Study of Color Stability of Tall Oil Fatty Acids through Isolation and Characterization of Minor Constituents[1,2]

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ABSTRACT

Minor constituents in high quality tall oil fatty acids have been isolated successfully by liquid column chromatography, using silicic acid as the adsorbent. The minor constituents contained two types of compounds: those which were noneffective and those which were effective in causing the darkening of tall oil fatty acids during heating. The former consisted of *trans*-3,5-dimethoxystilbene and rosin acids. The latter was separated into numerous fractions by the combination of chemical methods, silicic acid column chromatography, and low temperature fractional crystallization. The fractions were characterized by functional group analyses, chemical reactions, and UV and IR spectrometric methods. Most of the fractions contained two-three times as much oxygen in the molecule as the original sample and were highly oxidized fatty acids. They had mol wt ranging 300-551 and contained double bonds, carbonyl, ester, peroxide, and hydroxyl groups. The effect of these minor constituents upon the color stability of tall oil fatty acids during heating was postulated as being due to the hydroxyl groups located in the α-position to the double bond in the molecule.

INTRODUCTION

Tall oil fatty acids are an important by-product of the kraft or sulfate pulping process. The current production of tall oil fatty acids in the U.S. is over 370 million lb/year, which is ca. 40% of the U.S. production of all fatty acids. Even though the demand for tall oil fatty acids continues to increase, and more uses for them constantly are being found, their use is hampered by some defects, one of which is the development of a dark color during heating or manufacturing of their derivatives.

Commerical tall oil fatty acids (CTOFA), even those of the highest quality, contain two types of minor constituents: those which are noneffective and those which are effective in causing the darkening of tall oil fatty acids during heating. The former is composed of *trans*-3,5-dimethoxystilbene (1) and rosin acids. The present paper reports the isolation, fractionation, and identification of the minor constituents which are responsible for the development of dark color during heating of CTOFA.

EXPERIMENTAL PROCEDURES

Material Used

The sample of high quality CTOFA used in this investigation had the specification of 1.5% (maximum) of rosin acids and 1.5% (maximum) of unsaponifiables. Its approximate composition was as follows: palmitic acid, trace; stearic acid, 1.5%; C_{18}:1 acid, 51.0%; C_{18}:2 acid, 40.0%; C_{18}:3 acid, 3.0%; rosin acids, 1.0%; unsaponifiables, 1.0%; and unknowns, 2.0% (K.T. Zilch, personal communication).

Isolation and Fractionation of Minor Constituents

The isolation and fractionation of minor constituents from 7500 g high quality CTOFA are essentially the same as reported previously (1) and are summarized in Figure 1.

Attempts to fractionate the ether eluted acidic and nonacidic minor constituents by stepwise gradient elution liquid column chromatography did not yield satisfactory resolution. Therefore, these fractions first were treated by the low temperature solvent fractional crystallization

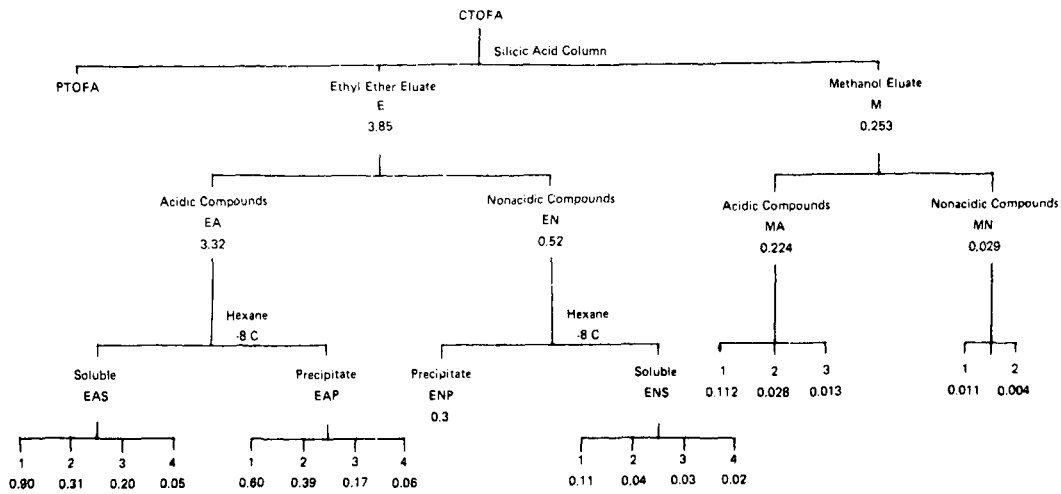

FIG. 1. Isolation and fractionation of minor constituents of commercial tall oil fatty acids (CTOFA). All numbers indicate percent by wt of the original sample. PTOFA = purified tall oil fatty acids, EAS = ether eluted acidic solubles, EAP = ether eluted acidic precipitate, ENS = ether eluted nonacidic precipitate, ENS = ether eluted nonacidic solubles, MA = methanol eluted acidic compounds, and MN = methanol eluted nonacidic compounds.

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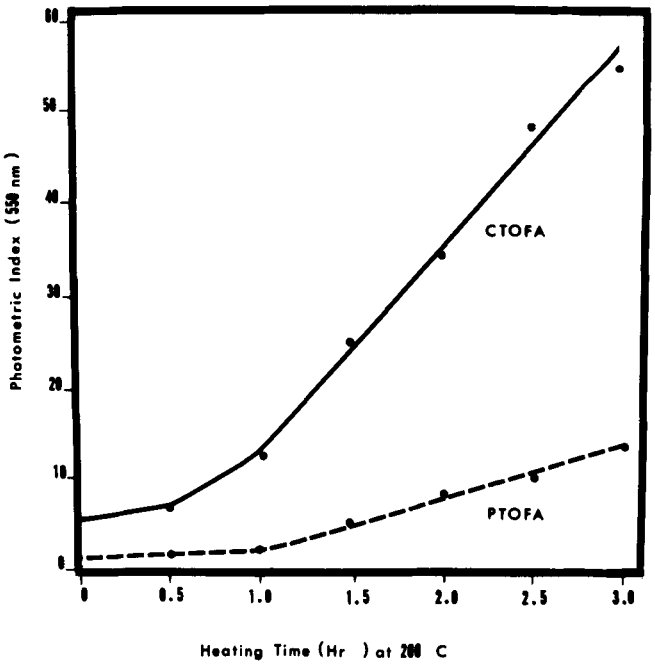

FIG. 2. Effect of minor constituents upon the color stability of tall oil fatty acids. CTOFA = commercial tall oil fatty acids and PTOFA = purified tall oil fatty acids.

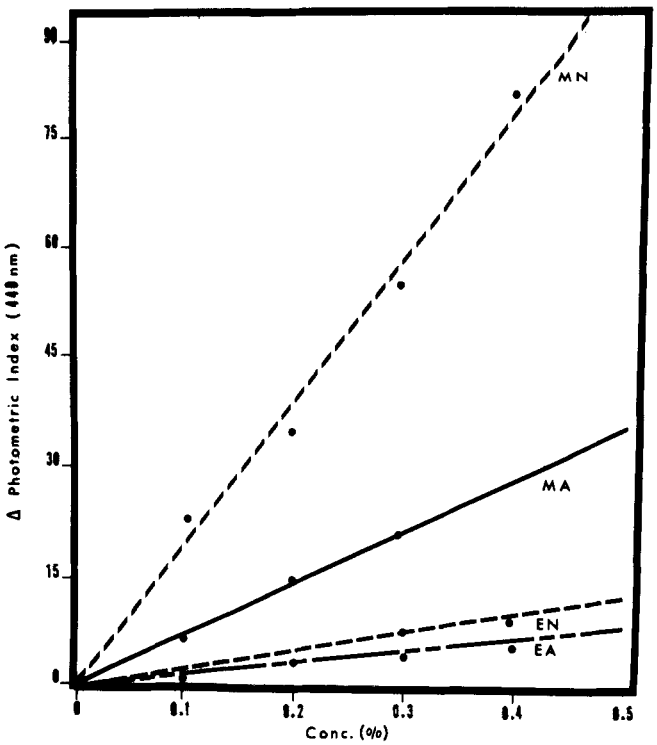

FIG. 3. Effects of the four groups of minor constituents upon the color stability of purified tall oil fatty acids. MN = methanol eluted nonacidic minor constituents, MA = methanol eluted acidic minor constituents, EN = ethyl ether eluted nonacidic minor constituents, and EA = ethyl ether eluted acidic minor constituents. Conc. = concentration.

method. The ether eluted acidic portion was dissolved in hexane (H) at a ratio of 1 to 7 and kept at -8 C for 12 hr. The precipitate thus formed was filtered to yield an ether eluted acidic precipitate (EAP) and ether eluted acidic solubles (EAS). The ether eluted nonacidic fraction was treated similarly at a sample to H ratio of 1 to 8, and ether eluted nonacidic solubles (ENS) and ether eluted nonacidic precipitate (ENP) were obtained.

The EAS, EAP, ENS, methanol (M) eluted acidic compounds (MA), and M eluted nonacidic compounds (MN) were fractionated further into subfractions with automatic liquid column chromatography. ENP later was found to be *trans*-3,5-dimethoxystilbene (1). A Uviscan III UV detector (Buchler Instruments, Fort Lee, N.J.) was connected to a chromatographic column for automatic recording of the chromatogram. The 1 x 23 in. column used was packed with 120 g silicic acid saturated with H and could tolerate a pressure of 100 psi. A constant flow pump (Cheminert Metering Pump from Chromatronix, Inc., Berkeley, Calif.) was used to control the flow rate at 120 ml/hr, regardless of back pressure in the column.

The chromatography for the above samples was done by the following solvent systems. EAS: sample size 1 g, solvent system, 70% H + 30% ethyl ether (E), 50% H + 50% E, 100% M; EAP: sample size 1 g, solvent system 50% H + 50% E, 100% E, 100% M; ENS: sample size 0.5 g, solvent system, 100% H, 50% H + 50% E, 100% M; MA: sample size 10 g, solvent system 100% E, 90% E + 10% M, 50% E + 50% M, 100% M; and MN: sample size 1 g, solvent system, 100% E, 50% E + 50% M, 100% M. The fractions were collected by an automatic fraction collector (Fractomat, Buchler Instruments).

Test for Thermal Stability of Fatty Acids

A cylindrical aluminum block, 127 mm high and 127 mm in diameter, was drilled around the circumference with 16 holes which could fit snugly 20 x 150 mm test tubes. Sample (5 g) was heated under air in the test tube. A hole for the insertion of a thermometer was drilled into the center of the aluminum block. The aluminum block was heated by a hot plate oven which consisted of an oven (AY 106X1A, Thermolyne Corp., Dubuque, Iowa) and a hot plate (HP A1915B, type 1900). The temperature of the aluminum block could be controlled within ± 1 C. A hole of the same diameter as the aluminum block was cut at the top of the oven so that test tubes containing the fatty acids to be tested could be inserted into the aluminum block.

Analytical Methods

Iodine value, peroxide value, double bonds, and free fatty acids were determined according to AOCS Official Methods (2). Saponification value and hydroxyl value were determined according to the method described by Mehlenbacher (3). Carbonyl value was analyzed by the methods of Bhalerao, et al. (4). Mol wt was determined with a Mechrolab vapor pressure osmometer, model 301A (Hewlett Packard, Avondale, Pa.), using M as the solvent. Elemental analyses was carried out by Schwarzkopf Micro Analytical Laboratory, Woodside, N.Y. IR studies were carried out with a Beckman IR-8 spectrophotometer. Color stability measurement was determined according to AOCS Official Method TD 2a-64 (2), using a Beckman DB-G spectrophotometer.

Reduction of Hydroperoxide to Hydroxyl Group

Sample (1 g) EAP-2, was dissolved in 20 ml E in an Erlenmeyer flask, and the flask was placed in an ice water bath. A 10% aqueous solution of sodium bisulfite (6 ml) slowly was added at room temperature, and the mixture was stirred occasionally over a 4 hr period. The aqueous layer was separated and discarded, and the ether solution was washed several times with water, dried over anhydrous calcium sulfate, filtered, and the solvent removed under vacuum (5).

Hydrogenation with Pt-C and H_2

MA-2 was hydrogenated mildly with Pt-C (5% platinum on carbon from Engelhard Industries, Newark, N.J.) according to the methods of Alexander and Cope (6). MA-2 (1 g) was dissolved in 50 ml M in a 100 ml round bottom flask.

TABLE I

Elemental Composition of Fractions of Minor Constituents of Tall Oil Fatty Acids

Fraction[a]	Carbon (%)	Hydrogen (%)	Oxygen (%)	Nitrogen (%)	Sulfur (%)
EAS-1	79.24	11.70	9.06	0.005	0.001
EAS-4	68.44	10.18	21.38	--	--
EAP-1	76.11	10.60	13.29	--	--
EAP-4	59.60	9.81	30.59	--	--
ENS-1	79.61	9.66	10.73	--	--
ENS-4	65.69	9.25	25.06	--	--
MA-1	76.02	11.17	12.81	--	--
MA-2	66.34	9.74	23.92	--	--
MN-1	77.80	10.07	12.13	0.004	0.001

[a]EAS = ether eluted acidic solubles, EAP = ether eluted acidic precipitate, ENS = ether eluted nonacidic solubles, MA = methanol eluted acidic compounds, and MN = methanol eluted nonacidic compounds.

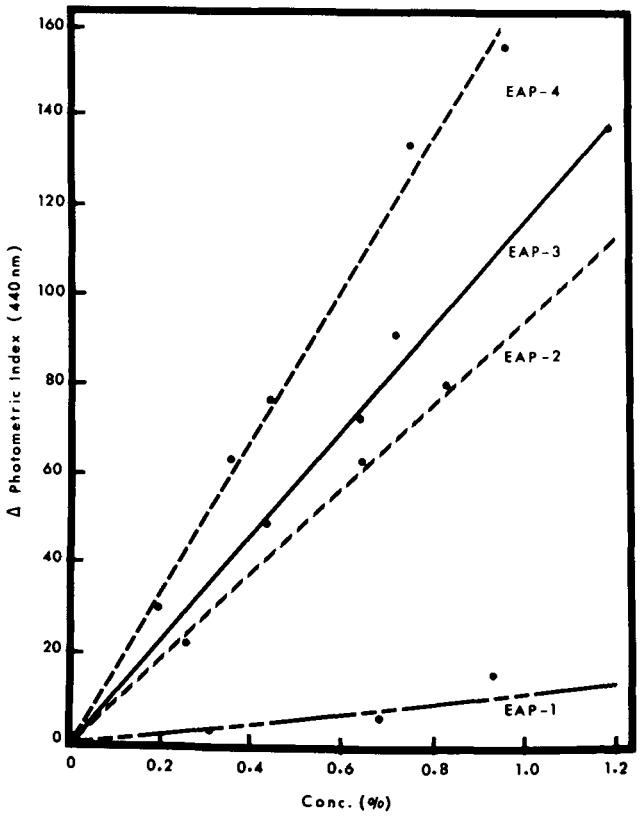

FIG. 4. Effect of subfractions of ether eluted acidic precipitate (EAP) minor constituents on the color stability of tall oil fatty acids. Conc. = concentration.

Pt-C (200 mg) was added to the solution and swirled to form a suspension. Hydrogen gas was introduced into the mixture at room temperature under atmospheric pressure. The mixture consumed hydrogen rapidly during the first hr, and the reaction was continued for 30 min after the hydrogen consumption had stopped. The hydrogenation took 6 hr after the addition of the catalyst. The reaction mixture was filtered and the catalyst washed with 50 ml M. The solvent was removed under vacuum to yield 940 mg hydrogenated product.

Reduction with $LiAlH_4$

The hydrogenated MA-2 was reduced further by $LiAlH_4$ using the method described by Frankel, et al. (7). $LiAlH_4$ (600 mg) was dissolved in 300 ml anhydrous E poured into a 100 ml, 2 necked round bottom flask. One neck was connected to a water cooling condenser protected by a drying tube, and the other neck was connected to a 100 ml reagent addition funnel. A solution of 520 mg hydrogenation MA-2 in 30 ml anhydrous E was added drop by drop into the $LiAlH_4$ solution, while it was stirred with a magnetic stirrer. The reaction mixture was stirred for 40 hr at room temperature. The $LiAlH_4$ then was inactivated with the addition of 30 ml E saturated with water. After acidification with 20 ml 5% HCl, the hydrogenated product was extracted into E, dried with sodium sulfate, and the solvent removed under vacuum.

The sample, MA-2, was reduced in the same manner.

RESULTS AND DISCUSSION

Isolation and Fractionation of Minor Constituents

Activated silicic acid effectively removed the minor constituents from the CTOFA. Activated carbon and alumina were found to be inefficient. The eluate from the silicic acid column was almost colorless. The colored materials originally present in CTOFA were absorbed by the silicic acid to form two bands: a dark red band on the top and a yellowish red one beneath it.

The ether eluted minor constituents (EA + EN) were 3.85% and the M eluted minor constituents (MA + MN) were 0.25% by wt of the original sample. The total amount of minor constituents was, therefore, 4.1%. Further separation of the 4 groups of minor constituents yielded a total of 17 fractions. The amount of each of the fractions is shown in Figure 1. It was found that fraction EAS-1 was composed mainly of rosin acids by its IR spectrum and liquid and gas chromatograms. ENP was found to be *trans*-3,5-dimethoxystilbene (1). These two fractions contributed little to the adverse effect upon the color stability of tall oil fatty acids.

Role of Minor Constituents in Color Stability

The color stability of CTOFA was improved drastically after the sample was passed through a silicic acid column (Fig. 2). The presence of trace amounts of the minor constituents did not greatly increase the initial color of the tall oil fatty acids. However, during heating, the purified tall oil fatty acids (PTOFA) maintained a light color while the CTOFA developed a dark color.

Effect of Four Groups of Minor Constituents upon Color Stability

The adverse effect of the four groups of minor constituents upon the color stability of tall oil fatty acids was tested by adding various amounts of each of these four fractions to PTOFA and then heating the mixture for 1 hr at 200 C (Fig. 3). The degree of darkening during heating was proportional to the amount of the minor constituents added. The M eluted minor constituents were more detrimental to the color stability of tall oil fatty acids than the E eluted minor constituents. However, the amount of M

TABLE II

Chemical Analysis of Fractions of Minor Constituents in Tall Oil Fatty Acids

Fraction[a]	Mol wt	-COOH mol	-COOR mol	-OH mol	-C=O mol	-C=C- mol	Peroxide mol
EAS-1	300	1.09	0.02	0.16	0.02	1.63	0.01
EAS-2	325	0.99	0.10	0.24	0.16	1.72	0.02
EAS-3	353	1.00	0.15	0.57	0.30	1.56	0.04
EAS-4	458	1.47	0.24	1.32	0.31	1.73	0.02
EAP-1	336	0.94	0.10	0.40	0.25	1.28	0.06
EAP-2	551	1.32	0.58	0.70	0.50	1.82	0.10
EAP-3	492	1.18	0.61	1.80	0.52	1.68	0.18
EAP-4	310	1.02	0.01	2.90	0.42	1.52	0.02
EN-1	339	0	0.22	0.33	0.27	1.28	0.03
EN-2	286	0	0.20	0.82	0.21	1.22	0.02
EN-3	354	0	0.37	1.97	0.61	1.49	0.13
EN-4	405	0	0.54	2.62	0.55	1.71	0.02
MA-1	314	0.96	0.00	0.50	0.23	1.65	0.03
MA-2	443	0.95	0.19	2.90	0.45	1.50	0.06
MN-1	341	0	0.45	1.51	0.50	1.43	0.08
MN-2	317	0	0.54	2.54	--	1.42	--

[a]EAS = ether eluted acidic solubles, EAP = ether eluted acidic precipitate, EN = ether eluted nonacidic, MA = methanol eluted acidic compounds, and MN = methanol eluted nonacidic compounds.

eluted minor constituents (0.25%) was much less than the E eluted minor constituents (3.85%). Consequently, the latter probably plays a more important role in the color stability of CTOFA. It also can be seen that the nonacidic minor constituents were more effective than the acidic minor constituents in causing darkening during heating.

To investigate whether or not there was a synergistic effect among different minor constituents, mixtures of MA + MN, MA + EN, EA + EN, and EA + MN were added at the same concentration level to PTOFA. The results of the color stability test showed no synergistic effect among the four groups of minor constituents.

Characterization of Chromatographic Fractions of Minor Constituents

Elemental analysis of the fractions of minor constituents indicated that they contained practically no nitrogen or sulfur (Table I). It also showed that the amount of oxygen/molecule increased with the increasing polarity in each group. For example, the ratio of the wt of oxygen to the wt of carbon for EAS-1 was 0.11; but for EAS-4 was 0.31; EAP-1 was 0.17; but EAP-4 was 0.51; ENS-1 was 0.14; but ENS was 0.39; MA-1 was 0.11; but MA-2 was 0.36. It also was found that the higher the oxygen content, the greater was its effect in causing darkening of PTOFA during heating. For example, EAP-4 with 30.59 oxygen, had significantly stronger effect in causing darkening than EAP-1 with 13.29% oxygen (Fig. 4).

The results of chemical analysis of the fractions of minor constituents are shown in Table II. The fact that most of the analysis did not result in whole numbers indicated that these fractions were still not pure compounds. The mol wt of the fractions of the minor constituents were generally equivalent to those of oxidized C_{18} fatty acids. Some fractions also may contain a carbon chain shorter than C_{18} which may have been the result from the cleavage of the chain by thermal oxidative decomposition. Since the mol wt of such acids was around 284, the fractions of the minor constituents with mol wt around 320 may be formed by adding 1 or 2 oxygen atoms to monomers during oxidation. Those with mol wt around 450 were probably mixtures of monomers and dimers.

The fractions were quite polar and rich in hydroxyl groups. As the polarity of the solvent used to elute the fraction from the silicic acid was increased, its content of hydroxyl groups also was increased. Peroxide value of these fractions indicated that some autoxidation did take place.

The fractions of the minor constituents had colors ranging from light yellow to dark red and viscosity from fluid to highly viscous. Both the color and viscosity of the fractions increased with the polarity of the solvent used for their elution.

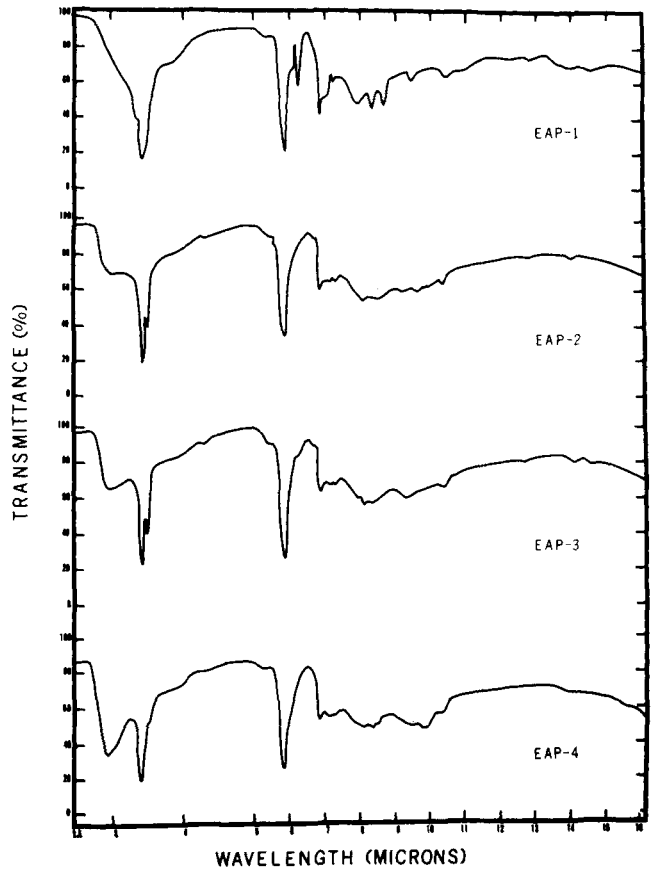

FIG. 5. IR spectra of ether eluted acidic precipitate (EAP) subfractions of minor constituents.

The increase in hydroxyl groups in the fractions eluted with solvents with increasing polarity was confirmed by IR spectrometric analysis. The hydroxyl absorption at 2.9 μ was increased in intensity from EAP-1 to EAP-4 (Fig. 5). Since the absorption of the hydroxyl group at 2.9 μ was due to intermolecular hydrogen bonding, and since such bonding would increase viscosity, the IR spectra of the fractions also may offer an explanation for the increase in vis-

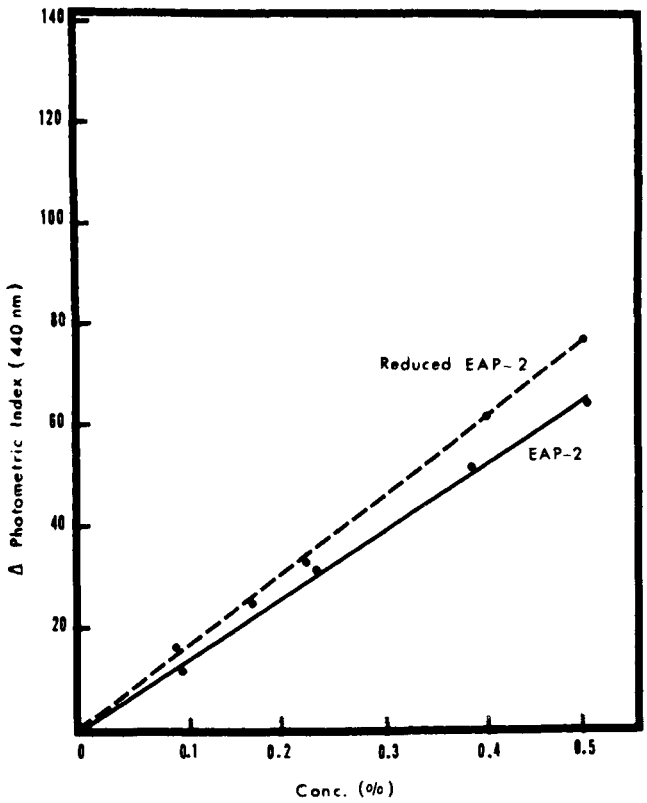

FIG. 6. Effect of reduction of hydroperoxide to hydroxyl groups in EAP-2 upon the color stability of purified tall oil fatty acids. EAP = ether eluted acidic precipitate and conc. = concentration.

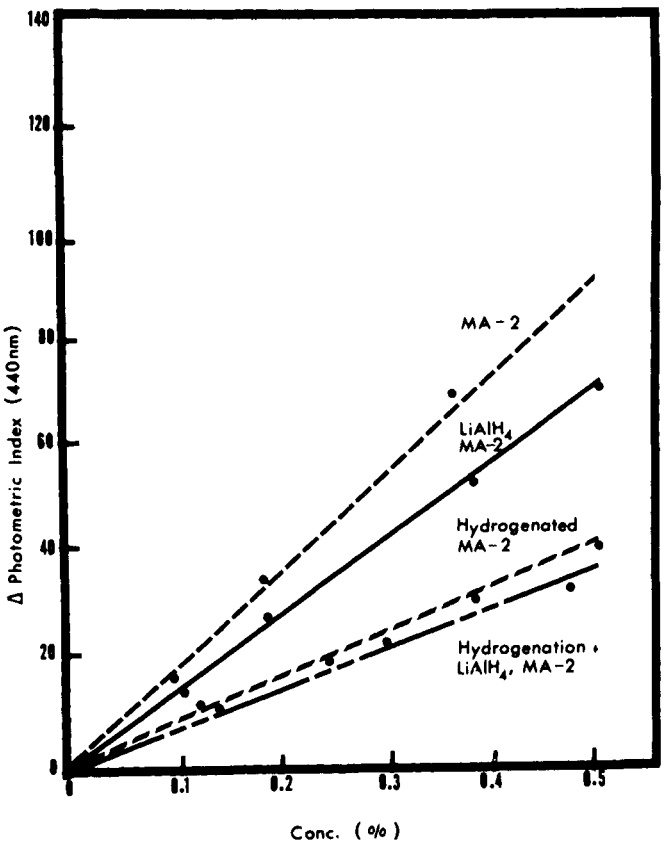

FIG. 7. Effects of fraction MA-2 after being reduced by different methods upon the color stability of purified tall oil fatty acids. $LiAlH_4$ = lithium aluminum hydride, MA = methanol eluted acidic minor constituents, and conc. = concentration.

cosity with the increase in polarity of the solvent used to elute the fraction.

The hydroxyl group absorption at 2.9 μ and the small absorption of *trans*-double bond at 10.3 μ were observed in the acidic, as well as nonacidic, fractions of the minor constituents. They were absent in the IR spectrum of PTOFA. Similar observations were reported previously by Frankel, et al., (7) and Knight, et al. (8). The absorptions at 10.3 and 2.9 μ constantly were observed in oxidized fatty esters but not in fresh samples.

The present results appeared to establish a causal relationship between the hydroxyl groups of the minor constituents and their effect upon the color stability of tall oil fatty acids. Figure 4 demonstrated that, when the polarity of the solved used to elute the fraction was increased, as from EAP-1 to EAP-4, the darkening effect upon PTOFA during heating also was increased. At the same time, the hydroxyl group content of the fractions also was increased, as evidenced both by chemical and IR analyses (Table II and Figure 5).

The following reduction of functional group studies further established that not all the hydroxyl groups in a molecule could accelerate the darkening of TOFA during heating. Only those hydroxyl groups which are at the α-position to a double bond have an adverse effect upon the color stability of tall oil fatty acids. When the hydroperoxide in fraction EAP-2 was reduced to a hydroxyl group, its adverse effect upon the color stability of PTOFA was increased (Fig. 6). The hydroxyl group formed by the reduction of the hydroperoxide group is most likely to be at the α-position to the double bond, because the hydroperoxide of fatty acids formed by autoxidation is usually at such a position.

The effect of the different methods of hydrogenation upon MA-2 to the color stability of tall oill fatty acids is shown in Figure 7.

The calculated amount of hydrogen required to saturate the double bond in 1 g MA-2 was 76 ml. However, only 65 ml hydrogen were consumed by the sample. Since hydroperoxyl groups should be hydrogenated before the double bonds, 3 ml hydrogen were required by 1 g MA-2 for this purpose. The amount of the double bond thus hydrogenated was, therefore, only 80%.

That the autoxidized fatty acids were difficult to hydrogenate completely was reported by previous workers. Frankel, et al., (7) reported that the dimerized fatty ester formed by autoxidation was not completely hydrogenated, even though the dimers were hydrogenated catalytically in a Parr shaker in an ethyl acetate solution at 50-60 C, 30 psi hydrogen pressure with 3-6% platinum oxide catalyst for 2 hr.

MA-2 was lighter in color and less viscous after hydrogenation. The adverse effect of hydrogenated MA-2 upon the color stability was decreased significantly after the catalytic hydrogenation. This may be explained by the fact that the double bond immediately next to the hydroxyl group is hydrogenated, and, thus, the arrangement of a hydroxyl group α to the double bond was destroyed.

MA-2 also was reduced with lithium aluminum hydride until the absorption at 5.8 μ due to carbonyl groups had disappeared completely. The IR spectrum of the lithium aluminum hydride reduced MA-2 showed the disappearance of the C=O stretching vibration band at about 5.8 μ and an increase in the intensity of the O-H stretching vibration band at 2.9 μ. This indicated that reduction with lithium aluminum hydride converted carbonyl, carboxyl, and ester groups into hydroxyl groups. A test of the effect of $LiAlH_4$ reduced MA-2 upon the color stability of PTOFA revealed that the darkening was decreased slightly, indicating that the adverse effect of the minor constituents upon color stability also was caused partially by the functional groups

which were reducible by lithium aluminum hydride. Since the reduction of MA-2 with lithium aluminum hydride increased the hydroxyl group content and decreased the adverse effect upon the color stability, it further was confirmed that no all hydroxyl groups could cause the darkening of PTOFA during heating.

In an attempt to see if the adverse effect of the minor constituents upon color stability could be eliminated completely if all the function groups were reduced hydrogenated MA-2 was reduced further with lithium aluminum hydride. Theoretically, this sould produce a simple saturated fatty alcohol. However, MA-2 reduced in such a manner still had a slight effect upon color stability. This was probably due to the incompleteness of the catalytic hydrogenation.

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