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Identification by mass spectrometry of new compounds arising from the reactions involving malvidin-3-glucoside-(O)-catechin, catechin and malvidin-3-glucoside

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RATIONALE: The aim of this work was to study the putative reactions that could occur in red wines between malvidin-3-glucoside-(O)-catechin (mv3glc-(O)-cat) adduct and catechin (cat) or malvidin-3-glucoside (mv3glc) in presence of acetaldehyde.
METHODS: Mv3glc-(O)-cat adduct (1 mM) was incubated with catechin (or mv3glc) in the presence of acetaldehyde (molar ratio of 1:4:10) in 12% ethanol/water at pH 3.2, protected from light and placed in the oven at 30 °C. The formation of the new compounds was monitored by liquid chromatography-diode-array detection/electrospray ionization mass spectrometry (LC-DAD/ESI-MS) analysis in the positive and negative ion mode.
RESULTS: The LC-DAD/ESI-MS characterization allowed the confirmation of the structures of the methylmethine-linked cat-mv3glc-(O)-cat ($[M–H]^-$ *m/z* 1097) and mv3glc-mv3glc-(O)-cat ($[M]^+$ *m/z* 1301) adducts.
CONCLUSIONS: The studies performed in model solutions showed that the colorless mv3glc-(O)-cat adduct can undergo some of the characteristic reactions of anthocyanins and flavan-3-ols that occur in red wine in the presence of acetaldehyde forming new methylmethine-bridged compounds. Copyright © 2012 John Wiley & Sons, Ltd.

Red wine is a complex matrix where polyphenolic compounds such as anthocyanins and flavan-3-ols (e.g. catechins and procyanidins) play the major role in its organoleptic properties (color and flavor). However, these compounds are very susceptible to undergo transformations during processing and storage. Therefore, several chemical reactions take place between anthocyanins, flavan-3-ols and also with small molecules (pyruvic acid, acetaldehyde, acetoacetic acid, etc.) yielding new stable anthocyanin-derived pigments (e.g. pyranoanthocyanins like vitisin A, vitisin B, methylpyranoanthocyanin, etc.) which contribute to the modification of the sensorial properties of red wines.[1–3] Among the several reactions involving red wine polyphenols, direct and acetaldehyde-mediated condensations between anthocyanins and flavan-3-ols have been widely studied.[4–12] One of the first reactions described in red wines was the polymerization reaction between anthocyanins and flavan-3-ols mediated by acetaldehyde,[4,5,7,13] which is essentially a by-product of ethanol oxidation.[14] Acetaldehyde could also arise from yeast metabolism during alcoholic fermentation. This kind of reaction leads to the formation of the purple-red anthocyanin-flavan-3-ol pigments linked by methylmethine bridges (being their λ_{max} is batochromatically shifted from genuine anthocyanins).

Direct reactions between anthocyanins and flavan-3-ols originate the dimeric-type flavanol-(4,8)-anthocyanin (F-A) and anthocyanin-(4,8)-flavanol (A-F) adducts. The characterization and formation pathway of F-A adducts in red wines is well documented in the literature and seems to be coherent among the authors, in opposition to the case of A-F pigments. In fact, the formation of A-F adducts in red wines is described in the literature through a mechanism in which a nucleophilic attack of flavanols (C-6/C-8) occurs towards the electropositive C-4 of anthocyanin giving rise to a colorless product (flavene structure). This adduct could further evolve to the colorless bicyclic form (additional interflavanolic linkage type-A, A-(O)-F) or undergo oxidation to give the red pigment A^+-F, which could dehydrate to the orange-yellow xanthylium salt.[10,12,15–17] The formation of a colorless flavene-type structure in red wines was described for the first time by Jurd in 1967.[18] The flavene form was later detected in model solutions by UV–vis and mass spectrometry.[19] Besides this adduct, the xanthylium form was also detected by UV–vis spectroscopy.[20,21] The bicyclic form of A-F adducts (additional ether linkage type-A) was identified by nuclear magnetic resonance (NMR) and their presence was confirmed in red wines.[11,17,22]

This work aimed to study the reactivity of the malvidin-3-glucoside-(O)-catechin adduct (previously synthesized) with catechin or mv3glc, both acetaldehyde-mediated, in order to simulate putative reactions that take place in red wines.

EXPERIMENTAL

Reagents

(+)-Catechin was purchased from Sigma-Aldrich® (Madrid, Spain). Malvidin-3-glucoside (mv3glc) was isolated from a young red table wine (*Vitis vinifera* L. cv. Touriga Nacional) by semi-preparative HPLC using a reversed-phase C18

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column (250 mm × 4.6 mm i.d.) as reported elsewhere,[8] and its structure and purity were confirmed by NMR. TSK Toyopearl gel HW-40(S) was purchased from Tosoh (Tokyo, Japan). Acetaldehyde was obtained from Fluka Chemika (Buchs, Switzerland).

Hemisynthesis of malvidin-3-glucoside-(O)-catechin

In order to maximize the yield of malvidin-3-glucoside-(O)-catechin, several experimental conditions were studied, namely pH, solvent, the temperature/time binomial and molar ratio of malvidin-3-glucoside and (+)-catechin. A solution containing mv3glc (2.3 mM):(+)-catechin (molar ratio of 1:20) was prepared in water (200 mL) at pH 2.5 (adjusted with dilute HCl or NaOH), protected from light and placed in the oven at 50 °C. The formation of the malvidin-3-glucoside-(O)-catechin compound was monitored every day by high-performance liquid chromatography (HPLC) with diode-array detection (DAD) at 280 nm. After 7 days of incubation, the synthetic reaction was stopped and the mixture purified.

Purification of malvidin-3-glucoside-(O)-catechin

The reaction mixture was extracted with ethyl acetate in order to remove the catechins and other organic impurities. The aqueous phase containing the desired compound was deposited onto a medium-porosity sintered glass funnel loaded with silica-C18 (reversed phase) connected to standard vacuum filtration glassware. The solution was washed with water and then eluted with methanol acidified with 2% HCl, yielding a more concentrated volume. Next, semi-preparative HPLC was performed in order to isolate the malvidin-3-glucoside-(O)-catechin compound. The final purification was made by column chromatography using TSK Toyopearl HW-40 (S) gel (250 mm × 16 mm i.d.) connected to a UV detector coupled to a computer. The flow rate was regulated at 0.8 mL/min using a peristaltic pump. The elution was performed with 70% aqueous acidified methanol and the detection wavelength was set to 280 nm. After removal of methanol on a rotary evaporator, the compound was freeze-dried and stored at −18 °C. The compound was further characterized by LC-DAD/ESI-MS and its structure and purity assessed by NMR analyses. The NMR data fits exactly with the that reported in the literature.[17]

Reactions of malvidin-3-glucoside-(O)-catechin with catechin (or mv3glc) mediated by acetaldehyde

Two solutions containing mv3glc-(O)-cat (1 mM):catechin (or mv3glc):acetaldehyde (molar ratio of 1:4:10) were prepared in 12% ethanol/water at pH 3.2 (adjusted with dilute HCl or NaOH), protected from light and placed in the oven at 30 °C. The formation of the new compounds was monitored by HPLC with DAD and by LC-DAD/ESI-MS analysis in positive and negative ion mode.

HPLC

The samples were analyzed by HPLC (Merck-Hitachi L-7100) on a 150 mm × 4.6 mm i.d. reversed-phase C18 column (Merck, Darmstadt, Germany) thermostatted at 25 °C; detection was carried out at 280 nm using a diode-array detector (Merck-Hitachi L-7450A). Solvents were (A) water/formic acid 9:1 (v/v) and (B) acetonitrile, with the following gradient: 10–35% B over 50 min at a flow rate of 0.5 mL/min. The sample injection volume was 20 µL. The chromatographic column was washed with 100% B during 10 min and then stabilized with the initial conditions during another 10 min.

The semi-preparative HPLC system (Elite LaChrom) was composed of a L-2130 quaternary pump, a manual injector with loop of 1 mL and a L-2420 UV-vis detector. The stationary phase was composed of a reversed-phase C18 column (Merck, Darmstadt, Germany) (250 mm × 4.6 mm i.d., 5 µm pore size) and the eluents were (A) water/formic acid 9:1 (v/v) and (B) acetonitrile, with the following gradient: 10–35% B over 50 min at a flow rate of 1 mL/min. Detection wavelength was set to 280 nm.

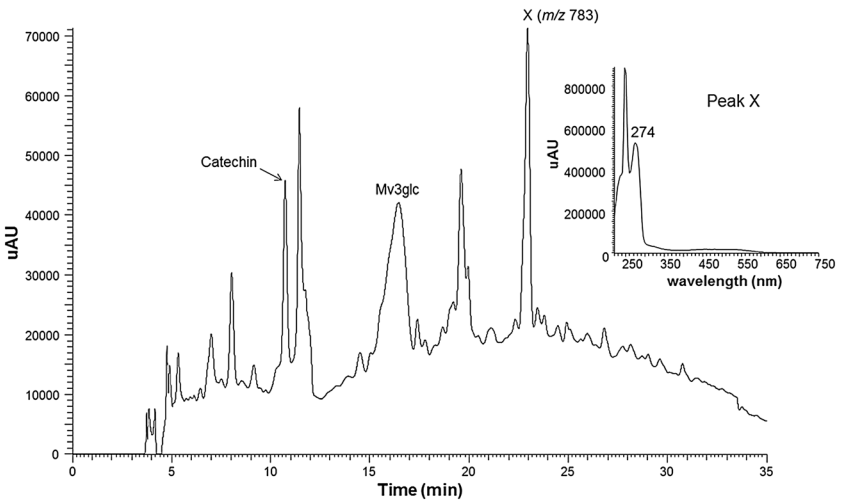

Figure 1. PDA chromatogram obtained from the LC-DAD analysis of the aqueous fraction obtained from the reaction between malvidin-3-glucoside and catechin. Inset: UV-vis spectrum of peak X (*m/z* 783).

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Table 1. Mass data of peak X (mv3glc-(O)-cat) obtained from the ESI-MS analysis in the positive ion mode

Compound	$[M+H]^+$ *m/z*	MS^2 fragments
mv3glc-(O)-cat	783	451 (−332) 469 (−314) 495 (−288) 621 (−162) 631 (−152) 657 (−126) 747 (−36)

LC-DAD/ESI-MS analysis

A Finnigan Surveyor series liquid chromatograph, equipped with a Thermo Finnigan (Hypersil Gold) reversed-phase column (150 mm × 4.6 mm, 5 μm, C18) thermostatted at 25 °C, was used. The samples were analyzed using the same solvents, gradients, injection volume and flow rate referred above for HPLC analysis. Double-online detection was done by a photodiode spectrophotometer and mass spectrometry. The mass detector was a Finnigan LCQ DECA XP MAX (Finnigan Corp., San Jose, CA, USA) quadrupole ion trap equipped with an atmospheric pressure ionization (API) source, using an electrospray ionization (ESI) interface. The vaporizer and the capillary voltages were 5 kV and 4 V, respectively. The capillary temperature was set at 325 °C. Nitrogen was used both as sheath and auxiliary gas at flow rates of 90 and 25, respectively (in arbitrary units). Spectra were recorded in positive and negative ion mode between *m/z* 250 and 1500.

RESULTS AND DISCUSSION

Hemisynthesis of malvidin-3-glucoside-(O)-catechin

The reaction of malvidin-3-glucoside (mv3glc) with (+)-catechin (molar ratio 1:20) was performed in order to synthesize the mv3glc-catechin dimeric structure. The reaction was followed by HLPC and after 7 days of incubation the reaction was stopped. After the work-up and the purification procedures the sample was analyzed by LC-DAD/MS in the positive ion mode (Fig. 1).

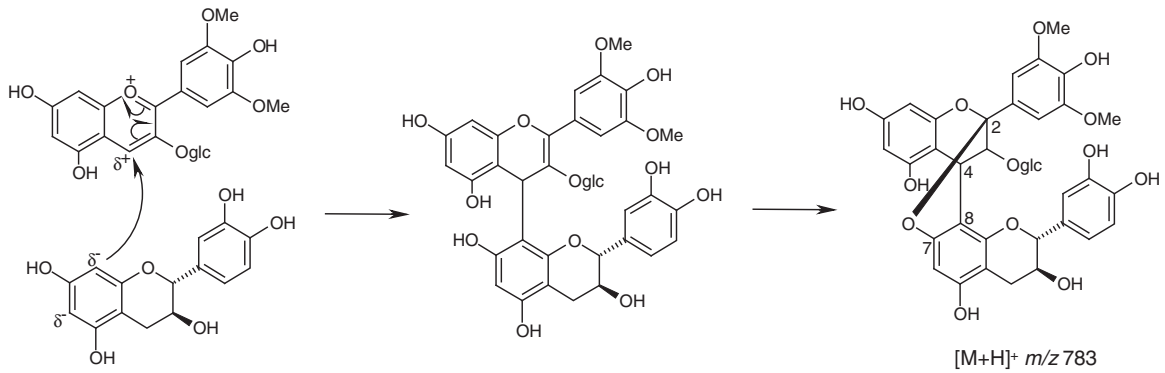

Figure 2. Formation pathway of the mv3glc-(O)-cat adduct (*m/z* 783).

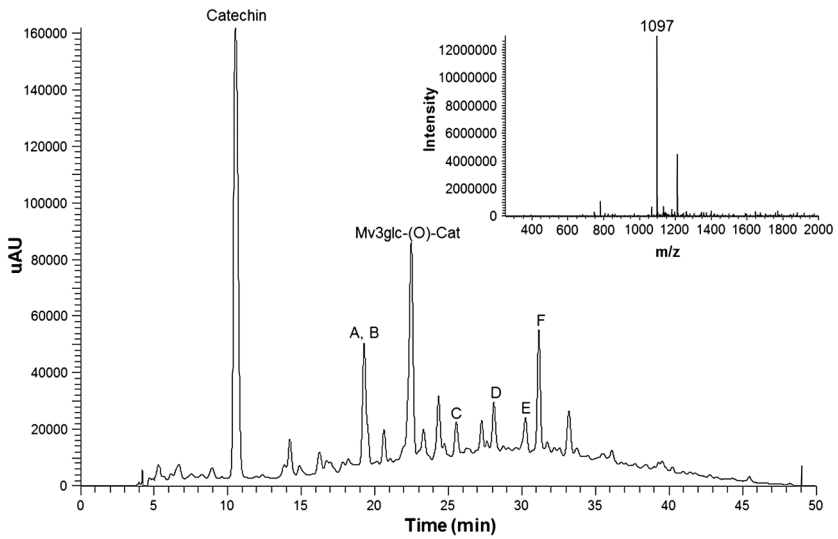

Figure 3. PDA chromatogram obtained from the LC-DAD analysis in the negative ion mode of the reaction of mv3glc-(O)-cat, (+)-catechin and acetaldehyde (1:4:10). Inset: Full LC/MS spectra of peaks A–D (*m/z* 1097).

The PDA chromatogram showed that the chromatographic peak identified as peak X presents a pseudo-molecular ion ($[M+H]^+$) at *m/z* 783 and MS^2 fragments that agree with the structure of the compound constituted by a mv3glc moiety linked to a catechin molecule (Table 1).

The major MS^2 fragments obtained were as follows: *m/z* 631 resulting from retro-Diels-Alder (RDA) fission of the catechin unit ($[M+H-152]^+$), *m/z* 621 resulting from the loss of a glucose residue ($[M+H-162]^+$) and *m/z* 657 corresponding to the loss of 126 u ($[M+H-126]^+$) and its aglycone, *m/z* 495 ($[M+H-126-162]^+$).

The UV-vis spectrum of peak X (λ_{max} 274 nm) revealed that it is a colorless compound. After the compound isolation by semi-preparative HPLC, the full ^1H and ^{13}C NMR characterization confirmed that it corresponds to a mv3glc-(4,8)-catechin dimeric structure in its bicyclic form (additional ether linkage type-A, C2-O-C7), which agrees with the data already published for the mv3glc-(O)-(−)-epicatechin compound.[17] This kind of structure suggests that the formation pathway of this dimeric compound preferentially evolves towards to the formation of the bicyclic form (more stable structure) rather than to the cationic flavylium form (Fig. 2).

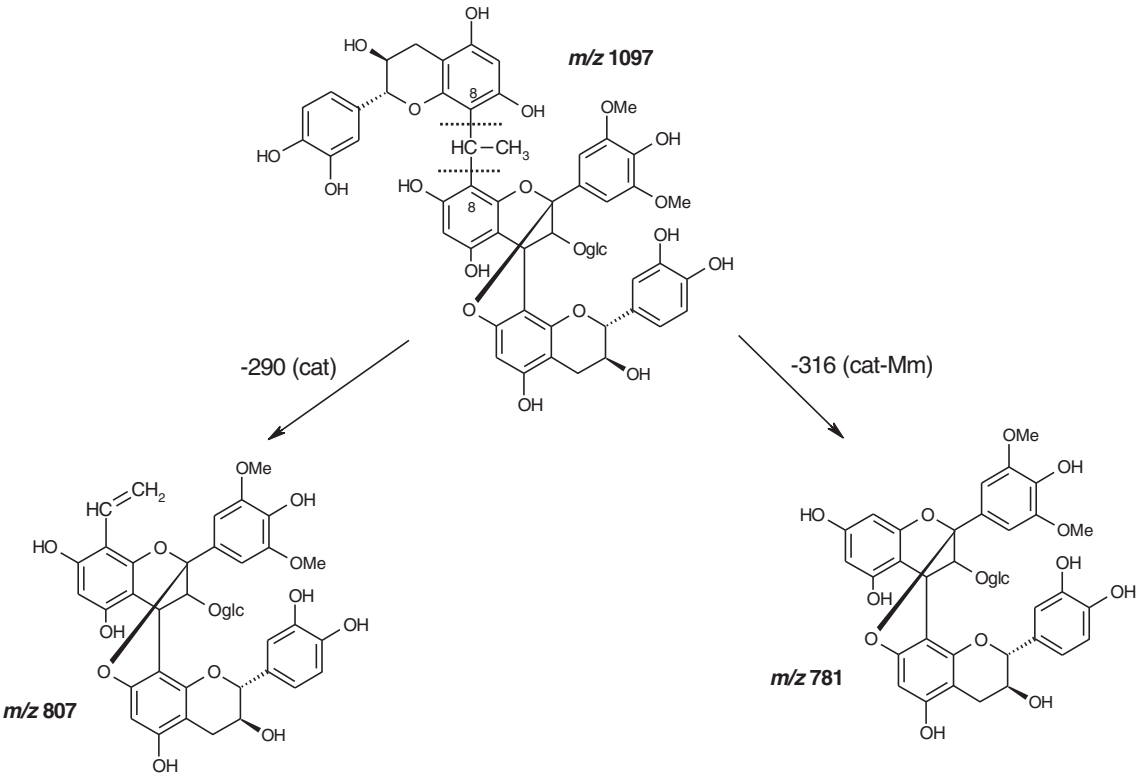

Figure 4. Fragmentation pattern of the cat-(8,8)-Mm-mv3glc-(O)-cat adduct in the negative ion mode.

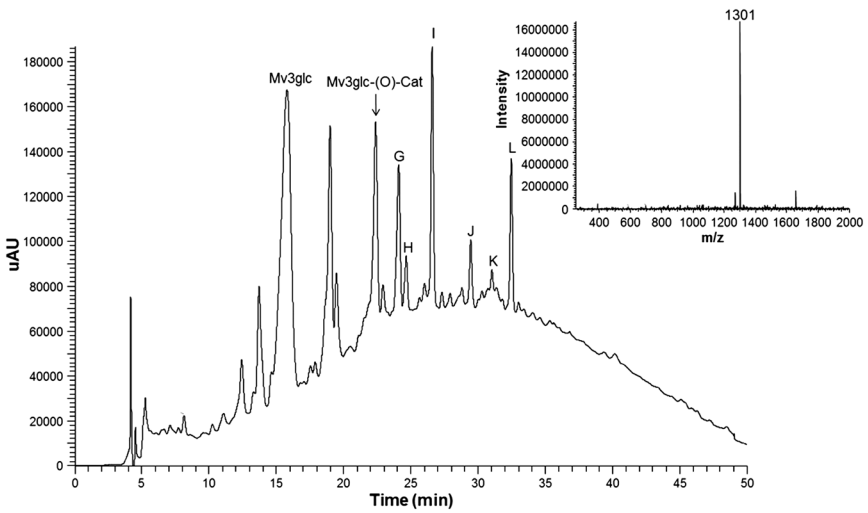

Figure 5. PDA chromatogram obtained from the LC-DAD analysis of the reaction mv3glc-(O)-cat, mv3glc and acetaldehyde (1:4:10). Inset: Full LC/MS spectra of peaks G–L (*m/z* 1301).

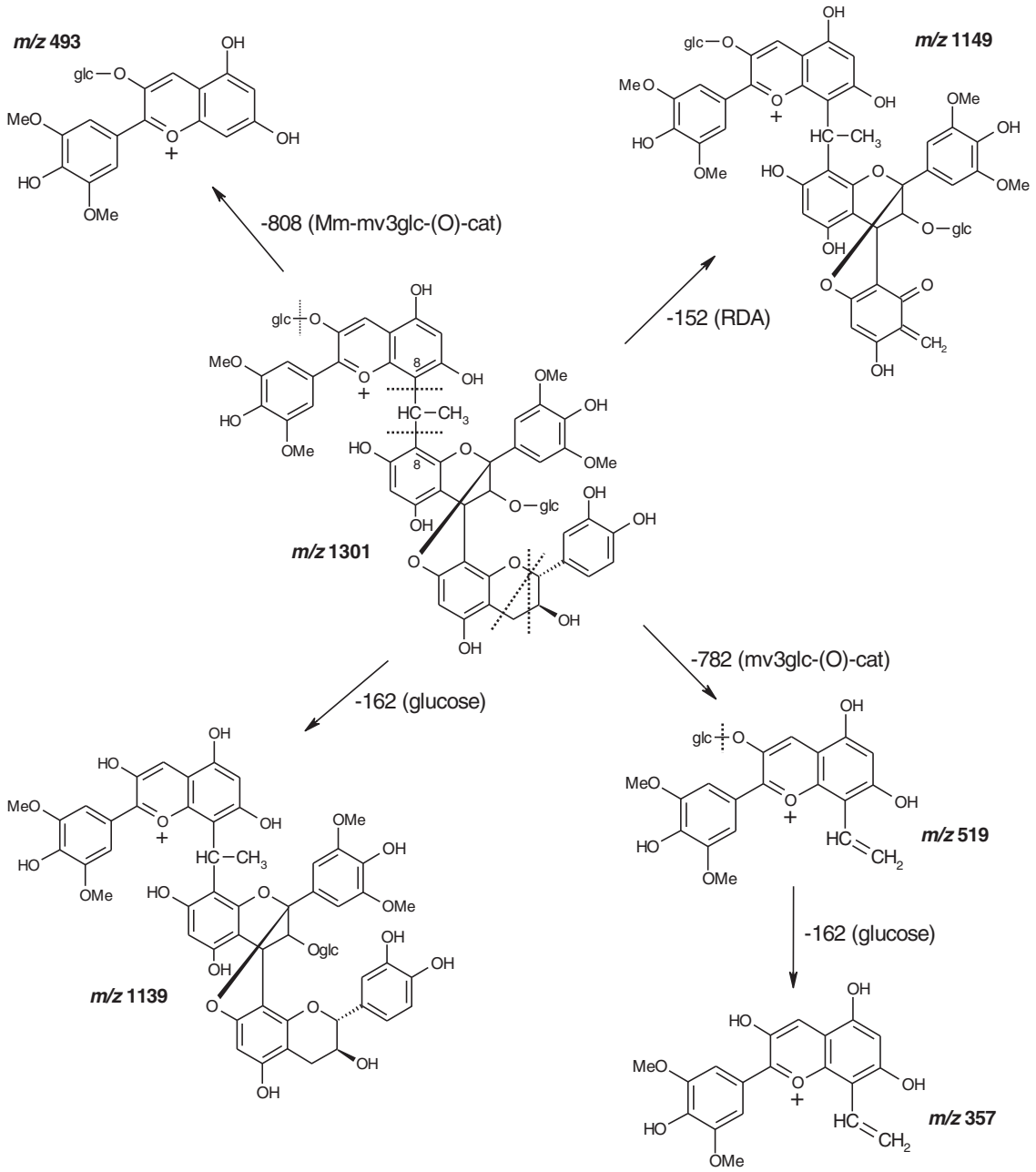

Figure 6. Fragmentation pattern of the mv3glc-(8,8)-Mm-mv3glc-(O)-cat adduct in the positive ion mode.

Hemisynthesis of malvidin-3-glucoside-(O)-catechin-derived compounds

It is well known that in red wines or in model solutions, flavan-3-ols and anthocyanins, in the presence of acetaldehyde, condense with each other through methylmethine (Mm) bridges.[23–26] According to the mechanism reported in the literature,[7] in acidic medium acetaldehyde originates the respective carbocation which readily undergoes nucleophilic attacks from the C-6/C-8 (preferentially) catechin positions that have a high negative charge density due to the presence of hydroxyl groups in *ortho* and *para* positions.[27] Then a dehydration occurs giving rise to the formation of an intermediate carbocation which then undergoes nucleophilic attacks from the C-6/C-8 (preferentially) of the mv3glc (hydrated form) leading to the formation of the adduct linked by the methylmethine bridge. This type of compounds contribute to the evolution of red wine color during ageing and storage and that is why their occurrence and formation have been widely studied.

In this work, reactions of malvidin-3-glucoside-(O)-catechin with catechin and with malvidin-3-glucoside mediated by acetaldehyde were separately performed in order to investigate the formation of compounds with methylmethine bridges. It is expected that each reaction will occur by the formation of several positional isomers at C-6 or C-8 of the upper unit methylmethine linked to two C-6 positions or one C-8 position of the lower unit. Furthermore, two diastereoisomers may be obtained for each positional isomer differing in the configuration of the asymmetric carbon of the methylmethine bridge (*R*/*S*).[6,28,29]

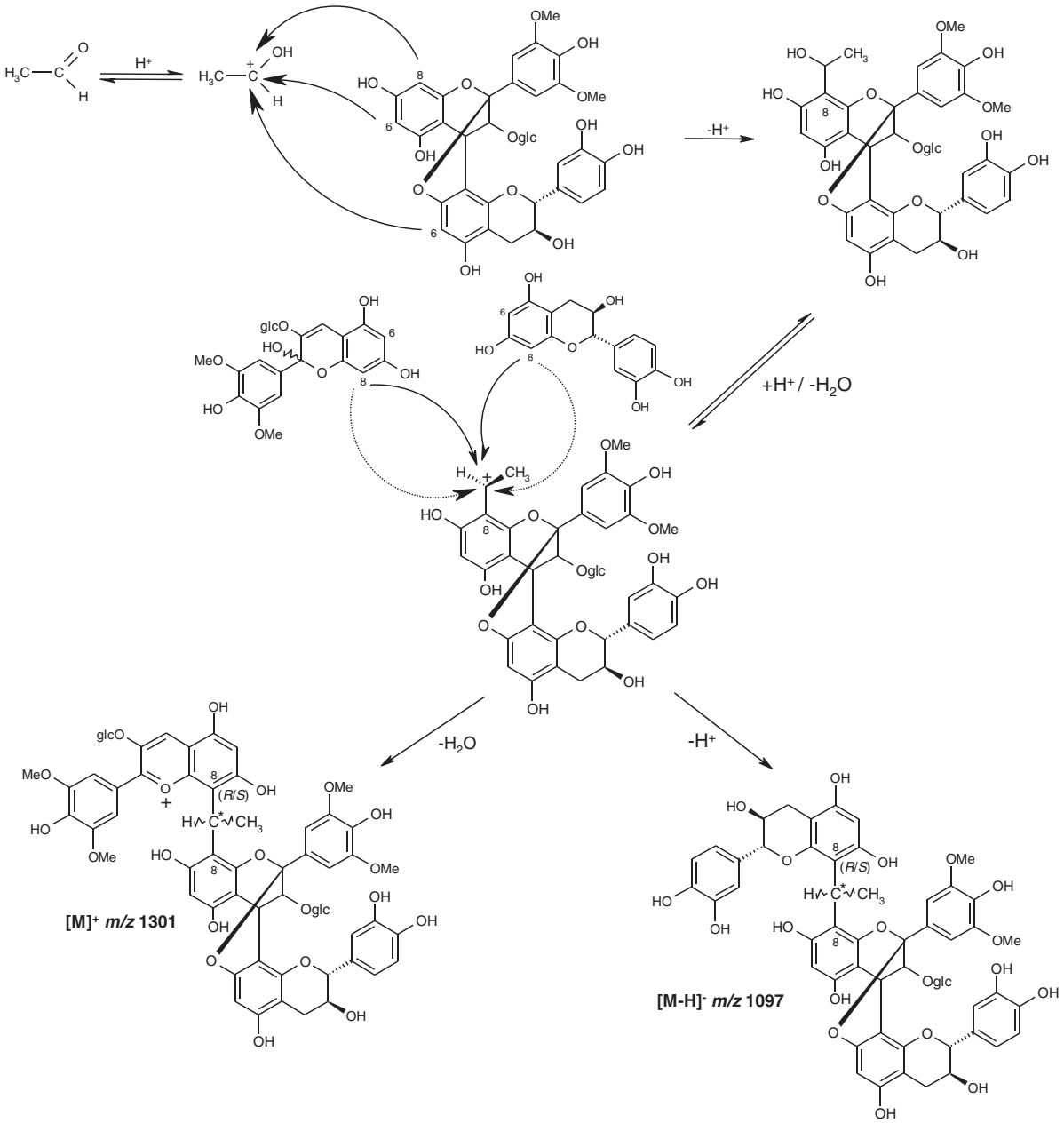

Figure 7. Proposed reaction mechanisms between mv3glc-(O)-cat and catechin, and between mv3glc-(O)-cat and mv3glc, both mediated by acetaldehyde.

Reaction between malvidin-3-glucoside-(O)-catechin and catechin mediated by acetaldehyde

The evolution of the reaction between mv3glc-(O)-cat and catechin mediated by acetaldehyde was followed by HLPC. LC-DAD/MS analysis of the reaction mixture in the negative ion mode was performed and six chromatographic peaks (peaks A–D, Fig. 3) with the same pseudo-molecular ion ($[M–H]^-$ *m/z* 1097) were detected.

The six chromatographic peaks detected with *m/z* 1097 should correspond to the six positional isomers that are expected to be formed. The pseudo-molecular ions and the respective MS^2 fragmentations patterns are consistent with the structure composed by a catechin molecule and a mv3glc-(O)-catechin adduct linked by a methylmethine bridge. The methymethine compounds formed are colorless shown by their UV-vis spectra (λ_{max} 277 nm). The two most common signals in the fragmentation pattern of the pseudo-molecular ion were *m/z* 807 ($[M–H–290]^-$) and 781 ($[M–H–316]^-$) which correspond to the loss of a catechin moiety and a methylmethine-catechin unit, respectively (Fig. 4).

Reaction between malvidin-3-glucoside-(O)-catechin and mv3glc mediated by acetaldehyde

The reaction between mv3glc-(O)-cat and mv3glc mediated by acetaldehyde was analyzed by LC-DAD/MS and the chromatogram revealed six peaks (peaks G–L, Fig. 5) with the same molecular ion ($[M]^+$ *m/z* 1301).

The UV-vis spectra of these peaks revealed that the new compounds formed have a purple-red color (λ_{max} 544 nm) and their mass data is consistent with a compound composed

of the mv3glc (flavylium form) molecule and the mv3glc-(O)-cat adduct linked by a methylmethine bridge. Some molecular fragments released are in agreement with the proposed structure, namely *m/z* 1149 ([M–152]$^+$), 1139 ([M–162]$^+$), 519 ([M–782]$^+$), and 493 ([M–808]$^+$), which correspond to RDA fission, loss of a glucose residue, loss of the mv3glc-(O)-catechin adduct and loss of a methylmethine-mv3glc-(O)-cat moiety, respectively (Fig. 6).

Formation mechanism

According to the mechanism described in the literature,[7] in acidic medium acetaldehyde attacks the phloroglucinol rings of the mv3glc-(O)-cat which presents a negative charge density in carbons C-6/C-8 (preferentially), to give the respective ethanol-mv3glc-(O)-cat adduct. Further dehydration gives rise to the formation of an intermediate carbocation which then undergoes nucleophilic attacks from the C-6/C-8 of the mv3glc in hydrated form or from the C-6/C-8 of the catechin leading to the formation of the respective adducts linked by methylmethine bridges (Fig. 7).

The proposed structures with 8,8-methylmethine displayed in Fig. 7 are probably the ones that preferentially formed bridges because the C-8 carbon possesses a higher negative charge density than the C-6 position[27,30] and their formation should thus be favored. The formation of several compounds in low yields did not allow their isolation to proceed to complete the structural elucidation by NMR.

CONCLUSIONS

The studies performed in model solutions showed that the colorless mv3glc-(O)-cat adduct can undergo some of the characteristic reactions of anthocyanins and flavan-3-ols that occur in red wines in the presence of acetaldehyde forming methylmethine-bridged compounds. The structures of the resulting compounds were confirmed by their mass fragmentation patterns and a formation mechanism was proposed. These results bring more insights on the putative complexity of the polyphenolic composition of red wines and opens new pathways to discover more complex and polymeric compounds in such matrices.

Acknowledgements

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REFERENCES

[1] J. Bakker, C. F. Timberlake. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* **1997**, *45*, 35.
[2] H. Fulcrand, C. Benabdeljalil, J. Rigaud, V. Cheynier, M. Moutounet. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401.
[3] J. He, C. Santos-Buelga, A. M. S. Silva, N. Mateus, V. De Freitas. Isolation and structural characterization of new anthocyanin-derived yellow pigments in aged red wines. *J. Agric. Food. Chem.* **2006**, *54*, 9598.
[4] C. Dallas, J. M. Ricardo-Da-Silva, O. Laureano. Products formed in model wine solutions involving anthocyanins, procyanidin B-2, and acetaldehyde. *J. Agric. Food. Chem.* **1996**, *44*, 2402.
[5] N. E. Es-Safi, H. Fulcrand, V. Cheynier, M. Moutounet. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47*, 2096.
[6] J. C. Rivas-Gonzalo, S. Bravo-Haro, C. Santos-Buelga. Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. *J. Agric. Food Chem.* **1995**, *43*, 1444.
[7] C. F. Timberlake, P. Bridle. Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976**, *27*, 97.
[8] J. Pissarra, N. Mateus, J. C. Rivas-Gonzalo, C. Santos-Buelga, V. De Freitas. Reaction between malvidin 3-glucoside and (+)-catechin in model solutions containing different aldehydes. *J. Food Sci.* **2003**, *68*, 476.
[9] Y. Hayasaka, J. A. Kennedy. Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Aust. J. Grape Wine Res.* **2003**, *9*, 210.
[10] L. Jurd. Review of polyphenol condensation reactions and their possible ocurrence in aging of wines. *Am. J. Enol. Vitic.* **1969**, *20*, 195.
[11] S. Remy, H. Fulcrand, B. Labarbe, V. Cheynier, M. Moutounet. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* **2000**, *80*, 745.
[12] T. C. Somers. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175.
[13] E. Haslam, T. H. Lilley. Natural astringency in foodstuffs – a molecular interpretation. *Crit. Rev. Food Sci. Nutr.* **1988**, *27*, 1.
[14] H. L. Wildenradt, V. L. Singleton. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic.* **1974**, *25*, 119.
[15] M. Duenas, E. Salas, V. Cheynier, O. Dangles, H. Fulcrand. UV-visible spectroscopic investigation of the 8,8-methylmethine catechin-malvidin 3-glucoside pigments in aqueous solution: Structural transformations and molecular complexation with chlorogenic acid. *J. Agric. Food. Chem.* **2006**, *54*, 189.
[16] H. Liao, Y. Cai, E. Haslam. Polyphenols interactions. Anthocyanins: Copigmentation and colour changes in red wines. *J. Sci. Food Agric.* **1992**, *59*, 299.
[17] S. Remy-Tanneau, C. Le Guerneve, E. Meudec, V. Cheynier. Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry. *J. Agric. Food. Chem.* **2003**, *51*, 3592.
[18] L. Jurd. Anthocyanidins and related compounds. *XI. Catechin-flavylium salt condensation reactions. Tetrahedron* **1967**, *23*, 1057.
[19] M. Mirabel, C. Saucier, C. Guerra, Y. Glories. Copigmentation in model wine solutions: Occurrence and relation to wine aging. *Am. J. Enol. Vitic.* **1999**, *50*, 211.
[20] C. Santos-Buelga, S. Bravo-Haro, J. C. Rivas-Gonzalo. Interactions between catechin and malvidin-3-monoglucoside in model solutions. *Z. Lebensm.-Unters.-Forsch.* **1995**, 269.
[21] C. Santos-Buelga, E. M. Francia-Aricha, S. De Pascual-Teresa, J. C. Rivas-Gonzalo. Contribution to the identification of the pigments responsible for the browning of anthocyanin-flavanol solutions. *Eur. Food Res. Technol.* **1999**, *209*, 411.

[22] P. D. Bishop, C. W. Nagel. Characterization of the condensation product of malvidin-3,5-diglucoside and catechin. *J. Agric. Food. Chem.* **1984**, *32*, 1022.
[23] T. Escribano-Bailon, M. Alvarez-Garcia, J. C. Rivas-Gonzalo, F. J. Heredia, C. Santos-Buelga. Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin 3-*O*-glucoside and (+)-catechin. *J. Agric. Food. Chem.* **2001**, *49*, 1213.
[24] N. E. Es-Safi, V. Cheynier, M. Moutounet. Role of aldehydic derivatives in the condensation of phenolic compounds with emphasis on the sensorial properties of fruit-derived foods. *J. Agric. Food. Chem.* **2002**, *50*, 5571.
[25] N. E. Es-Safi, H. Fulcrand, V. Cheynier, M. Moutounet. Competition between (+)-catechin and (−)-epicatechin in acetaldehyde-induced polymerization of flavanols. *J. Agric. Food. Chem.* **1999**, *47*, 2088.
[26] C. Saucier, D. Little, Y. Glories. First evidence of acetaldehyde-flavanol condensation products in red wine. *Am. J. Enol. Vitic.* **1997**, *48*, 370.
[27] G. Bendz, O. Martensson, E. Nilsson. Studies of flavylium compounds. I. Some flavylium compounds and their properties. *Arkiv Kemi.* **1967**, *27*, 67.
[28] T. Escribano-Bailón, O. Dangles, R. Brouillard. Coupling reactions between flavylium ions and catechin. *Phytochemistry* **1996**, *41*, 1583.
[29] D. F. Lee, E. E. Swinny, G. P. Jones. NMR identification of ethyl-linked anthocyanin-flavanol pigments formed in model wine ferments. *Tetrahedron Lett.* **2004**, *45*, 1671.
[30] V. Atanasova, H. Fulcrand, C. Le Guerneve, W. Cheynier, M. Moutounet. Structure of a new dimeric acetaldehyde malvidin 3-glucoside condensation product. *Tetrahedron Lett.* **2002**, *43*, 6151.

