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HPLC−DAD−ESI-MS/MS Characterization of Pyranoanthocyanins Pigments Formed in Model Wine

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Supporting Information

ABSTRACT: Formation of wine pyranoanthocyanins in model wine was monitored by HPLC−DAD−ESI-MS/MS, using red grape skin extracts and wine fermentation metabolites (acetaldehyde, pyruvic and acetoacetic acids, and diacetyl) and also hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, and sinapic acids). Pyruvic acid and acetaldehyde reacted fast, the first reaching high product yield and the second inducing mainly pigment polymerization. In contrast, acetoacetic acid and diacetyl reacted slowly with poor product yields. Hydroxycinnamic acids progressively reacted without apparent formation of polymeric pigments, the reaction rate and yield increasing as the number of hydroxy/methoxy groups did. Substituent at C-10 strongly affected the visible maximum absorbance wavelength, whereas B-ring substitution pattern or sugar acylation exerted little effect. The 10-methylpyranoanthocyanins formed from acetoacetic acid were also found as side products in the formation of 10-carboxypyranoanthocyanins. Finally, we report for the first time on UV−vis and MS spectral data of 10-acetylpyranoanthocyanins formed from diacetyl, and their occurrence in commercial red wines is suggested.

KEYWORDS: pyranoanthocyanins, red wine, fermentation metabolites, diacetyl, hydroxycinnamic acids, LC−MS, UV−vis, vitisin

INTRODUCTION

In the last 2 decades the knowledge on anthocyanin-related red wine color chemistry has been importantly increased.[1] The earliest anthocyanin-derived compounds that were proposed and further found to occur in wine were polymeric pigments formed by reaction between anthocyanins and tannins. In addition, other structures of anthocyanin-derived pigments have been discovered, especially those called pyranoanthocyanins. Pyranoanthocyanins are a class of red wine pigment formed as early as during alcoholic fermentation and also over the aging of the wine. The general pathway of formation of pyranoanthocyanins involves an anthocyanin and a compound having a polarizable double bound (pyranoanthocyanin precursor) that react to give rise to a new pyrano ring (D-ring in Figure 1) fused to the anthocyanin molecule.[2] The first reported pyranoanthocyanins were those derived from some yeast metabolites (vitisin A from pyruvic acid, and vitisin B from acetaldehyde) but the list of possible nonanthocyanin reactants has notably increased, including nonphenolic and phenolic compounds. In recent years, some pyranoanthocyanins with the simplest structures (A-type vitisins or 10-carboxypyranoanthocyanins, and 10-methylpyranoanthocyanins) have been identified as the starting point for the formation of more complex pyranoanthocyanins,[3−7] or their transformation into other kinds of nonred pigments.[8,9]

The contribution of pyranoanthocyanin to total red wine color is still a matter of controversy, and one of the reasons is because only a few pyranoanthocyanins have been usually detected and quantified and there are only scarce data about their concentrations in wine.[10] Moreover, the unambiguous identification of the different pyranoanthocyanin types has been usually confirmed after isolation of the reaction product between only the main grape and wine anthocyanin (malvidin 3-glucoside) and the corresponding precursor, following the most favorable reaction conditions. However, the best pH and solvent conditions for the aforementioned synthesis reactions did not necessarily match those of real wine.[11−13] Because of the complex mixture of anthocyanins occurring in red wine, the formation of a great diversity of wine pyranoanthocyanins is expected, and interesting identification data for many of them are available.[14,15] However, as far as we know, there is not a systematic study of the chromatographic and spectral properties covering all the expected pyranoanthocyanin series according to the reactant precursor.

The aim of our work has been the contribution to a database of chromatographic (HPLC) and spectroscopic (DAD-UV−vis and MS/MS) data for a wide variety of most known kinds of pyranoanthocyanins that can be formed in wine. We developed the reactions between red grape skin extracts and pyranoanthocyanin precursors in a model wine medium (13% alcohol, v/v, and pH 3.5). We selected Syrah grapes because of their comparable proportions of both nonacylated and different acetylated and *p*-coumaroylated anthocyanins. With regard to pyranoanthocyanin precursors, we tried the reaction with known compounds of both nonphenolic (pyruvic acid, acetaldehyde, and acetoacetic acid) and phenolic (*p*-coumaric, caffeic, ferulic, and sinapic acids) types. Finally, we also assayed a previously unreported possible precursor, diacetyl, a secondary product of lactic acid bacteria metabolism.

MATERIALS AND METHODS

Chemicals. All solvents were of HPLC quality and water was of Milli-Q quality. Malvidin 3-glucoside (PhytoLab, Vestenbergsgreuth,

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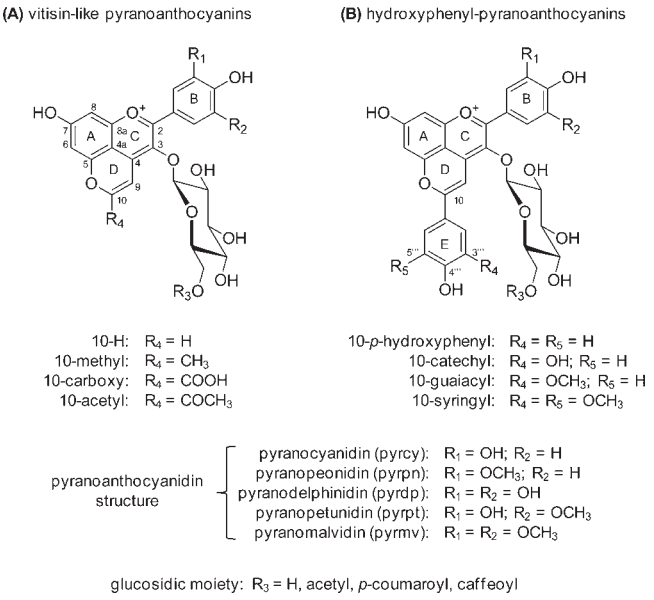

Figure 1. General structures of vitisin-like pyranoanthocyanins (A) and hydroxyphenylpyranoanthocyanins (B). Structural features: labeling of aromatic rings, substituent at C-10 (R_4 in D-ring for vitisin-like pyranoanthocyanins; R_4 and R_5 in E-ring for hydroxyphenylpyranoanthocyanins), B-ring substituent (R_1 and R_2), and type of acylation of the glucosidic moiety (R_3).

Germany) was used as standard for quantification of anthocyanins in red grape skin extracts. The following reagents were used as precursors for the formation of wine pyranoanthocyanins in model reactions: pyruvic acid (≥97%, Aldrich), acetaldehyde (≥99.5%, Fluka), lithium acetoacetate (≥90%, Fluka), 2,3-butanedione (≥99.0%, Fluka), caffeic acid (≥95%, Merck), *p*-coumaric acid (≥98.0%, Fluka), ferulic acid (≥99.0%, Fluka), and sinapic acid (≥99.5%, Phytolab, Vestenbergsgreuth, Germany).

Red Grape Skin Extracts. An amount of 1500 g of healthy red grapes (*Vitis vinifera* Cabernet Sauvignon, Petit Verdot, and Syrah cultivars), collected at technological maturity (estimated alcoholic strength of 13%, v/v), was finger pressed to remove the pulp and the seeds. The remaining skins were washed three times in water (Milli-Q) and softly dried twice by patting them between sheets of filter paper. The dried skins were extracting with 1000 mL of a mixture 50:48.5:1.5 (v/v) of methanol/water/formic acid[16] using a homogenizer (Heidolph DIAX 900) for 2 min, and the resulting slurry was centrifuged at 2500*g* at 5 °C for 15 min. After evaporation of methanol under reduced pressure (40 °C), the remaining aqueous solution was freeze-dried and the dried extract was stored (−18 °C) until use.

Model Reactions of Formation of Wine Pyranoanthocyanins. Wine pyranoanthocyanins formation was mimicked in model reactions using the grape skin extract containing anthocyanins and the corresponding reagent. Freeze-dried grape skin extract was dissolved in model wine (13% ethanolic water with 5 g/L of tartaric acid) to reach an anthocyanin concentration of 800 mg/L (as malvidin 3-glucoside) and the pH was then adjusted to 3.5. Syrah grape skin extract was used for all the model reactions, whereas Cabernet Sauvignon and Petit Verdot grape skin extracts were only used for repetitions of the reaction with pyruvic acid. The different reagents for inducing the formation of wine pyranoanthocyanins were individually added in a molar ratio 10:1 with regard to anthocyanins. Every model reaction was maintained at 30 °C in 60 mL screw-cap amber-glass bottles without headspace and was performed in triplicate. Monitoring of the reactions was extended over 9 weeks, and samples of 2.5 mL were weekly picking for HPLC analysis. To avoid oxidation, after each sampling small glass balls were added to replace the taken volume of reaction mixture, thus eliminating bottle headspace.

Analysis of Pyranoanthocyanins by HPLC−DAD−ESI-MS/MS. HPLC separation and identification of pyranoanthocyanins were performed on an Agilent 1100 Series system (Agilent), equipped with a DAD (G1315B) and LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS/MS) system and coupled to an Agilent Chem Station (version B.01.03) data-processing station. The mass spectra data were processed with the Agilent LC/MS Trap software (version 5.3). The wine samples were injected (50 μL) after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) on a reversed-phase column Zorbax Eclipse XDB-C18 (4.6 × 250 mm; 5 μm particle; Agilent), thermostated at 40 °C. The chromatographic conditions were adapted from the OIV method for analysis of anthocyanins in red wines,[17] and the detection wavelength was 510 nm. The 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 0.63 mL/min. The linear gradient for solvent B was 0 min, 6%; 15 min, 30%; 30 min, 50%; 35 min, 60%; 38 min, 60%; 46 min, 6%. For identification, ESI-MS/MS was used employing the following parameters: positive ion mode; dry gas, N_2, 11 mL/min; drying temperature, 350 °C; nebulizer, 65 psi; capillary, −2500 V; capillary exit offset, 70 V; skimmer 1, 20 V; skimmer 2, 6 V; scan range, 50−1200 *m*/*z*. Some previously obtained[10] standards of vitisin A (10-carboxypyranomalvidin 3-glucoside), pinotin A (10-catechylpyranomalvidin 3-glucoside), and 10-*p*-hydroxyphenylpyranomalvidin 3-glucoside were available for comparison and identification.

RESULTS AND DISCUSSION

Formation of Pyranoanthocyanins. Figure 1 show the different pyranoanthocyanin structures formed in the studied model reactions. It is possible to classify these pyranoanthocyanins into two basic types of structures: on one hand, those pyranoanthocyanins having nonphenolic substituent at C-10, the so-called vitisin-like pyranoanthocyanins, derived from the reaction of fermentation metabolites (acetaldehyde, pyruvic acid, acetoacetic acid, and diacetyl) with anthocyanins; on the other hand, hydroxyphenylpyranoanthocyanins or pyranoanthocyanins having an hydroxyphenyl-like moiety linked to C-10, as they were formed by reaction between anthocyanins and hydroxycinnamic acids present in wine (*p*-coumaric, caffeic, ferulic, and sinapic acids). Common names have been given to some pyranoanthocyanins (e.g., vitisin A, vitisin B, and pinotin A for the compounds formed by reaction of malvidin 3-glucoside with pyruvic acid, acetaldehyde, and caffeic acid, respectively). We suggest the following rules for naming these compounds: the basic structure formed by the rings A−B−C−D could be named as the pyrano-derivative of a given anthocyanidin (pyranoanthocyanidin; for instance, pyranomalvidin); the glucosidic moiety linked to the C-3 position [ring C; 3-glucoside or 3-(6″-acyl)glucosides] and the residue linked to the C-10 position (ring D) are then indicated as substituent of the basic pyranoanthocyanidin structure (e.g., 10-carboxypyranomalvidin 3-glucoside for the aforementioned vitisin A, or pyranomalvidin 3-glucoside for vitisin B). In the case of pyranoanthocyanins derived from hydroxycinnamic acids, the substituent at C-10 could be named as *p*-hydroxyphenyl (from *p*-coumaric acid; with 4‴-hydroxyphenyl as the C-10 substituent), catechyl (from caffeic acid; the residue resembles cathecol, with 3‴,4‴-dihydroxyphenyl as the C-10 substituent), guaiacyl (from ferulic acid; the residue resembles guaiacol, with a 3‴-methoxy-4‴-hydroxyphenyl as the C-10 substituent), and syringyl (fron sinapic acid; the residue resembles syringol, with 3‴,5‴-dimethoxy-4‴-hydroxyphenyl as the C-10

Table 1. HPLC Retention Times (min) of Vitisin-like Pyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-H, from acetaldehyde; 10-carboxy, from pyruvic acid; 10-acetyl, from diacetyl; 10-methyl, from acetoacetic acid)

pyranoanthocyanin structure[a]	10-H	10-carboxy	10-acetyl	10-methyl
pyrdp-3-glc	11.9	10.9	14.0	14.8
pyrdp-3-acglc	13.9	12.1	nd	17.1
pyrdp-3-cfglc	nd	14.1	nd	nd
pyrdp-3-cmglc	20.0	16.5	nd	nd
pyrcy-3-glc	14.5	13.4	nd	17.5
pyrcy-3-acglc	nd	15.2	nd	20.1
pyrcy-3-cfglc	nd	16.1	nd	nd
pyrcy-3-cmglc	22.5	19.5	nd	25.5
pyrpt-3-glc	16.3	15.0	18.1	19.1
pyrpt-3-acglc	18.4	16.5	20.4	21.4
pyrpt-3-cfglc	nd	18.1	nd	nd
pyrpt-3-cmglc	23.6	20.6	24.8	26.7
pyrpn-3-glc	18.8	17.6	20.2	21.7
pyrpn-3-acglc	21.5	19.7	23.1	24.5
pyrpn-3-cfglc	23.3	21.0	nd	26.1
pyrpn-3-cmglc	26.6	23.9	27.3	29.6
pyrmv-3-glc	20.2	18.7	22.1	23.0
pyrmv-3-acglc	22.3	20.5	24.5	25.4
pyrmv-3-cfglc	24.2	21.9	nd	27.1
pyrmv-3-cmglc	27.3	24.5	28.3	30.2

[a] pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

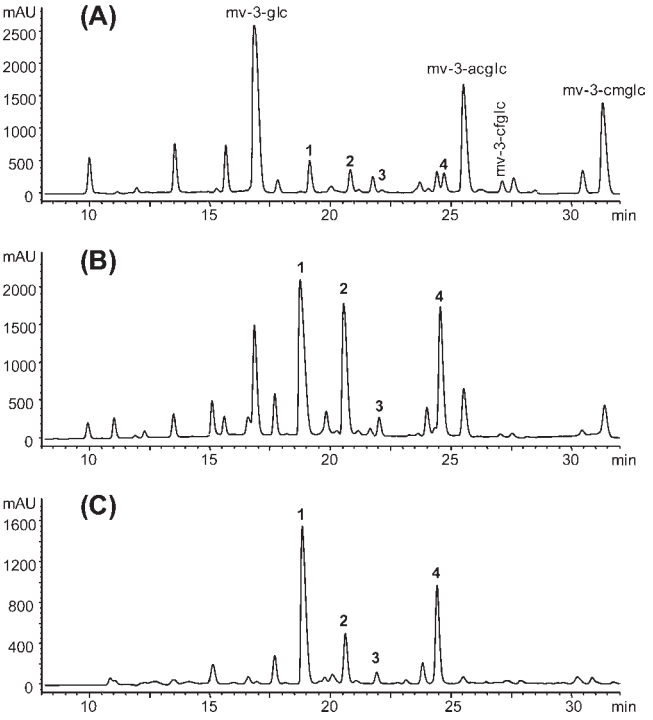

Figure 2. HPLC−DAD chromatograms (detection at 510 nm) for the reaction of pyruvic acid in a model red wine made from Syrah grape skin extract, after 1 day (A), 1 week (B), and 9 weeks (C) of reaction time at 30 °C. Peak assignations: mv-3-glc, mv-3-acglc, mv-3-cfglc, and mv-3-cmglc are the series of nonacyl, 6″-acetyl, 6″-caffeoyl, and 6″-*p*-coumaroyl derivatives of malvidin 3-glucoside; peaks 1−4 are the corresponding 10-carboxypyranoanthocyanins derived from the reaction between pyruvic acid and the aforementioned malvidin-type anthocyanins.

substituent); following these rules, pinotin A could be named as 10-guaiacylpyranomalvidin 3-glucoside.

With the exception of the vitisin-like pyranoanthocyanins derived from diacetyl (10-acetylpyranoanthocyanins), all the aforementioned types of compounds have been previously detected in model reactions and wines, especially those structures based on malvidin 3-glucoside. We are now reporting on the chromatographic and spectral (UV−vis and ESI-MS/MS) characteristics of almost all the possible structures that can be formed from each individual anthocyanin on the basis of the anthocyanidin B-ring substitution pattern and also the acylation or not of the glucosidic moiety. The selection of Syrah grape skin extract facilitated our objective because this grape cultivar contained high proportions of acylated anthocyanins (malvidin-based anthocyanins showed a molar distribution of 41.4% nonacylated, 21.4% acetylated, 33.2% *p*-coumaorylated, and 1.0% caffeoylated derivatives).

The formation of pyranoanthocyanins in model red wine followed two different trends: vitisin-like pyranoanthocyanins reached maximum yields in the first 1−2 weeks of reaction and further decayed, the initial anthocyanins being continuously decreasing until total disappearance. In contrast, the formation of hydroxyphenylpyranoanthocyanins was progressively increasing over time and parallel to anthocyanin decreasing, which not always totally disappeared after 9 weeks. In addition, remarkable differences in the yield of the reactions were observed in both cases. Moreover, the pyranoanthocyanin set formed from the same anthocyanidin structure (e.g., malvidin-type in their nonacylated and acylated forms) showed similar proportions than that initially found for the set of nonacylated and acylated anthocyanin precursors in grape skin extract [e.g., the set of 3-glucoside, 3-(6″-acetyl)glucoside, 3-(6″-*p*-coumaroyl)glucoside, and 3-(6″-caffeoyl)glucoside of malvidin] for all the reactions assayed. Therefore, the type of acylation of the sugar moiety linked to the anthocyanidin did not seem to exert an appreciable effect on the rate of formation of pyranoanthocyanins, although deeper investigation is needed to confirm this suggestion.

In parallel, we developed a control experiment using the same model wine but in the absence of any added reagent. We observed a disappearance of anthocyanins following a second-order polynomial evolution trend. The anthocyanin decrease was not total after the reaction time considered (34% of the initial anthocyanins still remained after 7 weeks of reaction) and developed without formation of any of the studied pyranoanthocyanins (see Supporting Information). In this control experiment, the decrease of anthocyanins was likely due to the formation of polymeric pigments following condensation of anthocyanins with mainly flavan-3-ols. In fact, direct condensation adduct of malvidin 3-glucoside and catechin was detected at *m/z* 781,[15] and precipitation of insoluble red-colored matter was observed.

Among vitisin-like pyranoantocyanin precursors, pyruvic acid reacted quickly and afforded the highest yield after 1 week (around 63%, based on the peak area of 10-carboxypyranomalvidin 3-glucoside with regard to the peak area corresponding to malvidin 3-glucoside at the beginning of the reaction), and all the

Table 2. HPLC Retention Times (nm) of Hydroxyphenyl-pyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-*p*-hydroxyphenyl, from *p*-coumaric acid; 10-catechyl, from caffeic acid; 10-guaiacyl, from ferulic acid; 10-syringyl, from sinapic acid)

pyranoanthocyanin structure[a]	10-*p*-hydroxyphenyl	10-catechyl	10-guaiacyl	10-syringyl
pyrdp-3-glc	25.6	22.5	26.7	27.9
pyrdp-3-cfglc	27.5	nd	28.5	nd
pyrdp-3-acglc	nd	24.2	28.6	29.5
pyrdp-3-cmglc	30.6	26.8	31.0	31.4
pyrcy-3-glc	28.9	25.8	30.1	31.3
pyrcy-3-cfglc	nd	27.6	32.1	nd
pyrcy-3-acglc	nd	27.9	32.3	33.3
pyrcy-3-cmglc	34.0	30.3	34.5	34.9
pyrpt-3-glc	30.5	27.3	31.7	33.0
pyrpt-3-cfglc	32.8	29.2	33.5	34.4
pyrpt-3-acglc	32.9	29.4	33.9	35.0
pyrpt-3-cmglc	35.5	32.2	35.9	36.6
pyrpn-3-glc	33.9	30.6	35.1	36.4
pyrpn-3-cfglc	36.2	32.9	37.0	38.1
pyrpn-3-acglc	36.6	33.2	37.6	38.7
pyrpn-3-cmglc	38.8	35.5	39.3	40.0
pyrmv-3-glc	35.0	31.7	36.1	37.5
pyrmv-3-cfglc	37.3	33.9	38.0	39.0
pyrmv-3-acglc	37.5	34.2	38.4	39.5
pyrmv-3-cmglc	39.5	36.3	40.0	40.6

[a] pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

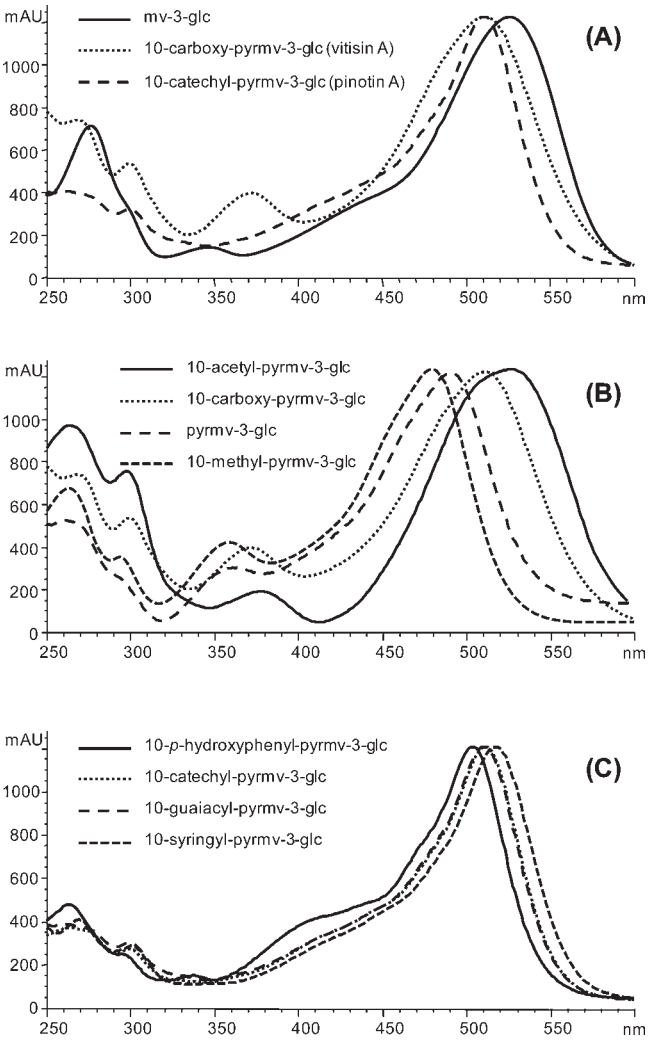

Figure 3. DAD online UV−vis spectra of malvidin 3-glucoside (mv-3-glc) vs its 10-carboxy- and 10-catechylpyranoanthocyanin derivatives (A), different vitisin-like pyranoanthocyanins derived from malvidin 3-glucoside (B), and different hydroxyphenyl-pyranoanthocyanins derived from malvidin 3-glucoside (C). Abbreviation: pyrmv-3-glc, pyranomalvidin 3-glucoside.

expected reaction products were detected by ESI-MS/MS (Table 1). Then, the concentration of 10-carboxypyranoanthocyanins was decreasing up to one-fifth of the maximum yield after 9 weeks (Figure 2) and the initial anthocyanins practically disappeared (0.5% remaining anthocyanins). Although the reactions with acetoacetic acid and diacetyl also developed with a quick and almost total disappearance of anthocyanins (less than 1% of initial concentrations remained after 9 weeks, on the basis of peak areas at 510 nm), they gave rise to poor yields (maximum yields around 2−3% after 1 week) and their reaction products were almost not detectable after 9 weeks, several of the expected compounds being undetectable if they derived from minor anthocyanins (Table 1). In the case of the reaction with acetaldehyde, the quick and total disappearance of anthocyanins was accompanied by an increase of polymeric pigments (broad and nonresolved chromatographic peak eluting in the time frame of 15−35 min, similar to that found in very old red wines; data not shown) with a scarce formation yield of B-type vitisins (maximum of only 0.3% after 2 weeks, on the basis of peak areas at 510 nm, and total disappearance after 8 weeks) and nondetection of some minor expected products. The latter results could likely be connected with the use of grape skin extracts that also contained flavan-3-ols, which can easily react with anthocyanins by mediation of acetaldehyde.[1] In fact, we were able to detect the formation of some of the possible adducts of malvidin-type anthocyanins with (epi)catechin linked by ethyl bridge (anthocyanin-flavan-3-ol condensation adducts mediated by acetaldehyde) in the first steps of the reaction with acetaldehyde, which originated signals under MS conditions for their molecular ions at *m*/*z* 809 (adduct from malvidin 3-glucoside), 851 [adduct from malvidin 3-(6″-acetyl)glucoside], and 955 [adduct from malvidin-(6″-*p*-coumaroyl)glucoside], which further fragmented under the MS/MS conditions as previously reported.[19]

With regard to hydroxyphenylpyranoanthocyanin formation reactions, the highest yield was reached by sinapic acid (66% after 6 weeks, based on peak areas at 510 nm, which decreased to half after 9 weeks with total disappearance of anthocyanins), followed by caffeic acid (yield of 43% after 9 weeks, with one-fourth of the initial anthocyanins still remaining), ferulic acid (maximum yield of 22% after 6 weeks, which decreased to half after 9 weeks with total disappearance of anthocyanins), and *p*-coumaric acid (maximum yield of 18% after 9 weeks with half of the initial anthocyanins still remaining). These results were in agreement with previously reported moderate enhancement of kinetics of reaction of disubstituted (caffeic and ferulic acids) and trisubstituted (sinapic acid) hydroxycinnamic acids compared to that of the monosubstituted one (*p*-coumaric acid) toward malvidin 3-glucoside.[12] With the exception of few of the very minor expected compounds [mainly 3-(6″-caffeoyl)glucosides], most hydroxyphenylpyranoanthocyanins

Table 3. Online DAD UV−Vis Maxima (sh, shoulder) of Vitisin-like Pyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-H, from acetaldehyde; 10-carboxy, from pyruvic acid; 10-acetyl, from diacetyl; 10-methyl, from acetoacetic acid)

pyranoanthocyanin structure[a]	10- H	10-carboxy	10-acetyl	10-methyl
pyrdp-3-glc	∼487	264 sh, 299, 368, 485 sh, 509	∼528	∼477
pyrdp-3-acglc	∼488	264 sh, 300, 368, 483 sh, 511	nd	nd
pyrdp-3-cfglc	nd	nd	nd	nd
pyrdp-3-cmglc	nd	270, 299, 314 sh, 364, 483 sh, 512	nd	nd
pyrcy-3-glc	nd	270, 296, 352, 485 sh, 504	nd	nd
pyrcy-3-acglc	nd	nd	nd	nd
pyrcy-3-cfglc	nd	nd	nd	nd
pyrcy-3-cmglc	nd	nd	nd	nd
pyrpt-3-glc	∼488	269, 298, 370, 485 sh, 510	∼530	264, 292 sh, 356, 458 sh, 477
pyrpt-3-acglc	∼494	∼512	nd	∼484
pyrpt-3-cfglc	nd	nd	nd	nd
pyrpt-3-cmglc	∼494	nd	nd	272 sh, 294, 314 sh, 460 sh, 482
pyrpn-3-glc	∼487	276, 294 sh, 354, 485 sh, 505	nd	276, 293 sh, 353, 455 sh, 475
pyrpn-3-acglc	∼486	276, 295 sh, 352, 485 sh, 507	∼528	∼478
pyrpn-3-cfglc	nd	277, 300 sh, 328, 485 sh, 508	nd	∼480
pyrpn-3-cmglc	∼485	283, 299 sh, 310, 485 sh, 509	280 sh, 299, 312, 505, 530 sh	277, 297, 318, 362 sh, 455 sh, 478
pyrmv-3-glc	265, 292 sh, 357, 470 sh, 490	269, 299, 371, 486 sh, 511	271, 300, 388, 512 sh, 531	264, 293, 356, 460 sh, 480
pyrmv-3-acglc	264, 294 sh, 359, 470 sh, 494	270, 300, 371, 487 sh, 514	264 sh, 305, 382, 515 sh, 535	262, 293, 360, 460 sh, 482
pyrmv-3-cfglc	∼498	276, 299, 323 sh, 368 sh, 486 sh, 514	nd	266 sh, 292 sh, 340, 354 sh, 460 sh, 483
pyrmv-3-cmglc	272 sh, 299, 312 sh, 470 sh, 497	279, 304, 312 sh, 373, 487 sh, 516	280 sh, 309, 322 sh, 380, 515 sh, 536	269, 297, 312, 360 sh, 460 sh, 482

[a] pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

could be detected by ESI-MS/MS, even in the case of the less reactive *p*-coumaric acid (Table 2).

Chromatographic Behavior of Pyranoanthocyanins. Under the reverse-phase chromatographic conditions used, the vitisin-like pyranoanthocyanins showed the following behavior (Table 1): the substituent at C-10 position in the D-ring conditioned the polarity of the pyranoanthocyanin structure, thus eluting in the increasing order of 10-carboxy- (A-type vitisins), 10-H- (B-type vitisins), 10-acetyl-, and 10-methylpyranoanthocyanins; for each kind of vitisin-like structure, the increasing elution order according to the glucosidic moiety was the same as that found for anthocyanins, namely, 3-glucoside, 3-(6″-acetyl)glucoside, 3-(6″-caffeoyl)glucoside, and 3-(6″-*p*-coumaroyl)glucoside; every vitisin-like pyranoanthocyanin derived from 6″-nonacylated anthocyanins eluted after their corresponding anthocyanidin 3-glucoside; in contrast, the vitisin-like pyranoanthocyanins derived from 6″-acylated anthocyanins eluted before their corresponding anthocyanidin 3-(6″-acyl)-glucoside, with acetyl, caffeoyl, or *p*-coumaroyl being the acyl group. The aforementioned behavior led to the elution of all the possible vitisin-like pyranoanthocyanins derived from a specific anthocyanidin structure within the time frame comprised between the chromatographic peaks corresponding to the anthocyanidin 3-glucoside and its 3-(6″-*p*-coumaroyl) glucoside, thus introducing a great complication to the chromatographic profile, as it is depicted in Figure 2B for the 10-carboxypyranoanthocyanins (A-type vitisins) derived from malvidin-type anthocyanins. In the latter example, malvidin-type anthocyanins eluted within the time frame of 16.8−31.3 min, whereas their corresponding 10-carboxypyranoanthocyanins eluted within 18.7−24.5 min, and their respective 10-H-, 10-acetyl-, and 10-methylpyranoanthocyanins did so within 20.2−27.3, 22.1−28.3, and 23.0−30.2 min, respectively. The accumulation of chromatographic peaks corresponding to the vitisin-like pyranoanthocyanins was especially high between the peaks corresponding to each pair of nonacylated and acetylated anthocyanidin 3-glucosides.

In contrast, each hydroxyphenylpyranoanthocyanin was always less polar and eluted later than the corresponding anthocyanin from which it was derived (Table 2). Moreover, each hydroxyphenylpyranoanthocyanin series eluted after the anthocyanin series from which it was derived; for instance, malvidin-type anthocyanins eluted within the frame time of 16.8−31.3 min, whereas the 10-catechylpyranoanthocyanins did within 31.7−36.3 min. However, a change in the elution order according to the glucosidic moiety was observed for all the hydroxyphenylpyranoanthocyanin series: 3-(6″-caffeoyl)glucosides eluted earlier than 3-(6″-acetyl)glucosides, when the elution order was the reverse for their respective anthocyanins under the same conditions. As seen for vitisin-like pyranoanthocyanins, the structure of the hydroxyphenyl substituent at C-10 of D-ring (the new E-ring) importantly conditioned the polarity and the elution order of the resulting pyranoanthocyanin: for a specific anthocyanin-derived hydroxyphenylpyranoanthocyanin series, the first eluting compound was always the 10-catechyl derivative (from caffeic acid), following by the 10-*p*-hydroxyphenyl (from *p*-coumaric acid), the

Table 4. Online DAD UV−Vis Maxima (sh, shoulder) of Hydroxyphenylpyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-*p*-hydroxyphenyl, from *p*-coumaric acid; 10-catechyl, from caffeic acid; 10-guaiacyl, from ferulic acid; 10-syringyl, from sinapic acid)

pyranoanthocyanin structure[a]	10-*p*-hydroxyphenyl	10-catechyl	10-guaiacyl	10-syringyl
pyrdp-3-glc	∼504	277, 300 sh, 415 sh, 485 sh, 509	261 sh, 277, 302, 420 sh, 485 sh, 511	267, 299, 420 sh, 485 sh, 514
pyrdp-3-cfglc	nd	nd	nd	nd
pyrdp-3-acglc	∼505	∼512	278, 303, 420 sh, 485 sh, 510	∼516
pyrdp-3-cmglc	nd	280, 298 sh, 314 sh, 415 sh, 485 sh, 510	262, 301 sh, 317, 420 sh, 485 sh, 512	271, 316 sh, 420 sh, 485 sh, 516
pyrcy-3-glc	274 sh, 415 sh, 475 sh, 500	∼508	∼506	nd
pyrcy-3-cfglc	nd	nd	nd	nd
pyrcy-3-acglc	nd	nd	nd	nd
pyrcy-3-cmglc	nd	255 sh, 280, 300 sh, 314 sh, 415 sh, 475 sh, 508	∼506	nd
pyrpt-3-glc	263, 295, 337, 415 sh, 475 sh, 504	261 sh, 275, 298 sh, 415 sh, 485 sh, 510	260, 277 sh, 301, 420 sh, 485 sh, 510	269, 300, 420 sh, 485 sh, 518
pyrpt-3-cfglc	nd	nd	∼510	nd
pyrpt-3-acglc	264, 298, 339, 415 sh, 475 sh, 505	260, 274 sh, 300, 415 sh, 485 sh, 511	262, 276 sh, 299, 420 sh, 485 sh, 512	274, 300, 420 sh, 485 sh, 520
pyrpt-3-cmglc	265, 299, 315, 415 sh, 475 sh, 504	∼511	261, 305, 316 sh, 420 sh, 485 sh, 513	272, 301, 316 sh, 420 sh, 485 sh, 520
pyrpn-3-glc	273 sh, 297 sh, 415 sh, 475 sh, 500	250, 277, 296, 415 sh, 475 sh, 506	258, 275, 299, 415 sh, 475 sh, 507	270, 300, 420 sh, 485 sh, 514
pyrpn-3-cfglc	∼502	255, 280, 297, 326 sh, 415 sh, 475 sh, 508	255 sh, 282, 300, 327, 415 sh, 475 sh, 510	nd
pyrpn-3-acglc	278, 298, 415 sh, 475 sh, 501	255, 277, 298, 415 sh, 475 sh, 507	258, 275, 299, 415 sh, 475 sh, 508	274, 301, 420 sh, 485 sh, 514
pyrpn-3-cmglc	277, 298, 314 sh, 415 sh, 475 sh, 502	253, 281, 314 sh, 415 sh, 475 sh, 508	256 sh, 281, 301, 314 sh, 415 sh, 475 sh, 509	281, 302, 314 sh, 420 sh, 485 sh, 514
pyrmv-3-glc	263, 295, 337, 415 sh, 475 sh, 504	261, 274 sh, 299, 415 sh, 485 sh, 511	263, 277 sh, 300, 420 sh, 485 sh, 513	269, 301, 420 sh, 485 sh, 518
pyrmv-3-cfglc	263, 298, 332, 415 sh, 475 sh, 506	258 sh, 280, 301, 326, 415 sh, 486 sh, 512	258, 278, 301, 328, 420 sh, 485 sh, 513	272, 299, 330 sh, 420 sh, 485 sh, 518
pyrmv-3-acglc	263, 296, 337, 415 sh, 475 sh, 506	262, 275 sh, 300, 415 sh, 486 sh, 512	263, 278 sh, 300, 420 sh, 485 sh, 513	269, 302, 420 sh, 485 sh, 519
pyrmv-3-cmglc	266, 299, 314 sh, 415 sh, 475 sh, 505	260 sh, 281 sh, 302, 314 sh, 415 sh, 486 sh, 512	263 sh, 281, 301, 314 sh, 420 sh, 485 sh, 513	273, 303, 316 sh, 420 sh, 485 sh, 519

[a] pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

10-guaiacyl (from ferulic acid), and the 10-syringyl (from sinapic acid) derivatives.

Online DAD UV−Vis Spectra of Pyranoanthocyanins. The two kinds of considered pyranoanthocyanins contain different chromophores having characteristic online UV−vis spectra, as depicted in Figure 3A for the 3-glucosides of 10-carboxypyranomalvidin and 10-catechylpyranomalvidin, and showing visible absorption maxima that were hypsochromically shifted with regard to their corresponding original anthocyanin (malvidin 3-glucoside).

UV−vis spectra of vitisin-like pyranoanthocyanins were characterized in their visible region by a maximum absorbance broad band in the red-absorption region (475−536 nm) that was not symmetrical, showing a shoulder on the left part of the band (455−515 nm), together with secondary bands near the yellow-absorption region (353−388 nm) and the UV-region (262−283 and 292−305 nm). The wavelength of the maximum absorbance in the red-absorption region of vitisin-like pyranoanthocyanins was very affected by the substituent linked to C-10 (Figure 3B, Table 3). In fact, this band showed maximum absorbance at 480 nm for the malvidin-based derivative having the most electron-donor substituent (methyl group), whereas bathochromic shifts up to 54 nm were observed for the corresponding derivatives having substituent with decreasing electron-donor (and parallel increasing electron-acceptor) capabilities, following the sequence of 10-methyl (absorption maximum in the range 475−484 nm), 10-hydrogen (absorption maximum in the range 485−498 nm), 10-carboxy (absorption maximum in the range 504−516 nm), and 10-acetyl (broad absorption maximum in the range 528−536 nm) groups.

Less important was the change induced by the B-ring substitution pattern on the maximum absorbance wavelength in the

Table 5. ESI-MS/MS (molecular ion; product ion) of Vitisin-like Pyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-H, from acetaldehyde; 10-carboxy, from pyruvic acid; 10-acetyl, from diacetyl; 10-methyl, from acetoacetic acid)

pyranoanthocyanin structure*	10- H	10-carboxy	10-acetyl	10-methyl
pyrdp-3-glc	489, 327	533, 371	531, 369	503, 341
pyrdp-3-acglc	531, 327	575, 371	nd	545, 341
pyrdp-3-cfglc	nd	695, 371	nd	nd
pyrdp-3-cmglc	635, 327	679, 371	nd	nd
pyrcy-3-glc	473, 311	517, 355	nd	487, 325
pyrcy-3-acglc	nd	559, 355	nd	529, 325
pyrcy-3-cfglc	nd	679, 355	nd	nd
pyrcy-3-cmglc	619	663, 355	nd	633, 325
pyrpt-3-glc	503, 341	547, 385	545, 383	517, 355
pyrpt-3-acglc	545, 341	589, 385	587, 383	559, 355
pyrpt-3-cfglc	nd	709, 385	nd	nd
pyrpt-3-cmglc	649, 341	693, 385	691, 383	663, 355
pyrpn-3-glc	487, 325	531, 369	529, 367	501, 339
pyrpn-3-acglc	529, 325	573, 369	571, 367	543, 339
pyrpn-3-cfglc	649	693, 369	nd	663, 339
pyrpn-3-cmglc	633, 325	677, 369	675, 367	647, 339
pyrmv-3-glc	517, 355	561, 399	559, 397	531, 369
pyrmv-3-acglc	559, 355	603, 399	601, 397	573, 369
pyrmv-3-cfglc	679, 355	723, 399	nd	693, 369
pyrmv-3-cmglc	663, 355	707, 399	705, 397	677, 369

* pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

Table 6. ESI-MS/MS (molecular ion; product ion) of Hydroxyphenylpyranoanthocyanin Series Formed in Model Wine from Different Reagents (10-*p*-hydroxyphenyl, from *p*-coumaric acid; 10-catechyl, from caffeic acid; 10-guaiacyl, from ferulic acid; 10-syringyl, from sinapic acid)

MS/MS	10-*p*-hydroxyphenyl	10-catechyl	10-guaiacyl	10-syringyl
pyrdp-3-glc	581	597, 435	611, 449	641, 479
pyrdp-3-cfglc	743	nd	773	nd
pyrdp-3-acglc	nd	639, 435	653, 449	683, 479
pyrdp-3-cmglc	727	743, 435	757, 449	787, 479
pyrcy-3-glc	565, 403	581, 419	595, 433	625
pyrcy-3-cfglc	nd	743	757	nd
pyrcy-3-acglc	nd	623, 419	637, 433	667, 463
pyrcy-3-cmglc	711	727, 419	741, 433	771, 463
pyrpt-3-glc	595, 433	611, 449	625, 463	655, 493
pyrpt-3-cfglc	757, 433	773	787, 463	817, 493
pyrpt-3-acglc	637, 433	653, 449	667, 463	697, 493
pyrpt-3-cmglc	741, 433	757, 449	771, 463	801, 493
pyrpn-3-glc	579, 417	595, 433	609, 447	639, 477
pyrpn-3-cfglc	741, 417	757, 433	771, 447	801, 477
pyrpn-3-acglc	621, 417	637, 433	651, 447	681, 477
pyrpn-3-cmglc	725, 417	741, 433	755, 447	785, 477
pyrmv-3-glc	609, 447	625, 463	639, 477	669, 507
pyrmv-3-cfglc	771, 447	787, 463	801, 477	831, 507
pyrmv-3-acglc	651, 447	667, 463	681, 477	711, 507
pyrmv-3-cmglc	755, 447	771, 463	785, 477	815, 507

* pyrdp, pyranodelphinidin; pyrcy, pyranocyanidin; pyrpt, pyranopetunidin; pyrpn, pranopeonidin; pyrmv, pyranomalvidin; glc, glucoside; acglc, 6″-acetyl glucoside; cfglc, 6″-caffeoyl glucoside; cmglc, 6″-*p*-coumaroyl glucoside; nd, not detected.

visible region. The vitisin-like pyranoanthocyanins with trisubstituted B-ring (pyranodelphinidin-, pyranopetunidin-, and pyranomalvidin-types) showed visible maxima that were bathochromically shifted up to 12 nm (3−12 nm according to the C-10 substituent) with regard to those of the pyranoanthocyanins with disubstituted B-ring (pyranocyanidin- and pyranopeonidin-types) (Table 3), similar to the effects reported for the anthocyanins from which they are derived.[18] In addition, the acylation of the 3-glucosidic moiety only induced expected changes in the UV−vis spectra of an individual vitisin-like pyranoanthocyanin series when the acylating residues were 6″-*p*-coumaroyl and 6″-caffeoyl, thus appearing as extra shoulders at around 312−314 and 322−340 nm (Table 3), respectively, as has been similarly reported for acylated anthocyanins.[18]

In contrast, hydroxyphenylpyranoanthocyanins showed a sharper band in the red-absorption region (500−520 nm) together with two shoulders in the orange-brown absorption regions of 475−486 and 415−420 nm (Figure 3C, Table 4) and also other UV-region absorption maxima or shoulders (250−263, 275−280, 295−301 nm). In addition, the substitution pattern of the hydroxyphenyl moiety (E-ring) affected the wavelength of maximum absorbance in the red-absorption region, and successive bathochromic shifts of 6−7 nm were observed, following the sequence of monosubstituted (10-*p*-hydroxyphenylpyranoanthocyanins, 500−506 nm), disubstituted (10-catechylpyranoanthocyanins, 506−512 nm; 10-guaiacylpyranoanthocyanins, 506−513 nm), and trisubstituted (10-syringylpyranoanthocyanins, 514−520 nm). The shoulder at 415−420 nm was more pronounced in the case of 10-*p*-hydroxyphenylpyranoanthocyanins. Less than described for vitisin-like pyranoanthocyanins, the B-ring substitution pattern of hydroxyphenylpyranoanthocyanins also affected the red-absorption region maximum: a bathochromic shift of only 3−5 nm for trisubstituted structures (pyranodelphinidin-, pyrananopetunidin-, and pyranomalvidin-types) with regard to disubstituted ones (pyranocyanidin- and pyranopeonidin-types). Finally, the acylation of the glucose residue only introduced change in the UV-absorption region in the expected cases of *p*-coumaroyl and caffeoyl acylating groups, similarly to that seen for vitisin-like pyranoanthocyanins.

ESI-MS/MS Spectra of Pyranoanthocyanins. The soft positive ionization conditions provided by the electrospary ionization (ESI) chamber, together with the use of an acidic elution solvent, allowed the formation of molecular ions for all kinds of pyranoanthocyanins in their flavylium-like form. Further fragmentation in the ion trap (MS/MS) led to the loss of the entire glucosidic moiety, whether it was acylated or not (no intermediate loss of the acyl residue of the glucosidic moiety is observed). This behavior is typical for anthocyanins (anthocyanidin 3-glucosides) and it has been also reported for the pyranoanthocyanins formed from them.[15] Tables 5 and 6 summarize the molecular (MS conditions) and product ions detected when possible (enough signal intensity for the molecular ion to be isolated in the ion trap and fragmented under MS/MS conditions), for the studied pyranoanthocyanin series. These data were in agreement with the expected m/z values for each pyranoanthocyanin series

and were used, in combination with UV−vis data, for identification. By means of extracted ion chromatograms (EIC) at m/z values of the expected pyranoanthocyanidin aglycons, it was possible to identify each of the pyranoanthocyanidin-based series for every combination of C-10 substituent and B-ring substitution pattern.

MS detectors have high sensitivity and they allow the analysis of single compounds in complex mixtures without previous isolation (extracted ion chromatograms). However, our results showed the necessity of using MS/MS experiments because some pairs of different pyranoanthocyanin structures were isomeric [the 3-(6″-*p*-coumaroyl)- and the 3-(6″-caffeoyl)glucosides of the pairs pyranodelphinidin/pyranocyanidin and pyranopetunidin/pyranomalvidin of all the pyranoanthocyanins studied; the 3-glucosides of 10-acetyl-pyranoanthocyanins and the 3-(6″-acetyl)glucosides of their respective pyranoanthocyanins] and had the same m/z value for their molecular ions but different m/z values for their product ions (Tables 5 and 6). Therefore, the use of single-quadrupole MS detectors cannot distinguish between such pairs of compounds, especially in the case of vitisin-like pyranoanthocyanins because their retention times were also quite close (Table 1). On the other hand, even working under MS/MS conditions, MS detectors were not enough for unequivocal identification of each of the very wide sort of pyranoanthocyanin structures. As can be seen in Tables 5 and 6, many pyranoanthocyanin series (including all the nonacylated and acylated 3-glucosides) showed the same molecular and product ions (for instance, pyranopeonidins and 10-methylpyranocyanidins show the common aglycon m/z value, 325, and 10-*p*-hydroxyphenyl-pyranodelphinidins and 10-catechylpyranocyanidins show the common aglycon m/z value of 419). In all the aforementioned cases, the UV−vis information afforded by DAD-detector and the retention times obtained by the separated experiments developed with each individual pyranoanthocyanin precursors were necessary to differentiate among the above remarked coincidences. The use of ion trap instruments able to perform MS^n experiments with $n \geq 3$ could help with the unambiguous identification of the aforementioned pairs of compounds of identical molecular weight, really having an add-on value for other researchers.

Tentative Identification of New Pyranoanthocyanin Structures. In this work, a new kind of pyranoanthocyanin structure has been found. As far as we know, the 10-acetylpyranoanthocyanins derived from diacetyl (butanedione) has not been previously reported. We tried this compound because it was expected to be present in red wines as a lactic acid bacteria metabolite and it presented the chemical requisites for reacting with anthocyanins to form pyranoanthocyanins (a polarizable double bound in its enolic form). We found that diacetyl reacted in a model wine, giving rise to the expected 10-acetylpyranoanthocyanins. However, the yield of the reaction was poor and we were only able to detect by ESI-MS/MS a few of the expected components of the complete series (Table 6). It is remarkable that the UV−vis spectra of this new kind of pyranoanthocyanins showed a visible maximum at around 528−536 nm (Figure 3B and Table 3), a value quite similar to that of the anthocyanins from which it was formed. Therefore, these new pigments can be described as red-purple pigments and not as orange pigments as other pyranoanthocyanins usually are. Despite of the low capability of diacetyl to react with anthocyanins and the little amounts expected for this metabolite in wines, it is noteworthy that we found MS evidence of the occurrence of 10-acetylpyranomalvidins in real samples of red wines that developed

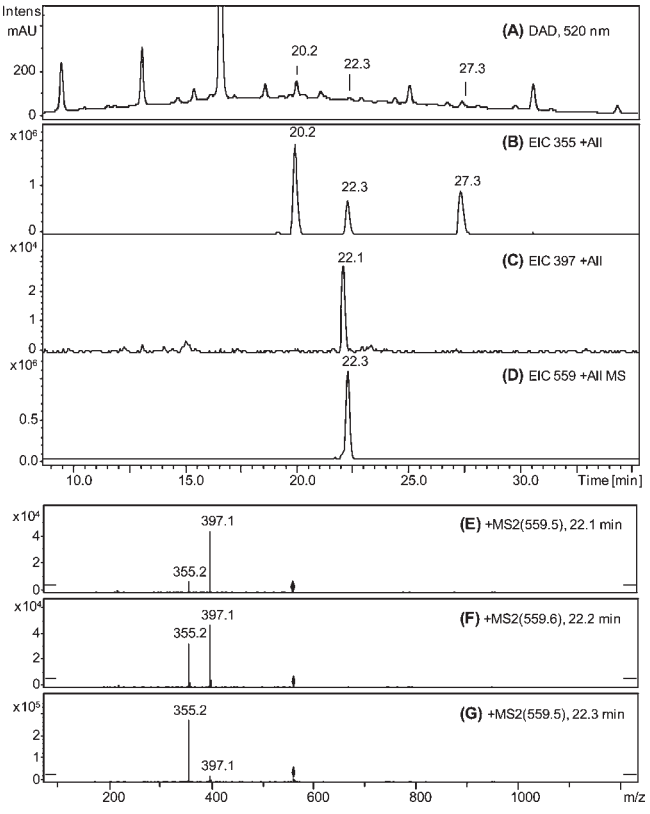

Figure 4. HPLC−DAD−ESI-MS/MS analysis of a 2-year-old Tempranillo commercial red wine. DAD-chromatogram at 520 nm (A). Extracted ion chromatogram (EIC) at m/z 355 (pyrmv) showing the peaks assigned to the 3-glc (20.2 min), 3-acglc (22.3 min), and 3-cmglc (27.3 min) derivatives (B); EIC at m/z 397 (10-acetyl-pyrmv) showing the peak assigned to its 3-glc (22.1 min) derivative (C); and EIC at m/z 559 corresponding to the molecular ions of the isomers 10-acetyl-pyrmv-3-glc and pyrmv-3-acglc (D). MS/MS spectra of peaks corresponding to 10-acetyl-pyrmv-3-glc (E) and pyrmv-3-acglc (G) and their overlapping zone (F). Abbreviations: pyrmv, pyranoamalvidin; 3-glc, 3-glucoside; 3-acglc, 3-(6″-acetyl)glucoside; 3-cmglc, 3-(6″-*p*-coumaroyl)glucoside.

malolactic fermentation (Figure 4), thus suggesting that their formation in red wine is possible. The identification of 10-acetylpyranoanthocyanins in wines was difficult because of their low concentrations. In addition, the expected major 10-acetylpyranomalvidin 3-glucoside partially coeluted with its isomer 10-pyranomalvidin 3-(6″-acetyl)glucoside under the chromatographic conditions we used (Figure 4C,D), and its occurrence was confirmed by MS/MS experiments (Figure 4E−G): the isolated molecular ions (m/z 559) were fragmented into two product ions, one corresponding to 10-acetylpyranomalvidin aglycon (m/z 397) and the other to pyranomalvidin aglycon (m/z 355), the ratio between these two product ions varying accordingly to the elution time.

Finally, one apparently new pyranoanthocyanin structure was found in the experiments using pyruvic acid. In this case, a second set of signals was found in the corresponding extracted ion chromatograms at the expected m/z values of the pyranocyanidin and pyranopeonidin aglycons (355 and 369, respectively). These new signals showed the same molecular and product ions as their corresponding 10-carboxypyranoanthocyanins, thus indicating that they were isomers. In addition, the retention times and UV−vis spectra of these new compounds were identical to that

described for 10-methylpyranoanthocyanins with petunidin- and malvidin-based structures, respectively, which showed coincident m/z values for their molecular and product ions (Table 5). Moreover, the series of the other 10-methylpyranoanthocyanins (delphinidin-, cyanidin-, and peonidin-based structures) was also found in the reaction mixture with pyruvic acid when appropriate m/z common aglycon values (341, 325, and 339, respectively) were selected to obtain extracted ion chromatograms. We repeated the reaction of pyruvic acid with grape skin extracts from other grape cultivars (Cabernet Sauvignon and Petit Verdot) with similar results (data not shown). Bearing in mind the low amounts in which acetoacetic acid occurs in wine and the poor yield of its reaction in the model wine reaction studied in this work, it could be also suggested that the occurrence of 10-methylpyranoanthocyanins in wine could be related to an important extent to the formation of 10-carboxypyranoanthocyanins. In fact, the isolation and identification of 10-methylpyranoanthocyanins were made for the first time from aged Port wines,[13] a type of red wine that is characterized by high contents of pyruvic acid and 10-carboxypyranoanthocyanins due to its special winemaking technique. In recent years, the reactivity of 10-carboxypyranoanthocyanins has been reported, leading to the formation of new wine pigments: the so-called portisins (reaction products of 10-carboxypyranoanthocyanins with vinylflavanols, vinylphenols, and hydroxycinnamic acids with the loss of the 10-carboxy group),[3−6] a new family of pyranoanthocyanin dimers with a turquoise color;[7] or the new yellowish pigments (called oxovitisins) produced by oxidative decarboxylation of 10-carboxypyranoanthocyanins.[8,9]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Control model wine evolution over the reaction time. This material is available free of charge via the Internet at http://pubs.acs.org.

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