

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, NEW YORK AGRICULTURAL EXPERIMENT STATION]

A CONTRIBUTION TO THE CHEMISTRY OF GRAPE PIGMENTS IV. THE ANTHOCYANS IN ISABELLA GRAPES¹

BY R. J. ANDERSON AND FRED P. NABENHAUER

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Introduction

During the past few years the pigments occurring in a number of cultivated American grapes have been investigated in this Laboratory. It has been found that the anthocyanins isolated from dark blue grapes such as Concord,² Norton and Clinton,³ representing *Vitis labrusca*, *V. aestivalis* and *V. riparia* consist principally of monoglucosides of monomethyl delphinidin. The pigment occurring in Seibel grapes,⁴ a hybrid of *Vitis aestivalis*, *V. rupestris* with *V. vinifera*, was identical with the anthocyanin, oenin, found by Willstätter and Zollinger⁵ in the dark blue European grape, *Vitis vinifera*.

It appeared of interest to determine whether other hybrids of American grapes with *V. vinifera* also had inherited the pigment peculiar to the European grape. The Isabella was selected as another example of such a cross. The origin of the Isabella is unknown but it has been propagated in this country since about 1816. According to Hedrick⁶ it is hybrid representing *Vitis labrusca* and *Vitis vinifera*.

The investigation has shown that the pigment occurring in the Isabella is identical with that found in the Seibel grape and it appears to be identical with the anthocyanin isolated by Willstätter and Zollinger from the dark blue European grape.

The chloride of the glucoside crystallized in dark, reddish-brown prisms from a mixture of methyl alcohol and dil. hydrochloric acid. It could be recrystallized easily from the same solvents and the composition corresponded to the formula $C_{23}H_{25}O_{12}Cl + 4H_2O$.

The sugar-free pigment, anthocyanidin or oenin chloride, $C_{17}H_{15}O_7Cl + 1.5H_2O$, was obtained in beautiful, prismatic crystals when the glucoside was hydrolyzed with boiling dil. hydrochloric acid.

The work of Willstätter and Zollinger⁵ has shown that oenin contains two methoxy groups and that on fusion with potassium hydroxide, phloroglucinol and a methyl ether of gallic acid were formed. According to the

¹ Presented before the Division of Biological Chemistry of the American Chemical Society at the Baltimore Meeting, April, 1925.

² Anderson, *J. Biol. Chem.*, **57**, 795 (1923).

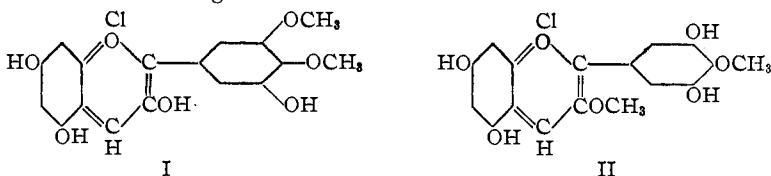
³ Anderson and Nabenhauer, *ibid.*, **61**, 97 (1924).

⁴ Anderson, *ibid.*, **61**, 685 (1924).

⁵ Willstätter and Zollinger, *Ann.*, **408**, 83 (1915).

⁶ Hedrick, "Grapes of New York," N. Y. Agr. Exp. Sta., **1908**.

same authors the constitution of oenicin chloride may be represented by either of the following formulas:

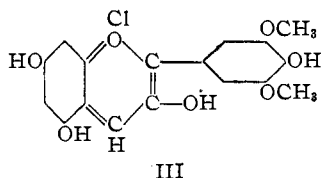


The positions of the methoxy groups were not known but it was believed that Formula II was the most likely.⁷ The interesting syntheses that have been made recently by Pratt and Robinson⁸ of various anthocyanidins have not thrown any light upon the position of the methoxy groups in the natural anthocyan pigments.

It has been noticed in this Laboratory when anthocyanidin is fused with potassium hydroxide that phloroglucinol may be isolated without difficulty but we have not succeeded in obtaining any crystalline derivative of gallic acid by this method. It was found, however, that the acetyl derivative of anthocyanidin⁸ gave on oxidation with neutral permanganate a derivative of gallic acid. The oxidation product could not be identified but it was found that all of the methoxy groups were attached to the gallic acid part of the molecule.

Similar experiments were tried with the anthocyanidin from Isabella grapes. It was found to be more convenient to acetylate the anthocyanidin in pyridine solution by addition of acetyl chloride according to the method of Einhorn and Hollandt.⁹ Unfortunately, the acetyl derivative does not crystallize from any of the usual solvents. It is precipitated on addition of water as a fine, amorphous powder with a faint pinkish-straw color. The amount of acetic acid obtained after hydrolysis and distillation indicates that four acetyl groups had been introduced.

The amorphous acetyl derivative was oxidized in aqueous suspension with neutral permanganate and also with potassium permanganate in acetone solution. In both cases the same oxidation product was obtained, a substance that crystallized in dense, colorless prisms and which proved to be an acetyl derivative of syringic acid. On saponification, syringic acid was obtained which agreed completely in properties with synthetic syringic acid prepared according to the methods of Perkin and Weizmann¹⁰ and Bogert and Ehrlich.¹¹



⁷ Willstätter and Zollinger, *Ann.*, **412**, 195 (1916).

⁸ Pratt and Robinson, *J. Chem. Soc.*, **127**, 166, 1128, 1182 (1925).

⁹ Einhorn and Hollandt, *Ann.*, **301**, 95 (1898).

¹⁰ Perkin and Weizmann, *J. Chem. Soc.*, **89**, 1649 (1906).

¹¹ Bogert and Ehrlich, *THIS JOURNAL*, **41**, 798 (1919).

Since syringic acid is 3,5-dimethoxy-4-hydroxybenzoic acid it follows that the constitution of oenin chloride must be represented by Formula III.

Experimental Part

Precipitation of the Pigment as Picrate.—The grape skins were separated from the pulp by hand and the material was worked up exactly as described before.⁴ From 150 kg. of grapes 29.3 kg. of pressed skins was obtained which yielded 134.7 g. of crude picrate.

Preparation of the Glucoside.—The crude picrate was digested in 900 cc. of methyl alcohol containing 150 cc. of 20% hydrochloric acid dissolved in methyl alcohol. A small amount of insoluble matter was filtered off and the clear, intensely dark red solution was precipitated by adding 6 liters of anhydrous ether. The amorphous, dark red precipitate was collected on a Büchner funnel, washed with ether and dried. It weighed 28.6 g. For the purpose of removing the last traces of picric acid the amorphous glucoside was powdered and digested in 300 cc. of anhydrous ether which contained 10 cc. of 20% hydrochloric acid in methyl alcohol. After the mixture had been shaken for two hours it was filtered and the solid was washed with ether until the washings left no residue on evaporation. The substance, after drying in a vacuum desiccator, weighed 28.3 g.

The Ferric Chloride Reaction.—An alcoholic solution of the glucoside gave no characteristic color reaction with ferric chloride, differing in this respect from the glucosides isolated from Concord, Norton and Clinton grapes. Addition of ferric chloride to an alcoholic solution of the glucoside gave only a deep wine color which was quite permanent but in aqueous solution the color quickly faded to pale straw.

Crystallization of the Glucoside.—The amorphous glucoside, 3 g., was dissolved in 10 cc. of methyl alcohol. The solution was filtered, mixed with 10 cc. of 5 *N* hydrochloric acid and allowed to stand in a loosely covered dish. After two hours, crystals had begun to form, and after standing for four hours the crystals were filtered off, washed with ethyl alcohol and dried in the air. The substance, which weighed 1.9 g., consisted of uniform prisms. In reflected light the crystals showed a beautiful bronze luster. The substance was recrystallized by dissolving in 10 cc. of methyl alcohol and adding 10 cc. of 5 *N* hydrochloric acid. The substance again separated in prismatic crystals which, after they had been filtered off, washed and dried, weighed 1.7 g. The crystals contained a trace of ash which evidently consisted of potassium chloride and it was necessary to recrystallize four times before the preparation was ash-free.

The substance was dried at 105° in a vacuum over phosphorus pentoxide before it was analyzed.

Anal. Subs., 0.1817: CO₂, 0.3485; H₂O, 0.0771. Subs. 0.2396: AgCl, 0.0664. Calcd. for C₂₃H₂₅O₁₂Cl (528.5): C, 52.22; H, 4.73; Cl, 6.71. Found: C, 52.31; H, 4.74; Cl, 6.85.

The loss in weight on drying was 12.51 and 11.45% which corresponds to 4 molecules of water of crystallization. Calculated for 4H₂O: 11.99%.

The methoxyl determination was made according to the Zeisel method.

Methoxyl. Subs., 0.1903: AgI, 0.1312. Calcd. for 2CH₃O: CH₃O, 11.73. Found: 9.10.

Hydrolysis of the Glucoside

Preparation of Anthocyanidin Chloride.—The amorphous glucoside, 10.6 g., was dissolved in 150 cc. of water; 150 cc. of concd. hydrochloric acid was added. The

solution was heated to boiling and boiled for five minutes. Prismatic crystals began to separate after the solution had boiled for one minute. After the reaction mixture had stood in the ice box overnight the crystals were filtered off, washed with dil. hydrochloric acid and dried in a vacuum over sulfuric acid and potassium hydroxide. The product consisted of uniform prismatic crystals that appeared to be perfectly homogeneous. The substance weighed 5.8 g. It was analyzed after it had been dried at 105°.

Anal. Subs., 0.1731: CO₂, 0.3542; H₂O, 0.0644. Subs., 0.3012: AgCl, 0.1139. Calcd. for C₁₇H₁₅O₇Cl (366.5): C, 55.66; H, 4.09; Cl, 9.68. Found: C, 55.80; H, 4.16; Cl, 9.35.

Methoxyl. Subs., 0.1944: AgI, 0.1814. Calcd. for 2CH₃O: CH₃O, 16.91. Found (Zeisel): 12.32.

The loss in weight of three samples on drying was 6.27, 6.02 and 6.23%, respectively, which is equivalent to 1.5 molecules of water of crystallization. (Calculated for 1.5 H₂O, 6.86%.)

Isolation of Glucose as Phenylglucosazone.—Phenylglucosazone was obtained from the filtrate from the anthocyanidin chloride by the method described before.⁴ The yellow, needle-shaped crystals weighed 0.7 g. and melted with decomposition at 206°.

Reactions of Anthocyanin and Anthocyanidin.—The reactions of the glucoside and of the sugar-free pigment were identical with those described for the pigments obtained from Seibel grapes.⁴

Preparation of the Acetyl Derivative of the Anthocyanidin.—The acetyl derivative was prepared by the method of Einhorn and Hollandt.⁹ The anthocyanidin chloride, 6 g., was dissolved in 30 cc. of pyridine and while the solution was cooled in ice water 9 cc. of acetyl chloride was added gradually during constant stirring. After standing overnight the reaction mixture was poured into 500 cc. of water acidified with hydrochloric acid. The lumpy precipitate was filtered off, dissolved in pyridine and again precipitated by pouring the solution slowly into 500 cc. of dil. hydrochloric acid. The acetyl derivative separated as a fine, amorphous precipitate of a faint pinkish-straw color. It was filtered off, washed with water and dried in a vacuum over sulfuric acid. The product, which weighed 7.5 g., was free from chlorine and nitrogen. It was easily soluble in pyridine and in acetone, giving brown solutions. It dissolved sparingly in hot ethyl or methyl alcohol but was insoluble in water or dilute acids. The substance was rather easily soluble in dilute alkali with development of a green color that appeared red in transmitted light; on heating the liquid, the green changed to brown. When the substance was heated with dilute acids the color turned bright red, evidently due to hydrolysis. The solution in alcohol, which was brown, also turned bright red when the liquid was heated with hydrochloric acid. It was impossible to obtain the substance in crystalline form.

The acetyl derivative contained 4 acetyl groups as determined by hydrolysis with 30% phosphoric acid and titration of the acetic acid that distilled.

Anal. Subs., 0.4709, 0.4546: 36.86, 36.96 cc. of 0.1 N KOH soln. Calcd. for 4CH₃CO: CH₃CO: 34.46. Found: 33.65, 34.95.

Oxidation of the Acetyl Derivative by Neutral Permanganate.—The acetyl derivative, 7.5 g., was ground to a fine suspension with 800 cc. of water, and 15 g. of magnesium sulfate was added. The mixture was kept at room temperature and stirred with a turbine while 26 g. of powdered potassium permanganate was added in small portions in the course of three hours. After standing overnight the solution was colorless. It was acidified with sulfuric acid and the manganese dioxide formed was dissolved by adding sulfur dioxide. A small quantity of unaltered acetyl derivative was filtered off, washed with water and dried. It weighed 0.9 g.

The filtrate, containing the oxidation products, was extracted with five portions of ether and the latter was removed by distillation. An oily residue remained which solidified when water was added. The substance was dissolved by the addition of a little sodium bicarbonate. The solution was filtered, diluted to 200 cc. with water and acidified with hydrochloric acid, when a slight cloudiness was produced and a small amount of resinous material separated. The solution was warmed to 50°, shaken with Norite, filtered and concentrated to about 40 cc. by distillation in a vacuum. Crystals began to separate during the concentration, and after the solution had cooled the crystals were filtered off, washed with water and dried in a vacuum desiccator. The mother liquors on further concentration deposited a small amount of crystals which were collected and added to the first lot. The crystals which weighed 0.65 g., were twice recrystallized from water after treatment with Norite and 0.3 g. of colorless, stout prisms was obtained. When heated slowly in a capillary tube the substance began to soften at 187° and melted to a colorless fluid at 189°.

A further quantity of the same substance was obtained on oxidizing 5.4 g. of the acetyl derivative with potassium permanganate in acetone solution. In this case the acetylsyringic acid was contained in the manganese dioxide precipitate. It was isolated as described above and purified by crystallization from water. The substance separated in dense, colorless prisms that sintered at 187° and melted at 190°. The pure crystals weighed 0.25 g.

The crystalline substance obtained as described above should be an acetyl derivative of dimethylgallic acid. The investigation showed that it was the acetyl derivative of syringic acid.

Saponification of Acetylsyringic Acid.—In a preliminary experiment 0.1 g. of crystals, obtained from the mother liquors, was refluxed for one and a half hours with 10 cc. of water and 3 cc. of phosphoric acid. When the solution cooled, long, silky needles separated which were filtered off and washed with water. The crystals were dissolved in hot water, treated with Norite and allowed to crystallize. Long, delicate, colorless needles were obtained which melted at 208°.

A quantitative determination of the acetic acid was made by hydrolyzing the substance with 30% phosphoric acid and titrating the acetic acid in the distillate. 0.5496 g. substance required 22.43 cc. of 0.1 *N* potassium hydroxide solution. Calculated for $1\text{C}_9\text{H}_9\text{CO}_2$: 22.89 cc.

The amount of acetic acid recovered is therefore about 98% and the results indicate that only one acetyl group was present.

Syringic Acid.—Long, fine, colorless needles crystallized from the acid solution mentioned above after the acetic acid had been distilled. After the solution had stood at 0° for some time, the crystals were filtered off and washed with water. The substance after drying in a vacuum over sulfuric acid weighed 0.4 g. The recovery was therefore nearly quantitative, since the calculated yield of syringic acid is 0.41 g.

The substance was dissolved in hot water, the solution treated with Norite and the reprecipitated material twice recrystallized from water. Colorless, silky needles were obtained that weighed 0.3 g. and melted at 209–210°. There was no loss in weight on drying at 105°.

Anal. Subs., 0.1092: CO_2 , 0.2175; H_2O , 0.0494. Calcd. for $\text{C}_9\text{H}_{10}\text{O}_5$ (198): C, 54.54; H, 5.05. Found: C, 54.32; H, 5.06.

Methoxyl. Subs., 0.0708: AgI, 0.1661. Calcd. for $\text{C}_7\text{H}_4\text{O}_3(\text{O}.\text{CH}_3)_2$ (198): CH_3O , 31.31. Found (Zeisel): 30.99.

The analytical results reported above indicate that the oxidation product obtained from the acetyl derivative of anthocyanidin is a dimethyl ether of gallic acid, and the melting point indicates that this substance is syringic acid.

The identification was completed by synthesizing syringic acid and its acetyl derivative and comparing the properties of these compounds with those of the oxidation products described above. The melting points of the synthetic preparations were found to be several degrees higher than given in the literature but agreed completely with those of the oxidation products.

Various melting points have been reported for syringic acid. Körner¹² who first prepared this acid stated that it melted at 202°. The same melting point was reported by Gadamer¹³ while Power¹⁴ obtained a sample of syringic acid that melted at 198–199°. Synthetic syringic acid prepared by Graebe and Martz¹⁵ melted at 204°. Bogert and Isham¹⁶ and also Bogert and Ehrlich¹¹ described synthetic syringic acid which melted at 204°. Bogert and Isham¹⁶ stated that the acid had a characteristic aromatic odor.

Preparation of Synthetic Syringic Acid

Gallic acid was methylated by the method of Perkin and Weizmann.¹⁰ The trimethylgallic acid crystallized in colorless needles and it melted at 167–186°. Syringic acid was obtained by partially hydrolyzing 6 g. of trimethylgallic acid as described by Bogert and Ehrlich.¹¹ The substance was dissolved in hot water, treated with Norite and twice recrystallized from water. Long, colorless, delicate needles were obtained that weighed 2.2 g. The preparation was entirely odorless and the aqueous solution gave with ferric chloride a faint brownish-yellow color. The acid, which did not contain any water of crystallization, melted at 210–211° and the melt solidified at 183°.

When some of this product was mixed with the acid obtained by oxidation the mixture melted at 210–211° and solidified at 183°.

Synthetic Acetylsyringic Acid.—Although acetylsyringic acid was prepared by Gadamer¹⁷ nearly 30 years ago we could not find any mention of this substance in the reference books of organic chemistry. The product described by the above-mentioned author melted at 181–183° and it had been prepared from syringic acid obtained from syringin according to the method of Körner.¹²

The acetyl derivative of syringic acid may be easily prepared by boiling syringic acid with acetic anhydride. It is only slightly soluble in cold water and crystallizes in stout prisms or in dense, irregular plates, depending upon the concentration of the solution.

One g. of syringic acid was boiled for one and a half hours with 15 cc. of acetic anhydride. The solution was evaporated to dryness under reduced pressure and the residue was dissolved in 125 cc. of hot water, treated with Norite, and twice recrystallized from water. The odorless and colorless crystals weighed 0.65 g. There was no loss in weight on drying at 105°. When heated in a capillary tube the substance melted at 190–191° and solidified at 182–181°.

When some of this substance was mixed with the acetylsyringic acid obtained by oxidation, the mixture melted at 190–191° and the melt solidified at 182–181°.

Summary

1. The pigment occurring in Isabella grapes has been examined.
2. The pigment was found to be a monoglucoside, $C_{23}H_{25}O_{12}Cl + 4H_2O$,

¹² Körner, *Gazz. chim. ital.*, **18**, 215 (1888).

¹³ Gadamer, *Ber.*, **30**, 2330 (1897).

¹⁴ Power, *Pharm. J.*, **1901**, 275. *Chem. Centr.*, **1901**, II, 725.

¹⁵ Graebe and Martz, *Ber.*, **36**, 215 (1903); *Ann.*, **340**, 213 (1905).

¹⁶ Bogert and Isham, *THIS JOURNAL*, **36**, 514 (1914).

¹⁷ Gadamer, *Arch. Pharm.*, **235**, 570 (1897).

that crystallized in prisms. It appears to be identical with the anthocyanin, oenin, which occurs in the dark blue European grape, *Vitis vinifera*.

3. The anthocyanidin chloride, $C_{17}H_{15}O_7Cl + 1.5H_2O$, prepared by hydrolyzing the glucoside, crystallizes in prisms and is apparently identical with oenidin.

4. It is interesting to notice that in crosses between American and European grapes the pigment peculiar to *Vitis vinifera* is inherited by the hybrid.

5. When the acetyl derivative of the anthocyanidin was oxidized with neutral permanganate, acetylsyringic acid was formed and by saponification of the latter syringic acid was obtained. This determines the constitution of oenidin chloride.

GENEVA, NEW YORK

NEW BOOKS

Inorganic Quantitative Analysis. By HAROLD A. FALES, Ph.D., Associate Professor of Chemistry at Columbia University. The Century Company, New York, 1925. xii + 493 pp. 49 figs. 20.5×13.5 cm. Price, \$3.50.

This is an outstanding example of a modern type of textbook which aims at presenting the subject of quantitative analysis from a physicochemical standpoint.

According to the preface it was "the aim of the author in preparing this work to apply the principles of Physical Chemistry to the theory of Quantitative Analysis. . . ." "Perhaps the dominant feature of the text is the manner in which it presents the theory." "The customary division of the subject into gravimetric and volumetric methods. . . has been abandoned, and in its place the development of the subject is based on the fundamental principles which are involved; these are presented in the following order: (1) precision, (2) weighing, (3) measurement of volumes, (4) neutralization, (5) solubility product, (6) oxidation-reduction and (7) evolution and measurement of gases."

With the exception of No. 7, the discussion of the theory of the above topics is comprehensive and thorough, the best treatment of the subjects from this standpoint that the reviewer has seen. The physicochemical explanations are presented in a quantitative way with the conventional mathematical equations and illustrated with graphs and tables.

No 7 is not handled on a par with the other topics. Instead of being a comprehensive discussion of the evolution and measurement of gases, such as the author might well have written, it contains merely the methods for determining carbon dioxide in soluble carbonates and for total carbon in steel.

At the end of each chapter is a list of problems with answers.

In a work so frankly physicochemical in character, one would expect to