

Crystalline Tannin from the Bark of *Acer Spicatum**

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The bark of *Acer spicatum* has been reported as an adulterant of, or substitute for, *Viburnum opulus* (1), but heretofore no information concerning its chemical constituents has been presented. *Acer spicatum* is not mentioned by Wehmer (2), and the only reference to it is that by St. John (3), which relates to the recognition of it in galenical preparations by means of a color test. In connection with a chemical study of *Viburnum opulus* it seemed advisable to investigate *Acer spicatum* bark in order to determine if the chemical constituents of the two drugs were at all similar.

From a preliminary chemical examination of the bark of *Acer spicatum*¹ by a general method (4), it soon became apparent that the main constituent was a seemingly amorphous tanning, which is by no means true of *Viburnum opulus* (5, 6). It was finally found upon further purification of the apparently amorphous tannin that a crystalline compound, $C_{20}H_{20}O_{13} \cdot 2H_2O$, could be isolated from it. This compound exhibited certain characteristics of a true tannin, e. g., precipitation of alkaloids and gelatin, and the tanning of goldbeater's skin. The goldbeater's skin test, originally devised by Atkinson and Hazleton (7) and later modified by Price (8), is considered by Nierenstein (9) to be the only true criterion of a natural organic tannin.

This tannin appeared to be identical in physical and chemical properties with crystalline acer-tannin isolated from the leaves of *Acer ginuala* by Perkin and Uyeda (10). Like acer-tannin, it hydrolyzed to form one molecule of aceritol and two molecules of gallic acid. Freudenberg and Rogers (11) have recently shown that aceritol and poly-

galitol from *Polygala senega* are identical. Accordingly, the aceritol from *Acer spicatum* tannin was identified by comparison with polygalitol isolated from *Polygala senega* root. An octoacetyl derivative, $C_{36}H_{32}O_{21}$, was also prepared which was similar in physical and chemical properties to the corresponding acer-tannin derivative prepared by Perkin and Uyeda (10). Moreover the tannin methylated by means of diazomethane yielded a crystalline hexamethyl derivative, $C_{26}H_{32}O_{13}$, a compound which Perkin and Uyeda were unable to obtain in a crystalline condition. Hydrolysis of the methylated tannin by the method of Fischer and Freudenberg (12), while not conclusive, indicated the presence of trimethyl gallic acid and the absence of dimethyl gallic acid in the hydrolysate.

EXPERIMENTAL

Isolation of Crystalline Tannin.—Seven Kg. of granulated *Acer spicatum* bark² were exhausted by extraction with 95% alcohol in a Lloyd extraction apparatus. The alcoholic extract was concentrated to a syrup-like consistence which weighed 1.6 Kg. To this extract were added with vigorous stirring 4.5 l. of water. The mixture was filtered, and the filtrate after standing 12 hours had deposited 55 Gm. of a red amorphous material. Upon longer standing, an additional 190 Gm. of this material was obtained. The 245 Gm. were dissolved in hot water, and the solution partially decolorized with charcoal. After several recrystallizations from water, 44 Gm. of colorless crystals melting at 165–166° with decomposition were obtained. $[\alpha]_D^{27} = +17.5^\circ$ (2.5% in acetone).

Analysis: Calcd. for $C_{20}H_{20}O_{13} \cdot 2H_2O$: C, 47.62; H, 4.76.

Found: C, 47.67, 47.47; H, 4.92, 5.01.

The tannin, air-dried at 100°, did not lose water of crystallization. However, when dried at 135–140°, it lost 7.05% of its weight. Calcd. for $C_{20}H_{20}O_{13} \cdot 2H_2O$: H_2O , 7.14%.

Analysis: Calcd. for $C_{20}H_{20}O_{13}$: C, 51.28; H, 4.28.
Found: C, 51.02, 51.38; H, 4.49, 4.51.

The intensity of color on goldbeater's skin (8) produced by a 0.2% aqueous solution of the tannin from *Acer spicatum* was about the same as that exhibited when a 0.2% aqueous solution of gall-tannin was used. Likewise, the precipitation effects upon aqueous salts of brucine, cinchonidine and strychnine, and upon a 1% aqueous solution of

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² Purchased from the S. B. Penick Company.

gelatin in 10% sodium chloride were almost identical.

Hydrolysis of the Tannin.—Since attempts to hydrolyze the tannin by the method of Fischer and Freudenberg (13) did not yield quantitative results, the following method was finally devised. A mixture of 3 Gm. of tannin and 150 cc. of 5% sulfuric acid was heated on the steam-bath during 24 hours, cooled, partially neutralized with sodium carbonate (8 Gm.), concentrated to a volume of 50 cc. and placed in a refrigerator. After 48 hours the gallic acid which had precipitated was removed by filtration. The gallic acid in the filtrate was extracted by shaking with three 15-cc. portions of ethyl acetate. The extracts were combined, washed with water, shaken with sodium chloride and evaporated. The combined gallic acid residues were dried in a vacuum desiccator over phosphorus pentoxide. Yield, 2.19 Gm. Calculated for $C_{20}H_{20}O_{13} \cdot 2H_2O$, 2.24 Gm. After one recrystallization from water the gallic acid melted at 238–240° with decomposition. A mixture of gallic acid (Eastman) showed no depression in melting point.

The aqueous solutions were combined, neutralized to Congo Red paper with sodium carbonate and evaporated to dryness. The residue was extracted with 150 cc. of absolute alcohol, and the extract evaporated to dryness. Yield, 0.78 Gm. Calculated for $C_{20}H_{20}O_{13} \cdot 2H_2O$, 0.98 Gm. Recrystallized from alcohol the aceritol melted at 143–144°. When mixed with polygalitol isolated from *Polygala senega* by the method of Shinoda, Sato and Sato (14), there was no depression in melting point.

Acetylation of the Tannin.—Two Gm. of tannin from *Acer spicatum* were heated during 2 hours under a reflux condenser with a mixture of 10 cc. of anhydrous pyridine and 8 cc. of acetic anhydride. The solution was then cooled to room temperature and poured into 50 cc. of a 5% aqueous solution of sulfuric acid to which ice had been added. The acetylated tannin was extracted from this by means of ether, the ethereal solution was washed several times with water, dried with anhydrous sodium sulfate and the ether removed by distillation. The residue, recrystallized once from methyl alcohol, formed ball-like masses of microcrystalline needles melting at 155–156°. Yield, 2.5 Gm. Calculated, 3.17 Gm.

Analysis: Calcd. for $C_{36}H_{36}O_{31}$: C, 53.73; H, 4.47; CH_3CO —(acetyl group), 42.77.
Found: C, 53.58, 53.86; H, 4.47, 4.61; CH_3CO —, 43.14, 43.30.

Methylation of the Tannin.—Two Gm. of the tannin were dissolved in 15 cc. of absolute methyl alcohol. To this solution was added an excess of an ether solution of diazomethane prepared from phenylnitrosourethane by the method of von Pechmann (15), and the mixture was placed in a refrigerator. After 12 hours, the white crystalline methyl derivative was removed by filtration. Yield, 1.26 Gm. Calculated, 2.21 Gm. The melting

point after recrystallization from a mixture of methyl alcohol and ether was 172–174°, with preliminary softening.

Analysis:³ Calcd. for $C_{26}H_{32}O_{13}$: C, 56.52; H, 5.79; $-OCH_3$, 33.69.
Found: C, 56.15, 56.47; H, 6.07, 5.94; $-OCH_3$, 34.01, 33.91.

Hydrolysis of the methylated tannin with 10% methyl alcoholic sodium hydroxide (12) yielded a small amount of a white substance which was insoluble in a mixture of equal parts of carbon tetrachloride and chloroform. Presumably this substance was trimethylgallic acid.⁴ However, it was impossible to obtain quantitative results from the hydrolysis of the methylated tannin.

SUMMARY AND CONCLUSIONS

1. A crystalline tannin has been isolated from the bark of *Acer spicatum*.
2. Both the tannin and the products of hydrolysis are similar in chemical and physical properties to acer-tannin and its products of hydrolysis.
3. A crystalline octoacetyl derivative of the tannin has been prepared. This derivative is similar in chemical and physical properties to the octoacetyl derivative of acer-tannin obtained from *Acer ginuala* leaves.
4. A crystalline hexamethyl derivative of the tannin from *Acer spicatum* has been prepared, but a crystalline methyl derivative of acer-tannin obtained from *Acer ginuala* has not been described.
5. Since diazomethane seldom, if ever, attacks alcoholic hydroxyl groups, the preparation of a hexamethyl derivative indicates strongly that the original tannin must possess a structure including six phenolic hydroxyl groups. This can be explained only upon the assumption that the original tannin molecule is composed of aceritol to which are attached two separate galloyl nuclei, and not one meta- or paragalloyl nucleus.
6. It is believed that evidence has been presented to indicate strongly that the tannin from *Acer spicatum* bark and the tannin obtained from *Acer ginuala* leaves are identical.

³ Analyses of the methylated tannin were done in the Laboratory of Microchemistry of Dr. Carl Tiedecke, 366 Fifth Avenue, New York.

⁴ Cf. ref. 12.

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2-Alkylmercurithiopyridine-5-Carboxylic Acids^{*,†}

Preparation of, and Stability of Their Solutions

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INTRODUCTION

In 1922 Kharasch (1) and Vieth (2) described some mercury compounds prepared by reacting a mercurial of the type $R\ Hg\ X$ with a mercapto acid of the type $R'\ S\ H$ where R' is a group containing an alkyl or aryl carboxylic or sulfonic acid. The resulting compounds, having the general formula $R\ Hg\ S\ R'\ Ac$ could be dissolved in solutions of sodium bicarbonate or alkali metal hydroxides. Later Kharasch (3) applied the same general method to the preparation of alkylmercurithio compounds more stable in character and suitable for medicinal application.

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† From the Research Laboratories of The Maltbie Chemical Company.

The preparation, antiseptic efficiency and toxicity of a series of alkyl derivatives were reported by Waldo (4) in 1931. One derivative, ethylmercurithiosalicylate, has since found widespread use as an external antiseptic.

Alkylmercurimercapto compounds, in general, when dissolved in aqueous solutions in the form of their alkali metal salts acquire medicinally undesirable properties, presumably as a result of the splitting-off of the alkylmercuri radical at the sulfur linkage, due to the oxidation of the mercapto compound or residue to the disulfide form. Although oxidation proceeds slowly at room temperature, in the presence of minute concentrations of certain metal ions, such as copper, iron and manganese acting as metallic catalysts, the normal oxidation is accelerated, resulting in marked deterioration over a period of several days. Kharasch (5) was able to stabilize aqueous solutions of these compounds by the addition of small amounts of aliphatic amines and diamines, the latter type of compound being most effective.

This paper describes alkylmercuri derivatives of 2-mercaptopyridine-5-carboxylic acid. Stability tests carried out at room temperature and at 60° C. on aqueous solutions of sodium 2-ethylmercurithiopyridine-5-carboxylate indicate that compounds of this type are substantially resistant to oxidation, even in the presence of metal ion catalysts.

EXPERIMENTAL

PREPARATION OF 2-ALKYLMERCURITHIOPYRIDINE-5-CARBOXYLIC ACIDS

An excess of alkylmercuric chloride, prepared by the Grignard reaction (6), was added to an alkaline alcoholic solution of 2-thiopyridine-5-carboxylic acid. After refluxing for 1 hour the solution was concentrated to one-half volume, diluted with water and filtered. The mercury compound was then precipitated with acetic acid and recrystallized from alcohol.

The compounds were analyzed for mercury by oxidizing with fuming nitric and sulfuric acids, then with permanganate, and titrating with potassium thiocyanate.

STABILITY OF SOLUTIONS

Oxidation Tests.—Stock solutions of the mercury compounds were prepared by dissolving 2 Gm. of the alkylmercurithiopyridine carboxylic acid in the theoretical quantity of 1% sodium hydroxide solu-