[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE

The Crystalline Coloring Compounds in Redwood Extract[2]

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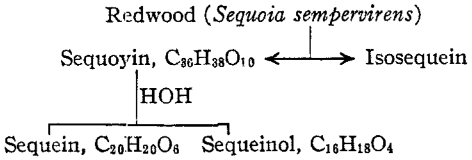
The heartwood of freshly cut green redwood is usually of a straw color. Soon after the surface is exposed to the atmosphere a red to brown discoloration develops. This paper describes the isolation and several properties of two crystalline coloring substances which, together with certain of their derivatives, may be responsible for the red and brown colors in seasoned redwood. In naming these compounds an attempt was made to conform as nearly as possible to the nomenclature used by Perkin and Everest[3] on coloring matter of similar nature.

The compounds and the relationship of a few derivatives are



One of the compounds, *sequoyin*, crystallizes when pure in white or pink flakes, melting at 214° (uncorr.). The other substance, *isosequein*, has been obtained from only two redwood specimens, but its presence is apparent in most redwood liquors. It crystallizes in clusters of red needles, melting sharply at 188°. Its isolation is made difficult by the presence of a relatively large amount of gums, tannins, phlobaphenes and other soluble material in redwood. All treatments that remove these impurities also remove the color compound.

Sequoyin appears to be best obtained by lixiviating green or seasoned redwood in the form of sawdust or chips with distilled water at room temperature. The product is concentrated to a thick sirup by distillation under reduced pressure. The distillate thus obtained is colorless and apparently is free from any appreciable quantity of volatile material. The black residue in the distilling flask is extracted several times with ether. Upon evaporation of the ether, crystallization takes place, usually within a few days. The ether extract from most samples of sawdust crystallized within twenty-four hours, while other extracts from a different source of wood failed to crystallize satisfactorily when given several weeks. The best yield of crystals is obtained from sound wood that has been recently felled. Acetic acid is invariably a component of the ether extract

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(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

(2) This is part of a paper presented at the seventy-ninth annual meeting of the American Chemical Society at Atlanta, Ga., April 7-11, 1930.

(3) Perkin and Everest, "The Natural Organic Colouring Matters," Longmans, Green & Co., London, 1918.

After crystallization of the concentrated ether extract has occurred, the crystals are purified by dissolving them in hot alcohol and precipitating once or twice with benzene and finally with water. The crystals thus obtained are thin, glistening flakes, odorless and tasteless. They are slightly soluble in cold water, more freely in hot water, slightly soluble in ether, soluble in glacial acetic acid and very soluble in alcohol. They dissolve readily in dilute alkalies, giving a bright red which gradually changes at the surface to a brown, forming at the same time a brown amorphous precipitate. This brown material exists in considerable quantity in redwood liquors. In cold concentrated sulfuric acid, the crystals dissolve with a deep red color. They burn with a luminous flame, giving an odor of a phenol and leave no ash.

Anal. Found: C, 68.7, 68.4, 68.4; H, 6.13, 6.07, 6.17; nitrogen, sulfur and halogen are absent.

Molecular weight determinations with absolute alcohol by the ebullioscopic method, with glacial acetic acid by the cryoscopic method, and with camphor by the method of Rast,[4] did not give consistent values.

With lead acetate and with stannous chloride, sequoyin gives a white colloidal precipitate that is not stable in the presence of alkali; with ferric chloride a permanent green is produced. It reduces Tollens' ammoniacal silver nitrate reagent very readily but gives no precipitate of cuprous oxide with Fehling's solution. Methoxyl (OCH_3), carboxyl ($COOH$) and carbonyl (CO) groups apparently are absent. Acetylation with acetic anhydride at 100° yields white microscopic crystals melting at 104 to 106°.

The percentage acetyl (CH_3CO) of these crystals, as determined by the method of Perkin,[5] equals 35.8 to 35.3%.

The properties just described fail to correspond with those of any substance previously described in the literature. The empirical formula suggested for sequoyin is $C_{36}H_{38}O_{10}$. More information in favor of this formula will appear later in this report. Calcd. for $C_{36}H_{38}O_{10}$: C, 68.6; H, 6.08; CH_3CO for octaacetate, 35.7.

By dry distillation or fusion with potassium hydroxide, a dark brown oil having a decided phenolic odor is obtained. It has not been characterized, as the amount obtained was very small, and appears to be a mixture of two or more phenolic substances. Fusion with glycerin at 200 to 210° for three-quarters of an hour gives two compounds melting at 185 and 216°, respectively.

Preparation of Sequein and Sequeinol.—Upon heating sequoyin with 5% sulfuric acid for several hours, two crystalline compounds may be obtained. They appear together or separately upon cooling the solution. They are separated from one another by fractional crystallization; sequein, which is the more soluble in alcohol, crystallizes in long slender white needles that melt sharply at 190° (uncorr.) when pure. The other, sequeinol, crystallizes from hot dilute alcohol in short needles that melt with some decomposition at 242° (uncorr.).

Sequein has properties similar to the parent substance. It has been obtained in yields up to 50.0%. It crystallizes from slightly acid solutions with water of crystallization, which it loses at 100 to 105°. The crystals are white, but after drying in a desiccator they become brown externally. When white they are tasteless, but after becoming brown they are bitter. Heated at temperatures above its melting point it decomposes and leaves a bulky carbonaceous residue. It is only slightly soluble in cold water, more so in hot water and is dissolved with facility by alcohol. Sodium bicarbonate solution dissolves it incompletely. Sequein could be used as an indicator, its tincture being very sensitive to free alkalies and alkaline salts, with which it gives a bright red, while with acids it is yellow. It exhibits phenolic properties, adds bromine,

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(4) Rast, *Ber.*, **55B**, 1051–1054 (1922).

(5) Perkin, *J. Chem. Soc.*, **87**, 107 (1905).

and an alcoholic solution produces a transient green with ferric chloride. Basic lead acetate yields a violet precipitate, which gradually darkens on exposure to the air. Neutral lead acetate produces a slight reddish precipitate, the filtrate of which is a bright red. Stannous chloride gives a permanent rose-colored precipitate. An ammoniacal solution becomes a bright cherry red, ultimately changing to a brown. Upon the addition of acetic acid to this brown solution a brown precipitate is deposited. This precipitate fuses at temperatures over 250° and burns completely without the formation of ash. It gives a deeper shade of red with alkalies than the original compound. With potassium permanganate, sequein is oxidized to a brown humus-like substance that is devoid of any color-producing properties, while with nitric acid, oxalic acid (m. p. 99°) has been obtained. Sequein gives no precipitate with Fehling's solution, nor is it affected by phenylhydrazine. With hydrogen chloride in a dry ethereal solution, it forms a red oil from which the crystals (m. p. 190°) are again obtained upon bringing in contact with water. Wool mordanted with potassium bichromate is dyed a reddish-brown.

Anal. Found: C, 67.40, 67.36; H, 5.63, 5.59.

Molecular weight determinations by the method of Rast using camphor as a solvent gave 356 and 358; by the ebullioscopic method using absolute alcohol as a solvent the values 345 and 336 were obtained.

The above analyses, therefore, correspond to a compound of the empirical formula $C_{20}H_{20}O_6$.

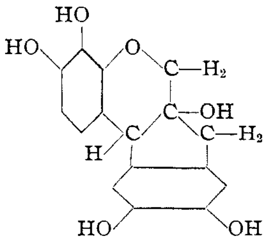
Calculated for $C_{20}H_{20}O_6$: C, 67.38; H, 5.66; molecular weight, 356.16.

Preparation of Sequein Hexaacetate.—Sequein is acetylated by boiling with acetic anhydride with or without the aid of a catalyst, such as sulfuric acid, and also with acetic anhydride and pyridine at room temperatures. Upon adding water to the cooled reaction mixture, the acetylated derivative separates out. After recrystallizing from hot alcohol it may melt at 124 or at 114°. The lower-melting derivative is the one most commonly obtained. The difference in melting points is undoubtedly due to the formation of two isomers.

Analyses on the acetylated derivative, m. p. 114°: Found: C, 62.62, 62.71; H 5.43, 5.41; acetyl (CH_3CO), 42.1, 42.2.

Molecular weight, average of several determinations, gave 607. These values calculated for a hexaacetate compound. Calcd. for $C_{20}H_{14}O_6 \cdot 6(CH_3CO)$: C, 63.13; H, 5.32; CH_3CO, 42.4; molecular weight, 608.25.

Attempts to characterize the products of degradation of sequein were not successful in that only a small amount of the pure compound was available. Fusion with potassium hydroxide, or dry distillation with diminished pressure, yields a brown oil having a very pronounced phenolic odor. During dry distillation orange-colored crystals may be seen momentarily in the receiving flask before dissolving in the distillate.



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The residue from the hydrolysis of the acetylated product does not again give the original compound. Upon extracting the acid residue with ether, a red film is formed when the solvent is evaporated. This material dissolves readily in dilute sodium hydroxide with a purple color and is precipitated as a brown oil upon the addition of an acid. All attempts to induce it to crystallize have failed. Upon exposure to the air it gradually darkens to a black granular mass.

Of the naturally occurring coloring principles the properties of sequein approach those of the dihydropyran group, of which hematoxylin and brasilin are the most important representatives. Brasilin is obtained from *Caesalpinia braziliensis*, while hematoxylin is derived from *Hematoxylon campeachianum*. The constitutional formula of hematoxylin accepted by W. H. Perkin and his co-workers after a series of extensive researches is shown as (A). On oxidation, hematoxylin gives rise to the actual dyestuff hematein, which dissolves in alkalies with a deep red color that is destroyed by excessive oxidation. Sequein has a larger molecule than hematoxylin, by reason of one or more side chains.

On the Properties of Sequeinol.—Sequeinol is soluble in alcohol and is insoluble in cold water, sodium carbonate solutions or carbon tetrachloride. With dilute alkalies it dissolves readily without color and is precipitated from alkaline solutions as microscopic needles with carbon dioxide or with dilute acids. It contains, therefore, no carboxyl group, but has phenolic properties. A clear emerald color is obtained with ferric chloride in 95% alcohol. Fused with finely ground potassium hydroxide at 220° for twenty minutes, it retains its original melting point, 242°.

Anal. Found: C, 69.97, 69.97; H, 6.55, 6.53.

Molecular weight by the ebullioscopic method using absolute alcohol as a solvent gave 280 and 288.

These values correspond for a compound with the empirical formula $C_{16}H_{18}O_4$: Calcd. for $C_{16}H_{18}O_4$: C, 70.03; H, 6.61; molecular weight, 274.14.

Preparation of Sequeinol Tetraacetate.—Heating with acetic anhydride in a water-bath with or without sodium acetate gave a product which crystallized from hot alcohol in short needles melting at 176 to 177° (uncorr.). The percentage acetyl of these crystals when determined by the method of Perkin was 38.51. Molecular weight determinations gave 446 and 466. Calcd. for $C_{16}H_{14}O_4 \cdot 4(CH_3CO)$: CH_3CO, 38.68; molecular weight, 442.2.

Results indicate that there are four hydroxyl groups in sequeinol. No compound with like properties has been found in the literature.

On dry distillation under reduced pressure an amber-colored oil is obtained as a distillate. It is insoluble in water, benzene and carbon tetrachloride, but is dissolved with facility by alcohol or ether. An aqueous suspension with ferric chloride gives a transient yellow-green color. With the addition of dilute sodium bicarbonate to this ferric chloride solution a red color is formed which indicates the presence of catechol.[6]

From the residue remaining from the acetyl determinations of sequeinol, small hard brown crystals are obtained. They melt at 218°, burn with a luminous flame, and leave no ash. They are insoluble in water, soluble in hot alcohol, give no color with ferric chloride and are precipitated instantly from alkaline solutions with acids.

On the Constitution of Sequoyin.—The acid filtrate from the hydrolysis of sequoyin reveals nothing of importance. It contains no reducing material, which demonstrates the absence of sugars in sequoyin.

Sequeinol apparently is not a decomposition product of sequein. Further treatment of sequein with sulfuric acid under the conditions for the breaking up of sequoyin gave no additional sequeinol. A formula of the pyran type for sequein provides for a half acetal linkage with sequeinol in sequoyin. A labile half acetal linkage is the most logical to explain the splitting of the sequoyin molecule with a dilute mineral acid to form two compounds, both of which have no free carboxyl group.

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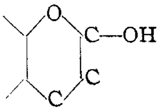
(6) Allen, "Commercial Organic Analysis," 5th ed., Blakiston, Philadelphia, 1925, p. 338.

Sequein is, therefore, concluded to have a hydroxyl group on the carbon atom adjoining the oxygen in the pyran ring. Upon acetylating such a compound, the ring structure is very likely to be broken. This explains the formation of sequein hexaacetate, and also why sequein is not recovered again after the hydrolysis of sequein hexaacetate.



Sequoyin having the half acetal structure just mentioned should, therefore, have two of its ten oxygen atoms unaffected by acetylation. This is confirmed by experimental evidence in that the octaacetate derivative is obtained upon acetylation.

The summation of sequein and sequeinol less one H_2O taken up on hydrolysis is equivalent to the formula $C_{36}H_{36}O_9$. Our analysis of sequoyin demonstrates that it possesses the formula $C_{36}H_{38}O_{10}$. One molecule of water must, therefore, be lost during the process of hydrolysis.

On the Properties of Isosequein.—This compound was obtained from only two redwood specimens in the green condition. The amount available for experimental investigation was small. After repeated crystallizations from hot alcohol it was finally obtained in clusters of red needles, which melt sharply at 188°. A dilute solution gives a violet color gradually darkening with alkalies, which is of similar intensity and sensitivity to that produced when proportionately dilute unpurified redwood extracts are treated with alkali. In acid solutions it is yellow. It possesses phenolic properties, producing a dark green with ferric chloride.

Molecular weight determinations by the method of Rast gave 348 and 353. Structurally it is believed to be related to sequein.

Summary

Two crystalline red color-producing compounds, sequoyin and isosequein, have been isolated from the heartwood of redwood. These two compounds, together with their decomposition products, are believed to be largely responsible for the color changes in redwood. Through hydrolysis with dilute acids sequein and sequeinol have been obtained from sequoyin. The compounds do not appear to have been described in the literature previously.

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