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Preliminary Study of Oaklins, a New Class of Brick-Red Catechinpyrylium Pigments Resulting from the Reaction between Catechin and Wood Aldehydes

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Several structurally related pigments were found to result from the reaction between catechin and coniferaldehyde/sinapaldehyde extracted from oak wood. Their structures were tentatively identified by mass spectrometry, and their formation was studied in different pH and temperature conditions for several days. They were all found to have a characteristic catechinpyrylium core, thereby constituting a new class of compounds named as oaklins. One of the main oaklins was also detected in a commercial table red wine aged in oak barrels.

KEYWORDS: Oaklins; brick-red pigments; catechin; sinapaldehyde; coniferaldehyde; oak wood; red wine

INTRODUCTION

The use of oak (e.g., barrels and chips) in wine storage during the first years of aging is a procedure widely employed by winemakers, strengthening the wine organoleptic characteristics and contributing to its stability. Nevertheless, it must be carried out with caution to obtain better quality and well-balanced wines (*1*).

Aging in oak wood allows wine to extract a series of phenolic and volatile compounds, depending upon the characteristics of the oak and the contact time between wine and wood. The factors that affect the pool of oak extractives are the species and geographical origin of the wood (*2*, *3*), as well as its processing, especially the method to obtain the staves, their seasoning, and the degree of oak toasting (*4*−*7*).

Among the potential compounds extractable from oaks, furanic (e.g., furfural and hydroxymethylfurfural), benzoic (e.g., hydroxybenzaldehyde and vanillin), and cinnamic aldehydes (e.g., coniferaldehyde and sinapaldehyde) are of particular interest because they can interact with some wine components (e.g., catechins and anthocyanins), hence contributing directly or indirectly to color and other sensory changes. In fact, several studies have demonstrated that different aldehydes can serve as intermediaries in reactions involving catechin and anthocyanins or even react directly with catechin, yielding a multiplicity of pigments with different color characteristics (*8*−*14*). Recently, the formation of a new red/orange (λ_{max} at 500 nm) catechinpyrylium-derived pigment has been shown for the first time resulting from the direct reaction between catechin and sinapaldehyde, one of the main oak wood aldehydes (*15*). Accordingly, analogous compounds can be expected to form through the reaction of oak-derived aldehydes with one or several units of catechin. Nevertheless, the existence of this type of pigment in wines aged in oak systems is yet to be reported, as well as their possible role in color changes observed during the aging period.

This work deals with the formation and structural study of new brick-red pigments resulting from the reaction between catechin and an extract solution rich in oak aldehydes, especially sinapaldehyde and coniferaldehyde. The likely presence of these pigments in a red wine aged in oak barrels was also investigated.

MATERIALS AND METHODS

Standards. Catechin was purchased from Sigma−Aldrich, Inc. (St. Louis, MO).

Extraction of Oak Wood Aldehydes. Oak (*Quercus robur)* chips (160 g) were incubated with 1 L of 12% aqueous ethanol adjusted to pH 3.5 set with 2% HCl during 15 h at room temperature and with periodic stirring. Aldehydes were extracted from the hydroalcoholic extract using ethyl acetate (3 × 350 mL) at pH 7. The solvent was evaporated in a rotator evaporator at 30 °C, and the residue was stored at −18 °C until use.

Formation of Catechinpyrylium-Derived Pigments. The oak extract was incubated with catechin (8 mM) in 50 mL of a 12% hidroalcoholic solution at pH 1.5. This model solution was kept at 35 °C and protected from light, and the formation of new compounds was followed by HPLC-DAD.

Analysis of the Formation of Catechinpyrylium-Derived Pigments at Different pH Values and Different Temperatures. Catechin (8 mM) was incubated with coniferaldehyde (0.7 mM) and sinapaldehyde (0.7 mM) in 5 mL of 12% hidroalcoholic solutions at different

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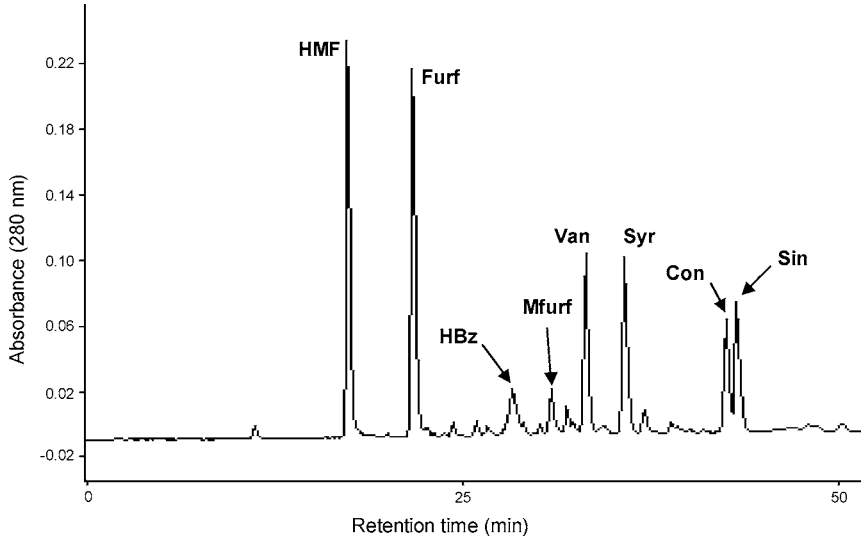

Figure 1. HPLC chromatogram recorded at 280 nm of the oak extract. HMF, hydroxymethylfurfural (0.13 g/kg of oak); Furf, furfural (0.13 g/kg); Mfurf, methylfurfural (0.02 g/kg); HBz, hydroxybenzaldehyde (0.02 g/kg); Van, vanillin (0.11 g/kg); Syr, syringaldehyde (0.10 g/kg); Con, coniferaldehyde (0.08 g/kg); Sin, sinapaldehyde (0.28 g/kg).

pH values (3.5, 2.5, and 1.5). These model solutions were kept at two different temperatures (25 and 35 °C) and protected from light, and the formation of new compounds was followed by HPLC-DAD. The analysis were done in duplicate for each sample.

HPLC Analysis. The extract of oak aldehydes was analyzed by HPLC using a 250 × 4.6 mm i.d. reversed-phase C18 column (Knauer, Berlin, Germany) at 40 °C. Solvents were (A) water/formic acid (98:2) and (B) methanol/water/formic acid (70:28:2), and separation was performed using the following gradient: isocratic 100% A over 3 min, 100% A to 60% A over 22 min, 60% A to 40% B over 18 min, isocratic 40% A over 12 min, 40% A to 20% A over 5 min, and isocratic 20% A over 5 min, at a flow rate of 1 mL/min. Peak separation was monitored at 280 nm using a L-7450 DAD detector (Knauer, Berlin, Germany), and the quantification of each aldehyde was done using standard curves made with reference compounds.

The sample containing catechin and the oak extract and the samples containing catechin and coniferaldehyde and sinapaldehyde were analyzed by HPLC-DAD using a 250 × 4.6 mm i.d. reversed-phase C18 column at 25 °C. Solvents were (A) water/formic acid (95:5) and (B) acetonitrile. The elution gradient was performed using a K-1001 Knauer pump from 10 to 35% B for 50 min at a flow rate of 1.5 mL/min.

Fractionation of Catechinpyrylium-Derived Pigments. The reaction extract was applied on a 200 × 40 mm polyamide resin > 80−100 mesh (Balin Petrol Chemical Co. Ltd., Hunan, China) column, and it was progressively eluted with 2% aqueous HCl with increasing percentages of methanol in water to obtain different fractions: F1, 40% of methanol; F2, 50%; F3, 60%; and F4, 70%. The criterion used for changing the percentages of methanol was the decrease of the color intensity of the solution eluted from the column. The solvent of each fraction was partially evaporated in a rotator evaporator at 30 °C, and the samples were freeze-dried and stored at −18 °C until use. These fractions were analyzed directly by LC/DAD−MS.

Fractionation of Red Wine Aged in Oak. A 4-year-old commercial table red wine produced by Barros, Almeida, and Ca., Vinhos, S.A using typical varieties of Douro Demarked Region and aged for a year in oak barrels was chosen for this study. A total of 500 mL of previously evaporated wine (to remove ethanol) was directly applied into a 10 cm diameter medium-porosity sintered glass funnel with polyamide resin connected to standard vacuum filtration glassware and gradually eluted with increasing percentages of acidified methanol (20, 40, 60, and 80%). The solvent of the three latter fractions was evaporated in a rotatory evaporator at 30 °C, and the samples were individually applied into a 250 × 16 mm i.d. TSK Toyopearl gel HW-40(S) (Tosoh, Japan) column and eluted with 70% aqueous methanol for 3 h, the period in which different fractions were collected. The solvent of each fraction was partially evaporated in a rotator evaporator at 30 °C, and the samples were freeze-dried and stored at −18 °C until use.

LC−MS Conditions. A Hewlett−Packard 1100 series liquid chromatograph, equipped with an 150 × 4.6 mm, 5 μm AQUA reversed-phase C18 column (Phenomenex, Torrance, CA) thermostated at 35 °C was used. Solvents were (A) aqueous 0.1% trifluoroacetic acid and (B) acetonitrile, using the gradient as reported in the literature (*16*). The capillary voltage was 3 V, and the capillary temperature was 190 °C. Spectra were recorded in positive ion mode between *m*/*z* 120 and 1500. The mass spectrometer was programmed to do a series of three scans: a full mass, a MS^2 of the most intense ion, and a MS^3 of the most intense ion in the second scan, using a relative collision energy of 45.

RESULTS AND DISCUSSION

Analysis of the oak extract by HPLC revealed the presence of several aldehydes (**Figure 1**), identified according to their retention times and UV/vis spectra compared to standards. The main peaks corresponded to hydroxymethylfurfural and furfural. Apart from these furanic aldehydes, methylfurfural, benzoic aldehydes (hydroxybenzaldehyde, vanillin, and syringaldehyde), and cinnamic aldehydes (coniferaldehyde and sinapaldehyde) were also detected. These aldehydes have already been reported in oak by several authors (*7*, *17*−*21*).

It is well-known that aldehydes such as acetaldehyde, propionaldehyde, benzaldehyde, furfural, hydroxymethylfurfural, and glyoxylic acid react with flavanol compounds and also with catechins and anthocyanins, giving rise to several compounds such as catechin-alkyl-anthocyanin adducts, catechin adducts, and xanthylium salts (*16*, *22*) in acidic conditions. Thus, it was expected that oak-derived aldehydes could also react with flavanol compounds yielding new compounds.

In fact, after only 1 day, the reaction between the aldehyde-rich oak extract and catechin at pH 1.5 led to the formation of several new compounds (**Figure 2**). Practically all of these new compounds showed maximum absorption in the visible range between 480 and 520 nm, which confers on them an orange/red color. The λ_{max} in the visible spectra of each compound is given in **Table 1**.

The reaction products were fractioned on polyamide resin with increasing percentages of methanol, and four fractions with different pigment composition were obtained. Enlargement of

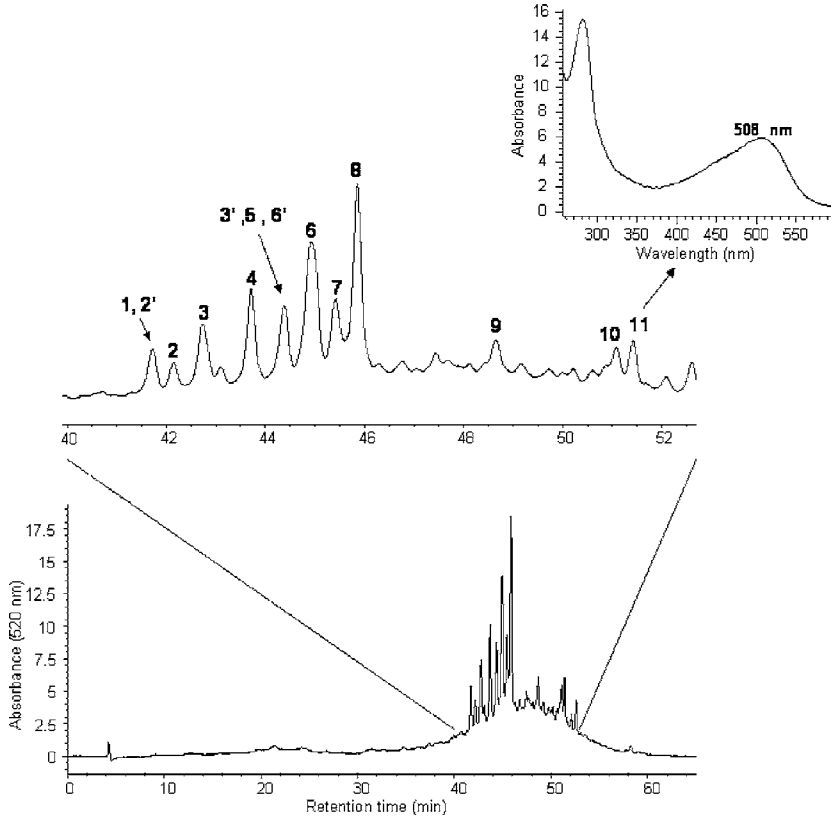

Figure 2. HPLC chromatogram recorded at 520 nm of the reaction between catechin and the aldehyde-rich oak extract. UV/vis spectrum of peak 11.

Table 1. Pigments Detected in the Different Fractions by LC–MS Analysis Resulting from the Reaction between Catechin and the Extraction Solution Rich in Oak Aldehydes[a]

peak number	Rt (min)	λ_{max} vis (nm)	peak identity	*m*/*z* MS	*m*/*z* MS^2	fragments	*m*/*z* MS^3	fragments	fractions
1	41.7	510	unidentified	699	547	RDA	395	RDA	not detected[b]
2	42.1	516	11-SCP-6-sinapyl alcohol 1	687	535	RDA			F1
2′	41.7	516	11-SCP-6-sinapyl alcohol 2	687	535	RDA			F1
3	42.6	500	11-GCP-9-cat 1	739	721	loss of H_2O	297	RDA	F3
					569	loss of H_2O and RDA			
					449	loss of cat			
3′	44.3	498	11-GCP-9-cat 2	739	721	loss of H_2O	297	RDA	F3, F4
					569	loss of H_2O and RDA			
					449	loss of cat			
4	43.6	511	11-SCP-9-cat	769	751	loss of H_2O	327	RDA	F3
					599	loss of H_2O and RDA			
					479	loss of cat			
5	44.4	490	unidentified	607	243	[M-364]			not detected[b]
6	44.9	494	11-GCP 1	449	297	RDA	282	loss of CH_3	F2, F3, F4
							269	loss of CO	
6′	44.3	494	11-GCP 2	449	297	RDA	282	loss of CH_3	F2, F3, F4
							269	loss of CO	
7	45.3	503	11-SCP 1	479	327	RDA	299	loss of CO	F2, F3, F4
8	45.7	500	11-SCP 2	479	327	RDA	299	loss of CO	F3, F4
9	48.6	488	unidentified	901	749	RDA	597	RDA	F3, F4
10	51.1	499	GCP-ethyl-cat 1	765	475	loss of cat	323	RDA	F3, F4
10′	50.5	499	GCP-ethyl-cat 2	765	475	loss of cat	323	RDA	F4
11	51.3	510	SCP-ethyl-cat 1	795	505	loss of cat	353	RDA	F3, F4
11′	51.0	507	SCP-ethyl-cat 2	795	505	loss of cat	353	RDA	F4

[a] SCP, syringylcatechinpyrylium; GCP, guaiacylcatechinpyrylium; cat, catechin; RDA, retro Diels–Alder. [b] Eluted from the toyopearl column with a lower percentage of methanol.

the respective chromatograms, in the zone between 40 and 52 min, of each fraction is presented in **Figure 3**. The molecular weight and mass spectra of these pigments were determined by LC−DAD/ESI−MS, and the results are summarized in **Table 1**. The mass spectra and the respective MS^2 and MS^3 fragmentations of peaks 4 and 6 are presented in **Figure 4** as an example.

Catechinpyrylium Derivates. A brick-red pigment (λ_{max} at 500 nm) with a molecular ion $[M]^+$ at *m*/*z* 479 and with a major fragment at *m*/*z* 327 ($[M-152]^+$) resulting from a RDA (retro Diels−Alder) fragmentation was detected in fractions F3 and F4 (peak 8). The ion at *m*/*z* 327 was also fragmented and yielded another fragment at *m*/*z* 299 (loss of CO, $[M-28]^+$) which was detected in the MS^3 spectra. These results and the retention times

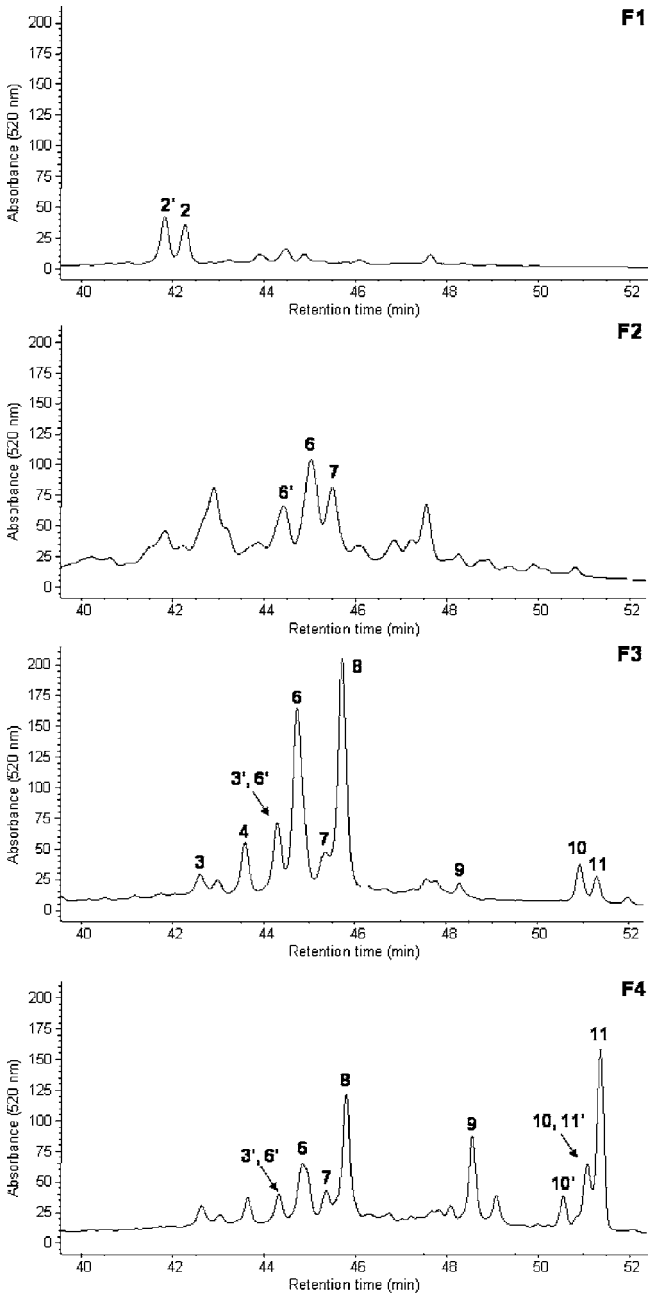

Figure 3. Part of the HPLC chromatograms recorded at 520 nm of fractions F1, F2, F3, and F4.

are consistent with the previously reported data for the brick-red catechinpyrylium-derived pigment formed from the reaction between catechin and sinapaldehyde (*15*). The phenylbenzopyrylium system (rings A, D, and E) is analogous to the one present in anthocyanidins, having extended conjugation of the π electrons throughout this system at the origin of its maximum absorption at 500 nm (**Figure 5A**). This pigment is named here as 11-syringylcatechinpyrylium, having the syringyl ring attached at the 11 position of the catechinpyrylium system. A mechanism of formation has been already proposed for this pigment (*15*).

In fractions F2, F3, and F4, a relatively small peak with the same molecular ion (*m*/*z* at 479) and fragmentation pattern as 11-syringylcatechinpyrylium was detected (peak 7). This compound is thought to correspond to an isomer of the 11-syringylcatechinpyrylium, probably involving a formation mechanism analogous to the one described in the literature but with the nucleophilic attack by position C6 of the catechin. The structure suggested for this compound is presented in **Figure 5B**.

Peak 6 (λ_{max} at 495 nm) detected in fractions F2, F3, and F4 displayed a molecular ion $[M]^+$ at *m*/*z* 449, releasing a major MS^2 fragment at *m*/*z* 297 (RDA, $[M-152]^+$). Further MS^3 fragmentation yielded two other ions at *m*/*z* 282 (loss of CH_3, $[M-15]^+$) and at *m*/*z* 269 (loss of CO, $[M-28]^+$). These results suggest that peak 6 corresponds to a 11-guaiacylcatechinpyrylium (**Figure 5A**) resulting from the reaction between catechin and coniferaldehyde, according to the same mechanism described in the literature for 11-syringylcatechinpyrylium. By analogy with syringylcatechinpyrylium, a smaller peak with the same mass spectrometric characteristics as 11-guaiacylcatechinpyrylium was also detected in fractions F2, F3, and F4 (peak 6′), which was similarly attributed to the 11-guaiacylcatechinpyrylium isomer formed by the attack of C6 of catechin.

A different pigment (λ_{max} at 515 nm) was detected in fraction F1 (peak 2). This compound was found to have a molecular ion $[M]^+$ at *m*/*z* 687 that yielded a MS^2 characteristic fragment at *m*/*z* 535 ($[M-152]^+$) corresponding to a RDA fragmentation. These mass data are consistent with the structure of a compound that contains a sinapyl alcohol group linked to the previous 11-syringylcatechinpyrylium pigment (**Figure 5C**). Its formation mechanism could involve the protonated sinapaldehyde carbocation, which induces an electrophilic attack at the free position C6 of ring A of the syringylcatechinpyrylium pigment to form the 11-syringylcatechinpyrylium-6-sinapyl alcohol adduct. Peak 2′ in fraction F1 was found to have a similar MS pattern but a different retention time. It may result from the reaction between the isomeric 11-syringylcatechinpyrylium pigment and sinapaldehyde by an equivalent mechanism. Similar pigments resulting from the reaction between syringylcatechinpyrylium and coniferaldehyde or between guaiacylcatechinpyrylium and coniferaldehyde/sinapaldehyde could be expected to form, but they were not detected.

An additional red/orange pigment (λ_{max} at 500 nm) with a molecular ion at *m*/*z* 739 was detected in fraction F3 (peak 3). This compound displayed three major fragment ions in the MS^2 spectrum at *m*/*z* 721 (loss of a water molecule from the catechin moiety, $[M-18]^+$), 569 (RDA and loss of a water molecule, $[M-152-18]^+$), and 449 (loss of a catechin moiety, $[M-290]^+$). The most intense ion at *m*/*z* 569 was fragmented and yielded a major MS^3 fragment ion at *m*/*z* 297 ($[M-272]^+$), which corresponds to the loss of the catechin moiety that had previously lost a water molecule. These data are consistent with the structure of the 11-guaiacylcatechinpyrylium pigment directly linked to a catechin moiety (i.e., 11-guaiacylcatechinpyrylium-9-catechin 1, **Figure 5D**). A possible mechanism for its formation is in part analogous to the one proposed for the formation of the syringylcatechinpyrylium/guaiacylcatechinpyrylium pigments. In fact, the first steps are identical until the formation of the intermediary carbocation, followed by a nucleophilic addition of C8 of a catechin molecule to form the aldehyde-linked catechin adduct. This adduct will suffer an intramolecular nucleophilic attack by the hydroxyl group at C7 in the vinyl C3′ of the bridge group. A final oxidation step yields the new syringylcatechinpyrylium/guaiacylcatechinpyrylium linked to a catechin moiety at C9. A second pigment with an identical molecular ion and fragmentation pattern as peak 3 but a different retention time was also detected in fractions F3 and F4 (peak 3′). It may correspond to the adduct isomer formed according to a similar mechanism as the one described before,

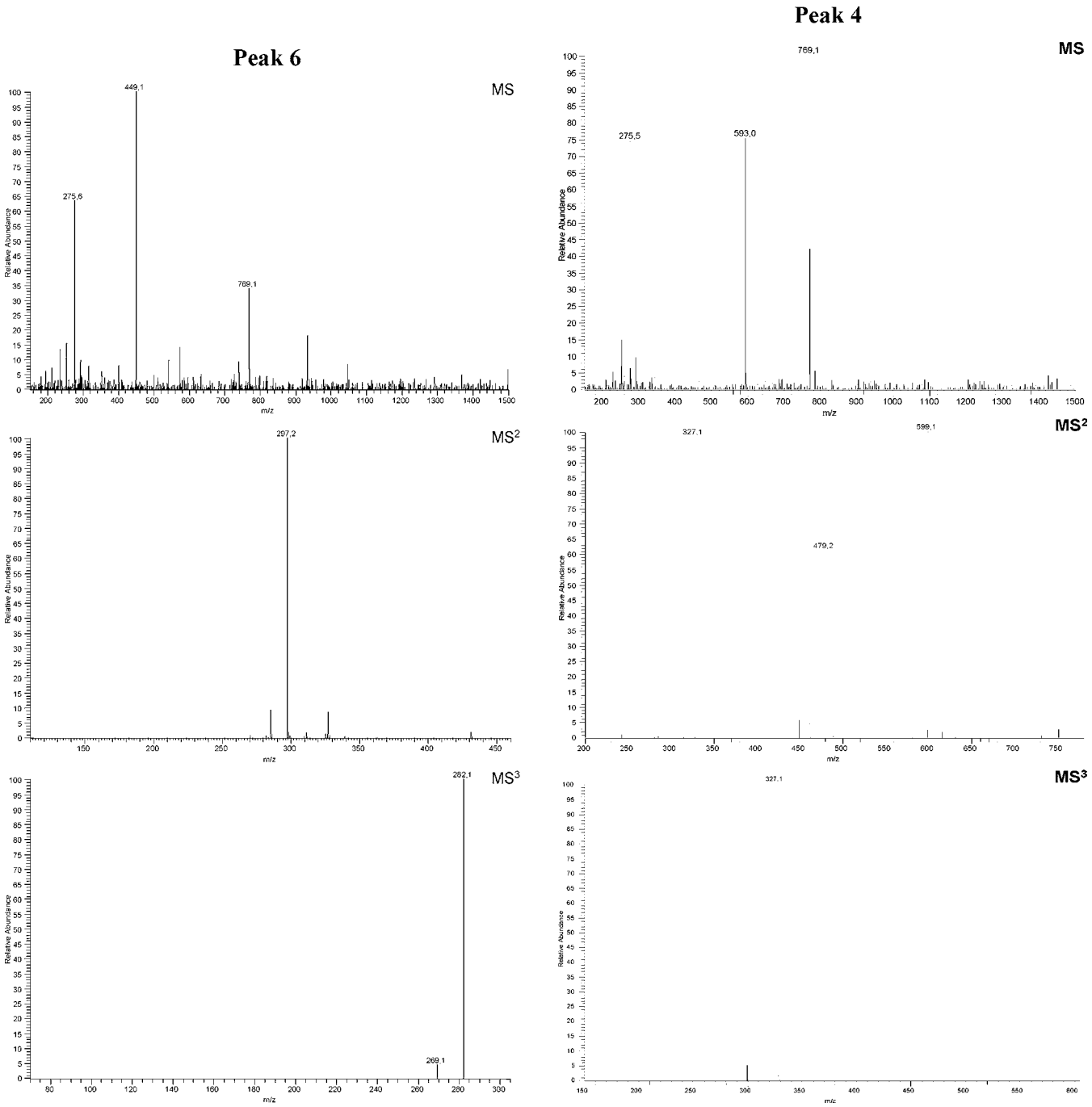

Figure 4. Mass spectra and respective MS^2 and MS^3 fragmentations for peaks 6 and 4.

but in which the intermediary carbocation is linked to the catechin in position C6 (i.e., 11-guaiacylcatechinpyrylium-9-catechin 2).

A similar pigment with a molecular ion at *m*/*z* 769 was detected in fraction F3 (peak 4), displaying an analogous fragmentation pattern to the 11-guaiacylcatechinpyrylium-9-catechin adduct, thus probably corresponding to the 11-syringylcatechinpyrylium-9-catechin adduct (**Figure 5D**). Only one isomer was detected.

Fractions F3 and F4 contained another brick-red pigment (peak 10) with a molecular ion at *m*/*z* 765 and a major fragment at *m*/*z* 475 (loss of a catechin moiety, $[M-290]^+$) in the MS^2 spectrum, which releases a MS^3 fragment at *m*/*z* 323 ($[M-152]^+$, RDA). This compound may correspond to 11-guaiacylcatechinpyrylium linked to a catechin unit through an ethyl bridge (**Figure 5E**). Similarly, a compound with a molecular ion at *m*/*z* 795 was also detected in fractions F3 and F4 (peak 11), with an analogous fragmentation pattern to peak 10, which is consistent with an 11-syringylcatechinpyrylium-ethyl-catechin adduct (**Figure 5E**). The hypothetic mechanism for the formation of these pigments should be analogous to the one proposed for the formation of ethyl-linked catechin adducts involving acetaldehyde (*10*), which would arise from oxidation of ethanol present in the solution (*23*).

The formation of isomers of these pigments was expected to occur because of the presence of an asymmetric carbon (C*) in the ethyl linkage, leading to formation of two diasteroisomeric structures (*R* and *S*). This may explain the presence in fraction F4 of two pairs of chromatographic peaks (10/10′ and 11/11′) with similar MS data, corresponding to guaiacylcatechinpyrylium/syringylcatechinpyrylium-ethyl-catechin diasteroisomers.

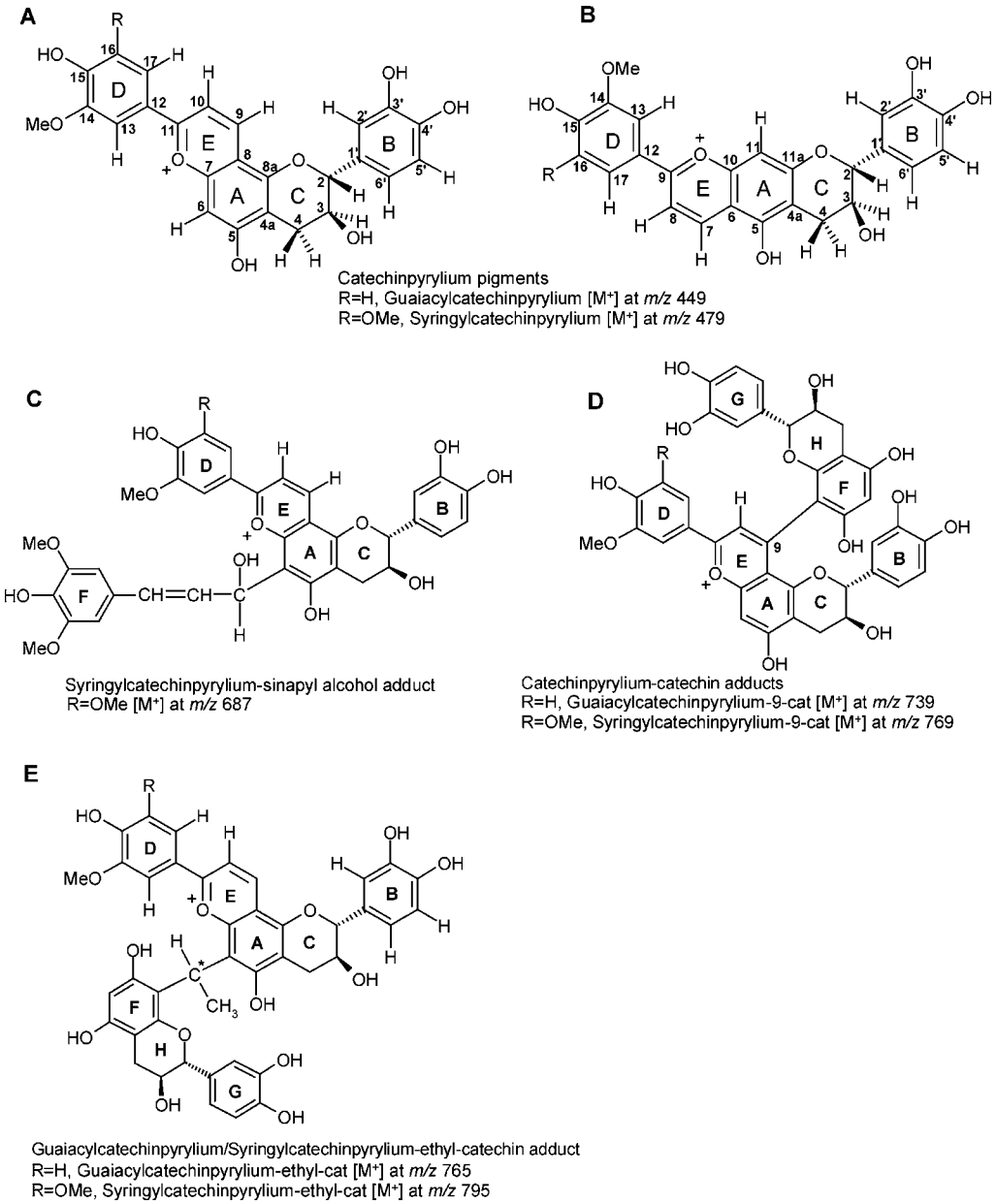

Figure 5. Structures of the catechinpyrylium pigments resulting from the reaction between catechin and coniferaldehyde (R = H, guaiacylcatechinpyrylium) or sinapaldehyde (R = OMe, syringylcatechinpyrylium).

All of the aforementioned pigments are proposed to result from the reaction between catechin and oak-derived aldehydes, thus constituting a new class of related brick-red pigments that are proposed here to be named as oaklins. This new family is characterized by a catechinpyrylium core composed of A, B, C, and E rings that constitute the chromophoric group. Oaklins may differ between them by the presence of an aromatic group (e.g., syringyl and guaiacyl) in ring D and also by the presence of catechin moieties.

Peaks 1, 5, and 9 were detected in model solution, but their structures were not tentatively identified. However, a major fragment $[M-152]^+$ in the MS^2 spectrum was detected for compounds corresponding to peaks 1 and 9, corresponding to a RDA fission, which can be indicative of the presence of a catechin moiety. This fragment releases a MS^3 fragment $[M-152]^+$, corresponding to a second RDA fragmentation. This may indicate the existence of two catechin moieties in their structures. The fragment $[M-364]^+$ detected for peak 5 could not be attributed. The fact that compounds corresponding to peaks 1 and 5 were eluted from the Toyopearl column with a lower percentage of methanol implies that these pigments may have different structures when compared to oaklins.

Formation of Catechinpyrylium Derivates at Different pH Values and at Different Temperatures. The formation of a great variety of oaklins in the model solution with catechin and the oak extract was unambiguous, indicating that these pigments could also occur in wines aged in oak barrels. However, the pH of this solution (1.5) was quite different from the usual pH of wine (~3.5). It is well-known that the pH and temperature are two very important factors influencing the formation of different compounds in wine matrixes. Therefore, the formation of oaklins at different pH values (3.5, 2.5, and 1.5) and at two different temperatures (25 and 35 °C) was investigated. Consequently, the evolution of the two main oaklins previously described (syringylcatechinpyrylium and guaiacylcatechinpyrylium) was accompanied during several days, and the results are presented in **Table 2**.

Table 2. Evolution of the Syringylcatechinpyrylium and Guaiacylcatechinpyrylium Concentration at Different pH Values and Temperatures

		concentration of oaklins (mg/L) in different experimental conditions					
		25 °C			35 °C		
compound	day	1.5	2.5	3.5	1.5	2.5	3.5
syringylcatechinpyrylium	1	0.42	0.08	0	0.50	0.20	0.08
	6	0.41	0.32	0.16	*a*	0.35	0.21
	8	0.32	0.32	0.17	*a*	0.40	0.24
	15	*a*	0.37	0.12	*a*	0.39	0.33
guaiacylcatechinpyrylium	1	0.73	0.08	0	1.02	0.25	0.07
	6	1.90	0.40	0.17	1.36	0.42	0.34
	8	1.80	0.43	0.16	0.83	0.52	0.50
	15	1.11	0.63	0.23	0.73	0.67	0.76

[a] Traces.

Both pigments formed in every solution after 1 day of reaction, except for the reaction at pH 3.5 and 25 °C, which only revealed the presence of oaklins at day 6. Guaiacylcatechinpyrylium and syringylcatechinpyrylium formed more rapidly at pH 1.5 and 35 °C but started decreasing much faster in these conditions than at pH 3.5 and 25 °C. These results were expected because lower pH values and higher temperatures should favor the formation of the coniferaldehyde/sinapaldehyde carbocations, which are involved in the mechanism of formation of guaiacylcatechinpyrylium and syringylcatechinpyrylium. Guaiacylcatechinpyrylium was present in slightly higher amounts than syringylcatechinpyrylium in solutions at pH 2.5 and 3.5, while, at pH 1.5, guaiacylcatechinpyrylium was present at higher concentrations.

Analysis of Oaklins in Red Wine. The formation of syringylcatechinpyrylium and guaiacylcatechinpyrylium at pH 3.5 and 25 °C was indicative of the possibility of the existence of this type of pigment in wines aged in oak barrels. To evaluate it, a 4-year-old commercial table red wine aged in oak barrels during a year was analyzed by HPLC-DAD-MS. The elution zone corresponding to the oaklins in the chromatogram of the wine was very complex because many other anthocyanin-derived pigments coeluted in the same chromatographic region showing more intense peaks. This did not allow the determination of the mass spectra of the peaks supposedly corresponding to the oaklins.

Further fractionation of the wine was performed on polyamide resin and toyopearl gel to obtain simpler pigment fractions that allowed the detection of the oaklins by LC−MS. The full mass chromatograms of these fractions displayed several molecular ion peaks supposedly corresponding to different oaklins, presenting molecular ions and retention times analogous to the ones corresponding to syringylcatechinpyrylium, guaiacylcatechinpyrylium, and guaiacylcatechinpyrylium/syringylcatechinpyrylium catechin adducts, for example.

However and despite the extensive purification of the wine, the major part of these molecular ion peaks could not be individually selected because other compounds present in the obtained fractions were predominant. This did not allow the analysis of the fragmentation pattern of these pigments, thereby preventing further comparison to and subsequent identification of these pigments as the various oaklins detected in model solution. This can be due to presumably low amounts of these pigments in wines, easily surpassed by other pigments such as the original anthocyanins and derived compounds.

Nonetheless, one of these pigments could be individually and selectively detected in the wine fraction eluted with 80% methanol and collected from the Toyopearl column after 2 h and 20 min. This compound showed similar mass data and retention time to peak 6 present in the model solution, identified as 11-guaiacylcatechinpyrylium. In fact, it displayed a molecular ion $[M]^+$ at *m*/*z* 449 and a MS^2 and MS^3 fragmentation pattern involving a RDA fragmentation and subsequent loss of CH_3 and CO, respectively. These results indicate that this pigment is most likely a 11-guaiacylcatechinpyrylium, demonstrating the existence of one of the main oaklins in the analyzed red wine. Therefore, it is expected that several oaklins can occur in different red wines aged in oak barrels. Because of their intense red/orange color and although probably not occurring in high amounts, their presence may somehow contribute to some color changes observed during wine aging in oak barrels.

This preliminary study confirms the formation in model solutions of several red/orange pigments resulting from the reaction between catechin and aldehydes extracted from oak, namely, coniferaldehyde and sinapaldehyde. These compounds were formed at different pH values and at two distinct temperatures. The formation of compounds derived from other aldehydes present in the oak extract was not detected. These pigments were all found to have a characteristic catechinpyrylium core and consequent related structural features, which lead to their designation as oaklins.

Moreover, 11-guaiacylcatechinpyrylium, one of the main oaklins detected in the model solutions, was also found to occur in a commercial table red wine, thereby suggesting that other similar compounds are likely to occur in red wine. Even though these pigments are not thought to occur in high amounts, oaklins existing in wines aged in oak barrels may play a role in some of the color changes observed during the aging process. Nonetheless, further studies are still required to unequivocally detect other oaklins in wine samples and to understand their contribution to the color changes, as well as additional structural analysis, using NMR techniques, to confirm their structures.

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