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Resin Studies. II. A Study and Extension of the Liebermann Color Reaction for Abietic Acid

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Introduction.—Abietic acid enters into many color reactions, *e. g.*, it gives brilliant colorations with antimony pentachloride,[1] phenol and bromine in carbon tetrachloride solution,[2] methyl or ethyl sulfate[3] and sulfuric acid in the presence of acetic anhydride.[4] Liebermann's test is the most characteristic and is carried out by dissolving a few mg. of the sample in 2–3 cc. of warm acetic anhydride and adding a few drops of concentrated sulfuric acid to the cooled solution. A violet or purplish-red color appears instantly but changes rather quickly through reddish-brown to yellowish-brown. The chemistry of the Liebermann test has not been elucidated. As an approach to this problem there are discussed in this paper the various factors which influence the color production observed in the test, the effect on the colorations of the substitution of other reagents, and the possible structural relationship existing between abietic acid and other compounds of definitely known structure which give the same colorations with the same reagents.

Experimental

Abietic acid was prepared from American "I" wood rosin by Steele's method:[5] acid and saponification no., 185.4; $[\alpha]^{25}_{\mathrm{D}}$ −75.5 (5% in ethyl alcohol); m. p. 158°. Eastman organic reagents were redistilled and used without further purification. Some of the terpenes used in the tests were generously donated by Schimmel and Co.

The Unmodified Liebermann Reaction.—The colors produced when sulfuric acid is added to an acetic anhydride solution of abietic acid depend on the concentration of the solution, the temperature, and the amount of mineral acid added. The following table summarizes the results obtained by adding 0.05 cc. of 95% sulfuric acid to 2 cc. of solutions of the concentrations indicated. The numbers above the arrows show approximately the number of seconds required for the color change.

Concn. of abietic acid	Colorations (25°)
>0.3 *M*	Blue $\xrightarrow{45}$ violet-blue $\xrightarrow{75}$ violet $\longrightarrow$ brown
.2 *M*	Blue $\xrightarrow{17}$ greenish-blue $\xrightarrow{163}$ green-black $\longrightarrow$ brown
.1 *M*	Violet-blue $\xrightarrow{8}$ blue $\xrightarrow{12}$ green $\longrightarrow$ brown
.05 *M*	Purple $\xrightarrow{4}$ violet $\xrightarrow{4}$ green-blue $\xrightarrow{9}$ green $\longrightarrow$ brown
.01 *M*	Reddish-violet $\xrightarrow{25}$ brown-violet $\xrightarrow{30}$ brown

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(1) Steinle and Kahlenberg, *J. Biol. Chem.*, **67**, 425 (1926).

(2) Hicks, *Ind. Eng. Chem.*, **3**, 86 (1911).

(3) Sans, *Ann. chim. anal. appl.*, **14**, 140 (1909).

(4) Liebermann, *Ber.*, **17**, 1884 (1884). Unimportant modifications have been suggested by Storch [*Ber. osteri. Ges. chem. Ind.*, **9**, 93 (1887)]; Morawski [*Chem. Z.*, **12**, 1321 (1888)], and Burchard [Dissertation, Rostock, 1889].

(5) Steele, THIS JOURNAL, **44**, 1333 (1922).

The reaction mixture developed a green or bluish-green fluorescence which was most pronounced in the dilute solutions. At higher temperatures the first color was of shorter duration, *e. g.*, at 100° there was only a momentary flash of the initial color and the intermediate shades did not appear at all. At low temperatures the colors were only slightly less fugitive than at room temperature. Sulfuric acid in excess of a few drops per 2 cc. of abietic acid solution renders the test less delicate due to side reactions and the heat produced on mixing acetic anhydride and the mineral acid. This latter effect can be avoided by dissolving the anhydride in the sulfuric acid before adding it to the abietic acid solution. Practically any concentration of sulfuric acid can be used for the test since the anhydride dehydrates the dilute acid. The colorations are also produced by adding acetic anhydride and sulfuric acid to a solution of abietic acid in an inert solvent. Burchard[4] used chloroform but other polyhalides, alkyl halides, hydrocarbons and carboxylic acids can also be used. The reaction products separated as a tar from those solvents in which sulfuric acid is insoluble. It was not possible to extract the colored intermediates by any of the great number of solvents tried. The colorations were instantly destroyed by water.

The Test with Reagents Other than Acetic Anhydride and Sulfuric Acid.—The anhydrides propionic to heptanoic, and also lauric, and chloroacetic, succinic, benzoic, phthalic and maleic anhydrides were substituted for the acetic anhydride. The colorations produced on addition of sulfuric acid to these solutions[6] were practically the same as those obtained with acetic anhydride solutions.

On addition of 0.05 cc. of sulfuric acid to 2 cc. of 0.3 *M* solutions of abietic acid in the acyl chlorides acetyl to heptanoyl, and also lauroyl, the following series of colors was obtained: greenish-brown ⟶ purplish-blue or blue ⟶ purple. The purple slowly passed to brown or reddish-brown. With the acyl chlorides the color changes occurred more slowly than with the anhydrides. Acyl chloride solutions of abietic acid darken on standing. The anhydride solutions remain colorless for months. With benzoyl chloride a strikingly brilliant series of colors was produced: greenish-brown ⟶ greenish-blue ⟶ indigo-blue ⟶ purple. The final color was a dull brown. Benzoyl chloride should replace acetic anhydride in the Liebermann reaction for abietic acid.

Solutions of abietic acid in acetamide and propionamide yielded pink to dark red colors on treatment with sulfuric acid. No characteristic colorations were obtained with benzene sulfonyl chloride as the solvent.

Solutions of abietic acid in the ethyl esters of the acids acetic to lauric were colored pale yellow by the addition of sulfuric acid. The color slowly darkened to a bright strawberry-red which was stable for several minutes. Alkyl halides, ethers, alcohols, hydrocarbons, carboxylic acids, ketones, aldehydes, etc., were used as solvents for the abietic acid. Sulfuric acid produced only dull red or brown colors in these solutions.

Although acetic anhydride is replaceable in the Liebermann test by other anhydrides and by acyl chlorides, the function of the sulfuric acid is apparently unique. Solutions of abietic acid in acetic anhydride and in all of the other solvents mentioned above were treated with other concentrated acids and condensing agents. Without exception, either no coloration, or only a red or reddish-brown coloration, was obtained with H_3PO_4, HPO_3, $HClO_4$, P_2O_5, HCl gas, $(C_2H_5)_2SO_4$, $NaHSO_4$, $ZnCl_2$, $AlCl_3$ and anhydrous CH_3COONa.

The roles of the anhydride or acyl halide and the sulfuric acid may be interchanged. With sulfuric acid as solvent for the abietic acid the addition of RCOCl or $(RCO)_2O$ to a freshly prepared solution immediately produced the characteristic colorations at the interface of the two liquids. Under these conditions the colors were exceedingly fugitive and the mixture quickly became reddish-brown. Since the colorations are produced immediately regardless of which reagent is used as the solvent, any reactions preceding

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(6) In the case of the solid anhydrides the sulfuric acid was added to the molten mixture.

the color formation must occur practically instantly on contact of the compounds involved. The few reactions which are known to occur between the compounds entering into the Liebermann test require considerable time for completion and can therefore be of no significance in the color production. It is possible that abietic acid and the anhydride or acyl chloride react to form a molecular compound as rapidly as the abietic acid dissolves. The colorations produced on the addition of sulfuric acid would then depend on the reaction or rearrangement of this molecular compound to form a color producing configuration. Many compounds, *e. g.*, the chalcones, exhibit this phenomenon (halochromism)[7] in the presence of concentrated sulfuric acid, which has been used for comparing the halochromatic properties of a large variety of compounds.[8] To explain the colorations produced by bufonin with the Liebermann reagents, Wieland and Weil assumed that acetic anhydride adds directly to a double bond. This addition product, after rearrangement, reacts with sulfuric acid to form "halochromic ketone sulfates."[9]

Attempts were made to isolate the colored substances formed in the Liebermann reaction and also to determine the fate of the abietic acid in the reaction. It was not possible to stabilize the colored products and the substances isolated were invariably mixtures of unchanged abietic acid and unworkable tarry material. This part of the work is being continued.

The Possible Structural Significance of the Liebermann Test for Abietic Acid.—The Liebermann reagents have been applied to a fairly large number of compounds. Other anhydrides and the acyl chlorides have not been used before in this test. The following list, which includes those compounds producing some coloration when treated in acetic anhydride solution with a few drops of concentrated sulfuric acid, has been compiled from Beilstein and the original literature: 1,4-dihydrobenzene, 1,3-dihydro-2,3-dimethylbenzene, 1,3-dihydro-1,3-dimethylbenzene, 2-isopropenyl-1-methylcyclopentene, 1-(α-methylallyl)-cyclohexene, $\Delta^{1,8(9)}$-*o*-menthadiene, $\Delta^{2,4}$-*p*-menthadiene, 4-isopropenyl-1,2,4-trimethylcyclohexene, 3-(Δ^{2}-butenyl)-2,4,4-trimethylcyclohexene, 3-(α-hydroxyisopropyl)-2,2-methylcyclopentene, 3(or 4-)-(α-hydroxyisopropyl)-1-(or 2-)-methylcyclopentene, Δ^{1}-8-*o*-menthenol, 3-(α-hydroxyisopropyl)-1-methylcyclopentanol produce red (or rose, carmine, etc.) colorations. 4-Isopropenyl-3-methylcyclopentene, $\Delta^{3,8(9)}$-*m*-menthadiene, $\Delta^{6,8(9)}$-*m*-menthadiene (?) or isocarvestrene, $\Delta^{4,6}$-*m*-menthadiene, Δ^{5}-8-*m*-menthenol, Δ^{2}-1,2-dimethylcyclohexenol or cantharenol, 1-(α-hydroxyisobutyl)-3-methylcyclohexene, diresorcin (at 100°), ethyldiresorcin, produce violet colorations. $\Delta^{1,8(9)}$-*m*-menthadiene or sylvestrene, carvestrene (*dl*-sylvestrene), *d*- and *dl*-$\Delta^{2,8(9)}$-*m*-menthadiene, Δ^{1}-8-*m*-menthenol, Δ^{2}-*m*-menthenol, carveprene (Aschan), produce blue colorations. $\Delta^{4,8(9)}$-*o*-menthadiene, Δ^{6}-8-*o*-menthenol, 1,4-dihydro-1,4-diisopropenylbenzene, 3,4-dihydro-1-isopropenylnaphthalene, 3,4-dihydro-1-(α-hydroxyisopropyl)-naphthalene, 1,2,3,4-tetrahydro-1-isopropenylnaphthalene, produce brown, yellow or green colorations.

According to Aschan[10] only structure I produces the blue color. Structure VI (below) symbolizes the only known *m*-menthadiene with both double bonds in the ring. This compound gives a violet color in the Liebermann test.[11] Monocyclic terpenes with the isopropenyl group ortho or para to the methyl group do not give characteristic colorations with the Liebermann reagents. Many other compounds of unknown or incompletely deter-

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(7) Dilthey, Neuhaus and Schommer, *J. prakt. Chem.*, **123**, 235 (1929).

(8) Pfeiffer, "Organische Molekulverbindungen," 2d ed., p. 68.

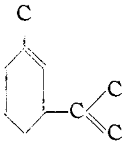
(9) Wieland and Weil, *Ber.*, **46**, 3315 (1913).

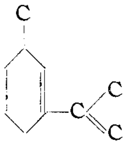
(10) Aschan, "Naphthenverbindungen, Terpene und Campherarten," 1929, p. 121.

(11) Henderson and Smeaton, *J. Chem. Soc.*, **117**, 144 (1920).

mined structure also give colorations with acetic anhydride and sulfuric acid, *e. g.*, cholesterol and some other sterols, certain of the bile acids, bufonin, lupeol, octahydroazulen, certain sesquiterpenes, arnidiol, brein, etc.

The above list is necessarily incomplete, but it is significant that with one important exception (VI), most of the compounds of known structure which give blue or violet colors with acetic anhydride and sulfuric acid are *m*-menthadienes containing the isopropenyl group and *m*-menthenols which can give, through dehydration, menthadienes containing this side chain. The location of the second double bond in the ring can vary since all of the following configurations are represented in the compounds giving blue or violet colors


I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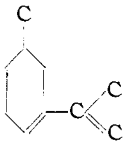
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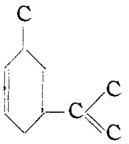
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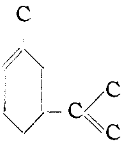
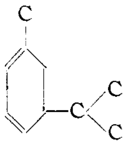

V


VI

It is shown above that pure abietic acid in acetic anhydride solution gives a blue or violet initial color[12] when treated with a little sulfuric acid. This suggests the possibility that abietic acid contains the *m*-menthadiene configuration which is apparently responsible for the same colorations observed in the test with other compounds of practically certain structure. Derivatively, however, abietic acid is apparently a hydrogenated methylretene carboxylic acid, and consequently should contain an isopropyl group. The products obtained by ozonization and permanganate oxidation of the compound include isovaleric acid, isobutyric acid and acetone. The formation of these compounds is obviously most conveniently explained by the presence of an isopropyl group. When abietic acid is heated with sulfur a 17% yield of retene (1-methyl-7-isopropylphenanthrene) is obtained.[13] With selenium a 70% yield of retene is produced.[14] In the temperature range in which these reactions were carried out (250–350°) hydrogen selenide decomposes into its elements forming an equilibrium mixture containing only 25% of hydrogen selenide,[15] so that dehydrogenation of the ring system proceeds in a medium which could reduce an unsaturated side chain. At the same temperatures hydrogen sulfide is much less decomposed.

Although L. Ruzicka[16] and others have pointed out that the reactions with sulfur and selenium are drastic ones and not unattended by the possibility of rearrangement or other change, the unaltered retene configuration is retained in most of the structures proposed for abietic acid,

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(12) With abietic acid and probably with many other compounds the intermediate violet or reddish shades are probably mixtures of the initial blue color with the final reddish-brown products.

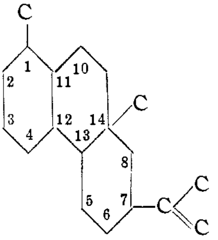
(13) Vesterberg, *Ber.*, **36**, 4200 (1903).

(14) Diels and Karstens, *ibid.*, **B60**, 2323 (1927).

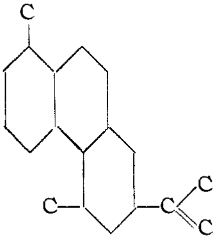
(15) Bodländer, *Z. physik. Chem.*, **29**, 429 (1899).

(16) Ruzicka, *Bull. l'inst. pin.*, **59**, 115 (1929).

but there is no general agreement on the positions assigned to the second methyl group, the carboxyl group and the two unsaturated linkages.[17] If the isopropyl group in the abietic acid carbon framework is replaced by the isopropenyl group[18] and the second methyl group allocated to a position meta to this side-chain, two structures for the compound are possible:



VII



VIII

By analogy with the menthadiene structures discussed above, the second double bond must be located in the ring to which the isopropenyl group is attached. This bond may have any of the positions indicated in structures I to V (above), excepting the positions between C_{13} and C_{14} and between C_8 and C_{14} in formula VIII. Although two positions are possible for the methyl group, C_5 is the only "free" meta position. However, since this methyl group is eliminated in the reaction with sulfur and selenium, attachment to a quaternary carbon like C_{14} is suggested. It is not intended to infer the structure of a compound, or part of a compound, from the observation of a color test. Considering, however, the uncertain mechanism of the reaction between sulfur or selenium and abietic acid, the conflicting conclusions deducible from the chemical degradation of the compound, and the remarkable specificity of the Liebermann reagents for the $\Delta^{x,8(9)}$-*m*-menthadiene grouping, it is not unreasonable to regard the reaction as a piece of chemical evidence which indicates the possibility of similarity between this latter type of structure and part of the abietic acid molecule.

Summary

A brilliant methylene-blue color is the most characteristic of the colorations produced when sulfuric acid is added to an acetic anhydride solution of abietic acid.

Other anhydrides and acyl chlorides can replace acetic anhydride in the test. Benzoyl chloride gives the most brilliant series of colorations.

On the basis of the Liebermann color reaction for abietic acid it is suggested that abietic acid may contain the $\Delta^{x,8(9)}$-*m*-menthadiene grouping.

Philadelphia, Pennsylvania

Received August 9, 1932
Published April 6, 1933

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(17) Margosches, Fuchs and Ruzicka, *Chem. Umschau Fette, Öle, Wachse Harze*, **36**, 115 (1929); Ruzicka, Goldberg, Huyser and Seidal, *Helv. Chim. Acta*, **14**, 545 (1931); Rouin, *Bull. l'inst. pin.*, **59**, 124 (1929); Dubourg, *ibid.*, **59**, 138 (1929); Aschan, *Fenno-Chem.*, **1**, 18 (1929).

(18) The following construction of the color test leaves the position of the carboxyl group indeterminable, hence it has been omitted from formulas VII and VIII.