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Changes in Proanthocyanidin Chain Length in Winelike Model Solutions

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Reactions of seed and skin proanthocyanidins in the presence or absence of (−)-epicatechin were followed in winelike solutions over 53 days at 30 °C. Proanthocyanidins were separated from flavanol monomers by sequential elution from a Sep Pak cartridge, and changes in proanthocyanidin composition were monitored by thiolysis analysis of the proanthocyanidin fraction. In solutions containing no free (−)-epicatechin, trace amounts of monomers were released and important losses of proanthocyanidins were measured, but their average composition and mean degree of polymerization (mDP) were hardly modified. In the presence of (−)-epicatechin, the mDP value decreased and oligomeric proanthocyanidins accumulated throughout the incubation while losses of total units were dramatically reduced. Our data indicate that interflavanic bond cleavage of proanthocyanidins occurred under mild acidic conditions such as encountered in wine and that the resulting carbocation proceeded to unknown species. The latter reaction did not take place in the presence of (−)-epicatechin. Epicatechin added to the intermediate carbocation, thus being incorporated as the end unit of a shorter proanthocyanidin chain. The results of this study are discussed in relation to the loss of astringency reported during wine aging.

KEYWORDS: Grape; *Vitis vinifera*; proanthocyanidins; flavanol monomers; tannins; acid-catalyzed cleavage; wines; astringency

INTRODUCTION

Phenolic compounds are responsible for essential organoleptic features of red wine. Thus, the color and astringency of young red wines are, respectively, due to anthocyanin pigments (under their red flavylium form) and to proanthocyanidins (condensed tannins), extracted from grape during the maceration phase. Both types of compounds are rather unstable species that undergo various types of chemical reactions, resulting in color and taste changes, as the wine ages (*1*−*3*). In particular, the loss of astringency occurring during wine storage is usually ascribed to polymerization of proanthocyanidins or to the formation of higher molecular weight proanthocyanidin−anthocyanin adducts. The mechanism of astringency perception is still poorly understood but is believed to involve interactions of astringent substances with salivary proteins (*4*), eventually leading to precipitation, reduced saliva viscosity, and hence increased friction (*5*−*7*). Astringency of proanthocyanidins has been reported to increase with chain length, up to the decamer level, and to decrease beyond this value, as the polymers become insoluble (*8*). However, the latter assumption has to be ruled out as higher molecular weight proanthocyanidins (>20 flavanol units) were soluble in wine and were selectively precipitated out by addition of fining proteins to wine (*9*−*10*). This indicates that they interact readily with proteins, thus suggesting that they are particularly astringent. Moreover, analysis of red wine phenolics by liquid chromatography−mass spectrometry (LC−MS) showed that a major part of tannin−anthocyanin adducts in wine were actually oligomeric rather than polymeric species (*11*). In particular, dimeric flavanol−anthocyanin adducts (T−A^+) were detected in a two year old Cabernet Sauvignon wine by LC−MS and the position of the anthocyanin as the terminal unit was confirmed by thiolysis (*11*). Such compounds presumably arise from the addition of an anthocyanin onto a carbocation resulting from cleavage of a proanthocyanidin interflavanic linkage. Their occurrence in wine suggests that the acid-catalyzed C−C bond-breaking characteristic of proanthocyanidin chemistry takes place during wine aging, in agreement with earlier findings (*12*). Reduction rather than an increase of the average proanthocyanidin molecular weight may thus be responsible for red wine deastringency. It also implies that the anthocyanin reacts under its nucleophilic hemiketal form and

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can be replaced with another flavanol unit, as shown earlier in other nucleophilic addition processes (*13*, *14*). In white wine, reactions of flavanol monomers with proanthocyanidin polymers have been reported to yield dimeric proanthocyanidins whereas incubation of polymers alone led to precipitation (*12*). The purpose of our work was to confirm the postulated C−C bond-breaking and C−C bond-making process in winelike model systems and to monitor changes in proanthocyanidin chain length that may be responsible for red wine deastringency.

MATERIALS AND METHODS

Chemicals and Thiolysis Standards. All organic solvents were high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). (+)-Catechin and (−)-epicatechin were purchased from Sigma (St. Louis, MO), and phenylmethanethiol was purchased from Fluka (Buchs, Switzerland). Other standards such as (−)-epicatechin-3-gallate, (−)-epigallocatechin, and the benzylthioether derivatives of (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-gallate, and (−)-epigallocatechin were isolated as described previously (*15*).

Preparation of Grape Polymeric Proanthocyanidins. Seeds and skins from *Vitis vinifera* cv. Shiraz harvested at the INRA experimental station of Pech-Rouge were manually separated. After the grapes were peeled, the skins were immediately frozen in liquid nitrogen. The seeds and skins were ground under liquid nitrogen using a Dangoumau blender (Prolabo, France). The resulting powders were then extracted with acetone/water (60:40, v/v). The supernatants recovered after centrifugation were concentrated under vacuum at 30 °C to give the seed and skin crude extracts. Lipophilic compounds were removed from seed extract by hexane liquid/liquid extraction. Both total proanthocyanidin extracts were freeze-dried.

Each extract was separately fractionated on Toyopearl TSK HW-50(F) gel from Tosoh Corp. (Tokyo, Japan) packed in a semipreparative scale column (200 mm × 25 mm), using the chromatographic conditions previously described (*15*). Monomers and oligomers were eluted with 5 bed volumes of ethanol/water (55:45, v/v) containing 0.05% trifluoroacetic acid. The polymeric proanthocyanidins were desorbed from the column using 3 bed volumes of acetone/water (60:40, v/v). Seed and skin polymeric fractions were concentrated under vacuum.

Thiolysis. Fractions to be analyzed were dissolved in methanol, mixed with an equal volume of thiolytic reagent (5% solution of phenylmethanethiol in methanol containing 0.2 M HCl), and heated for 2 min at 90 °C. The released units were analyzed by HPLC under the conditions previously described (*15*). Quantification of each terminal and extension unit was based on peak areas at 280 nm and calibration with external standards purified in our laboratory (*15*).

The mean degree of polymerization (mDP), galloylation rate, and percentage of epigallocatechin units were calculated as the molar ratio of total units to terminal units, molar ratio of galloylated units to total units, and molar ratio of epigallocatechin units to total units, respectively.

Preparation of the Reaction Media. The winelike model solution consisted of ethanol/water (12:88 v/v), adjusted to pH 3.2 by adding concentrated formic acid, and containing 0.5 g/L $Na_2S_2O_5$ to prevent oxidation reactions.

Four solutions were prepared by dissolving, respectively, seed proanthocyanidins (so as to obtain a final concentration of 3.7 mM ± 0.4 of constitutive units), seed proanthocyanidins (3.7 mM ± 0.4) plus (−)-epicatechin (6.1 mM ± 0.3), skin proanthocyanidins (2.8 mM ± 0.1), and skin proanthocyanidins (2.7 mM ± 0.1) plus (−)-epicatechin (2.5 mM ± 0.1) in the winelike solution. Proanthocyanidin concentrations, determined by thiolysis, are expressed in millimolar of constitutive units. The ratio of (−)-epicatechin to upper proanthocyanidin units equalled roughly one in the case of skin proanthocyanidins whereas it was about two in the case of seed proanthocyanidins.

The four solutions were prepared in triplicate and maintained at 30 °C throughout the incubation in sealed tubes. Two additional sets of seed procyanidin control solutions were prepared as described above except that $Na_2S_2O_5$ was omitted in one of them.

Fractionation of Monomeric and Polymeric Proanthocyanidins. Aliquots (0.5 mL) of the different samples were taken from each of the six tubes after 0, 21, 32, and 53 days of incubation and loaded onto preconditioned Sep Pak tC18 cartridges (Environmental model, Waters**,** Milford, MA). Elution was started with 5 mL of water. After the samples were dried under a N_2 stream, flavanol monomers were eluted with 5 mL of diethyl ether and proanthocyanidin oligomers and polymers were recovered from the cartridge by eluting with 5 mL of methanol.

Monitoring of Acid-Catalyzed Cleavage of Polymeric Proanthocyanidins. Changes in proanthocyanidins composition were monitored by analysis of the diethyl ether and methanol fractions recovered from the Sep Pak cartridge. The former fraction was taken to dryness, dissolved in methanol, and analyzed directly by HPLC whereas the latter was submitted to HPLC analysis before and after thiolysis.

Direct HPLC analyses were performed using a Waters Millenium HPLC−DAD system (Milford, MA) as described by Souquet et al. (*16*). The column consisted of a reversed-phase Lichrospher 100-RP 18 (Merck, Darmstadt, Germany) (250 mm × 4 mm i.d.) protected with a guard column of the same material and was equilibrated at 1 mL/min in a mixture of solvent A (water/formic acid 95:5 v/v) and solvent B (acetonitrile/water/formic acid 80:15:5 v/v/v) in a 97/3 ratio. Elution was performed using the following binary gradient: isocratic for 7 min with 3% B, from 3 to 40% B in 33 min, and from 40 to 50% B in 5 min. After it was washed with 90% of B, the column was reequilibrated in the starting mobile phase. The elution was monitored on a Waters 996 photodiode array detector and Millenium 32 software.

Electrospray ionization (ESI)−MS analyses were performed on a Sciex API I plus (Sciex, Thornhill, Ontario, Canada) simple quadrupole mass spectrometer operated in negative-ion mode with a needle potential of −4 kV and an orifice potential of −60 V. Proanthocyanidin fractions were analyzed either by direct infusion or by LC/ESI−MS as described by Souquet et al. (*16*).

Proanthocyanidins in methanolic solution containing 1% formic acid were submitted to direct infusion with a constant flow rate of 10 μL/min under the following conditions: spectra were obtained between *m*/*z* 500 and 2400 with a step size of 0.2 amu and a dwell time of 1 ms.

LC/ESI−MS analyses were performed under the following conditions: the masses were scanned between *m*/*z* 280 and 1200 in steps of 0.3 amu and with a dwell time of 0.9 ms. Chromatographic separation was performed on a C18 Nucleosil column (250 mm × 2 mm, 3 μm, Macherey-Nagel, Hoerdt, Germany) protected by a guard column filled with the same material. Elution was monitored at 280 nm on a 785A UV detector. A binary gradient was carried out at 280 μL/min with solvent A (water/formic acid 98:2 v/v) and solvent B (acetonitrile/water/formic acid 80:18:2 v/v/v). The elution patterns were as follows: isocratic for 10 min with 10% B, 10 to 15% B in 11 min, and from 15 to 40% in 25 min. After it was washed, the column was reequilibrated in the starting mobile phase. The eluant was split between the mass detector and the UV detector in a 1/3 ratio.

RESULTS AND DISCUSSION

Preparation and Characterization of Grape Polymeric Proanthocyanidins. Proanthocyanidin fractions devoid of monomeric phenols were obtained from grape seed and skin extracts by means of low-pressure chromatography on Toyopearl TSK HW-50(F).

Thiolysis of the seed proanthocyanidin fraction released (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-gallate, and the corresponding benzylthioethers, a result in accordance with previous studies on seed tannins (*17*−*19*). A mDP (molar ratio of total units to end units) of 9.5 ± 0.3 and a galloylation rate (molar ratio of galloylated units to total units) of 21.9 ± 0.25% were calculated for this polymeric fraction. These values are in the range of already published data concerning seed proanthocyanidin features (*17*−*19*).

Thiolysis of the skin proanthocyanidin extract released the benzylthioether derivative of (−)-epigallocatechin in addition

to the above-listed units. This observation is in agreement with the fact that skin proanthocyanidins are composed of both procyanidin and prodelphinidin units (*15*, *20*). The polymeric fraction of skin proanthocyanidins was characterized by a mDP of 36 ± 1.0, a molar galloylation rate of 5.7 ± 0.17%, and a percentage of epigallocatechin units of 25.8 ± 0.15%, which is in accordance with previous results (*15*, *18*).

The concentrations of proanthocyanidin units determined by thiolysis of the seed and skin extracts represented 69 ± 1.1% and 57 ± 0.4%, respectively, of the weighed material. These thiolysis yields are similar to those obtained for isolated compounds (around 70%) (*21*), indicating that both fractions were reasonably pure.

Direct HPLC−DAD analysis of both extracts confirmed the absence of flavanol monomers. Furthermore, this analysis revealed that the skin proanthocyanidin extract contained less than 0.5% of anthocyanins (expressed as malvidin-3-O-glucoside equivalents).

Changes in Proanthocyanidin Composition in the Presence or Absence of Epicatechin. Winelike model solutions of skin and seed proanthocyanidins containing (−)-epicatechin were prepared so that the ratio of flavanol monomer to the extension subunits was, respectively, equimolar and 2-fold equimolar. Proanthocyanidin solutions, without addition of monomeric species, were used as control solutions. Separation of monomeric and polymeric species was a prerequisite to study changes in proanthocyanidin composition under both conditions. Consequently, a quick separation method was adapted from that described by Sun et al. (*22*). The ability of this method to resolve monomeric and polymeric forms of proanthocyanidins by sequential elution with diethyl ether and methanol was checked by HPLC−DAD analysis of the eluted fractions obtained from freshly prepared solutions of proanthocyanidins and (−)-epicatechin.

Thiolysis of the diethyl ether fraction yielded some catechin, due to epimerization of (−)-epicatechin, but no benzylthioether derivatives, confirming that this fraction contained no proanthocyanidins. None of the monomers could be distinguished on the HPLC−DAD profile of the methanol fraction, meaning that they were mostly recovered in the diethyl ether fraction.

The Sep Pak fractionation method was thus applied to all solutions to separate free flavanol monomers, eluted with diethyl ether, from proanthocyanidins, which were recovered with methanol. No monomer was detected in the diethyl fraction from the control solutions at initial time, confirming their absence in both proanthocyanidin extracts.

Changes in the mDP of the proanthocyanidins present in each solution are presented in **Figure 1**.

In the control solution containing seed proanthocyanidins, a slight decrease of the mDP (from 9 to 8 after a 53 day reaction) was observed. Low amounts of free monomers were detected in the diethyl ether fractions obtained from this solution after incubation, suggesting that some cleavage of interflavanic C−C bonds had taken place. A larger decrease of mDP (from DP 36 to 25 after a 53 day reaction) was observed in the case of skin proanthocyanidins. No monomers were detected in the solutions, probably due to the much lower concentration of end units in these polymers. In the solutions containing (−)-epicatechin, the mDP values decreased to approximately 4, irrespective of proanthocyanidin origin. This may arise from cleavage of the interflavanic bonds followed by nucleophilic addition of (−)-epicatechin to the resulting carbocations as proposed earlier (*12*, *23*).

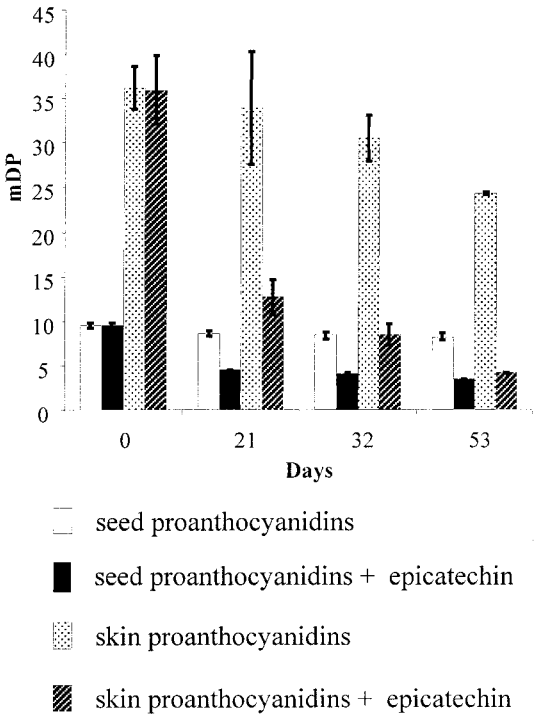

Figure 1. Changes in the mDP of seed and skin proanthocyanidins in winelike solutions in the presence or absence of (–)-epicatechin over the reaction time. Error bars represent ± standard deviation around the mean of three replicates.

In both control solutions, after 53 days of incubation, approximately 50% loss of proanthocyanidin units was observed. The observed losses are unlikely to be due to oxidative reactions, which should have been prevented by the presence of $Na_2S_2O_5$ in the solutions. However, the ESI−MS spectra obtained by direct injection showed, together with the masses corresponding to oligomeric proanthocyanidins, the presence of species differing from proanthocyanidins by an excess of 80 or 40 amu, respectively, corresponding to mono and doubly charged sulfite−proanthocyanidin adducts. The formation of these adducts is probably through the interflavanic bond cleavage followed by a nucleophilic addition of SO_3H on the resulting carbocation as described by Foo and co-workers (*24*). Thus, these adducts also represent diagnostic molecules of the interflavanic bond-breaking process. To evaluate the importance of those adducts in the losses observed, another series of control solutions without $Na_2S_2O_5$ were prepared and analyzed over the course of the time. The losses observed in this series were the same as in control solutions containing $Na_2S_2O_5$. Furthermore, analysis of the HPLC chromatograms obtained after thiolysis of the solutions containing sulfite adducts did not reveal the presence of any additional peaks, suggesting that they did not resist thiolysis under our experimental conditions.

Consequently, the observed losses are more likely to result from conversion of products of interflavanic bond cleavage to poorly defined species, usually referred to as phlobaphenes *(25, 26)* since our experimental conditions (i.e., aqueous media, low temperature, and protection from oxidation) are known to facilitate these reactions (*25*).

As the same general trends are observed for seed and skin proanthocyanidins solutions, changes in the concentrations of free monomers, extension units, and end units are illustrated only for the grape skin proanthocyanidin solutions (**Figure 2**). In the control solutions, the concentration of terminal subunits remained constant throughout the incubation whereas that of

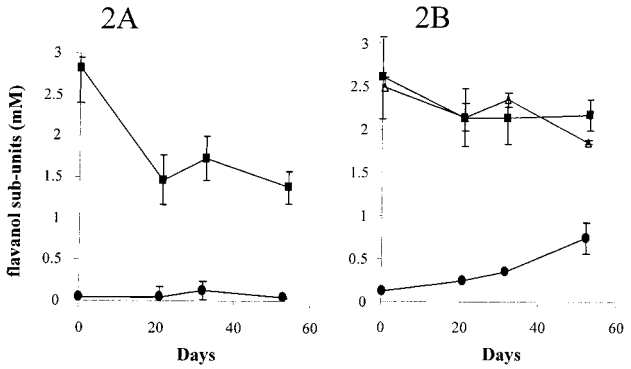

Figure 2. Changes in free flavanol monomers (△), proanthocyanidin end units (●), and proanthocyanidin extension and upper units (■) in winelike solutions containing skin proanthocyanidins. (**A**) Control solutions; (**B**) solutions containing (–)-epicatechin. Error bars represent ± standard deviation around the mean of three repetitions.

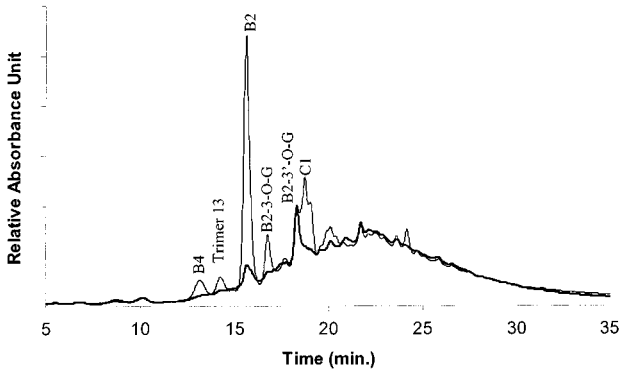

Figure 3. Comparison of the UV chromatograms (280 nm) of the methanol fractions obtained from control seed proanthocyanidin solutions (bold trace) and solutions containing seed proanthocyanidin and (–)-epicatechin, after 53 days of incubation. B2 stands for (epicatechin (4β-8) epicatechin), B2-3-gallate stands for (epicatechin 3-gallate (4β-8) epicatechin), B4 stands for (catechin (4β-8) epicatechin), B2-3′-O-gallate stands for (epicatechin (4β-8) epicatechin 3-gallate), C1 stands for (epicatechin (4β-8) epicatechin (4β-8) epicatechin), and "trimer 13" stands for (epicatechin (4β-6) epicatechin (4β-8) epicatechin).

extension units dropped. Preferential involvement of the latter in the observed proanthocyanidin losses is in agreement with the postulated phlobaphene formation process.

In solutions containing (−)-epicatechin, the total flavanol content decreased only slightly (less than 10% loss) over the time course of the reaction. The concentration of extension units remained almost stable, indicating that trapping of the carbocation intermediates by the available flavanol monomer prevented them from proceeding to sulfite adducts or degradation products. Formation of phlobaphenes has been reported to be similarly prevented via the addition of other nucleophiles such as phloroglucinol (*27*) or sulfhydryl groups of proteins (*26*). Sulfite−proanthocyanidin adducts were present only in trace amounts in epicatechin-containing solutions, indicating that (−)-epicatechin reacted preferentially with the formed carbocations. The amount of free (−)-epicatechin declined throughout the incubation (from 6.1 ± 0.3 to 4.39 ± 0.4 and from 2.8 ± 0.1 to 1.85 ± 0.03, respectively, in the seed and in the skin proanthocyanidin solutions), but this was compensated by an equivalent increase in the concentration of end units, confirming that (−)-epicatechin was gradually incorporated in proanthocyanidins as the terminal unit. Although only free (−)-epicatechin was initally present, the concentration of catechin end units also increased, peaking at 0.34 and 0.18 mM, respectively, for seed and skin polymers after 53 days of reaction. The total increase of terminal subunits concentration was due ∼75% to (−)-epicatechin and for the remaining 25% to catechin. This proportion corresponds to the rate of epimerization of (−)-epicatechin to catechin measured under our thiolysis conditions, so that it can be reasonably assumed that the increase of terminal units arises from the added (−)-epicatechin.

Identification of the Main Species Formed. Further analysis of the oligomeric proanthocyanidins formed under the conditions described above was carried out by LC−ESI−MS. The comparison of UV chromatograms (**Figure 3**) of the methanol fractions obtained from both control and (−)-epicatechin-containing solutions permitted us to screen the main species formed under the reaction conditions. On the basis of their retention time and on the mass signals, the main dimeric and trimeric forms were identified. B2 (epicatechin (4β-8) epicatechin) and B2-3-gallate (epicatechin 3-gallate (4β-8) epicatechin) were identified as the major dimers formed in the solution containing grape seed procyanidins and (−)-epicatechin after 53 days of incubation. B4 (catechin (4β-8) epicatechin) was also formed but to a lower extent. B2-3′-O-gallate (epicatechin (4β-8) epicatechin 3-gallate), which is one of the main grape seed procyanidins (*25*), was present in equivalent amounts in both types of solutions. Concerning trimers, C1, (epicatechin (4β-8) epicatechin (4β-8) epicatechin), appeared to be the dominant molecule. Another trimer eluting at around 14′ was tentatively identified on the basis of its retention time, as epicatechin (4β-6) epicatechin (4β-8) epicatechin referred to as "trimer 13" in earlier studies (*28*).

DISCUSSION

The above results clearly demonstrate that under mild acidic conditions such as encountered in wine, proanthocyanidins undergo spontaneous cleavage of their interflavanic bonds (**Figure 4**; **1**), in agreement with earlier findings (*12*, *23*). The resulting carbocations are subjected to nucleophilic addition of another flavanol molecule (**Figure 4**; **2a**,**b**) or proceed to poorly defined molecules, often referred to as phlobaphenes in the literature (**Figure 4**; **3**). Evidence of the addition of flavanol monomers onto the intermediate carbocation (**Figure 4**; **2b**) is brought by the decline of free monomers and concomitant increase of terminal units in solutions containing free (−)-epicatechin. This process results in a reduction of the average proanthocyanidin chain length and ultimately in the accumulation of oligomeric proanthocyanidins. The rate of the C−C bond-breaking process is primarily determined by pH (*23*, *26*). The kinetics of subsequent reactions are expected to depend on the ratio of polymers to monomeric species.

Besides, it is now well-established that anthocyanins, under their hemiketal form which is prominent at wine pH, can act as nucleophiles and compete with flavanols in various reactions (*14*, *29*), such as addition to *o*-quinones (*30*) and acetaldehyde-induced condensation processes (*13*, *31*). A mechanism involving nucleophilic addition of anthocyanins to the carbocations released by proanthocyanidin C−C bond cleavage can similarly be postulated to explain the formation of the lower molecular weight T−A^+ pigments recently shown to occur in wine (*11*). In the absence of flavanol monomers and anthocyanins, important losses of proanthocyanidins were measured. These involve mostly upper and extension units, suggesting that the C−C bond cleavage took place but some of the intermediate carbocations released proceeded to unknown phlobaphene

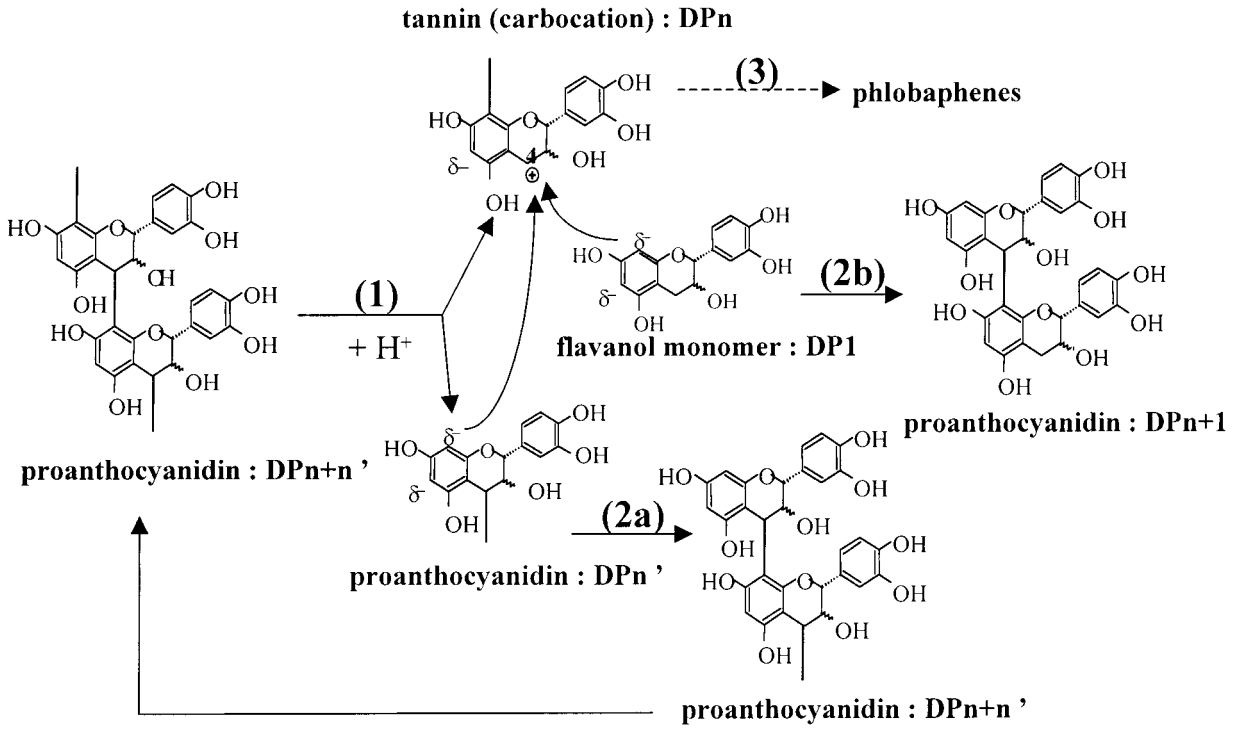

Figure 4. Scheme of the different routes of evolution of the proanthocyanidins in the presence or absence of free flavanol monomers.

molecules, in agreement with earlier findings (*26*). No increase of the mean DP was observed. However, the possibility that the original proanthocyanidins have proceeded to both higher and lower molecular weight species cannot be ruled out. In fact, it is likely that all proanthocyanidins present randomly added to the cleavage reaction products, yielding both higher and lower molecular weight species. Nevertheless, proanthocyanidins were much less reactive than flavanol monomers in the nucleophilic addition process since, in solutions containing excess of (−)-epicatechin, the terminal chain composition of the proanthocyanidins gradually changed to be predominantly (−)-epicatechin. This may be related to the smaller number of nucleophilic sites per constitutive unit in the polymers or possibly due to steric hindrance.

The significance of the three types of reactions (i.e., formation of lower molecular weight procyanidins, formation of proanthocyanidin−anthocyanin adducts, and conversion to phlobaphenes), with respect to astringency changes, remains to be investigated. Although the relationships between the structure and the way proanthocyanidins are perceived are only partly understood, the increase of astringency with the chain length of procyanidin oligomers is well-documented (*32*−*34*). Besides, smaller molecular weight procyanidins have been reported to contribute more bitterness than larger oligomers. Therefore, conversion of proanthocyanidins to lower molecular weight species can be reasonably expected to result in lower astringency and possibly also in increased bitterness. Conversion of proanthocyanidins to proanthocyanidin−anthocyanin adducts has been speculated to induce further astringency loss (*2*), but this still requires confirmation.

In control solutions (in the absence of flavanol monomers), no precipitation was observed over the time of the reaction whereas it had been reported that incubation of proanthocyanidins in white wine led to the formation of a precipitate (*12*). This suggests that the precipitate observed in white wine was due to formation of insoluble complexes between proanthocyanidin reaction products and other wine components such as proteins rather than to insolubilization of the products themselves. Selective precipitation of higher molecular weight proanthocyanidins in the presence of proteins has been demonstrated (*9*−*10*). A mechanism involving nucleophilic addition of proteins to the intermediate carbocation has also been related to the formation of haze and precipitates (*26*).

Reactions of proanthocyanidins in the absence of monomeric species are also likely to influence astringency. On one hand, as the resulting products may form insoluble complexes with other wine macromolecules, they probably also interact with salivary proteins, mediating an increase in overall astringency. On the other hand, their elimination through spontaneous precipitation may contribute to the astringency decline observed during wine aging.

Finally, the C−C bond-breaking and C−C bond-making process specific of proanthocyanidin chemistry can be used as a tool to produce oligomers from polymers. Preparation of polymeric proanthocyanidins is rather easy using Toyopearl TSK HW-50(F) gel while oligomeric proanthocyanidins are more difficult to isolate, in particular in the case of skin and wine proanthocyanidins, because they coelute with other polyphenolics (such as anthocyanins and flavonols). By using this chemical reaction, oligomeric proanthocyanidins free from other contaminating phenolics can be obtained from polymeric fractions.

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