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Received for review January 3, 1955. Accepted May 24, 1955.

WINE PRODUCTION

Skin Pigments of the Cabernet Sauvignon Grape and Related Progeny

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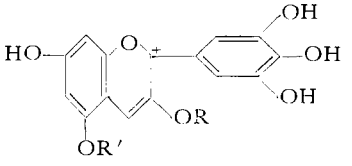
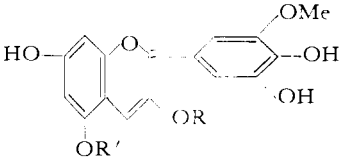
The skin pigments of the Cabernet Sauvignon grape have been separated by paper chromatography and identified as malvidin, two glucosides of malvidin, a petunidin glucoside, a delphinidin glucoside, and a complex diglucoside of malvidin. Evidence is presented to indicate that the two malvidin glucosides are the 3-glucoside and the 3,5-diglucoside, and that the delphinidin and petunidin pigments are the 3,5-diglucosides. The malvidin diglucoside was the most abundant. The pigments of progeny of Cabernet Sauvignon and Carignane grapes were qualitatively identical with those of the Cabernet Sauvignon. The order of appearance of the anthocyan pigments in the maturing Cabernet Sauvignon grape was: malvidin diglucoside, malvidin monoglucoside and delphinidin glucoside, petunidin glucoside, and free malvidin, contrary to the hypothesis that in the developing fruit or flower anthocyanins are formed at the expense of the corresponding anthocyanidin.

THE VISUAL APPEAL of many table grape varieties and many wines depends upon the attractive red colors of the anthocyan pigments present. The term "anthocyan" is used here to refer to both anthocyanin- and anthocyanidin-type pigments. Among the many factors influencing the shade and intensity of the red color, perhaps the most important is the composition of the pigment mixture in the grape or in the wine. Classical analytical techniques have often given incomplete information concerning the pigment mixtures. A detailed knowledge of the composition and inheritance pattern of the skin pigments in grapes is of particular value to the geneticist in the development of new grape varieties most likely to show desirable color characteristics.

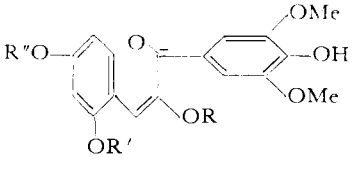
The first precise investigations of grape pigments were reported by Willstätter and Zollinger (*18, 19*), who characterized the anthocyan pigments of the "dark blue north Italian grape" (*V. vinifera*) as mainly "oenin" (malvidin-3-glucoside) with small amounts of the aglycone, malvidin, and malvidin diglucoside. These authors also presented evidence (*19*) that the skin pigments of *V. riparia* consisted mainly of the glucoside of a monomethyl derivative of delphinidin and a small amount of a dimethyl delphinidin derivative.



R = R′ = H, delphinidin
R′ = H, R = glucose, delphinidin-3-glucoside
R′ = R = glucose, delphinidin-3,5-diglucoside



R = R′ = H, petunidin
R′ = H, R = glucose, petunidin-3-glucoside
R = R′ = glucose, petunidin-3,5-diglucoside



R = R′ = R″ = H, malvidin
R = R′ = H, R″ = Me, hirsutidin
R′ = R″ = H, R = glucose, malvidin-3-glucoside (oenin)
R″ = H, R = R′ = glucose, malvidin-3,5-diglucoside (malvin)

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During the period 1923 to 1928, Anderson published the results of his extensive investigations of the anthocyan pigments in some of the grape varieties grown in New York State. He found (*1*, *4*) the anthocyanin pigments of the Norton, Concord, and Clinton grapes to be the same.

He also concluded that the pigmentation in these grapes was the same as that found by Willstätter in *V. riparia*. Anderson (*2*) found that the Seibel 78 and 2044 grapes, crosses between *V. vinifera* and an American grape, had the same pigment, oenin chloride, as the *V. vinifera* variety investigated by Willstätter. The methoxyl determinations were somewhat lower than required for a dimethoxy compound, however, so the presence of a small amount of a monomethyl delphinidin derivative was postulated. The Isabella grape (*3*), presumably a hybrid of *V. vinifera* and *V. labrusca*, was shown to have the same skin pigments as the Seibel grapes. It was in this investigation that Anderson unequivocally established the structure of the anthocyanidin resulting from the acid hydrolysis of oenin to be that currently referred to as malvidin. The pigmentation of the Ives grape (*17*) proved to be the most complex studied by Anderson. The pigment was found to consist of a small amount of free anthocyanidin, a monoglucoside of malvidin, a monoglucoside of a monomethyl delphinidin, and a *p*-hydroxycinnamic acid derivative of an anthocyanin.

In 1931 Levy, Posternak, and Robinson (*9*) found malvidin as the major product of the hydrolysis of the skin pigment of the Fogaruna grape (*V. vinifera*), but also found petunidin and delphinidin to be present.

Cornforth (*8*) used the methods of Levy to identify oenin as the skin pigment of the Australian wild grape, *V. hypoglauca*. The anthocyanidin obtained on hydrolysis of the pigment contained little or no delphinidin.

Brown (*7*) was the first to identify a diglucoside as the major pigment of a grape variety. Using Robinson's (*16*) techniques, he identified petunidin-3,5-diglucoside as the pigment of the Hunt muscadine grape (*V. Rotundifolia* Michx.). He found no evidence of a monoglucoside.

Recently Ribéreau-Gayon (*11–14*) reported that the pigments in the Merlot and Cabernet Sauvignon grapes were identical and consisted mainly of a mixture of the monoglucosides of malvidin, petunidin, and delphinidin. He also reported that the pigments in the hybrid grapes, Seibel 7053 and 5455, were the diglucosides of the same anthocyanidins. The free aglycone content of the grapes was reported to be small or zero.

The present paper describes the results of experimental work on the nature and inheritance of the skin pigments of the Cabernet Sauvignon grape and related progeny which has been under way in this laboratory for some time. The Cabernet Sauvignon grape has, with skillful vinification, yielded some of the finest of California red table wines. It is also the variety that contributes the predominant character to the red wines of Bordeaux. In the investigations of the pigments present in the various Cabernet Sauvignon progeny only small amounts of material were available. This effectively prohibited the use of the classical techniques of Willstätter (*18*, *19*) and Robinson (*16*) in the investigation of those varieties. However, the separation of anthocyan pigments by paper chromatography as described by Bate-Smith (*5*, *6*) proved to be ideal for the separation of the many closely related pigments found in grapes. Although the larger amounts of pure pigments needed for classical analyses cannot be obtained easily from paper chromatograms, much information can be obtained from the standard procedures of paper chromatography involving the use of chromogenic sprays for identification purposes and comparisons of the R_f values of unknown pigments with authentic specimens using a variety of developing solvents. Paper chromatography is a rapid method for the separation of the grape pigments and requires only an extremely small amount of the pigment mixture. These advantages may greatly facilitate various types of investigations involving grape pigments. For example, the pigmentation of progeny can be identified without resorting to tedious and time-consuming back-crossing, or the changes in pigment content as grapes are processed into wines and the wines are aged can be followed and the results of technological modifications readily discerned.

Source and Preliminary Treatment of Grape Skins

All the grape varieties investigated were grown on the experimental plots of the Department of Viticulture of the University of California. The Cabernet Sauvignon and Carignane varieties were grown at Davis, while most of the progeny were grown at Riverside. Large amounts of fruit of the two varieties listed above were available for this study, but normally only one cluster of fruit was available for each of the various progeny investigated.

In each instance the fruit was skinned by hand and the skins were soaked three times in fresh distilled water for 5-minute periods. The washed skins were blotted by pressing between cheesecloth or paper towels until the blotter was barely tinged with color. The damp skins were then placed in polyethylene bags and quickly frozen. The frozen skin samples were maintained at 0° F. or less until used.

Extraction of Pigments and Chromatographic Techniques

When large amounts of material were available, the pigments were extracted and purified in the following manner. About 120 grams of freshly thawed skins were mixed with 250 ml. of cold 1% hydrochloric acid and blended for 3 minutes in a Waring Blendor. The slurry was filtered through cheesecloth and the residues were reblended with an additional 100 ml. of cold acid. The combined extracts were filtered through paper and saturated aqueous lead acetate solution was added to the filtrate until no further precipitation of lead salts occurred. The rich blue supernatant solution was discarded and the lead salts were washed with distilled water several times in order to remove all traces of glucose. The washed salts were then air-dried on a porous clay plate and kept in a tightly stoppered amber colored bottle. No change in the bright blue color of these salts was observed after several months' standing. In a normal run about 200 mg. of the air-dried lead salt of the pigments was treated with 1 or 2 ml. of 2% methanolic hydrochloric acid. The lead chloride precipitate was removed from the dark red solution by centrifugation and the clear solution was used for the chromatographic studies.

When only small amounts of grape skins were available, as in the case of the Cabernet Sauvignon progeny, about 2 grams of freshly thawed grape skins and 10 ml. of 2% methanolic hydrochloric acid were allowed to stand overnight in a stoppered Erlenmeyer flask in a refrigerator. The resulting cold extracts were then used as soon as possible.

In the chromatographic investigation of the pigments a suitable portion of the pigment extract (usually 3 to 10 μl.), obtained by either of the above techniques, was spotted 2 inches from the edge of a sheet of Whatman No. 1 filter paper. In some runs the extract was spotted on a 9 × 11 inch sheet of paper, which was formed into a cylinder and placed in a Smillie anaerobic culture jar, 5 inches in diameter and 12 inches high, for development. In other runs the extracts were spotted on a full size (18 × 22 inches) sheet of paper and the development was carried out in a Model A Chromatocab (Research Equipment Corp., Oakland, Calif.). In either case the development was allowed to proceed by the ascending technique for 8 to 12 hours, during which time the solvent front moved approximately 200 mm. The chromatograms were developed at room temperature in an insulated room where temperature variations were small.

In the investigations of the Cabernet Sauvignon pigments, where larger amounts of the separated pigments were desired for identification by suitable

Table I. Pigments of Cabernet Sauvignon Grape

Spot	R_f: Butanol–27% aqueous acetic acid[a]	R_f: Butanol–acetic acid–water, 4:1:5	Color: Air dried	Color: With polyphosphomolybdic acid[b]	Relative Amount
A	0.82	0.76	Lilac	Red	Trace
B	0.63	0.57	Pink	Red	Small
C	0.54	0.39	Pink	Red	Moderate
D	0.43	0.21	Pink-lilac	Red	Large
E	0.32	0.15	Mauve	Blue-violet	Small
F	0.25	0.11	Blue-lilac	Rich blue	Moderate

[a] R_f values may vary slightly from batch to batch because of method of preparation. Values reported are average of more than 100 determinations.

[b] *o*-Dihydroxy phenols have been reported (*15*) to give a blue color when sprayed with phosphomolybdic acid. This has been confirmed by the writers with compounds like 3,4,5-trihydroxybenzoic acid, and with authentic specimens of petunidin and delphinidin.

micromethods, the following technique was used. Several hundred microliters of pigment extract were placed in a horizontal line 2 inches from the bottom of an 18 × 22 inch sheet of Whatman No. 1 filter paper and the chromatogram was developed using butanol–acetic acid–water mixture by the ascending technique until the best separation of the pigments was obtained. The separated lines of pigments were cut out and eluted with the minimum amount of 2% methanolic hydrochloric acid overnight. The eluate was decanted and used as quickly as possible. When acid hydrolysis of the pigment was desired, an equal volume of 6 *N* hydrochloric acid was added to the eluate and the mixture boiled for 30 seconds after all the methanol had been expelled. The anthocyanidin moiety was then extracted with a small amount of *n*-amyl alcohol and the aqueous phase was saved for identification of the sugar moiety. When basic hydrolysis of the pigment was desired, the eluate was evaporated to dryness at room temperature and the residue taken up in a small amount of 1% hydrochloric acid. The solution was heated to boiling and 3*N* sodium hydroxide was added dropwise until the solution turned from red through green to yellow, after which the solution was boiled 15 to 30 seconds longer. The anthocyanin could then be reconstituted by the addition of concentrated hydrochloric acid to the basic hydrolyzate until the deep red color reappeared. This solution was carefully evaporated to dryness and the residue taken up in a small amount of 2% methanolic hydrochloric acid in order to separate the pigment from the large amount of sodium chloride formed in the above procedure.

The developer used most often in the chromatographic separation of the pigments was a mixture of butanol, acetic acid, and water prepared in one of the following two ways. The first, according to the method of Bate-Smith (*5*), consisted of mixing 4 parts of 1-butanol with 1 part of glacial acetic acid and 5 parts of water. The thoroughly agitated mixture was allowed to remain at the temperature at which it was to be used for at least 4 hours. No difference in behavior was observed for mixtures which equilibrated for periods varying from 4 to 72 hours. The organic phase was then removed and used as a developer until it gave poor separations or disparate R_f values. The second method consisted of mixing equal volumes of 1-butanol and 27% (by volume) aqueous acetic acid. If the resulting mixture was turbid, glacial acetic acid was added dropwise until the water-saturated mixture just cleared. This developer was used within 24 hours after preparation. A mixture of *m*-cresol, acetic acid, and water (*5*) was also used as a developer in some instances. This was prepared by mixing 50 parts of *m*-cresol with 2 parts of glacial acetic acid and 48 parts of water. The thoroughly agitated mixture was allowed to stand for 1 to 3 days and the organic phase then removed and used until it gave poor separations or disparate R_f values.

The sugar moieties of the anthocyanins were characterized chromatographically using the following two developers in addition to those mentioned above. A mixture of acetic acid, pyridine, and water was prepared by mixing 5 parts of glacial acetic acid with 3 parts of pyridine and 2 parts of water, or a mixture of 1-butanol, acetic acid, and water was prepared by mixing them in the ratio of 4:1:1. There was no phase separation in either of these developers and they were used soon after preparation. The sugar spots were developed by spraying the air-dried chromatogram with aniline hydrogen phthalate (*10*) and carefully redrying at 105° C. for not exceeding 5 minutes. The sugars show up as brown or pink spots, depending on the sugar and the time of heating.

Investigation of Cabernet Sauvignon Pigments

When the Cabernet Sauvignon pigment extract was chromatographed using either of the butanol–acetic acid–water developers previously described, six well defined pigment spots were observed. The R_f values, colors when air-dried, visual estimate of the relative amounts, and colors when sprayed with 2% aqueous phosphomolybdic acid for these spots are given in Table I.

The pigment in spot B (R_f 0.63) gave reactions typical of an anthocyanidin and was shown to be malvidin. The pigment could be extracted from a 1% hydrochloric acid solution by amyl alcohol. No sugar moiety was found when the pigment was boiled with acid. The pigment and an authentic sample of malvidin moved at R_f 0.61 in butanol–27% aqueous acetic acid, at R_f 0.57 in butanol–acetic acid–water (4:1:5), and at R_f 0.85 in cresol–acetic acid–water. In each case the color of the grape pigment was identical with that of the authentic malvidin sample.

Investigation of the pigment in spot C (R_f 0.54) indicated that it was malvidin-3-glucoside. Acid hydrolysis of the pigment gave an anthocyanidin and a sugar moiety identified chromatographically as glucose. The anthocyanidin from spot C, synthetic malvidin, and grape malvidin (spot B) when chromatographed under the same conditions all moved at R_f values of 0.58 to 0.61 in butanol–27% aqueous acetic acid, at R_f values of 0.54 to 0.56 in butanol–acetic acid–water (4:1:5), and at R_f values of 0.83 to 0.86 in cresol–acetic acid–water. In each case the color of the anthocyanidin from spot C was identical with that of malvidin. The sugar moiety of the anthocyanin was compared chromatographically with glucose, fructose, ribose, xylose, arabinose, galactose, rhamnose, and mannose in each of four of the developing solvents previously listed. In all cases only glucose moved at the same R_f as the unknown sugar. The R_f values obtained for glucose and the unknown sugar are listed in Table II.

The R_f value of the original anthocyanin using butanol–acetic acid–water (4:1:5) was 0.39. Bate-Smith (*5*) has reported an R_f value of 0.40 for malvidin-3-glucoside, using butanol–acetic acid–water (4:1:5) as the developing solvent after spotting the pigment on the paper in a 1% aqueous hydrochloric acid solution. In the present work the pigments were spotted on the paper in a 2% methanolic hydrochloric acid solution before development of the chromatogram. Bate-

Table II. R_f Values for Sugar Moiety from Spot C

	Butanol–27% Aqueous Acetic Acid	Butanol–Acetic Acid–Water (4:1:1)	Cresol–Acetic Acid–Water	Acetic Acid–Pyridine-Water
Unknown sugar	0.28	0.17	0.27	0.76
Glucose	0.27	0.18	0.27	0.76

Smith (*5*, *6*) has shown that the composition of the extracting solvent in which the pigment is applied to the paper affects the R_f values which are obtained. An increase in hydrochloric acid concentration, for example, caused a decrease in R_f value. Thus, because of the variations in conditions, the above R_f value and the other R_f values reported by Bate-Smith which are listed below cannot be expected to be strictly comparable to the R_f values obtained in the present investigation. However, a thorough consideration of the results obtained by Bate-Smith, and in particular a comparison of the R_f values obtained for two pigments which were chromatographed in both laboratories, indicate that the R_f values observed in the two laboratories are of the same order of magnitude, with the values observed in the present investigation expected to be slightly lower.

Acid hydrolysis of the pigment in spot D(R_f 0.42) also gave malvidin and only one sugar, identified chromatographically as glucose. The comparative R_f values for the anthocyanidin from spot D with synthetic malvidin and grape malvidin (spot B) and for the sugar moiety from spot D with glucose were similar to those listed in the discussion of the identification of the pigment in spot C. The position of this anthocyanin on the chromatogram indicated that the pigment molecule must contain two glucose units and the R_f value also indicated that the anthocyanin was a 3,5-diglucoside rather than a bioside. Bate-Smith (*5*) has pointed out that the fall in R_f value due to the addition of a sugar group is additive, but that the formation of a bioside causes a much smaller drop in R_f value than that of a diglucoside. The observed R_f value for the original anthocyanin in spot D using butanol–acetic acid–water (4:1:5) was 0.21, while the value reported by Bate-Smith (*5*) for malvin was 0.22. The experimental evidence thus indicated fairly conclusively that this grape pigment was malvidin-3,5-diglucoside (malvin).

The pigment in spot E (R_f 0.32) was hydrolyzed in acid solution to give an anthocyanidin and a single sugar moiety. The sugar was shown by comparative chromatography to be glucose, the comparative R_f values being similar to those listed in Table II. The anthocyanidin and the original anthocyanin gave evidence of two free adjacent hydroxyl groups when sprayed with a solution of phosphomolybdic acid. The anthocyanidin was chromatographically identical with a sample of synthetic petunidin; each moved with an R_f value of 0.55 in butanol–27% aqueous acetic acid and of 0.58 in cresol–acetic acid–water. The approximate R_f value of the anthocyanin in butanol–acetic acid–water (4:1:5) was 0.15, a value between the R_f values reported by Bate-Smith (*5*) for malvin, 0.22, and for delphinidin-3,5-diglucoside, 0.11. The R_f value also was less than the values reported by Bate-Smith for delphinidin-3-monoside, 0.16, and for malvidin-3-glucoside, 0.40. Since the methylation of a hydroxyl group causes a rise in R_f value (*5*), the R_f value of this grape anthocyanin should fall about halfway between the R_f values reported for the delphinidin and malvidin-3-glucosides if the grape pigment were petunidin-3-glucoside. Actually, the R_f value for the pigment in spot E falls about halfway between the values reported for malvin and delphinidin-3,5-diglucoside, which suggests that the pigment under consideration is a petunidin diglucoside. Unfortunately, no R_f values for petunidin mono- and diglucosides were available in the literature for reference and no authentic samples of petunidin glucosides were available in this laboratory for chromatographic comparison. This pigment was one of the least abundant ones in the grape skins, so that further attempts to identify it by classical means appeared to offer little chance of success.

Acid hydrolysis of the pigment in spot F (R_f 0.25) gave an anthocyanidin which was shown to be delphinidin, and glucose as the only sugar moiety. The sugar was identified as glucose by the methods described, in which R_f values similar to those listed in Table II were obtained. The anthocyanidin was chromatographed alongside a sample of synthetic delphinidin to give the following R_f values in the various solvents: butanol–27% aqueous acetic acid, 0.51, 0.50; butanol–acetic acid–water (4:1:5), 0.42, 0.42; cresol–acetic acid–water, 0.15, 0.16. Both the original anthocyanin and the anthocyanidin obtained by hydrolysis gave blue colors when sprayed with phosphomolybdic acid solution, indicating two free adjacent hydroxyl groups. When subjected to Robinson's (*16*) solvent partition techniques, the pigment behaved like a diglucoside. Although no delphinidin-3,5-diglucoside was available for comparison, the grape anthocyanin was chromatographed against an extract of eggplant skin which has been reported (*20*) to contain delphinidin-3-glucoside. The R_f values are shown in Table III. The grape anthocyanin was obviously not chromatographically identical with the pigment from the eggplant skins. The experimental evidence thus indicated that the grape anthocyanin was probably delphinidin-3,5-diglucoside rather than delphinidin-3-glucoside.

Table III. R_f Values in Investigation of Pigment in Spot F

	Butanol–Acetic Acid–Water (4:1:5)	Cresol–Acetic Acid–Water
Grape anthocyanin	0.11	0.05
Eggplant extract	0.16	0.09
Delphinidin-3-glucoside[a]	0.16	0.11
Delphinidin-3,5-diglucoside[a]	0.11	0.03

[a] R_f values reported by Bate-Smith (*5*).

The pigment spot at R_f 0.82, spot A, was extremely faint and attempts at identification were greatly hindered by the lack of material. The pigment failed to give evidence of two free adjacent hydroxyl groups and, as it moved ahead of malvidin on chromatography using butanol–27% aqueous acetic acid, was thought possibly to be a more highly methylated pigment than malvidin, such as hirsutidin. Chromatographic comparison with an authentic sample of hirsutidin (see Table IV) showed definitely that the grape pigment was not hirsutidin, however. Basic hydrolysis of the grape pigment at a spot A yielded another pigment which was chromatographically identical with the grape malvin of spot D. Acid hydrolysis of an eluted methanolic hydrochloric acid extract of this new material or acid hydrolysis of the original pigment at spot A gave an anthocyanidin which was chromatographically identical with synthetic malvidin. These results are summarized in Table IV.

When an aliquot of the material from the basic hydrolysis of the pigment in spot A was chromatographed using butanol–acetic acid–water (4:1:5) and the developed chromatogram was sprayed with an indicator solution, an acidic spot was observed at an R_f value of 0.48. The indicator used was a 0.2% solution of bromocresol blue in 95% ethyl alcohol in which the pH was adjusted to 6.5 by the addition of sodium hydroxide solution. This acid spot was compared

with a number of authentic acids which are commonly present in plant materials and was apparently chromatographically identical with malic acid as shown in Table IV. The reaction of this spot when sprayed with an aqueous ferric chloride solution and the appearance under ultraviolet light both before and after fuming with ammonia were also identical with the results observed with malic acid. It is difficult, however, to reconcile the comparatively low R_f values of malic acid and malvin with the high R_f value of the complex pigment of spot A. A study of molecular models shows that it is possible for malic acid to form a diester involving both sugar molecules in malvin. Formation of such a diester would result in the formation of an additional ring system, which might in turn be expected to decrease the solubility of the compound in water and thus lead to a greater R_f value when chromatographed.

Identification of Pigments in Cabernet Sauvignon Progeny and Related Crosses

Because a number of the Cabernet Sauvignon progeny whose pigments were investigated had the Carignane grape as one parent, the pigments of the Carignane grape were compared with those of the Cabernet Sauvignon. Chromatographic comparison using the three developers described showed the pigment content of these two varieties to be qualitatively identical. The same six pigment spots were observed for Carignane, with R_f values identical to those observed for the Cabernet Sauvignon pigments. There was some variation in the relative amounts of the various pigments in the two varieties, however.

The pigment content of 14 Cabernet Sauvignon selfs, 38 Carignane selfs, and 20 seedling progeny of Cabernet Sauvignon crossed with Carignane crossed with the white St. Emilion was investigated by chromatographic comparison with their colored parents. All colored progeny showed a qualitative pigment pattern identical with that of the parents. Six pigment spots were observed in each case with approximately the same R_f values as those previously given for the pigments in Cabernet Sauvignon. Some visual quantitative differences were observed both in the individual pigment content within a given cross and in the over-all pigment content between crosses, but these differences were not investigated further.

Order of Development of Pigments of Cabernet Sauvignon Grape

The mature Cabernet Sauvignon grape contains malvidin and two malvidin glucosides. In view of the evidence presented by Bate-Smith (*5*) that in *Coreopsis*, glucosides form in the maturing plant at the expense of aglycones, the order of appearance of the grape pigments as the color developed in the grape skin was investigated. A maturing cluster of Cabernet Sauvignon grapes was found which had berries at different stages of pigmentation, from completely green to the deep purple color of maturity. The berries were divided into five color classes based on the degree of color development, and the skins of these berries were examined chromatographically for pigment composition using butanol–acetic acid–water (4:1:5). The first anthocyanin pigment to appear was the malvidin diglucoside. As the berries ripened, the malvidin monoglucoside and the delphinidin glucoside were the next to appear at about the same time; then the petunidin glucoside appeared. Free malvidin was not observed in the grape skins until one week after the berries had developed the purple-red color of complete maturity. The time of appearance of the complex diglucoside of malvidin was not noted. This order of appearance is contrary to the observations of Bate-Smith in the case of *Coreopsis*, where the anthocyanins developed at the expense of the corresponding anthocyanidins.

Acknowledgment

The authors wish to acknowledge with gratitude the gift of synthetic specimens of malvidin, petunidin, and delphinidin from E. C. Spaeth, and of an authentic sample of hirsutidin-3,5-diglucoside from K. Zarudnaya. The authors are also indebted to H. P. Olmo and E. Hinreiner for many helpful suggestions and to H. P. Olmo for supplying and identifying the various Cabernet Sauvignon progeny which were investigated.

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Received for review March 7, 1955. Accepted May 23, 1955. Taken from the doctoral dissertation presented by Albert H. Bockiau, June 1954.

Table IV. Comparison of R_f Values Used in Investigation of Spot A

Material	Butanol–27% Aqueous Acetic Acid	Butanol–Acetic Acid–Water (4:1:5)	Cresol–Acetic Acid–Water
Spot A	0.81	0.76	0.89
Hirsutidin	0.62	0.55	1.00
Basic hydrolyzate from spot A	0.43	0.22	0.75
Grape malvin (spot D)	0.42	0.22	0.75
Anthocyanidin from spot A	0.59	0.55	0.86
Synthetic malvidin	0.59	0.54	0.86
Acidic moiety from basic hydrolyzate from Spot A	...	0.48	0.16
Malic acid	...	0.48	0.16