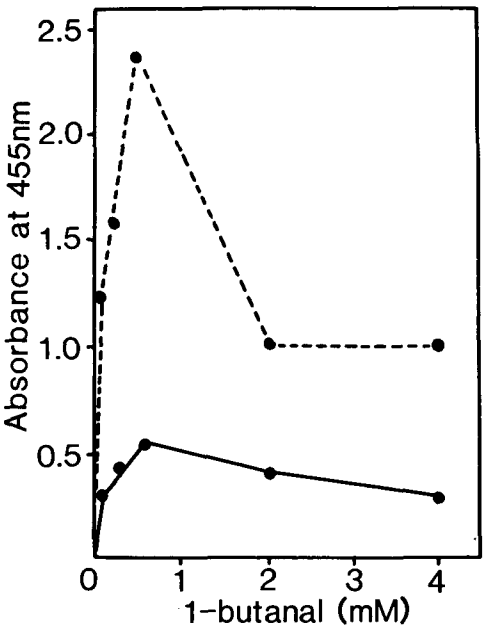


FIG. 1. Relationship between the amount of 1-butanal and absorbance at 455 nm of the reaction mixture in glacial acetic acid containing 0.5 mM TBA. The mixture was heated at 100 C for 15 min followed by standing at room temperature for 21 hr (- - -) or was heated at 100 C for 6 hr (——).

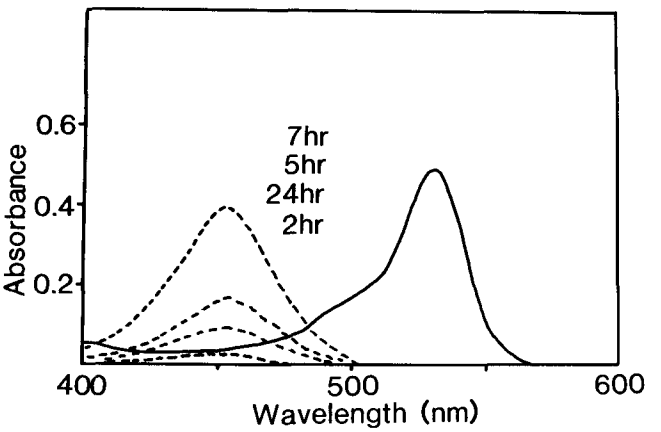


FIG. 2. Absorption spectra of the reaction mixture of 0.5 mM TBA and 0.5 mM 1-butanal in 15% acetic acid. The mixture was kept at 25 C for indicated periods (- - -) or was heated at 100 C for 6 hr (——).

red pigment with a maximum at 532 nm was formed (Fig. 2). The absorption spectrum of the red pigment was quite similar to that of the 2:1 adduct of TBA and malonaldehyde (12). It is likely that the red pigment was produced via the yellow pigment. Formation of the yellow and red pigments was suppressed completely when the reaction mixture was substituted with nitrogen gas, indicating that molecular oxygen is involved in the reaction.

TBA was reacted with various amounts of 1-butanal in 15% acetic acid at 100 C for 15 min or 6 hr (Fig. 3). Formation of the yellow pigment increased as the concentration of 1-butanal increased. It is, however,

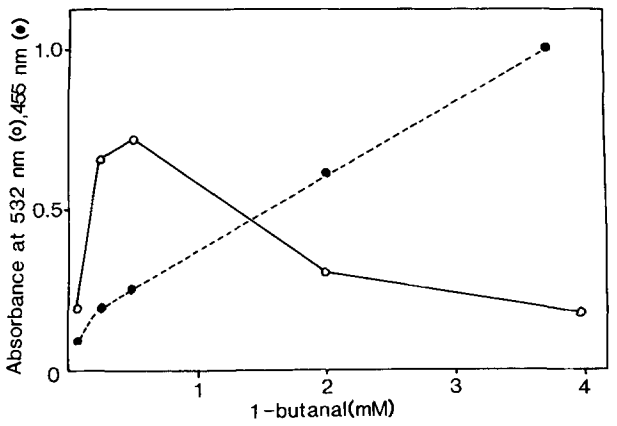


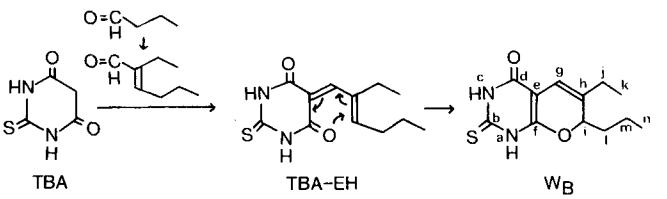
FIG. 3. Relationship between the amount of 1-butanal and absorbance at 455 and 532 nm of the reaction mixture in 15% acetic acid containing 0.5 mM TBA. The mixture was heated at 100 C for 15 min (- - -) or 6 hr (——), and the absorbance was measured immediately.

impossible to measure the amount of the yellow pigment, since the pigment is unstable. The maximal yield of the stable red pigment was obtained at about equimolar concentrations of 1-butanal. It is interesting that there is an optimal concentration of 1-butanal for the red pigment formation.

Reaction of TBA with 1-hexanal in 15% acetic acid gave the same results. Thus, the reaction of 0.5 mM TBA and 0.5 mM 1-hexanal at 25 C gradually produced an unstable yellow pigment and at 100 C for 6 hr produced a stable red pigment. As the concentration of 1-hexanal increased, formation of the yellow pigment increased, and the highest formation of the red pigment was observed in the reaction mixture containing nearly equal amounts of TBA and 1-hexanal. The reaction of TBA with 1-heptanal gave similar results.

Formation of colorless adducts in reaction of TBA and saturated aldehydes. We found that the reaction of TBA with the saturated aldehydes gave colorless adducts besides the yellow and red pigments. A mixture of TBA and a slight excess amount of 1-butanal in 15% acetic acid was heated at 100 C for 15 min under reflux. Colorless adduct W_B was isolated in a crystalline form from the chloroform extract of the yellow-colored reaction mixture. Absorption spectrum of W_B exhibited maxima at 293 and 336 nm. Elemental analysis and the mass spectrum of W_B revealed an empirical formula of $C_{12}H_{16}N_2O_2S$, suggesting that it was produced by dehydration of 1 mol TBA and 2 mol 1-butanal. Adduct W_B was produced in a much higher yield by reaction of TBA with 2-ethyl-2-hexenal, a compound produced by aldol condensation and dehydration of 2 mol 1-butanal, under the same reaction conditions. 2-Ethyl-2-hexenal could not, however, be produced from 1-butanal alone in 15% acetic acid heated at 100 C for 30 min. The unsaturated aldehyde may be produced in the presence of TBA as a catalyst.

Condensation of TBA with 2-ethyl-2-hexenal would give the 1:1 adduct (TBA-EH) shown in Scheme 1, as suggested in the reaction of TBA and aromatic aldehydes (14). While the empirical formula of adduct W_B supported the structure TBA-EH, mass spectral fragmentations, ^{1}H-NMR spectrum and ^{13}C-NMR spectrum did not support the structure. The compound in structure TBA-EH must be colored due to its high conjugation. The compound TBA-EH can be readily cyclized to its isomeric pyranopyrimidine, which may be colorless. Noise and off-resonance decoupling ^{13}C-NMR spectrum of W_B revealed the presence of five different primary, two different secondary, three different tertiary and two different quaternary carbons. All the carbon signals of W_B can be reasonably assigned as the pyranopyrimidine structure (Table 1). Two amide carbons in TBA-EH are symmetrically oriented and cannot be distinguished in ^{13}C-NMR spectrum, and the spectrum of TBA-EH would give only four different primary carbons. ^{1}H-NMR of W_B revealed 16 protons (Table 2). Two protons at 13.08 and 12.21 ppm were exchangeable and can be ascribed to the NH protons. Twelve alkyl protons due to ethyl and propyl groups appeared at the ppm values lower than 2.05, and they were assigned as listed in Table 2 by decoupling techniques. Characteristic signals, which appeared at 5.98 (singlet) and 5.01 ppm (doublet), can be assigned as the protons at the *g* position and the *i* position forming a pyran ring, respectively. The latter proton may be coupled with one of the adjacent hindered protons at the *l* position. If W_B had the noncyclized structure as TBA-EH, the proton signal at 5.01 ppm must be split into a triplet coupled with two adjacent protons. Mass spectral fragmentations of W_B gave an intense peak at m/e 209, indicating the loss of a propyl radical to leave a stable pyran radical. Thus, the structure of colorless adduct W_B was determined as 2-thio-4-oxo-6-ethyl-7-



SCHEME 1

TABLE 1

Noise and Off-Resonance Decoupling of ^{13}C-NMR Data of Adduct W_B (d_6-Dimethylsulfoxide)

Tetramethylsilane (ppm)		Assignment[a]
173.0	Singlet (C)	*d*
159.0	Singlet (C)	*b*
155.3	Singlet (C)	*f*
132.0	Singlet (C)	*e*
109.0	Doublet (CH)	*g*
92.2	Singlet (C)	*h*
81.9	Doublet (CH)	*i*
34.5	Triplet (CH_2)	
24.5	Triplet (CH_2)	*i,l,m*
17.4	Triplet (CH_2)	
13.6	Quartet (CH_3)	*k,n*
11.4	Quartet (CH_3)	

[a]See Scheme 1.

TABLE 2

^{1}H-NMR Data of Adduct W_B (d_6-Dimethylsulfoxide)

Tetramethylsilane (ppm)		Assignment[a]
13.08	1H Singlet	a,c
12.21	1H Singlet	
5.98	1H Singlet	g
5.01	1H Doublet (J=7.3 Hz)	i
2.05	2H Multiplet	j
1.73	1H Multiplet	l
1.51	2H Multiplet	l
1.39	1H Multiplet	m
1.04	3H Triplet	k
0.91	3H Triplet	n

[a]See Scheme 1.

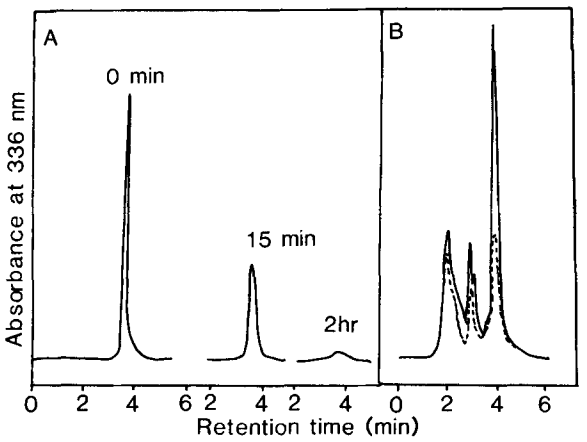


FIG. 4. HPLC of adduct W_B. Samples were analyzed by a Zorbax ODS column with an elution solvent, MeOH/H_2O (8:2, v/v), at a flow rate of 0.8 ml/min. (A) A solution of 2.4 mM W_B in 15% acetic acid was heated at 100 C for 15 min and 2 hr. The peaks due to the yellow and red pigments could not be detected under HPLC conditions. (B) A mixture of 2.5 mM TBA and 5 mM 1-butanal in 15% acetic acid was heated at 100 C for 15 min (——) and 6 hr (- - -). Peaks that eluted faster than that of W_B may be due to the yellow and red pigments.

n-propyl-1H,2H,3H,4H,7H pyrano[2,3-*d*]pyrimidine as shown in Scheme 1.

Colorless adducts W_{Hx} and W_{Hp} were produced by reaction of TBA with 1-hexanal or 1-heptanal, respectively. These adducts were found to be of the same types derived from 1 mol TBA and 2 mol of the corresponding aldehydes.

Formation and degradation of W_B in 15% acetic acid was monitored by use of HPLC (Fig. 4). When W_B was heated at 100 C in 15% acetic acid, it was readily degraded. Thus, the peak with a retention time of 3.6 min corresponding toW_B was reduced to 40% by 15-min heating and to 5% by 2-hr heating (Fig. 4A). When a mixture of 2.5 mM TBA and 5 mM 1-butanal in 15% acetic acid was heated, the peak corresponding to W_B appeared. Formation of W_B was higher at 15-min than at 6-hr heating (Fig. 4B). Formation of W_B was not affected by substituting the reaction mixture with nitrogen gas, indicating that the reaction does not require molecular oxygen.

Formation of the yellow and red pigments from the colorless adducts. Colorless adduct W_B was heated in 15% acetic acid at 100 C for 15 min and subsequently kept at room temperature. An unstable yellow pigment gradually formed in the solution (Fig. 5). When W_B was heated in 15% acetic acid at 100 C for 6 hr, a stable red pigment was produced (Fig. 5). It is likely that the red pigment was produced via the yellow pigment. Formation of the yellow and red pigments was suppressed completely when the solution was saturated with nitrogen gas before the reaction. Treatment of adduct W_{Hx} in 15% acetic acid gave similar results. Thus, the yellow pigment was produced with short heating and the red pigment with long heating. The results of the color development from the colorless adducts were in good agreement with those of the reaction of TBA and the saturated aldehydes described above. These observations support the idea that the formation of the yellow and red pigments in the reaction of TBA with saturated aldehydes proceeded via the intermediary colorless 1:2 adducts, i.e., W_B and W_{Hx}, under aerobic conditions. The red pigment may be formed via the yellow pigment (Scheme 2).

Comparison of the yellow and red pigments by HPLC. To obtain information on the identity of the yellow and

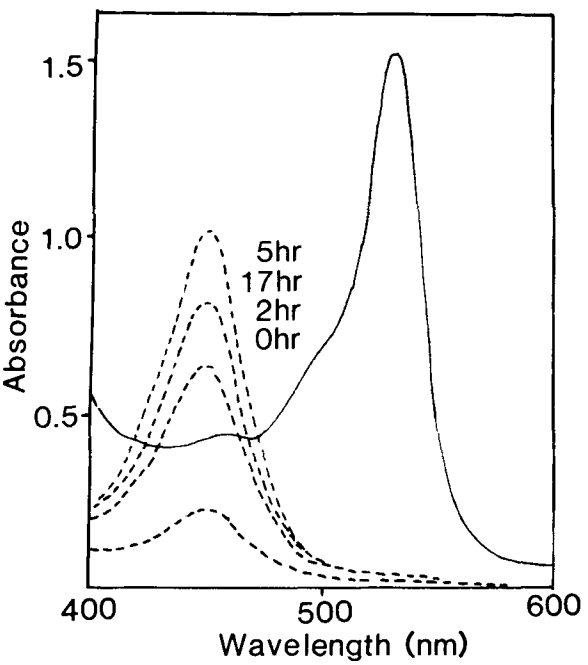


FIG. 5. Spectral changes of adduct W_B heated in 15% acetic acid. A mixture of 2.4 mM W_B in 15% acetic acid was heated at 100 C for 15 min followed by standing at room temperature for the indicated periods (- - -), or was heated at 100 C for 6 hr (——).

red pigments produced from 1-butanal, W_B and malonaldehyde, HPLC of the pigments was carried out (Fig. 6). The yellow pigment produced in the reaction of equimolar amounts of TBA with 1-butanal in 15% acetic acid (Fig. 6A-1) and that produced from W_B in 15% acetic acid (Fig. 6A-2) showed the same retention time at about 3.1 min, indicating that the pigments were identical. Chromatography of the red pigment derived

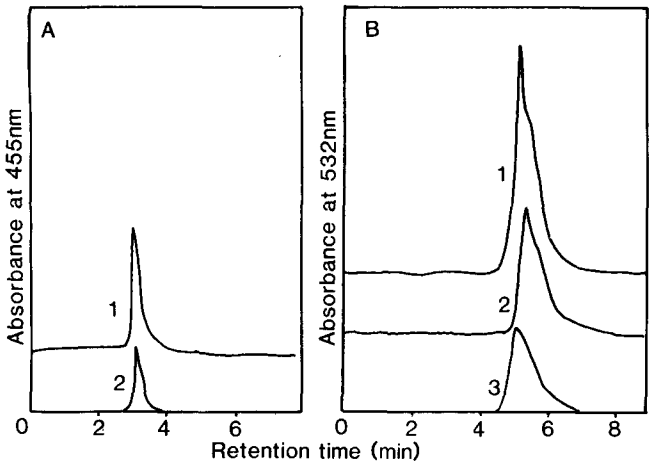
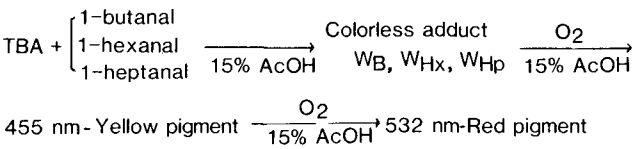


FIG. 6. HPLC of the yellow (A) and red (B) pigments. Samples were analyzed by a Zorbaz ODS column with an elution solvent, MeOH/0.04 M acetate buffer (pH 5.5) (4:6, v/v), at a flow rate of 0.8 ml/min. (A-1) A mixture of 1 mM TBA and 1 mM 1-butanal in 15% acetic acid was heated at 100 C for 15 min. (A-2) A mixture of 2.4 mM W_B in 15% acetic acid was heated at 100 C for 15 min. (B-1) A mixture of 8.7 mM TBA and 2.6 mM malonaldehyde bis(dimethyl-acetal) in 45% acetic acid was heated at 100 C for 20 min. (B-2) A mixture of 1 mM TBA and 1 mM 1-butanal in 15% acetic acid was heated at 100 C for 6 hr. (B-3) A mixture of 2.4 mM W_B in 15% acetic acid was heated at 100 C for 6 hr.

from the reaction of TBA and 1-butanal (Fig. 6B-2) and from W_B (Fig. 6B-3) revealed a broad peak with a retention time at about 5.1 min, again indicating identical pigments. The retention time was close to that of the red pigment derived from the reaction of TBA and malonaldehyde (Fig. 6B-1). Cochromatography of the red pigments derived from W_B and malonaldehyde revealed that the pigments were similar. Several attempts to differentiate the red pigments derived from these reactions in HPLC analysis under various conditions failed. It is interesting to note that the red pigments derived from 1-butanal, W_B and malonaldehyde showed the same absorption spectra with a maximum at 532 nm and the same retention times in HPLC analysis.

DISCUSSION

While the TBA reaction with malonaldehyde produces the red 2:1 adduct whose structure is unambiguously established (12,13), the reaction mechanisms of TBA with other aldehydes have not yet been elucidated. With respect to the reaction with aromatic aldehydes, Dox and Plaisance (14) obtained the yellow colored 1:1 adducts. The yellow pigment formed in the reaction mixture of TBA and the saturated aldehydes could not be isolated owing to its instability in aqueous media (6,7). Pryor (10) studied the reaction of TBA with 1-propanal. The yellow 450-nm pigment was produced first, and the red 532-nm pigment was subsequently produced in the presence of air. From the results, they tentatively suggested a mechanism. An initial 1:1 adduct at the 5 position of TBA is formed that undergoes autoxidation to give a 2-propenal-TBA adduct. This adduct then undergoes Michael addition of



SCHEME 2

a second molecule of TBA to give the red malonaldehyde adduct.

Our experiments demonstrated that the reaction of TBA with the saturated aldehydes progressed diversely. Formation of the yellow and red pigments depended on the reaction conditions. The yellow pigment was degraded in the presence of a large excess of saturated aldehydes in glacial acetic acid, probably due to subsequent reaction with the saturated aldehydes. Red pigment formation was observed only in the aqueous acetic acid and depended on the ratio of the reactants. The optimal ratio of TBA and the saturated aldehydes was about 1:1. The formation of the yellow and red pigments required molecular oxygen, indicating that some oxidative mechanisms are involved in the formation of these pigments.

The 1:1 reaction of TBA and the saturated aldehydes provided unexpected colorless adducts in the absence of molecular oxygen. Structural analysis of the colorless adducts suggested the following reaction pathway. Aldol condensation and dehydration of 2 mol of the saturated aldehydes initially gave the α,β-unsaturated aldehydes by the catalytic effect of TBA. The unsaturated aldehydes reacted with TBA to form the 1:1 adducts at the 5 position of TBA, which were in turn cyclized to colorless adducts with pyranopyrimidine structure (Scheme 1). The colorless adducts were converted into the yellow and red pigments in the presence of molecular oxygen (Scheme 2).

Patton and Kurtz (15) reported that α,β-unsaturated aldehydes give the yellow pigment and subsequently the red pigment in the presence of cupric ion or after standing exposed to air for several days, suggesting an autoxidation mechanism for the conversion. From the present results, the reaction of TBA with α,β-unsaturated aldehydes may proceed as illustrated in Schemes 1 and 2.

The absorption spectrum and the retention time in HPLC analysis of the red pigment produced in the reaction of TBA and 1-butanal or from colorless adduct W_B were close to those of malonaldehyde-TBA adduct. It appears unlikely, however, that the structure of the red pigment derived from W_B is the same as that of the malonaldehyde-TBA adduct. The structure of the red pigment derived from W_B must be closely related to that of the malonaldehyde-TBA adduct.

The reaction of TBA with the saturated aldehydes is of particular interest, since the red 532-nm color of the TBA reaction has been generally ascribed to malonaldehyde. Oxidized lipids contain a variety of saturated aldehydes (3), which may be involved in the development of the red color under the restricted TBA reaction conditions described in this paper.

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