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Investigations on Anthocyanins in Wines from *Vitis vinifera* cv. Pinotage: Factors Influencing the Formation of Pinotin A and Its Correlation with Wine Age

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Pinotage red wines were found to contain a reaction product of malvidin 3-glucoside and caffeic acid, the so-called pinotin A. A total of 50 Pinotage wines from the vintages 1996−2002 were analyzed for the content of pinotin A, malvidin 3-glucoside, caffeic acid, and caftaric acid. Statistical analyses were performed to reveal variations in the content of these compounds and to determine the factors that influence pinotin A formation during wine aging. An exponential increase of the concentration of this aging product was observed with prolonged storage time. The most rapid synthesis of pinotin A was observed in 2.5−4 year old wines, although at this age malvidin 3-glucoside is already degraded to a large extent. This phenomenon is explained by the increased ratio of caffeic acid/malvidin 3-glucoside, which strongly favors the formation of pinotin A and makes side reactions less likely. Pinotin A formation proceeds as long as a certain level of malvidin 3-glucoside is maintained in the wines. In wines >5−6 years old degradation or polymerization of pinotin A finally exceeds the rate of its de novo synthesis.

KEYWORDS: ***Vitis vinifera*; Pinotage; red wine; anthocyanins; malvidin 3-glucoside; caffeic acid; pinotin A; aging products**

INTRODUCTION

During wine aging the monomeric anthocyanins undergo various condensation reactions and the majority of them are finally incorporated into the heterogeneous and poorly characterized group of polymeric pigments (*1*). However, during the first steps in the formation of polymers monomeric anthocyanin derivatives as well as oligomeric pigments develop. An increasing number of these initial aging products have been isolated and structurally characterized in recent years. Vitisin A, a reaction product of malvidin 3-glucoside and pyruvic acid (formed by the action of wine yeasts during fermentation), was among the first aging products isolated from red wines (*2*, *3*). Several studies have shown that relatively high concentrations of vitisin A (5−6 mg/L) are present in wines shortly after fermentation. In the further course of wine aging (6−12 months of storage) the vitisin A content steadily declines (*4*−*7*). Further aging products evolve from the reactions of anthocyanins with flavanols and proanthocyanidins, thus forming ethyl-linked or vinyl-bridged oligomers (*8*−*11*). Quantitative data on their content in red table wines and port wines have not been published so far. It can only be assumed that the concentrations of individual compounds are rather low due to the high number of possible reaction products and the concomitant formation of polymeric pigments.

As the first member of yet another group of aging products, like the vitisins bearing an additional pyran ring between C-4 and the hydroxylic group attached to position 5 of the anthocyanin base structure, a malvidin 3-glucoside 4-vinylphenol adduct has been isolated and characterized (*12*). Structurally similar pyranoanthocyanins, namely the 4-vinylcatechol, 4-vinylguaiacol, and 4-vinylsyringol adducts of malvidin 3-glucoside, have been tentatively assigned after MS analysis of Shiraz red wines (*13*). These pigments were proposed to arise from the reaction of malvidin 3-glucoside with the respective 4-vinylphenols, which were themselves thought to be produced during fermentation by decarboxylation of the corresponding cinnamic acids through side activities of the wine yeast (*12*, *13*). For the first time we were recently able to isolate and structurally identify the adduct of malvidin 3-*O*-β-D-glucoside and 4-vinylcatechol, named pinotin A (*14*). Thorough investigations on the pathway of formation revealed that pinotin A is generated in a direct reaction between malvidin 3-glucoside and caffeic acid (**Figure 1**) (*15*). Similarly, we could show that the major route to the related 4-vinylphenol, 4-guaiacol, and 4-vinylsyringol adducts is through a direct reaction of malvidin 3-glucoside with coumaric acid, ferulic acid, and sinapic acid, respectively, without the need of prior decarboxylation of cinnamic acid derivatives by wine yeasts.

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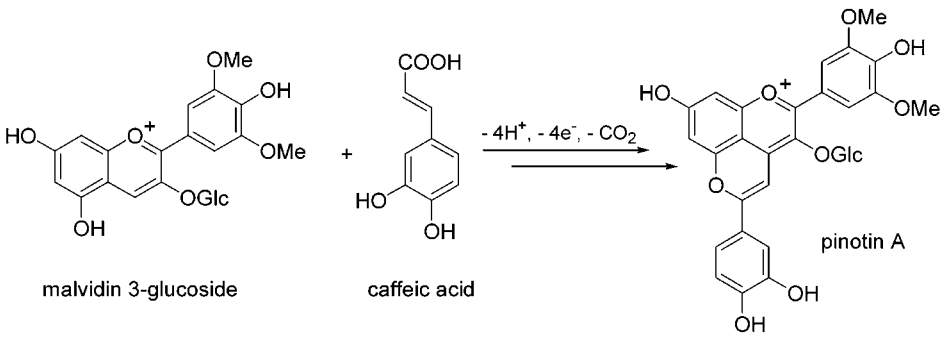

Figure 1. Formation and structure of pinotin A. For details on the reaction pathway refer to ref *15*.

Pinotin A is in no way unique to Pinotage wines, but occurs in all common red wine varieties in lower concentrations (unpublished data). In Pinotage wines its formation seems to be favored due to the unusually high concentration of caffeic acid, which we found to be typical for this variety. The slow chemical reaction pathway (*15*) leading to pinotin A together with its higher pH stability makes the pigment potentially attractive to be used as an aging indicator for red wines.

The aim of the present study was an investigation of factors influencing the formation of pinotin A in Pinotage red wines. Apart from wine age, the influence of the concentrations of the direct precursors caffeic acid and malvidin 3-glucoside as well as of the structurally related caftaric acid was examined.

MATERIALS AND METHODS

Chemicals. Caffeic acid (>99%) was purchased from Sigma-Aldrich (Germany). 2-*O*-Caffeoyltartaric acid (caftaric acid) was isolated from Riesling wine (*16*) and malvidin 3-glucoside from various red wines by countercurrent chromatography (CCC) (*17*, *18*). An authentic pinotin A reference standard was recently isolated from Pinotage wines by CCC and purified by semipreparative HPLC (*14*). Purity and identity of the isolated compounds were confirmed by HPLC-DAD, HPLC-ESI-MS^n, and NMR.

Wine Samples. A total of 50 Pinotage wines (vintages 1996 to 2002) were either purchased from local supermarkets and wine stores or obtained during routine import controls by German food control authorities.

Chromatographic Analysis. Wines were analyzed by HPLC with diode array detection. A PU-980 Intelligent HPLC pump equipped with a DG-980-50 three-line degasser, LG-980-02 ternary gradient unit, and MD-1510 multiwavelength detector were used (Jasco, Germany). Samples were injected via a Rheodyne 7175 injection valve (Techlab, Germany) equipped with a 20 μL loop, and separations were carried out on a 250 × 4.6 mm i.d., 4 μm, Synergi MaxRP-12 column (Phenomenex, Germany). Solvents were water/acetonitrile/formic acid (87:3:10, v/v/v, solvent A; 40:50:10, v/v/v, solvent B), and the flow rate was 0.5 mL/min. The linear gradient was from 6 to 20% B at 0−20 min, from 20 to 40% B at 20−35 min, from 40 to 60% B at 35−40 min, from 60 to 90% B at 40−45 min, and held at 90% B at 45−50 min.

Identity of the peaks was confirmed by comparison of retention time, UV/vis spectra, and mass spectrometric data (*15*) with authentic reference compounds.

For quantification, calibration curves for caffeic acid (at 323 nm), malvidin 3-glucoside, and pinotin A (at 510 nm) were obtained in the appropriate concentration ranges. Details on the calibration range, number of calibration points, and linear correlation coefficients are provided as Supporting Information. The calibration curve for caffeic acid was also applied to the quantification of caftaric acid taking into account the differing molecular weight. All wines were analyzed in autumn 2002 (i.e. a wine of vintage 2002 was 6 months old at the time of analysis).

Statistical Analysis. Statistical analysis was performed with STATISTICA version 6 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Qualitative and Quantitative Analysis of Wine Samples. A typical chromatogram of an 18 months old Pinotage wine is shown in **Figure 2**. Malvidin 3-glucoside and its acylated derivatives were the predominant anthocyanins, while the peonidin-derived pigments were only present in minor amounts. Apart from the anthocyanins regularly encountered in red wines, the aging product pinotin A (peak 8) eluted just prior to malvidin 3-(6″-coumaroylglucoside) (peak 9). It is noteworthy that depending on the chromatographic conditions applied (e.g. solvent systems, column material) these two peaks eluted together or even in reversed order. In addition, pinotin A could be mistaken for peonidin 3-(6″-coumaroylglucoside) (peak 7) during routine analysis of wines, if the peak identity is not supported by mass spectrometric data (*14*, *15*). By using a diode array detector pinotin A can be unequivocally identified as its visible absorbance maximum is hypsochromically shifted from 530 nm (typical for pigments with a malvidin aglycone) to 510 nm (*19*). Caffeic acid and caftaric acid were the major hydroxycinnamic acids encountered in Pinotage wines.

The concentrations of malvidin 3-glucoside, pinotin A, caffeic acid, and caftaric acid in all analyzed wines are summarized in **Table 1**. Average values and standard deviations sorted by vintage are presented in **Table 2** (years 1996, 1997, and 2002 are not included due to the low number of data sets).

Composition Changes with Wine Age. The box plots in **Figure 3** present side-by-side the distributions of malvidin 3-glucoside, pinotin A, caffeic acid, and caftaric acid in the wines of different ages.

Malvidin 3-Glucoside. An average of 48 mg/L of malvidin 3-glucoside were detected in 1.5 year old Pinotage wines, approximately half of the concentration found in a young wine (6 months old). The content of malvidin 3-glucoside decreased with age. Only 12 mg/L were left in wines stored for 4.5 years, and 5−6 mg/L remained in wines of the two preceding vintages. These values are largely concordant with data obtained for Cabernet Sauvignon wines produced in the period of 1987 to 2002 (*7*). The course of malvidin 3-glucoside degradation (**Figure 3**) was not linear but exponential, since the largest losses were observed in the first years. An analysis of variance (ANOVA) showed a significant difference in the log-transformed concentration of malvidin 3-glucoside for different vintages ($p < 0.0001$). Using Tukey-Kramer HSD pairwise comparisons for unequal sample sizes, cf. for example ref *20*, we detected significant differences in the average concentrations for vintages that were at least two years apart, and also between 2000 and 2001 ($p < 0.01$).

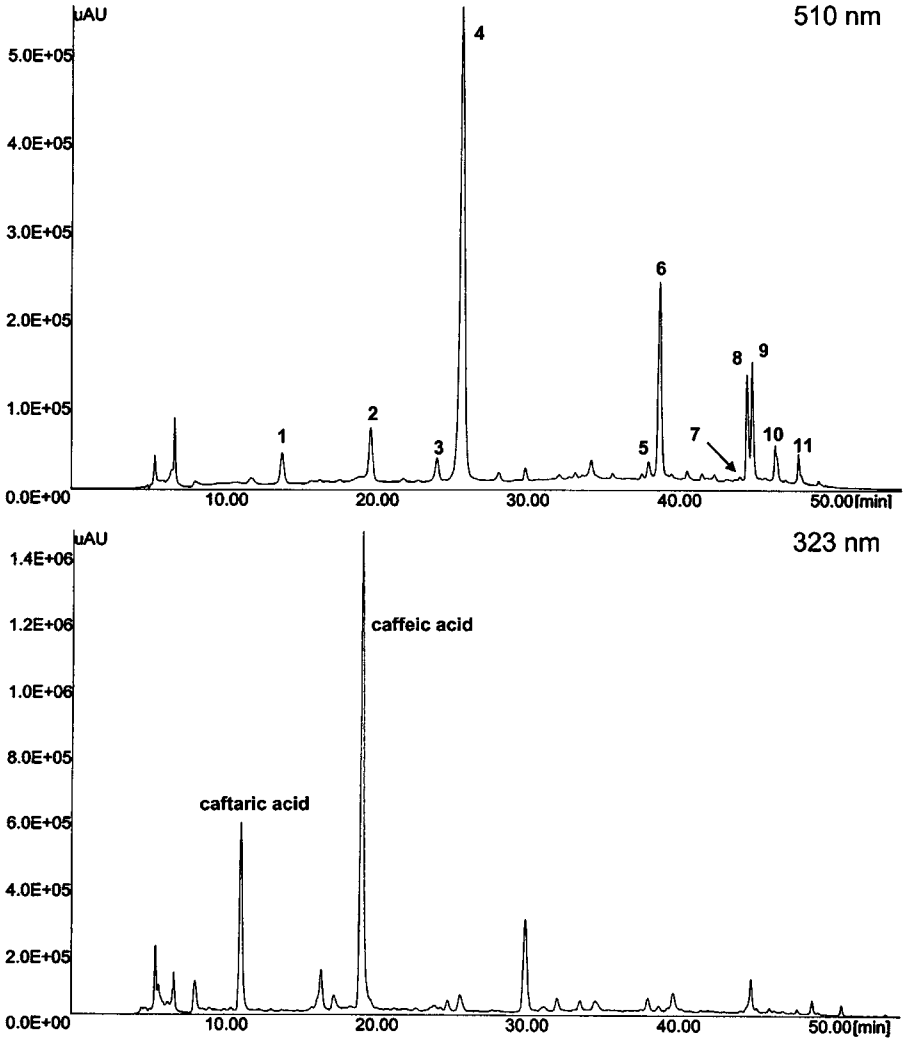

Figure 2. Typical HPLC chromatogram (extracted from diode array data) of an 18 months old Pinotage wine (vintage 2001). Anthocyanins were detected at 510 nm (top), cinnamic acids and derivatives at 323 nm (bottom): peak 1 = delphinidin 3-glucoside; peak 2 = petunidin 3-glucoside; peak 3 = peonidin 3-glucoside; peak 4 = malvidin 3-glucoside; peak 5 = peonidin 3-(6″-acetylglucoside); peak 6 = malvidin 3-(6″-acetylglucoside); peak 7 = peonidin 3-(6″-coumaroylglucoside); peak 8 = pinotin A; peak 9 = malvidin 3-(6″-coumaroylglucoside); peak 10 = 6″-acetylpinotin A; peak 11 = 6″-coumaroylpinotin A.

Most of the malvidin 3-glucoside initially present was converted into polymeric pigments (*1*, *21*), since no other discrete peaks were formed during aging. However, it can be assumed that various wine constituents (e.g. low molecular yeast metabolites, anthocyanins, flavanols) can serve as potential reaction partners for malvidin 3-glucoside (including intermolecular self-polymerization). As all of these compounds are at their peak concentration in young wines they enable a wide range of condensation reactions, thus explaining the rapid loss of malvidin 3-glucoside shortly after bottling (*22*). With wine aging, the concentration of the reactants decreases and the reaction slows down. The maximum rate of oxidative degradation of malvidin 3-glucoside would also occur in young wines, as the concentration of dissolved oxygen is at its peak level directly after bottling.

Pinotin A. The pinotin A concentration showed, in contrast to malvidin 3-glucoside, an exponential increase. Only 0.4 mg/L of pinotin A was present in a young wine (vintage 2002; 6 months old). The concentration increased with age and reached its maximum with an average concentration of 10.5 mg/L in wines of vintage 1998 (4.5 years old). Afterward, a degradation of pinotin A was observed, although due to the low number of data sets available for wines aged 5.5 years and older it was not possible to determine the exact time when the decrease set in. An ANOVA for the log-transformed concentrations of pinotin A showed that there are significant differences between different vintages, with older wines showing higher concentrations. Pairwise comparisons with Tukey-Kramer HSD for unequal sample sizes gave significant mean differences between 1998 vs 2000 and 1998 vs 2001 ($p < 0.02$). With larger sample sizes yearly differences would probably become more apparent.

Caffeic Acid and Caftaric Acid. Pinotage wines are characterized by unusually high concentrations of caffeic acid. The caffeic acid content in most common red wine varieties does not exceed 10 mg/L (*23*−*25*), whereas in Pinotage wines we detected as much as 77 mg/L, a value that was confirmed in a very recent study on Pinotage wines of various vintages by Rossouw and Marais (*26*). The amount of caftaric acid is also elevated. Landrault et al. (*25*) reported an average content of 51 mg/L in various French red wines, while Burns et al. (*27*) found mean values of 36 mg/L in red wines of different varieties and origins. The overall average caftaric acid concentration in the Pinotage wines examined in our survey was 66 mg/L, ranging from as low as 1.7 mg/L up to 164.2 mg/L. The concentration of caffeic

Table 1. Content [mg/L] of Malvidin 3-Glucoside, Pinotin A, Caffeic Acid, and Caftaric Acid in the 50 Analyzed Pinotage Wines from 1996 to 2002

sample	vintage	malvidin 3-glucoside	pinotin A	caffeic acid	caftaric acid
1	1996	6.90	1.13	11.92	85.89
2		6.06	2.74	12.50	82.78
3	1997	5.69	11.78	28.79	38.64
4		2.82	6.98	57.43	20.50
5	1998	10.09	10.80	36.85	3.25
6		18.30	5.66	28.54	70.58
7		14.73	14.10	37.33	39.11
8		9.56	17.93	33.78	36.42
9		13.31	4.37	10.43	127.68
10		8.89	9.96	33.21	44.05
11	1999	19.45	7.29	35.58	37.52
12		19.97	7.07	59.13	36.83
13		26.72	3.83	39.28	66.43
14		15.97	6.91	39.06	10.40
15		31.99	2.53	18.22	80.22
16		15.16	7.70	54.35	3.41
17		15.17	4.33	11.56	84.11
18		23.20	5.34	21.68	95.43
19	2000	38.95	1.19	6.92	106.35
20		25.02	4.86	59.38	38.94
21		21.05	10.08	32.94	25.83
22		9.65	1.76	14.81	81.85
23		18.04	2.41	17.25	68.66
24		23.72	2.86	77.06	9.83
25		7.89	2.33	20.73	164.17
26		26.00	4.82	28.12	101.01
27		36.56	3.58	48.78	67.23
28		35.12	3.62	32.98	70.46
29		40.84	2.65	44.28	67.34
30		25.53	2.91	20.21	81.35
31		25.05	2.30	57.94	1.72
32		38.36	2.02	20.18	98.95
33		22.70	4.10	50.36	66.29
34		40.80	2.25	30.23	96.20
35		22.79	5.29	53.46	3.80
36	2001	28.72	4.66	38.36	62.06
37		57.81	0.33	15.62	56.35
38		39.64	4.08	58.72	44.30
39		59.83	0.26	14.19	97.02
40		32.09	6.47	62.02	1.49
41		31.34	0.15	1.98	113.61
42		44.00	1.24	26.24	88.20
43		52.37	3.04	53.29	64.41
44		39.41	2.19	34.67	118.54
45		41.82	0.95	10.43	141.77
46		67.53	7.63	62.94	42.90
47		88.31	2.21	26.18	137.78
48		45.16	2.18	30.04	49.71
49		49.07	1.28	15.50	82.06
50	2002	84.87	0.42	8.93	133.38

acid stayed relatively constant during aging and the average content in the wines fluctuated only slightly between 30 and 36 mg/L. In contrast, the average amount of caftaric acid decreased (although not statistically significant) by approximately 35% after storage for 1.5−4.5 years. This observation can be explained by the fact that tartrate esters of hydroxycinnamic acids hydrolyze slowly during wine aging (*28*, *29*). Through this process a stable level of free caffeic acid is maintained, while at the same time the amount of caftaric acid is reduced. The ratio between caffeic acid and caftaric acid is also known to be influenced by winemaking technology. Grapes do not contain free caffeic acid, but the more water soluble caftaric acid (*30*, *31*). Elevated fermentation temperatures and especially the use of pectolytic enzymes with cinnamoyl esterase side activities would favor the release of caffeic acid from its precursor. Such enzymes are commonly applied during winemaking to improve the color extraction from the grapes. Details on the vinification procedure for the analyzed wines are not known; however, it can be concluded that wines which possess extraordinary high concentrations of free caffeic acid and at the same time only minor amounts of caftaric acid were subjected to such kinds of treatment (e.g. samples 5, 16, 24, 31, 35, and 40). Wines showing the reverse extreme, a high concentration of caftaric acid and only minor amounts of caffeic acid, were most likely produced at lower fermentation temperatures, not treated with enzymes or only with enzymes lacking cinnamoyl esterase activity (e.g. samples 9, 19, 41, 45, and 50). Our data suggest that Pinotage grapes provide a pool of approximately 400 μmol/L of caffeic acid, which is extracted during crushing and fermentation. The ratio between the free and the bound form then evolves depending on the vinification conditions applied and age (**Figure 4**).

Factors Influencing the Formation of Pinotin A. Young wines contain the highest concentrations of malvidin 3-glucoside, one of the two educts required for pinotin A formation (**Figure 1**). According to the law of mass action one would expect that a high concentration of malvidin 3-glucoside leads in turn to a rapid synthesis of pinotin A, but exactly the opposite is the case. The fastest degradation rates for malvidin 3-glucoside and the lowest concentrations of pinotin A were observed in wines up to 2.5 years of age, while the superproportional formation of pinotin A set in afterward, when the major part of the initially present malvidin 3-glucoside had already been consumed, obviously through other reaction pathways. A possible explanation for the rapid decrease of malvidin 3-glucoside in young wines due to the presence of numerous other reaction partners has already been mentioned. By taking into account that their overall initial concentration certainly exceeds the concentration of caffeic acid it can be explained why the formation of pinotin A cannot compete with these alternative reactions during the first years of storage. Hence, the majority of malvidin 3-glucoside is consumed elsewhere. With further aging the scenario changes. The concentration of the interfering reactants decreases, side reactions become less likely, and due to the lower concentration of malvidin 3-glucoside self-polymerization is reduced. At the same time the content of caffeic acid remains stable and the average molar ratio of caffeic acid to malvidin 3-glucoside climbs from 1.8:1 (after 1.5 years) to 6.6:1 (after 4.5 years) and strongly favors the direct reaction between these two components. This combination of a stable amount of caffeic acid with a sufficient concentration of malvidin 3-glucoside and less interference from other components can explain the exponential increase in pinotin A content in aged wines. Starting at a certain age, a linearly increasing amount of (replenishing) caffeic acid is slowly transformed into pinotin A. Therefore new pinotin A develops at a linear rate, and hence, the overall pinotin A concentration grows exponentially. Further evidence for this hypothesis is drawn from the fact that the proportion of malvidin 3-glucoside transformed into pinotin A was greatly enhanced in the older wines, confirming the lack of competing reactions. Between 1.5 and 2.5 years of age an average of 43.47 μmol/L (21.43 mg/L) of malvidin 3-glucoside degraded in the wines while only 1.36 μmol/L (0.85 mg/L) of pinotin A were formed. This low conversion rate of malvidin 3-glucoside into pinotin A increased from 3.1% to 28.4% during the following year and further to 45.1% between 3.5 and 4.5 years of age. The actual rates are probably even higher, as it should be taken into account that pinotin A is not

Table 2. Mean Values, Standard Deviations (SD), and Coefficients of Variation (CV) for Malvidin 3-Glucoside, Pinotin A, Caffeic Acid, and Caftaric Acid in the 45 Pinotage Wines from Vintages 1998–2001

		malvidin 3-glucoside			pinotin A			caffeic acid			caftaric acid		
vintage	*n*	mean [mg/L]	SD [mg/L]	CV [%]	mean [mg/L]	SD [mg/L]	CV [%]	mean [mg/L]	SD [mg/L]	CV [%]	mean [mg/L]	SD [mg/L]	CV [%]
1998	6	12.5	3.7	29.2	10.5	5.1	48.6	30.0	10.1	33.6	53.5	42.2	78.9
1999	8	21.0	6.0	28.8	5.6	1.9	33.9	34.9	16.9	48.5	51.8	34.8	67.1
2000	17	26.9	10.2	37.7	3.5	2.1	59.4	36.2	19.3	53.2	67.7	42.2	62.4
2001	14	48.4	16.1	33.3	2.6	2.3	88.9	32.2	20.3	63.2	78.6	40.2	51.1

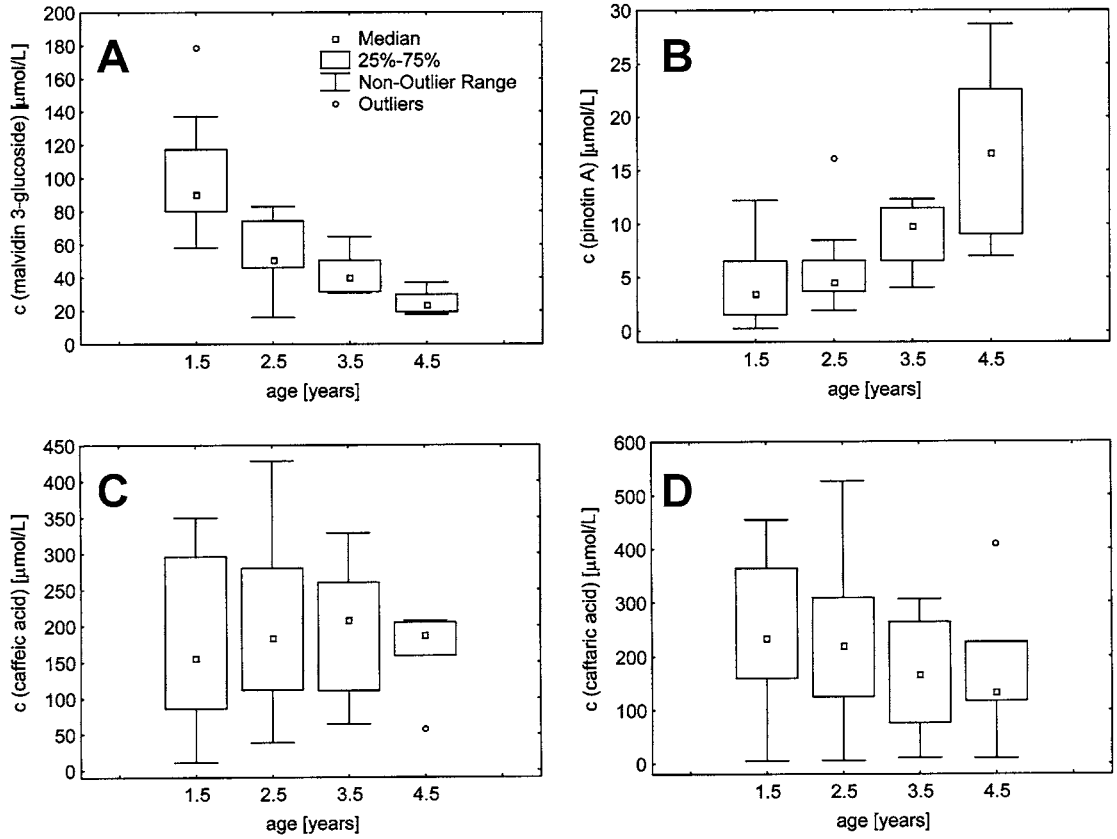

Figure 3. Concentration of (A) malvidin 3-glucoside, (B) pinotin A, (C) caffeic acid, and (D) caftaric acid in Pinotage wines aged for 1.5–4.5 years (vintages 1998–2001).

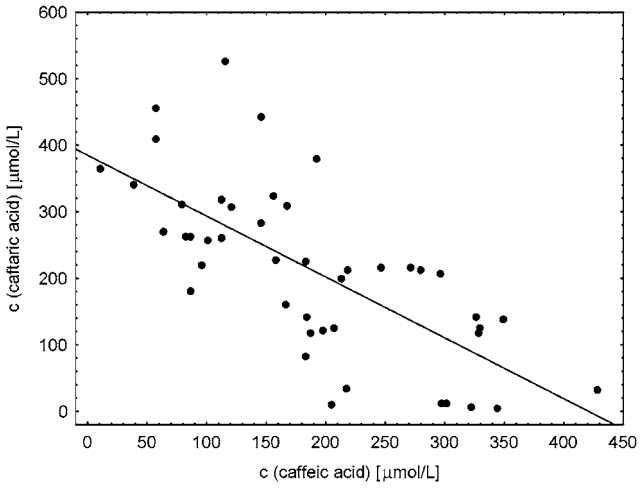

Figure 4. Relationship between caffeic acid and caftaric acid in the 45 Pinotage wine samples from 1998 to 2001.

necessarily a final product but can in turn be included into polymeric pigments, a process that takes place simultaneously with pinotin A formation. Although polymeric compounds containing a pinotin A substructure are not known, oligomeric compounds formed through reaction of vitisin A, another pyranoanthocyanin, with vinyl-flavanols in red port wines have recently been identified (*32*).

We attempted to quantitatively explain the pinotin A content using the concentrations of malvidin 3-glucoside, caffeic acid, and caftaric acid and the age of the wine. We transformed the concentrations by taking logarithms. Consequently, the data for log(pinotin A) resemble a normal distribution. Clearly, the pinotin A concentration increases with age, and both malvidin 3-glucoside and caffeic acid are necessary for its formation. However, a linear regression model using age and malvidin 3-glucoside provides a relatively poor fit ($R^2 = 39\%$). A model using age and caffeic acid behaves much better ($R^2 = 75\%$). Moreover, adding malvidin 3-glucoside or caftaric acid to the model does not significantly improve it (**Table 3**).

For a quantitative prediction of the pinotin A concentration (in μmol), we can use

$$\log(\text{pinotin A}) = -4.07 + 0.54 \times \text{age} + 0.84 \times \log(\text{caffeic acid})$$

Table 3. Model for the Quantitative Prediction of Pinotin A Concentration[a]

	estimated coeff	SD	*p* value
intercept	−4.07	0.57	<0.0001
age	0.54	0.07	<0.0001
log(caffeic acid)	0.84	0.11	<0.0001

[a] Regression summary: $R^2 = 75\%$. Response variable: log(pinotin A).

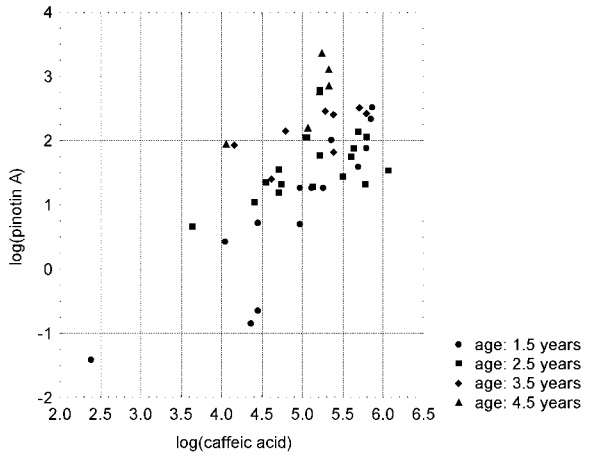

Figure 5. Correlation between caffeic acid and pinotin A (log-transformed concentrations). Vintage 2001 (age 1.5 years): $p < 0.0001$, $r = 0.89$. Vintage 2000 (age 2.5 years): $p = 0.017$, $r = 0.57$. Vintage 1999 (age 3.5 years): $p = 0.082$, $r = 0.65$ (only 8 data sets). Vintage 1998 (age 4.5 years): $p = 0.065$, $r = 0.78$ (only 6 data sets).

where the standard error of this model is 0.50, or the retransformed version:

$$\text{pinotin A (in } \mu\text{mol)} = \exp(-4.07 + 0.54 \times \text{age}) \times (\text{caffeic acid})^{0.84}$$

Our analysis shows that caffeic acid is a much stronger predictor of pinotin A concentration than malvidin 3-glucoside (in wines up to approximately five years of age) and the regression model indicates that the formation of pinotin A is largely controlled by caffeic acid. This hypothesis is affirmed by the strong correlation between the log-transformed concentrations of caffeic acid and pinotin A in the wines of different ages (**Figure 5**). The highly significant correlation between caffeic acid and pinotin A observed in the young vintage 2001 wine emphasizes the importance of a high caffeic acid concentration for pinotin A formation in the presence of competing reaction partners. Malvidin 3-glucoside only becomes crucial when it falls below a certain threshold, as the pinotin A content in red wines can only increase as long as the formation rate of pinotin A over-compensates the simultaneous incorporation into polymers. This also explains the drop in pinotin A concentration in the samples from vintage 1997 and 1996. When the level of malvidin 3-glucoside fell below a certain point, which according to our data was approximately between 5 and 10 mg/L, degradation of pinotin A was more pronounced and its content decreased.

Caftaric Acid. The final contribution of caftaric acid to pinotin A production is difficult to assess. A direct reaction of caftaric acid with malvidin 3-glucoside to form pinotin A, with simultaneous cleavage of the tartaric acid moiety, is impossible, as we demonstrated earlier that caffeic acid esters cannot undergo this reaction (*15*). Through hydrolysis of caftaric acid, however, the amount of free caffeic acid (which is initially absent in the must, as only a pool of caftaric acid is provided by the grapes) is increased. A high concentration of caftaric acid is therefore favorable for pinotin A formation. However, the beneficial effect of a big pool of caftaric acid would certainly not exceed the positive effect that a similarly high amount of free caffeic acid would have on pinotin A formation. The interdependency of the free and bound form of caffeic acid has been discussed already.

It should not be overlooked that caftaric acid could also have a negative impact on the reaction rate. Due to its caffeic acid substructure caftaric acid may shield the reactive positions of malvidin 3-glucoside and inhibit or delay the reaction and formation of covalent bonds with caffeic acid. From our data the extent of this factor cannot be assessed. Model reactions with various amounts of caftaric acid in solutions containing constant concentrations of malvidin 3-glucoside and caffeic acid would need to be performed under controlled conditions.

Our data clearly indicate that pinotin A formation depends to a larger extent on the concentration of caffeic acid than on malvidin 3-glucoside. The high amounts of malvidin 3-glucoside present in young wines are rapidly degraded by various side reactions and only a very small percentage was converted into pinotin A. The superproportional formation of pinotin A commences when interfering reactions become less likely due to a lower concentration of the reactants, while the caffeic acid level remains rather stable throughout the aging process. A minimum concentration of 5−10 mg/L of malvidin 3-glucoside was required to maintain a reaction rate high enough to compensate for the simultaneous incorporation of pinotin A into polymeric pigments. The pinotin A content can increase until the wines are approximately 5 to 6 years old. Pinotin A is therefore a potential chemical marker for wine age, but an exact prediction of the vintage would require a higher number of previously analyzed data sets.

Pinotage red wines were the ideal object for this study as they exhibit unusually high concentrations of caffeic acid. Nonetheless, the formation of pinotin A is not limited to this cultivar and will proceed in all wines containing free caffeic acid. In wines with higher amounts of other hydroxycinnamic acids, structurally similar pyranoanthocyanins may form following the same reaction mechanism. The content of the resulting aging products in wines from other varieties will be the subject of future investigations.

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Supporting Information Available: Details on the calibration range, number of calibration points, and linear correlation coefficients for malvidin 3-glucoside, pinotin A, caffeic acid, and caftaric acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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