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Identification of New Derivatives of 2-*S*-Glutathionylcaftaric Acid in Aged White Wines by HPLC-DAD-ESI-MS^n

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Glutathione, a natural occurring antioxidant, is a thiol-containing peptide present in grape must and wine. It is able to regenerate the *o*-diphenol group of enzymatically oxidized *trans*-caftaric acid, giving rise to 2-*S*-glutathionyl-*trans*-caftaric acid (also known as grape reaction product, GRP) and thus inhibiting the browning of wine. The amount of GRP present in a wine provides information on the oxidation history of the wine over its elaboration and aging. GRP has been proved to suffer hydrolysis in model solutions and wines. To know the actual content of glutathione involved in white wine browning inhibition as GRP, the GRP-derived products have been studied in 1-year-aged white wines by HPLC-DAD-ESI-MS^n. Online UV−vis spectra and pseudomolecular ions [(M + H)$^+$] obtained by LC-MS supported the formation of some of the expected GRP hydrolysis products, mainly 2-*S*-glutathionyl-*trans*-caffeic acid (*trans*-GSCf), together with minor 2-*S*-(cysteinylglycyl)-*trans*-caftaric acid, 2-*S*-(γ-glutamylcysteinyl)-*trans*-caftaric acid, and 2-*S*-cysteinyl-*trans*-caftaric acid. On the basis of UV−vis and LC-MS^2 spectra, new GRP derivatives in aged white wines have been tentatively characterized for the first time as three monoethyl esters of GRP (GRP-Et) and also the *cis* isomers of GRP, GSCf, and some of the GRP-Et.

KEYWORDS: Ethyl ester; glutathionylcaftaric acid; GRP; hydrolysis; isomerization; LC-MS; wine

INTRODUCTION

The oxidation of white wines is a well-known problem in the winemaking industry because the color properties of musts and wines could be modified. Enologists have traditionally recommended the preventive protection of must against oxidation, usually by the addition of sulfur dioxide, with the aim of avoiding browning development during bottle storage. However, strict regulations about sulfur dioxide employment exist in the food industry, because of its toxicity and allergenic effects on human health (*1*, *2*).

Phenolic compounds are widely recognized as good substrates for oxidation reactions. In white grape must, usually obtained as free-run juices (without pressing) or softly pressed juices, the main kinds of phenolic compounds are hydroxycinnamates, especially the predominant hydroxycinnamoyltartaric acids (*trans*-caftaric, *trans*-coutaric, and *cis*-coutaric acids) (*3*). The latter compounds have been identified as oxidation substrates and browning precursors in white wines (*4*, *5*). Once grape berries are crushed, *trans*-caftaric acid is released into the must together with grape polyphenol oxidase (PPO) that, in the presence of oxygen, induces the enzymatic production of the corresponding *o*-quinones (*6*). *o*-Quinones are unstable, very reactive, compounds, which easily condense with other phenolic compounds to form polymerized adducts (*7*, *8*). *o*-Quinones and their low molecular weight polymerized adducts are not colored compounds, but the increase in the condensation degree finally led to the formation of yellow to brown polymerized pigments in must (*9*).

It is also known that glutathione, a thiol-containing peptide present in grape must, helps prevent the enzymatic browning reaction. When an *o*-quinone is formed from caftaric (or coutaric) acid by means of grape PPO, glutathione can react with it, thus regenerating the *o*-diphenol group. The product of this reaction is 2-*S*-glutathionylcaftaric acid (also known as grape reaction product, GRP), which is nonoxidizable by grape PPO, and its formation avoids the browning of must that develops via the *o*-quinones (*10*, *11*). However, it can undergo an additional oxidation under the laccase action (*12*) from *Botrytis cinerea*, on botrytized grapes. The laccase oxidation of GRP yields the corresponding *o*-quinones which, in turn, can proceed to brown polymers and also gives rise to 2,5-di-*S*-glutathionylcaftaric acid in the presence of an excess of glutathione. As long as glutathione is available, GRP formation prevents the participation of *o*-quinones in coupled reactions leading to pigment formation. The behavior of glutathione toward enzymatic oxidation of must polyphenols constitutes the basis of the addition of glutathione and other thiol-containing compounds, such as cysteine, to fresh must as a prefermentative practice for preventing browning and the development of oxidative off-flavors in white wines (*13*). In addition, a recent work has highlighted the

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key role of glutathione with regard to the protection and enhancement of some thiol-related varietal aromas when white wines are produced under prefermentative oxidative conditions (*14*).

Although GRP is not generally active toward enzymatic oxidation, it is susceptible to hydrolysis. The independent hydrolysis of the two amide bonds of the glutathione moiety and the ester bond of the caftaric moiety has been studied in model solution, and some of the expected hydrolysis products have been found in wines (*11*).

The amount of GRP present in a wine provides information of the oxidation history suffered by the wine over its elaboration and aging, and it can be a marker of the technology employed over the winemaking process (*15*−*19*). However, GRP has been described as an oxidation product in grape must and wine that can be involved in subsequent reactions (e.g., hydrolysis reactions (*11*)) giving rise to GRP-derived compounds. Therefore, the measurement of the concentration of only GRP does not provide an actual estimation of how much GRP was formed during the winemaking process, because it is also present as GRP-derived products that need to be previously identified. Once GRP-derived products can be identified, the total GRP concentration could be obtained as the summation of remaining, nonreacted, GRP and the formed GRP derivatives. For this reason, the aim of this work was the identification by spectral characterization (UV−vis and ESI-MS^n spectroscopies) of the GRP-derived compounds occurring in white wines. Due to the development of reported or suspected reactions involving GRP (hydrolysis and, very likely, esterification and isomerization as well), we have focused our attention on 1-year-aged white wines in which these reactions could have already developed (*11*).

MATERIALS AND METHODS

Chemicals. Water (Milli-Q), HPLC-grade acetonitrile (Supergradient HPLC, Scharlab, Barcelona, Spain), and methanol (HPLC SG, SYMTA, Madrid, Spain) were used. Some commercial standards were available for identification (caffeic, *p*-coumaric, and ferulic acids, from Sigma-Aldrich), whereas a sample of *trans*-caftaric acid was a gift from Dr. Vrhovsek (IASMA, Italy).

Winemaking. Healthy grapes from *Vitis vinifera* cultivars Airén, Chardonnay, and Macabeo, grown in Ciudad Real (region of Castilla-La Mancha, Spain), were harvested at optimal ripening stage and separately processed at the experimental winery of the University of Castilla-La Mancha (Ciudad Real, Spain). After the grapes were destemmed and crushed in a bladder press, the three resulting musts (250 L each) were sulfited (100 mg/L of SO_2, added as $K_2S_2O_7$). After cold-settling at 4 °C for 48 h, the clean fraction of each must was racked and inoculated with *Saccharomyces cerevisiae* selected yeasts (UCLM S377, Fould-Springer, France) to induce alcoholic fermentation under temperature control at 18 °C. Two replicates (100 L each) of winemaking were carried out for each must. Alcoholic fermentation was monitored by density measurement and enzymatic fermentable sugar determination (Boehring Mannheim, Germany). Once alcoholic fermentation ended, the three single-cultivar white wines were filtered, sulfited (60 mg/L of SO_2) to prevent malolactic fermentation, and finally bottled. The bottles were aged for 1 year at 15 °C in darkness. OIV official methods (*20*) were used for wine conventional analysis and the control of prevention of malolactic fermentation.

Analysis of Hydroxycinnamic Acid Derivatives in Must and Wine by HPLC-DAD-ESI-MS^n. Prior to the HPLC analysis, must and white wine phenolic compounds were isolated by solid phase extraction (SPE) on reversed-phase C18 cartridges (Sep-Pak, 500 mg of adsorbent, Waters). After several experimental trials, the following conditions were set up to achieve the quantitative adsorption and further elution of phenolic compounds (data not shown): 2 mL of racked must or white wine was diluted with 2 mL of water and passed through the C18 cartridges, previously conditioned with 4 mL of methanol and 4 mL of water. After washing with 2 mL of water to remove nonadsorbed compounds (sugars and other nonphenolic polar compounds), phenolic compounds were eluted with 10 mL of methanol. The eluate was dried in a rotary evaporator (40 °C) and dissolved in 2 mL of the solvent A used in the HPLC analysis.

Table 1. Hydroxycinnamic Acid Derivatives Identified (+, Positive Identification) in Must and Young and 1-Year-Aged White Wines of the Three Grape Cultivars Studied[a]

peak	t_R (min)	assignation	must	young wine	aged wine
1	5.3	2-*S*-cysteinyl-*trans*-caftaric acid			+
2	5.6	2-*S*-(cysteinylglycyl)-*trans*-caftaric and 2-*S*-(γ-glutamylcysteinyl)-*trans*-caftaric acid			+
3	5.9	2-*S*-glutathionyl-*trans*-caftaric acid	+	+	+
4	6.2	*trans*-caftaric acid	+	+	+
5	7.7	2-*S*-glutathionyl-*cis*-caftaric acid	+	+	+
6	8.4	*trans*-coutaric acid	+	+	+
7	9.1	*cis*-coutaric acid	+	+	+
8	9.9	2-*S*-glutathionyl-*trans*-caffeic acid			+
9	10.6	*trans*-caffeic acid		+	+
10	11.1	*trans*-fertaric acid	+	+	+
11	11.9	*cis*-fertaric acid	+	+	+
12	12.4	*trans*-GRP-Et-1			+
13	12.6	2-*S*-glutathionyl-*cis*-caffeic acid		+	+
14	14.9	*cis*-GRP-Et-1			+
15	15.1	*trans*-*p*-coumaric acid		+	+
16	16.9	*trans*-GRP-Et-2			+
17	19.0	*trans*-GRP-Et-3			+
18	19.2	*trans*-ferulic acid		+	+

[a] All the same compounds were identified whatever the grape cultivar. GRP-Et means monoethyl ester as shown in **Figure 1**.

HPLC separation and identification of hydroxycinnamic acid derivatives were performed on an Agilent 1100 series system (Agilent, Waldbronn, Germany), equipped with a DAD photodiode detector (G1315B) and an LC-MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI/MS^n) system, both coupled to an Agilent Chem Station (version B.01.03) for data processing. The samples, after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Düren, Germany), were injected (50 μL) in duplicate on a reversed-phase column Zorbax Eclipse XDB-C18 (4.6 × 250 mm; 5 μm particle; Agilent), thermostated at 40 °C. Solvent A was water/acetonitrile/formic acid (87:3:10, v/v). Solvent B was water/acetonitrile/formic acid (40:50:10, v/v). The solvents were filtered through a 0.45 μm nylon Millipore filter. We used a previously described chromatographic method (*21*). The HPLC runs were made in duplicate for each sample.

For identification, the ESI-MS^n detector was used in positive mode for GRP and GRP-derived compounds and negative mode for hydroxycinnamic and hydroxycinnamoyltartaric acids, setting the following parameters (*21*, *22*): dry gas, N_2, 11 mL/min; drying temperature, 350 °C; nebulizer, 65 psi; capillary voltage, −2500 V (positive ionization mode) and +2500 V (negative ionization mode); target mass, m/z 600; compound stability, 40% (negative ionization mode) and 100% (positive ionization mode); trap drive level, 100%; and scan range, m/z 50−1200.

Empirical Molecular Calculations. Calculation of the MM2 minimum energy space conformation of protonated *trans*- and

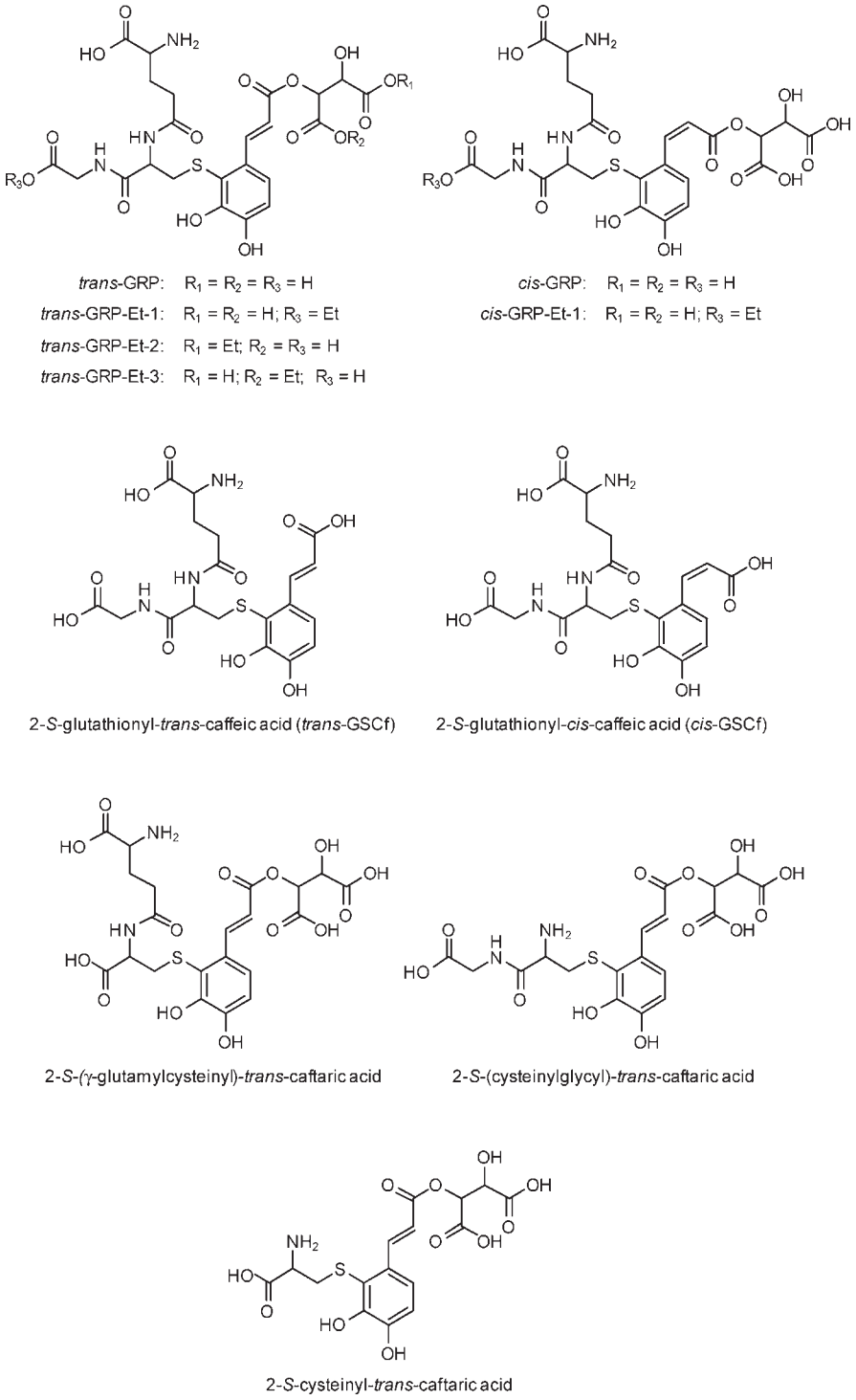

Figure 1. Structures of GRP (2-*S*-glutathionylcaftaric acid) and GRP-derived compounds identified in 1-year-aged white wines from the three grape cultivars. GRP-Et, monoethyl esters of GRP.

cis-GRP was performed using ChemBio3D Ultra software (Cambridge Soft, version 12.0).

RESULTS AND DISCUSSION

The identification of hydroxycinnamic acid derivatives found in must and young and 1-year-aged white wines led to the same results whatever the grape cultivar studied. As **Table 1** summarizes, the racked musts of the three grape cultivars contained variable proportions of the expected hydroxycinnamoyltartaric acids derived from caffeic, *p*-coumaric, and ferulic acids (caftaric, coutaric, and fertaric acids, respectively), and also 2-*S*-glutathionylcaftaric acid (the so-called GRP). In addition to the aforementioned compounds, free caffeic, *p*-coumaric, and ferulic acids, released by hydrolysis from their tartaric esters, were also found in the three single-cultivar wines just before bottling (young wines), in agreement with previous results (*3*). Finally, the three different 1-year-aged wines also showed the presence of the same newly formed compounds derived from GRP (**Figures 1** and **2a**): some of them were assigned as hydrolysis products [2-*S*-glutathionylcaffeic acid, 2-*S*-(cysteinylglycyl)-caftaric acid, 2-*S*-(γ-glutamylcysteinyl)-caftaric acid, and 2-*S*-cysteinylcaftaric acid], whereas others were assigned

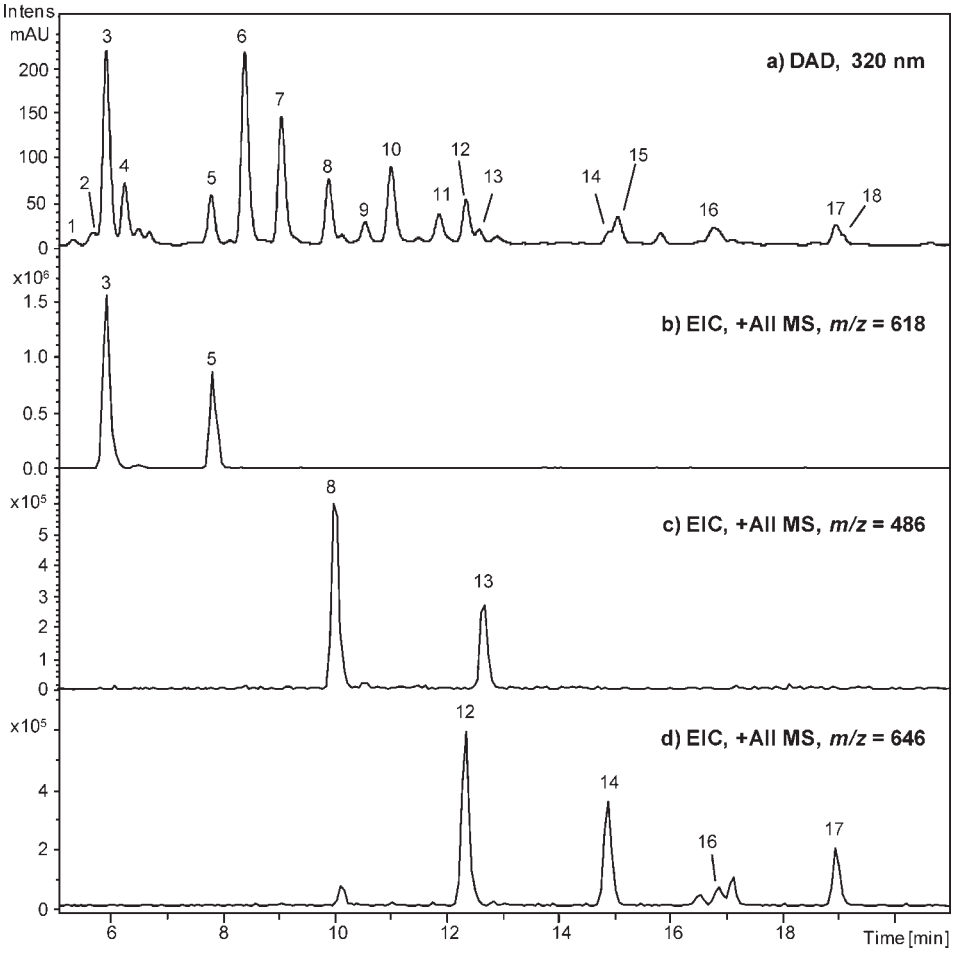

Figure 2. HPLC chromatograms of the hydroxycinnamic acid derivatives fraction of 1-year-aged Macabeo white wine: (**a**) DAD chromatogram at 320 nm; (**b**) extracted ion chromatogram (EIC) at *m*/*z* 618; (**c**) EIC at *m*/*z* 486; (**d**) EIC at *m*/*z* 646.

as ester reaction products between GRP and ethanol (GRP-Et-1, GRP-Et-2, and GRP-Et-3). All of the GRP-related compounds were assigned as the major expected *trans* isomers. In addition, GRP and some of its newly formed derivatives were found to occur also as their minor *cis* isomers.

GRP has been described as the *trans* isomer of 2-*S*-glutathionylcaftaric acid (*trans*-GRP). However, two peaks were observed in the extracted ion chromatogram (EIC) at the *m*/*z* value of its pseudomolecular ion ($[M + H]^+$, *m*/*z* 618, **Figure 2b**). The MS^2 fragmentation patterns for those two components differed only in the relative intensity of the product ions generated in the ion trap (**Figure 3a**,**b**). In addition, the corresponding peaks in the DAD chromatogram for these two GRP isomers (**Figure 2a**, peaks 3 and 5) showed different online UV−vis spectra. Whereas peak 3 showed the expected UV−vis spectrum for the *trans* isomer of GRP (*11*) with maximum absorption bands at 253 and 329 nm (**Figure 4a**), peak 5 showed a hypsochromically shifted absorption maximum at 316 nm, and the intensity of this band notably decreased in comparison to the intensity of the other band at 253 nm (**Figure 4b**). The hypsochromic shift shown by the compound eluting as peak 5 could be attributable to the *cis* isomer of GRP, on the basis of the reported values for the couples of *trans*/*cis* isomers of coutaric acid (314 vs 311 nm) and fertaric acid (326 vs 325 nm) and those reported for the *trans*/*cis* isomers of cinnamic acids in grapes (*23*−*25*), although in our case the observed shift was of much higher magnitude.

A tentative mechanism of the most favored fragmentations that GRP and it derivatives suffered in the ion trap in positive MS^2 conditions is given in **Figure 5**. The most favored fragmentations seem to be the breaking of the amide bonds (A- and B-type fragmentations) and the coupled breaking of the thioether bond and the carboxy group of the glycine unit of the glutathione moiety (C-type fragmentation), in addition to a less probable fragmentation following A-type fragmentation (D-type fragmentation), in agreement with previously suggested fragmentations for GRP (*26*). The main fragmentation suffered by *trans*-GRP was the C-type fragmentation (**Figure 3a**, signal at *m*/*z* 264, 100% relative intensity). However, the probability of a C-type fragmentation was considerably reduced (only 25% relative intensity) for the suggested *cis*-GRP (**Figure 3b**). A likely explanation for such a difference can be found by examination of the space conformation that the protonated *trans* and *cis* isomers of GRP can adopt in the gas phase. Empirical molecular calculations showed that the minimum energy molecular conformation for the pesudomolecular ion $[trans\text{-GRP} + H]^+$ makes very accessible the thioether bond for breaking in the ion trap by collision with He atoms (**Figure 6a**), whereas this bond is quite hidden for this collision in the case of $[cis\text{-GRP} + H]^+$ (**Figure 6b**). The MS^2 spectra of *trans*- and *cis*-GRP showed other minor signals corresponding to product ions generated by less favored fragmentations (**Figure 3a**,**b**). The suggested fragmentation mechanism (**Figure 7**) in this case begins with the breaking of the ester bond involving the tartaric acid moiety (F-type fragmentation) and follows with subsequent already suggested fragmentations involving amide bonds (A′-, B′-, and D′-type fragmentations), in addition to others (G- and H-type fragmentations). As far as we

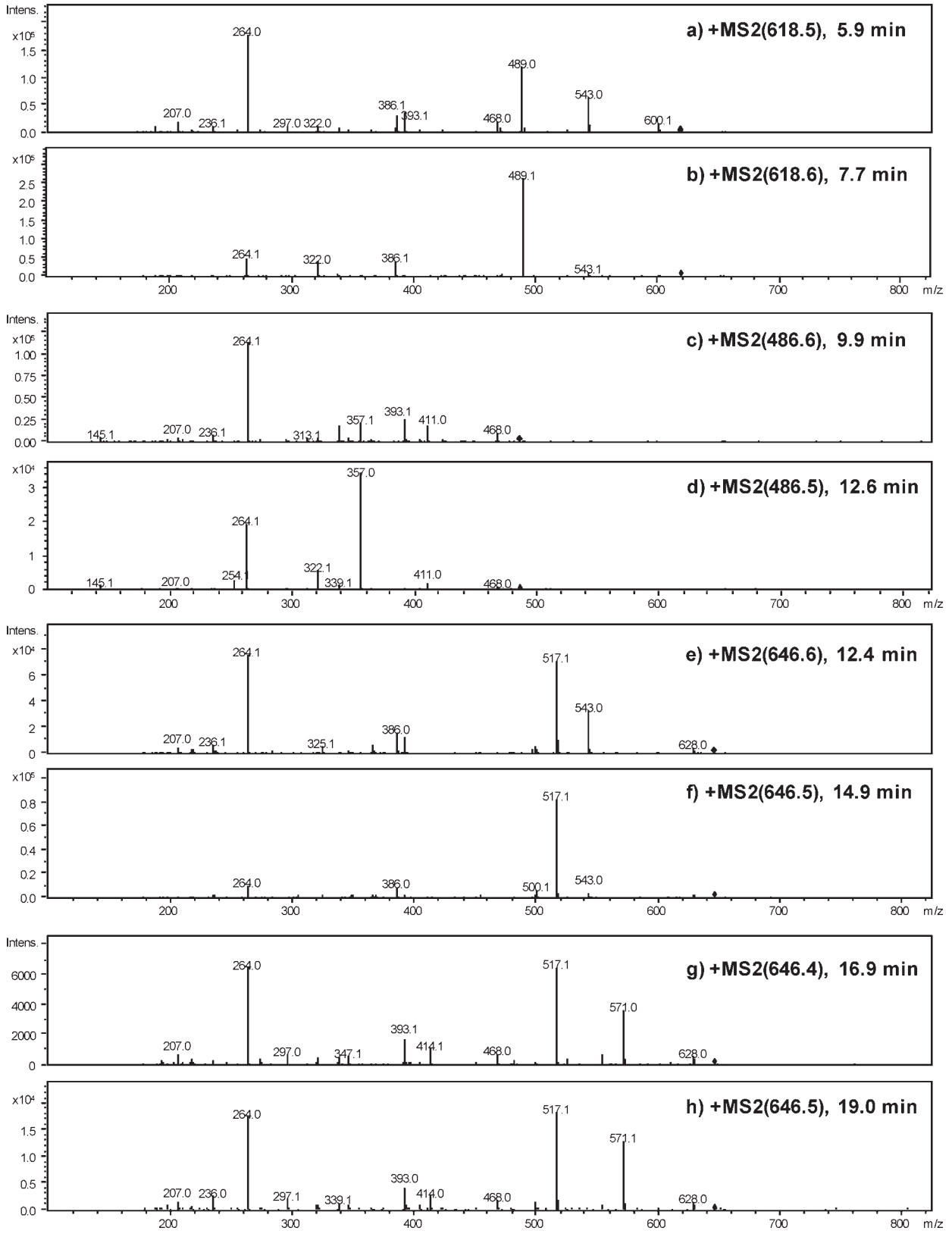

Figure 3. HPLC-ESI-MS^2 spectra corresponding to the following GRP derivatives found in 1-year-aged Macabeo white wine: (**a**) *trans*-GRP; (**b**) *cis*-GRP; (**c**) *trans*-GSCf; (**d**) *cis*-GSCf; (**e**) *trans*-GRP-Et-1; (**f**) *cis*-GRP-Et-1; (**g**) *trans*-GRP-Et-2; (**h**) *trans*-GRP-Et-3. Similar MS^2 spectra were obtained from Airén and Chardonnay 1-year-aged wines. For structure assignations see **Figure 1**.

know, the occurrence of *cis*-GRP in wine had not been previously reported.

In 1-year-aged white wines, new GRP-related peaks were observed in addition to the above-mentioned peaks. The two peaks detected in the EIC at *m*/*z* 486 (peaks 8 and 13, **Figure 2c**) generated main product ions in their respective MS^2 spectra (**Figure 3c**,**d**) that were in agreement with the *trans* and *cis* isomers of a molecular structure corresponding to a GRP hydrolysis product by loss of the tartaric acid moiety (2-*S*-glutathionylcaffeic acid, GSCf; **Figure 1**). According to the

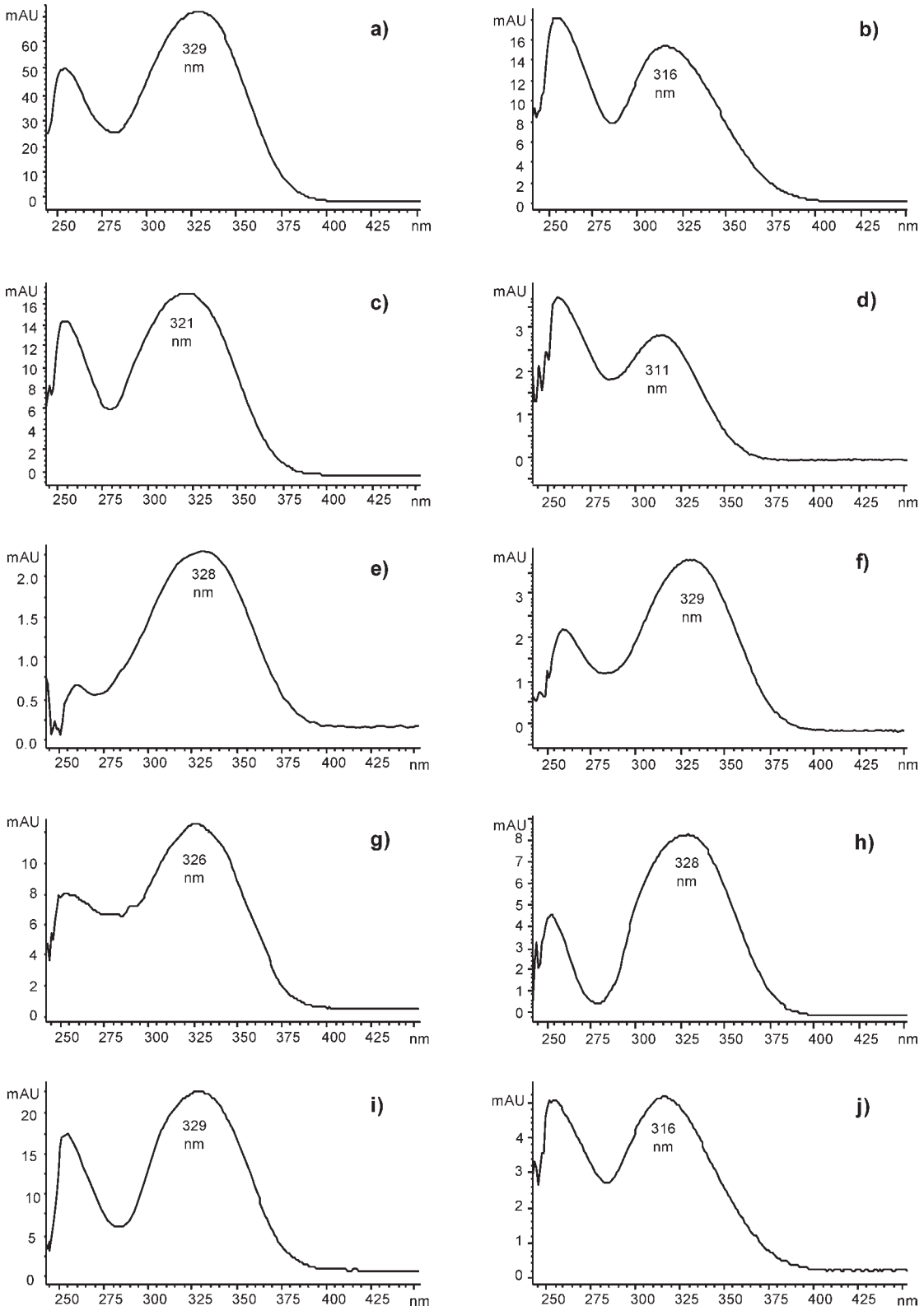

Figure 4. HPLC-DAD online UV−vis spectra corresponding to the following GRP derivatives found in 1-year-aged white wines: (**a**) *trans*-GRP; (**b**) *cis*-GRP; (**c**) *trans*-GSCf; (**d**) *cis*-GSCf; (**e**) 2-*S*-(cysteinylglycyl)-*trans*-caftaric acid and 2-*S*-(γ-glutamylcisteinyl)-*trans*-caftaric acid; (**f**) 2-*S*-cysteinyl-*trans*-caftaric acid; (**g**) *trans*-GRP-Et-2; (**h**) *trans*-GRP-Et-3; (**i**) *trans*-GRP-Et-1; (**j**) *cis*-GRP-Et-1. For structure assignations see **Figure 1**.

above discussion about GRP isomers, peak 8 could be assigned as the *trans* isomer because of the shape of its online UV−vis spectrum (**Figure 4c**) and the higher relative proportion of the MS^2 product ion at m/z 264 (**Figure 3c**); on the other hand, peak 13 was very minor, and both UV−vis (**Figure 4d**) and MS^2 spectra (**Figure 3d**) suggested it to be the *cis* isomer of GSCf. The lack of the tartaric acid moiety affected the UV−vis spectra, thus inducing a hypsochromic shift of the band around 320 nm that was of higher magnitude for the *trans* isomer (8 vs 5 nm for the *cis* isomer). In addition, the lack of the tartaric acid moiety made less important the influence on the relative intensity of the signal at m/z 264 due to the changes

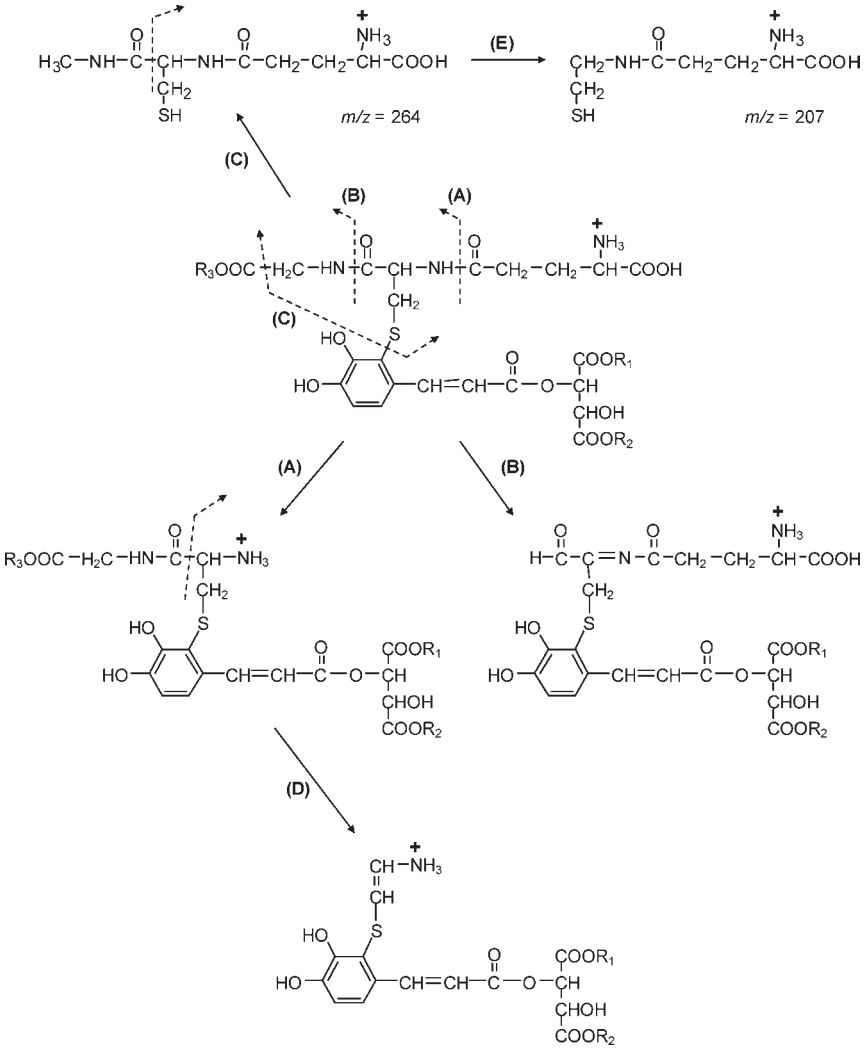

Figure 5. Tentative fragmentation mechanism suggested for the most favored fragmentations of GRP and GRP-derived compounds in positive ionization mode. R_1, R_2, and R_3 can be H or ethyl as shown in **Figure 1**. In the case of 2-*S*-glutathionylcaffeic acid (GSCf), A-, B-, and D-type fragmentations lead to the same type of product ions without the tartaric acid moiety (decrease of 132 units in the *m*/*z* ratio of the obtained signals for GRP).

of configuration in the double bond (*trans* to *cis*). In fact, *cis*-GSCf also gave a very intense signal at *m*/*z* 264 (57% relative intensity), and empirical molecular calculations showed that the thioether bond was similarly accessible to He collision in the ion trap as for *trans*-GSCf (data not shown). The hydrolysis product *trans*-GSCf had been previously described in model solutions and white wines of different vintages (*11*), but no report was found dealing with the occurrence of *cis*-GSCf. That study also described other GRP hydrolysis products resulting from the loss of amino acid residues or the combination of tartaric acid and amino acid losses. We obtained evidence supporting the occurrence of some of these GRP hydrolysis products in our 1-year-aged white wines. Peak 2 showed a UV−vis spectrum (**Figure 4e**) very similar to that of *trans*-GRP, and its MS spectrum showed two signals at *m*/*z* 489 and 561, which were assigned to the pseudomolecular ions of the *trans* isomers of 2-*S*-(cysteinylglycyl)caftaric acid and 2-*S*-(γ-glutamylcysteinyl)caftaric acid, respectively. In addition, another minor GRP hydrolysis product was observed eluting at 5.3 min (peak 1), and it was assigned as 2-*S*-cysteinyl-*trans*-caftaric acid on the basis of its UV−vis spectrum (**Figure 4f**) and the detection of a signal at *m*/*z* 432 in its MS spectrum. No MS^2 data were available for the three latter minor GRP hydrolysis compounds.

Finally, four peaks were also found in the EIC at *m*/*z* 646 (**Figure 2d**) of 1-year-aged wines, which could be attributed as monoethyl esters of GRP. Peaks 16 and 17 showed online UV−vis spectra very similar to one another (**Figure 4g**,**h**) and also to that of *trans*-GRP (**Figure 4a**). In addition, these two peaks generated almost identical MS^2 spectra (**Figure 3g**,**h**) that closely resembled the MS^2 spectrum of *trans*-GRP (**Figure 3a**), with the exception that product ions generated by the suggested A-, B-, and D-type fragmentations have increased by 28 units their *m*/*z* values. Because of the above-mentioned reasons, these two peaks were assigned as the *trans* isomers of the two possible monoethyl esters of GRP that can be formed by esterification of the tartaric acid moiety (*trans*-GRP-Et-2 and *trans*-GRP-Et-3, **Figure 1**). Moreover, the latter compounds gave rise to the same MS^2 minor signals as *trans*-GRP following the less likely fragmentations suggested in **Figure 7** (signals at *m*/*z* values of 468, 393, 339, 322, 297, and 236 in **Table 2**), thus reinforcing their suggested assignments. The other two peaks found in EIC at *m*/*z* 646, eluting at 12.4 and 14.9 min, were assigned as the couple of isomers *trans* (peak 12) and *cis* (peak 14), respectively, of the ethyl ester of GRP formed by esterification of the carboxyl group of the glycine unit of the glutathione moiety (*trans*-GRP-Et-1 and *cis*-GRP-Et-1, **Figure 1**). The assignation of *cis* and *trans* double bonds was based on their respective online UV−vis spectra

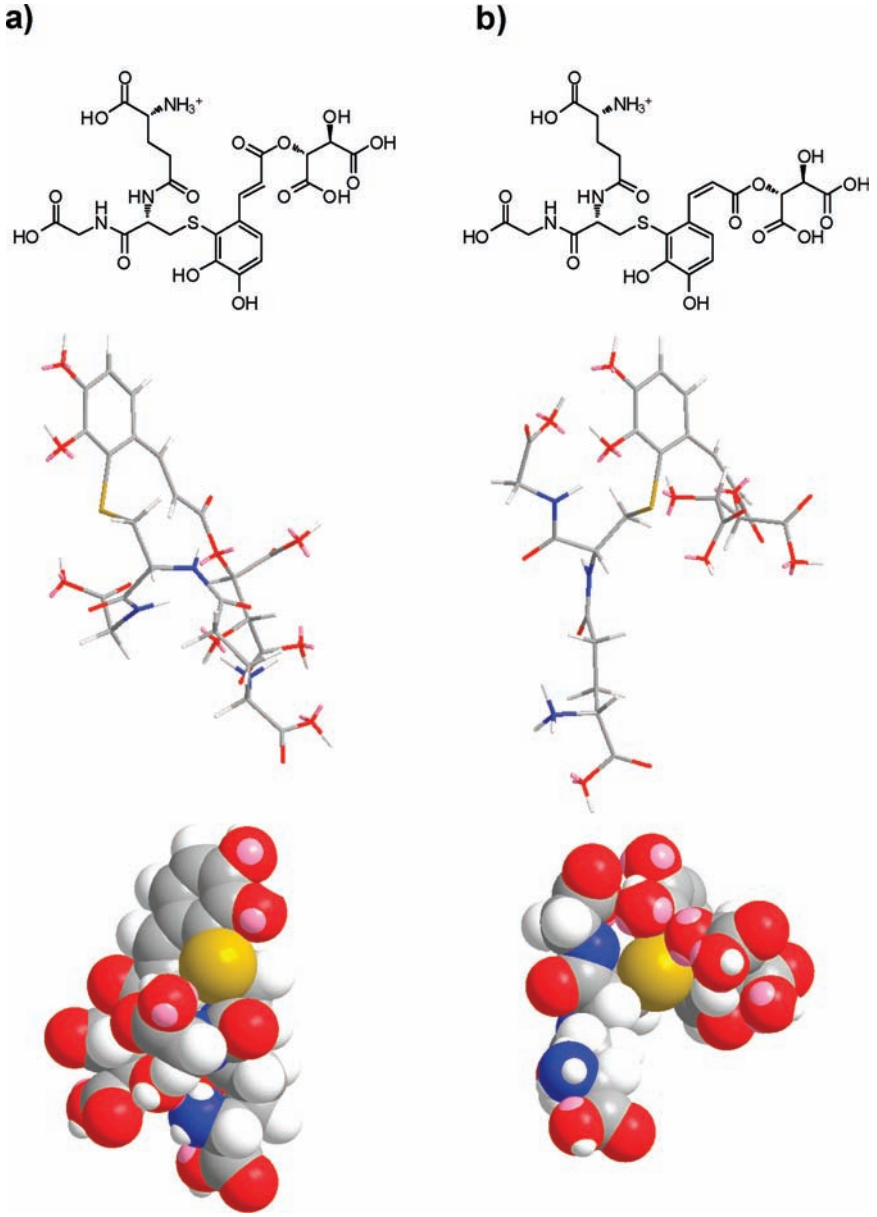

Figure 6. Stereochemical structures and minimum energy space conformations (sticks and space filling models) of (**a**) *trans*-GRP and (**b**) *cis*-GRP.

(**Figure 4i**,**j**, respectively) and also by paying attention to the relative intensity of the signal at *m*/*z* 264 in their respective MS^2 spectra (**Figure 3e**,**f**, respectively), as it was argued for the assignation suggested for *trans*- and *cis*-GRP. The location of the ethyl group was based on the presence of a product ion at *m*/*z* 543 of medium (*trans*-GRP-Et-1, **Figure 3e**) or low (*cis*-GRP-Et-1, **Figure 3f**) relative intensity, attributable to a B-type fragmentation, together with a signal at *m*/*z* 517 that was of very high relative intensity (92% for *trans*-GRP-Et-1, **Figure 3e**; 100% for *cis*-GRP-Et-1, **Figure 3f**), attributable to a product ion generated by a A-type fragmentation that retains the ethyl group (**Table 2**). The MS^2 signals assigned to the less favored fragmentations (F-, A′-, G-, and H-type; **Table 2**) were also in agreement with the location of the ethyl group on the glycine unit suggested for the latter GRP derivatives.

Formation of the oxidation marker GRP begins just during the must extraction from grapes and continues over the wine-making process. However, the formed GRP tends to disappear because it can be involved in subsequent reactions such as oxidation (less probable in the absence of laccase from *Botrytis cinerea*), hydrolysis, esterification, and *trans*/*cis* isomerization. The analysis by HPLC-DAD-ESI-MS^n of 1-year-aged white wines made from three grape cultivars allowed the detection and identification of GRP-derived products formed by hydrolysis, esterification, and *trans*/*cis* isomerization. The MS^2 and UV−vis spectra showed characteristic differences enough to distinguish between *trans* and *cis* isomers: the product ion signal at *m*/*z* 264 was the most intense for *trans* isomers, whereas this signal was minor for *cis* isomers; the *cis* isomers showed a considerable hypsochromic shift (10−13 nm) of their absorption maximum corresponding to the band attributable to the cinnamic residue (around 320 nm). Also, the MS^2 spectra allowed the detection and assignation of GRP hydrolysis products losing the tartaric acid moiety or some of the amino acid residues of the glutathione moiety. Moreover, the interpretation of the MS^2 spectra on the basis of the suggested fragmentation mechanisms has allowed the location of the ethyl residues in the four monoethyl esters of GRP that have been identified. In addition to the previously reported 2-*S*-glutathionyl-*trans*-caftaric acid (*trans*-GRP), 2-*S*-glutathionyl-*trans*-caffeic acid (*trans*-GSCf),

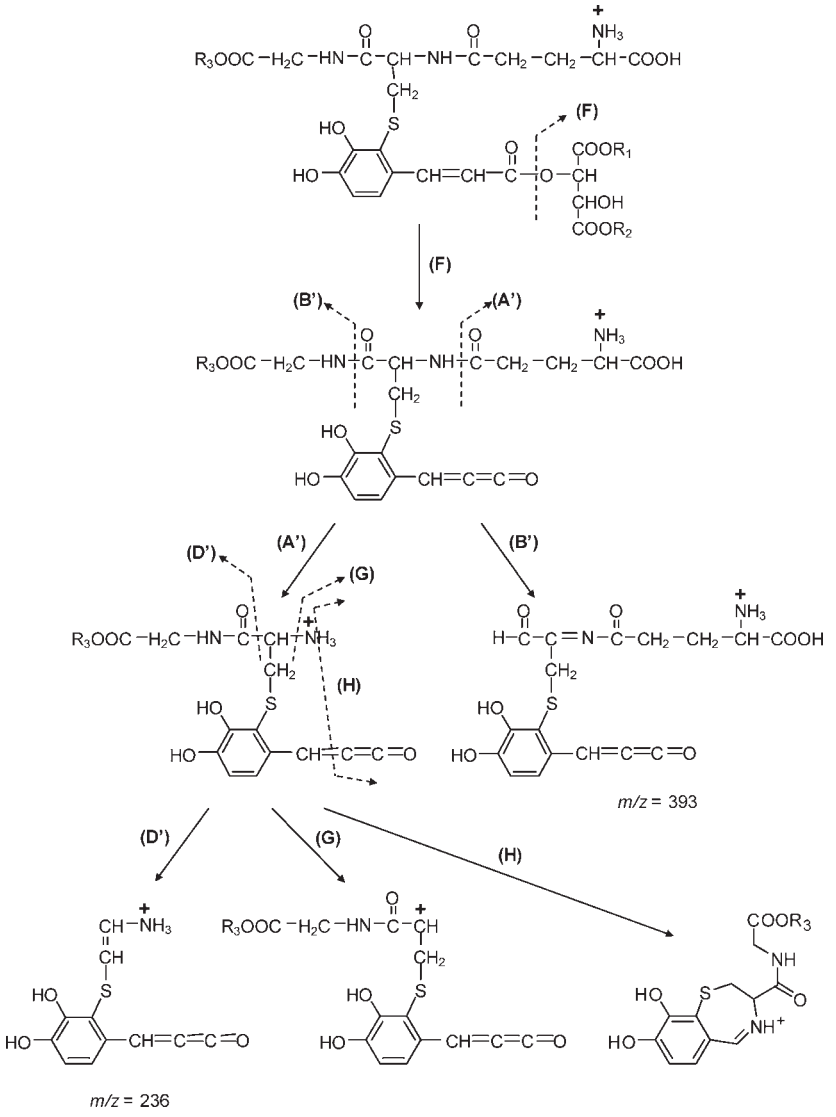

Figure 7. Tentative fragmentation mechanism suggested for the less favored fragmentations of GRP and GRP-derived compounds in positive ionization mode. R_1, R_2, and R_3 can be H or ethyl as shown in **Figure 1**. In the case of 2-*S*-glutathionylcaffeic acid (GSCf), the same kind of product ion as formed by F-type fragmentation from GRP-like compounds is produced by loss of water from the carboxyvinylphenol moiety.

Table 2. Product Ions (*m*/*z* Values) for Different GRP and GRP-Derived Compounds (Structures as in **Figure 1**) Identified in 1-Year-Aged White Wines, Following the Fragmentation Mechanisms Suggested in **Figures 5** and **7**

fragmentation	GRP	GSCf	GRP-Et-1	GRP-Et-2	GRP-Et-3
A	489	357	517	517	517
B	543	411	543	571	571
C	264	264	264	264	264
D	386	254	386	414	414
E	207	207	207	207	207
F	468	468	496	468	468
A′	339	339	367	339	339
B′	393	393	393	393	393
D′	236	236	236	236	236
G	322	322	350	322	322
H	297	297	325	297	297

2-*S*-(cysteinylglycyl)-*trans*-caftaric acid, 2-*S*-(γ-glutamylcysteinyl)-*trans*-caftaric acid and 2-*S*-cysteinyl-*trans*-caftaric acid, the following GRP-derivatives have been tentatively identified: *cis*-GRP, *cis*-GSCf, *trans*- and *cis*-GRP-Et-1, *trans*-GRP-Et-2, and *trans*-GRP-Et-3.

The use of GRP amounts as markers of the oxidation history of a white wine during its elaboration (including aging in bottle) needs the evaluation of all the forms in which GRP can exist. Although GRP was the main form within the GRP-type compounds in 1-year-aged white wines (**Table 3**), around one-third to one-fourth of GRP was found as *cis* isomer. Moreover, GRP-derived compounds (hydrolysis products and monoethyl esters) accounted for important percentages (total of 45−64%). These results suggest that GRP importantly evolves in aged white wines and that other GRP-type compounds must be also considered for an estimation of the total GRP content in those wines. Therefore, it can be suggested that the relative concentrations of GRP and the different GRP-derived compounds for each sample, as well as the ratios between some of them, could provide much more relevant information about the real oxidation history than a single "total GRP" value. The chromatographic conditions used in this work allowed a good separation of the GRP-derived compounds that could provide a proper quantification by DAD chromatograms at 320 nm. However, further corrections regarding the differences in molar absorptivity at 320 nm of the *trans* and *cis* isomers must be achieved for a more accurate quantification.

Table 3. Percentages (Mean Value ± Standard Deviation; $n = 2$) of GRP and GRP-Derived Compounds Occurring in 1-Year-Aged White Wines[a]

GRP or GRP-derived compound	Airén	Chardonnay	Macabeo
2-*S*-cysteinyl-*trans*-caftaric acid	1.02 ± 0.06	4.51 ± 0.19	1.71 ± 0.16
2-*S*-(cysteinylglycyl)-*trans*-caftaric acid + 2-*S*-(γ-glutamylcysteinyl)-*trans*-caftaric acid	2.10 ± 0.12	0.97 ± 0.01	3.43 ± 0.23
2-*S*-glutathionyl-*trans*-caftaric acid (*trans*-GRP)	37.37 ± 0.64	28.49 ± 1.06	43.85 ± 1.63
2-*S*-glutathionyl-*cis*-caftaric acid (*cis*-GRP)	13.45 ± 0.59	7.10 ± 0.27	10.81 ± 0.98
2-*S*-glutathionyl-*trans*-caffeic acid	14.57 ± 0.45	24.33 ± 0.44	14.87 ± 0.47
trans-GRP-Et-1	11.04 ± 0.18	11.16 ± 0.52	11.22 ± 0.10
2-*S*-glutathionyl-*cis*-caffeic acid	6.98 ± 0.25	10.01 ± 0.65	3.26 ± 0.69
cis-GRP-Et-1	3.18 ± 0.37	1.95 ± 0.13	2.29 ± 0.17
trans-GRP-Et-2	5.95 ± 0.11	5.31 ± 0.28	3.70 ± 0.49
trans-GRP-Et-3	4.37 ± 0.01	6.19 ± 0.25	4.88 ± 0.26

[a] Calculation based on the peak areas identified in the chromatograms obtained at 320 nm.

ABBREVIATIONS USED

GRP, grape reaction product, 2-*S*-glutathionylcaftaric acid; GSCf, 2-*S*-glutathionylcaffeic acid; GRP-Et-1, monoethyl ester of 2-*S*-glutathyonylcaftaric acid at the carboxy group of the glycine terminal unit of the glutathionyl moiety; GRP-Et-2 and GRP-Et-3, monoethyl esters of 2-*S*-glutathyonylcaftaric acid at the two carboxy groups of the caftaric acid moiety.

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