Chapter 9B
Flavanols, Flavonols and Dihydroflavonols

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9B.1 Structure and Occurrence in Grape

9B.1.1 General Structural Characteristics

Flavanols, flavonols, and dihydroflavonols, like the anthocyanins presented in Chapter 9A, belong to the flavonoid family. These compounds are phenolic compounds that share a common C6-C3-C6 skeleton consisting of two phenolic rings (named A and B) linked together by a heterocyclic pyran ring (C-ring) as shown in Fig. 9B.1. Among them, several classes can be distinguished on the basis of

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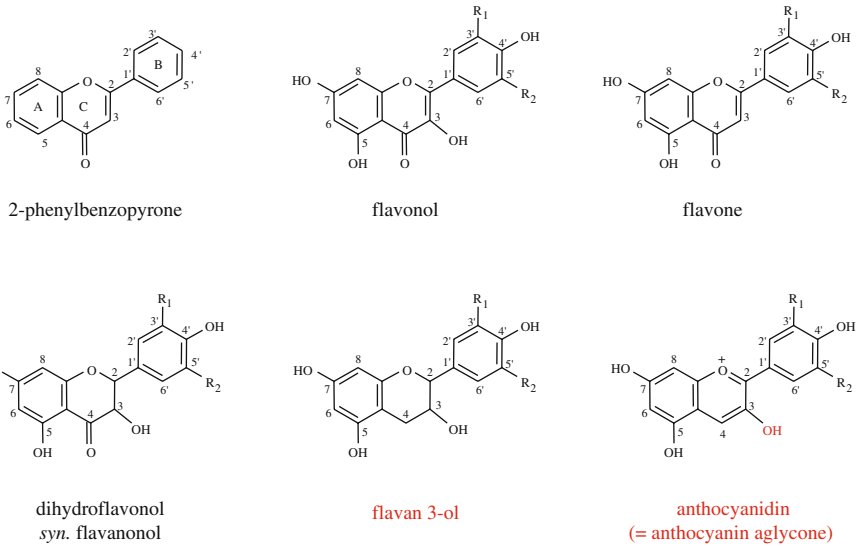

Fig. 9B.1 Chemical structures of flavonoids R1, R2 = H, OH, OCH3

the oxidation state of the C-ring. Flavonoids *sensu stricto* are based on the 2-phenylbenzopyrone structure characterized by a double link between carbons C2 and C3 and a ketone group in C4. They are represented in grapes by flavonols (that also have a hydroxyl group in C3), while flavones are also present in vine leaves. Flavonoids *sensu largo* comprise additional classes which do not show these characteristic features. Among them, anthocyanins, flavanols and dihydroflavonols are encountered in grapes. The first two groups are particularly abundant in grape and wine and essential to wine quality. Indeed, anthocyanins are the red pigments of grapes and responsible for the colour of red wines whereas flavanols contribute to taste (especially astringency and bitterness) and are also involved in the development of oxidative browning, haze and precipitates, as described in Chapter 9D.

Within each flavonoid class, further diversity results from modifications of their three ring skeleton, including

- hydroxylation
- methylation of some of the phenolic hydroxyls
- glycosylation: as illustrated in the case of anthocyanins, flavonoids can be glycosylated by various sugars (monomeric or oligomeric), usually by C-O-C glycosidic linkages. In some plant species, C-glycosylation is also encountered
- acylation of the alcoholic hydroxyl groups
- polymerisation, especially in the flavanol family

Grape flavonoids are hydroxylated on their C5 and C7 carbons, so that their A-ring is a phloroglucinol ring, and, on the B-ring, in the 4′ position. They can also be hydroxylated in 3′ or 3′ and 5′ positions and substituted by glycosidic or acyl groups on the alcoholic OH group in position 3.

9B.1.2 Flavanols

9B.1.2.1 Structure and Localisation

Grape flavanols, more accurately called flavan 3-ols as they are hydroxylated in the 3 position, are found as monomers but also as oligomers and polymers.

The major flavan 3-ol monomers in grape are (+)-catechin and its isomer, (–)-epicatechin, and, to a lesser extent, the gallic ester of (–)-epicatechin, (–)-epicatechin 3-gallate (Su and Singleton 1969) (Fig. 9B.2). Gallocatechin (Piretti et al. 1976; Czochanska et al. 1979b) has also been reported in *Vitis vinifera* and catechin 3-gallate (Lee and Jaworski 1987) and gallocatechin 3-gallate (Lee and Jaworski 1990) have been detected in some non-*Vinifera* varieties.

Flavanol oligomers and polymers are also called condensed tannins or proanthocyanidins. The term tannin refers to their capacity to interact or react with proteins and precipitate them out. When heated under acidic conditions, these molecules release red anthocyanidin pigments, hence the term proanthocyanidins. The term leucoanthocyanidin, also referring to this particular property, is sometimes encountered in the literature. However, this should be restricted to another group of compounds, flavan 3,4-diols, which are intermediates in the biosynthetic pathway leading to flavanols and anthocyanins (Stafford and Lester 1984; Nakajima et al. 2001; Abrahams et al. 2003) but have never been isolated from grapes, presumably due to their instability.

In B-type proanthocyanidins (Fig. 9B.2), the flavanol constitutive units are linked by C4-C8 and/or C4-C6 bonds, opening the possibility for branched structures.

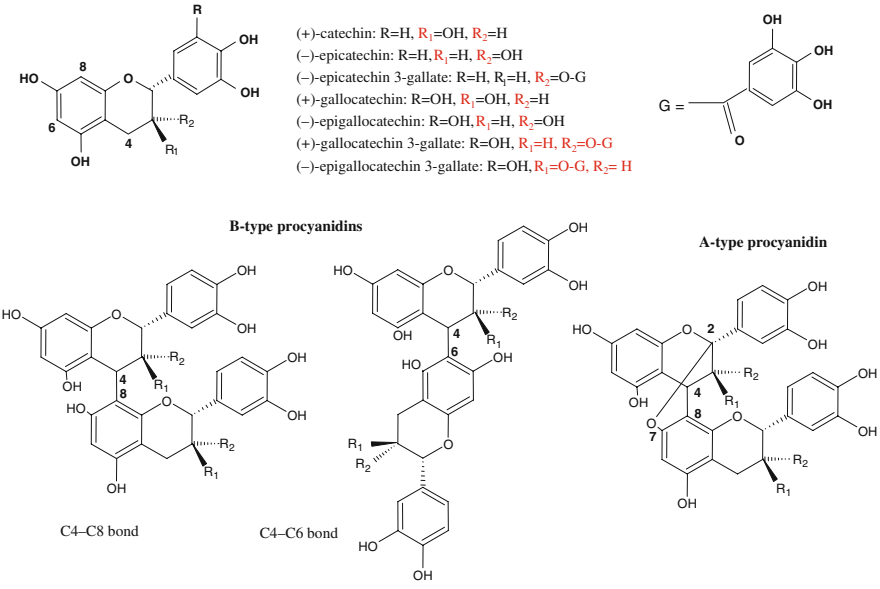

Fig. 9B.2 Structures of flavanol monomers and dimers

Double linkages, with C2-O-C7 or C2-O-C5 bond in addition to the C4-C6 or C4-C8 bond, give rise to A-type proanthocyanidins. Depending on the nature of the anthocyanidin released (see Chapter 9A), several groups of proanthocyanidins can be distinguished. Grape and wine proanthocyanidins belong to two of these groups, namely procyanidins and prodelphinidins that release cyanidin and delphinidin, respectively (Fig. 9B.3). Besides, mass signals that may correspond to methoxylated flavanol dimers have been detected in wine (Cooper and Marshall 2001) but their identification has not been confirmed. Finally, some of the constitutive units can be substituted (e.g. galloylated).

Flavanols are present in various vine plant tissues, including wood (Boukharta et al. 1988), leaves (Bogs et al. 2005; Tesnière et al. 2006), stems (Souquet et al. 2000), and fruit. Within the grape berry, they are particularly abundant in seeds and skins.

Table 9B.1 lists the flavanol dimers and trimers found in grape berries and the methods used for identification. Procyanidin dimers and trimers were first identified in seeds but they are also present in skins and stems with different distributions (Ricardo da Silva et al. 1991a) and trace amounts of B1 through B4 have been detected in pulp (Bourzeix et al. 1986). However, analysis of grape extracts by acid degradation and ^{13}C NMR established the presence of prodelphinidins along with the expected procyanidins (Czochanska et al. 1980). In agreement with this finding,

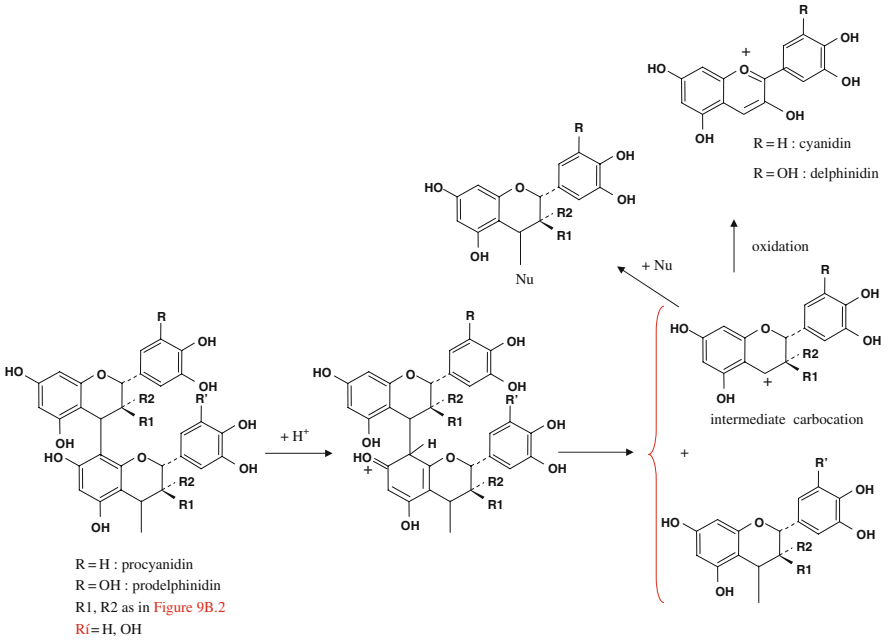

Fig. 9B.3 Acid-catalysed cleavage of proanthocyanidin interflavanic linkages and subsequent reactions, namely oxidation to anthocyanidin and nucleophilic (Nu) addition

Table 9B.1 Flavanol oligomers, flavonols and dihydroflavonols identified in grapes and wines, methods of detection

	Source	Reference	Detection
Flavanol dimers			
(–)-Epicatechin-(4β-8)-(+)-catechin (B1)	Berries	Weinges and Piretti (1971)	LC -IR, -^{1}H NMR, -MS
(–)-Epicatechin-(4α-8)-(–)-epicatechin (B2)	Berries	Weinges and Piretti (1971)	LC -IR, -^{1}H NMR, -MS
(+)-Catechin-(4α-8)-(+)-catechin (B3)	Berries	Weinges and Piretti (1971)	LC -IR, -^{1}H NMR, -MS
(+)-Catechin-(4α-8)-(–)-epicatechin (B4)	Berries	Weinges and Piretti (1971)	LC -IR, -^{1}H NMR, -MS
(–)-Epicatechin-(4α-6)-(–)-epicatechin (B5)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, −^{1}H NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(+)-Catechin-(4α-6)-(+)-catechin (B6)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, -^{1}H NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin-(4β-6)-(+)-catechin (B7)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, -^{1}H NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(+)-Catechin-(4α-6)-(–)-epicatechin (B8)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, -^{1}H NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epigallocatechin-(+)-catechin	Wine	Remy (1999)	HPLC-DAD, LC-MS
(–)-Epicatechin-(+)-gallocatechin	Wine	Remy (1999)	HPLC-DAD, LC-MS
(–)-Epicatechin-(+)-epigallocatechin	Wine	Remy (1999)	HPLC-DAD, LC-MS
(–)-Epicatechin 3-gallate-(4β-8)-(+)-catechin (B1 3-gallate)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol

Table 9B.1 (continued)

	Source	Reference	Detection
(–)-Epicatechin 3-gallate-(4β-8)-(–)-epicatechin (B2 3 gallate)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin-(4β-8)-(–)-epicatechin 3-gallate (B2 3' gallate)	Berries	Czochanska et al. (1979a)	PLC, -MS, -^{1}H NMR
(–)-Epicatechin-(4β-8)-(–)-epicatechin 3-gallate (B2 3' gallate)	Stem	Boukharta et al. (1988)	LC-,UV spectroscopy, -^{1}H NMR after enzymatic hydrolysis, acid hydrolysis, or with α-thiol
(+)-Catechin-(4α-8)-(–)-epicatechin 3-gallate (B4 3 gallate)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin 3-gallate-(4β-8)-(–)-epicatechin 3-gallate (B2 3, 3' digallate)	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
Flavanol trimers			
(–)-Epicatechin-(4β-8)- (–)-epicatechin-(4β-8)-(–)- epicatechin (C1)	Wine	Lea et al. (1979)	PC, TLC after toluene-thiol hydrolysis
(–)-Epicatechin-(4β-8)-(–)-epicatechin-(4β-8)-(+)-catechin	Wine	Lea et al. (1979)	PC, TLC after toluene-thiol hydrolysis
(–)-Epicatechin-(4β-8)-(–)-epicatechin-(4β-6)-(+)-catechin	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, – ^{1}H NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin-(4β-6)-(–)-epicatechin-(4β-8)-(–)-epicatechin	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin-(4β-8)-(–)-epicatechin-(4β-6)-(–)-epicatechin	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
(–)-Epicatechin-(4β-6)-(–)-epicatechin-(4β-8)-(+)-catechin	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol

Table 9B.1 (continued)

	Source	Reference	Detection
(–)-Epicatechin-(4β-8)-(–)-epicatechin 3-gallate-(4β-8)-(+)- catechin	Seeds	Ricardo da Silva et al. (1991c)	HPLC, TLC, NMR, FAB-MS after enzymatic hydrolysis, acid hydrolysis, partial acid-catalysed degradation with phloroglucinol or phenylmethanethiol
Flavonols			
Quercetin, (R1=OH, R2=H) 3-glucoside	Skin	Ribéreau-Gayon (1964)	PC- Fluorescence, -UV spectroscopy, hydrolysis
Quercetin 3-glucuronide	Skin	Ribéreau-Gayon (1964)	PC- Fluorescence, -UV spectroscopy, hydrolysis
Quercetin 3-glucosylgalactoside	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Quercetin 3-glucosylxyloside	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Quercetin 3-rhamnosylglucoside	Skin	Cantos et al. (2002)	HPLC-DAD, -MS-MS
Quercetin 3-rhamnosylglucoside	Leaves	Hmamouchi et al. (1996)	HPLC-UV spectrometry, -MS, -^{1}H and ^{13}C NMR, -TLC after hydrolysis
Kampferol (R1=R2=H) 3-glucoside	Skin	Ribéreau-Gayon (1964)	PC- Fluorescence, -UV spectroscopy, hydrolysis
Kampferol 3-glucuronide	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Kampferol 3-galactoside	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Kampferol 3-glucosylarabinoside	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Myricetin (R1=R2=OH) 3-glucoside	Skin	Ribéreau-Gayon (1964)	PC- Fluorescence, -UV spectroscopy, hydrolysis
Myricetin 3-glucuronide	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis

Table 9B.1 continued

	Source	Reference	Detection
Isorhamnetin (R1=OCH_3, R2=H) 3-glucoside	Skin	Cheynier and Rigaud (1986)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Luteolin (R1=R2=H, O-β gluc at C7) 7-glucoside	Leaves	Hmamouchi et al. (1996)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Apigenin (R1=OH R2=H, O-β gluc at C7) 7-glucoside	Leaves	Hmamouchi et al. (1996)	HPLC-UV spectrometry, -MS, -^{1}H NMR, -TLC after hydrolysis
Laricetin (R1=OCH_3, R2=OH) 3-glucoside and 3-galactoside	Skin	Mattivi et al. (2006)	HPLC-DAD, -MS before and after hydrolysis
Syringetin (R1=R2=OCH_3) 3-glucoside and 3-galactoside	Skin	Mattivi et al. (2006)	HPLC –DAD, -MS before and after hydrolysis
Dihydroflavonols			
Dihydroquercetin (R1=OH, R2=H) 3-rhamnoside (astilbin)	Skin	Trousdale and Singleton (1983)	LC-^{1}H NMR
Dihydroquercetin, (R1=OH, R2=H) 3-rhamnoside (astilbin)	Stem	Souquet et al. (2000)	LC-MS, ^{1}H NMR
Dihydroquercetin 3-galactoside	Skin	Masa et al. (2007)	HPLC-DAD, TLC, PC
Dihydroquercetin 3-glucoside	Skin	Masa et al. (2007)	HPLC-DAD, TLC, PC
Dihydrokampferol (R1=R2=H) 3-rhamnoside (engeletin)	Skin	Trousdale and Singleton (1983)	LC-^{1}H NMR
Dihydrokampferol 3-rhamnoside (engeletin)	Stem	Souquet et al. (2000)	LC-MS, – ^{1}H NMR

additional dimers containing gallocatechin and epigallocatechin units, either in the lower or in the upper position have been detected in wine (Fulcrand et al. 1999; de Pascual-Teresa et al. 2000).

The reversed-phase HPLC separation methods used for monomer and oligomer analysis become poorly resolutive as the molecular weight increases, owing to the large number of possible isomers distributed along the chromatographic profile and smaller amounts of each individual compound. Thus, detection of species beyond the tetramer by using this technique is almost impossible, especially in grape extracts which contain proanthocyanidins based on several constitutive units. Nevertheless, the prevalence of larger oligomers and polymers has been demonstrated in numerous plant species including grapes (Czochanska et al., 1980).

Alternative methods have been developed to characterize proanthocyanidins polymers. Some separation according to molecular weight can be achieved by adsorption chromatography on Sephadex (Lea and Timberlake 1974) or Fractogel (Derdelinckx and Jerumanis 1984; Ricardo da Silva et al. 1991c) columns or by gel permeation chromatography (Bae et al. 1994; Le Bourvellec et al. 2006; Yanagida et al. 1999) but the resolution is rather poor especially for polymers. Normal phase HPLC on silica (Rigaud et al. 1993; Yanagida et al. 2000) or diol (Kelm et al. 2006) columns improve separation of the polymeric proanthocyanidins and was successfully applied to grape seed (Prieur et al. 1994; Rigaud et al. 1993) and grape skin (Souquet et al. 1996) extracts. The elution profiles showed that grape seed proanthocyanidins consist of oligomers and lower molecular weight polymers while skin proanthocyanidins are polymeric material eluted as a large hump at the end of the chromatogram. However, no relationship between the retention time and the chain length could be established when comparing both extracts, due to their different compositions (Cheynier et al. 1999b). Proanthocyanidin polymers can be analysed by HPLC after acid catalysed cleavage in the presence of a nucleophilic agent. In these methods, the intermediate carbocations released from the upper units (initially substituted in C4) after cleavage of the interflavanic bonds react with the nucleophile (usually phloroglucinol or toluene-α-thiol) to yield an adduct while the other units are released as such (Fig. 9B.3). HPLC analysis of the reaction products thus gives access to the total amount of proanthocyanidins (calculated as the sum of released units), the nature and proportions of each constitutive unit and the mean degree of polymerisation (mDP).

Application of thiolysis to grape proanthocyanidins showed that those extracted from seeds are partly galloylated procyanidins (Prieur et al. 1994), based on epicatechin, catechin and epicatechin units, with degrees of polymerisation ranging from 1 to 16 in the fractions analysed. Those from skins (Souquet et al. 1996) consist of both procyanidins and prodelphinidins with mDP around 30 (up to 80 in the polymeric fractions). They contained epicatechin and epigallocatechin as their major constitutive units and very low levels of galloylated units (a few percent). Proanthocyanidins from stems (Souquet et al. 2000) and pulp (Mané et al. 2007b; Souquet et al. 2006) are also mixed procyanidin/prodelphinidin polymers with lower proportions of epigallocatechin units and higher levels of galloylation than skin proanthocyanidins. The mean degrees of polymerization calculated for pulp

proanthocyanidins (about 20) are also intermediate between those in seeds and skins in all analysed varieties (Mané et al. 2007b; Souquet et al. 2006). The flavanol concentration is higher in seeds than in skins and pulp contain only small amounts. However, the contribution of skins to the entire berry content may exceed that of seeds in some varieties (Mané et al. 2007a).

9B.1.2.2 Variability in Grape

Flavanol composition is developmental stage-, genetic- and growth condition-dependent. Skin flavanols are synthesised mainly during a few weeks after flowering (Kennedy et al. 2001; Downey et al. 2003a). The skin flavanol pool is considered as almost constant during ripening when expressed on a per berry basis, on a quantitative and qualitative point of view (Fournand et al. 2006). In contrast, other authors have described a decrease in the content of flavanol monomers and proanthocyanidins after veraison (Downey et al. 2003a; Kennedy et al. 2002), and a concomitant increase (Kennedy et al. 2001) or decrease (Downey et al. 2003a) of proanthocyanidin mDP. In seeds, accumulation of flavanols is a bit delayed when compared to skin, and maximal concentration is reached a few weeks after veraison (Bogs et al. 2005; Downey et al. 2003a). The concentration of flavanol monomers then decreases sharply (Downey et al. 2003a; Romeyer et al. 1986) while slight accumulation of procyanidin oligomers is observed (Romeyer et al. 1986). The total amount of proanthocyanidins that can be extracted by the usual solvents decreases over the same period. However, the additional units measured by subjecting the residue to acid catalysed cleavage compensate for this loss, suggesting that polymeric flavanols have been strongly absorbed on the plant cell wall material rather than degraded (Downey et al. 2003a).

Flavanol composition, like anthocyanin profiles (Roggero et al 1988; Mazza and Miniati 1993), is greatly affected by genetics. Reported values for skin mDP ranged from 16 (Muscat de Hambourg; Souquet et al. 2006) to 86 (Cabernet-Sauvignon; Monagas et al. 2003) and for seed from 3 (Pinot noir; Mané et al. 2007b) to 10 (Maccabeo; Souquet et al. 2006). It should however be emphasized that extreme values reported by different authors are also related to variations in the analysis protocols. For instance, some authors separate oligomers from polymers whereas others do not. The percentage of B-ring trihydroxylation of subunits from skin proanthocyanidins is also a variable parameter, with values as high as 31% for Cabernet-Sauvignon (Monagas et al. 2003) but of only 3% in Maccabeo (Souquet et al. 2006). Percentage of galloylation of skin and seed flavanols seems to be independent and ranged from 1.1 to 6.5 in skin and from 9.5 to 20.6 in seeds (Mané et al. 2007b; Souquet et al. 2006; Monagas et al. 2003). Comparison of total amount is more delicate since values are expressed differently among publications: per gram of tissue (dry or fresh) weight, per gram of entire berry, per berry. Values found in literature for total amount of skin tannins is between 1.76g/kg of berries for Mourvedre and almost 3.15 for Muscat de Hambourg (Downey et al. 2003a; Cortell et al. 2005; Fournand et al. 2006; Souquet et al. 2006; Mané et al. 2007b).This concentration seems independent of anthocyanin concentration even if the number of varieties studied is statistically rather small. Contribution of the seeds to the total

composition of the berry is also cultivar dependant; Maccabeo berries contain two times less proanthocyanidin in the seed than in the skin (Souquet et al. 2006) whereas seed contribution to the total flavanol pool of Pinot berries is greater than 60% (Mané et al. 2007b).

This effect can be modulated by environmental factors. Significant increases were found in skin proanthocyanidin content, proportion of (–)-epigallocatechin, and average DP in berries from zones with a low vine vigor (Cortell et al. 2005). In reaction to sun exposure, skin proanthocyanidin content tends to increase, particularly trihydroxylated subunits and mDP is enhanced (Downey et al. 2004; Cortell and Kennedy 2006). Shaded fruits reached a lower maximum in proanthocyanidin content than sun-exposed ones but the contents at harvest were similar. Most authors agree that water stress had only slight effects on tannin composition (Ojeda et al. 2002; Kennedy et al. 2002; Castellarin et al. 2006). Seed flavanol composition seems hardly affected by environmental factors.

9B.1.3 Flavonols and Dihydroflavonols

9B.1.3.1 Structure and Localisation

Flavonols, which play a protective role against UV radiations, are found in grape skins and in leaves. Some flavonols were also detected in pulp (Pereira et al. 2006), but none in the seeds (Rodriguez Montealegre et al. 2006).

The major flavonols in grape are 3-glycosides of quercetin that is dihydroxylated on the B-ring (Fig. 9B.1, R1=OH, R2=H), and especially its 3-glucoside and 3-glucuronide (Ribéreau-Gayon 1964; Wulf and Nagel 1976; Cheynier and Rigaud 1986; Downey et al. 2003b; Price et al. 1995). Other flavonols and dihydroflavonols (syn. flavanonols) were also identified in different parts of the plant as listed in Table 9B.1.

9B.1.3.2 Variability in Grape

The amount of flavonols in the berries also depends on the developmental stage, genetic and environmental factors. Flavonol biosynthesis occurs at flowering and again after veraison. Small amounts can be detected at the green stage but synthesis mainly occurs during ripening and a constant increase in flavonol content per berry is measured (Downey et al. 2003b).

Each cultivar possesses a specific flavonol and dihydroflavonol profile (quantitative and qualitative aspect) that could be used for taxonomic characterization (Mattivi et al. 2006; Masa et al. 2007). It was believed that white cultivars did not contain any methylated flavonol until Rodriguez Montealegre et al. (2006) detected small amounts of isorhamnetin glucoside in their skins. Based on a survey of 91 *Vitis vinifera* cultivars, Mattivi et al. (2006) concluded that isorhamnetin derivatives are present in small amounts in white cultivars whereas derivatives of myricetin, laricitrin and syringetin appear to be specific of red cultivars. However, myricetin was detected in some white Muscadine cultivars (*Vitis rotundifolia* sp.) (Talcott and

Lee 2002). The amount of flavonols ranged between 2 and 30mg/kg of berry for white skinned cultivars and 4 and 78mg/kg of berry for black skinned cultivars (Mattivi et al. 2006). Rodriguez Montealegre et al. (2006) agreed that red skinned cultivars contain more flavonols but they detected higher concentrations, up to 170mg/kg for Viognier berries or 200mg/kg for Shiraz berries.

Flavonol and especially quercetin levels in the skins of Pinot noir grapes showed a dramatic response to sun exposure (Price et al. 1995). The effect of bunch shading on flavonol accumulation was confirmed in Merlot (Spayd et al. 2002) and Syrah (Downey et al. 2004) while temperature had little or no effect. This suggests that biosynthesis of flavonols is light-induced, accordingly with the role of flavonols as UV-protectant (Cortell and Kennedy 2006; Downey et al. 2004; Spayd et al. 2002). This effect was maximum when shading was applied a few weeks before flowering, almost preventing any flavonol synthesis. When the treatment was applied after flowering, the amount of berry flavonols was 8–10 times lower than in the control berries (Cortell and Kennedy 2006; Downey et al. 2004). No detailed impact on the different grape flavonols in skins is available. The flavonol content in pulp also discriminated between shaded and light exposed berries, kaempferol and quercetin derivatives being more abundant in the pulp of sun exposed berries, and myricetin derivatives, along with other unidentified flavonols, in that of shaded berries (Pereira et al. 2006).

9B.2 Extraction into the Wine

Wine flavonoid composition depends not only on the grape composition but also on their extraction and subsequent reactions during the wine-making and aging process. Thus, white wines obtained by direct pressing with minimum skin contact contain mostly the flavonoids originating from pulp. In rosé and red wine technology, the procedures used to extract the anthocyanin pigments from the skins also result in increased extraction of other flavonoids from skins, seeds and eventually stems or leaves if present in the fermentation tank. Extraction continues until the wine is separated from the solid residue (marc or pomace) by racking or pressing. Its kinetics depend on the solubility of the compounds and on their accessibility within the berry tissues, which can be modulated by physiological factors such as the maturation stage. It is further influenced by other technological factors, including the concentration of alcohol and of sulfur dioxide in the liquid phase, the temperature and the extent of must homogenization. Consequently, the wine flavonoid composition is influenced by the duration of pre- and post-fermentation maceration phases and by treatments enhancing cell wall or berry degradation (e.g. use of pectinolytic enzymes).

9B.2.1 White Wines

Quercetin 3-glucuronide was the only flavonol detected in free run juices and wines (Alonso et al. 1986; Betes-Saura et al. 1996), along with trace amounts of kampferol

3-glucoside in Riesling wine (Baderschneider and Winterhalter, 2001). Its average concentration was found to be 0.5mg/L and 0.25mg/L, in free run juices and wines, respectively and 0.4mg/L in Cava sparkling wines. Champagne wines made from Pinot noir and Chardonnay contained quercetin aglycone and trace amounts of astilbin and engeletin (Chamkha et al. 2003) which have also been reported in other white wines (Trousdale and Singleton 1983). Finally, leaf contamination of the grape crush may result in increased flavonol concentration in wines (Somers and Ziemelis 1985).

Flavanol monomers and oligomers have been found in small amounts (a few mg/L) in white wines made without maceration (Cheynier et al. 1989b; Betes-Saura et al. 1996; Chamkha et al. 2003; Ricardo da Silva et al. 1993). Delays between harvest and pressing, especially if sulfur dioxide is added to prevent oxidation, as well as thorough pressing, result in increased concentrations of flavonoids in white musts and wines (Yokotstuka 1990; Somers and Pocock 1991). Skin contact before fermentation is sometimes used in white wine making to favour the extraction of volatile compounds and increase wine varietal character. This practice also resulted in an increase of flavanol concentration in wine (Cheynier et al. 1989b; Ricardo da Silva et al. 1993). Procyanidin B1 was the major dimer and galloylated dimers were present in very low amounts, suggesting that flavanols in white wine do not originate from seeds.

To our knowledge, proanthocyanidin polymers have not been analysed in white wines. In a recent study performed in our laboratory, no flavanol derivatives could be detected in Champagne wines after thiolysis (Mané 2007) although the pulp of all three Champagne cultivars contained about 20mg of proanthocyanidins per kg of berries (Mané et al. 2007b). This can be due to adsorption of the higher polymers on the grape cell wall material, as described for apple (Renard et al. 2001) or to oxidation during pressing of the must. Indeed, the role of enzymatic oxidation, catalyzed by the grape polyphenoloxidase (PPO) during obtention of white musts is well documented. Flavanol monomers are rather poor substrates for PPO and proanthocyanidins cannot be oxidized by the enzyme, presumably due to steric hindrance. However, all these compounds are readily oxidized by the quinones resulting from enzymatic oxidation of caffeoyl tartaric acid, the major substrate of PPO in grape (Cheynier et al. 1988; Cheynier and Ricardo Da Silva 1991). Increasing the level of oxygen exposure before fermentation resulted in much lower amounts of flavanols in wine, confirming the role of oxidation (Cheynier et al. 1989b; Ricardo da Silva et al. 1993).

9B.2.2 Red Wines

Anthocyanin content reaches a maximum early in fermentation (Nagel and Wulf 1979) whereas tannin extraction continues throughout pomace contact (Singleton and Draper 1964). Monitoring of red must flavonoid composition during maceration showed that the extraction of flavonols and of proanthocyanidins from skins roughly parallels that of anthocyanins while that of seed flavanols is slower (Cheynier et al. 1997b; Morel-Salmi et al. 2006). The initial rate of flavonoid

extraction from skins was not influenced by the alcohol content, although the final concentration reached in water was lower than in 6.5% or 13% ethanol whereas extraction of flavonoids from seeds increased with concentration of ethanol (Canals et al. 2005). Whether this is due to their chemical structure, seed procyanidins being less hydrophilic than other flavonoids, or to the different characteristics of both plant tissues is unknown.

Consequently, pre-fermentation maceration at low temperature increases the level of anthocyanins and flavanols from pulp and skins while post-fermentation maceration increases that of proanthocyanidins, as a result of enhanced extraction from seeds (Cheynier et al. 2006).

Treatments favouring contact between solid and liquid phases such as pumping over or punching down are traditionally used to enhance extraction. Alternative processes have been proposed more recently. These include physical processes such as thermovinification and flash release (in which the grapes are heated quickly at high temperature ($> 95^{\circ}C$) and then placed under vacuum, to produce instant vaporization and cooling) and the use of pectinolytic enzymes.

Flash release and thermovinification greatly accelerated extraction of all flavonoids from Grenache, Carignan or Mourvedre grapes and can be used to produce polyphenol-enriched juices (Morel-Salmi et al. 2006). Maceration after flash release treatment further increased extraction of flavonoids and especially of flavanols. After maceration, the concentration of flavanols in flash release treated wines was much higher than in the control wine while that of flavonols and anthocyanins was hardly modified. The use of pectinolytic enzymes results in increased juice yield (Berg 1959; Ough and Crowell 1979) along with increased browning of white wines (Berg 1959) and faster colour development (Ough et al. 1975), enhanced extraction of phenolic compounds and colour in red wines (Fernandez-Zurbano et al. 1999).

Numerous protocols have been proposed to estimate flavonoid extractibility from grape and relate it to ripeness and crop quality. Such approaches have shown that anthocyanin extraction increases as they accumulate in the berries (Canals et al. 2005; Fournand et al. 2006) but that their extraction yield is stable (Fournand et al. 2006). The rate of proanthocyanidin extraction from skins as well as the quantity extracted remain constant throughout berry development. Extracted proanthocyanidins showed lower mDP and lower rate of galloylation than those remaining in the marc. Although the extraction yield did not change, this selectivity was somewhat lower in riper grapes. Extraction of flavonols from shaded grapes appeared more efficient than from sun exposed grapes, as the concentration of flavonols in the simulated maceration was only decreased 2.5-fold (vs 8-fold in the grape skins) (Cortell and Kennedy 2006). The extraction rate of skin proanthocyanidins was higher from sun exposed than from shaded grapes but the concentrations in both extracts were similar (Cortell and Kennedy 2006).

Nevertheless, the proposed protocols do not simulate flavonoid extraction during the maceration and fermentation process, as they do not reproduce the effect of gradual alcohol accumulation, temperature increase and duration of the maceration phase.

The concentrations of anthocyanins and proanthocyanidins present in red Grenache wines at the end of fermentation represented about 30% and 50%, respectively, of their amounts in grape (Morel-Salmi et al. 2006). Extraction of the pomace allowed to recover most of the proanthocyanidins but hardly increased the yield of anthocyanins. This indicates that a major proportion of anthocyanins have been converted to other species and/or been irreversibly adsorbed on the solid material during fermentation, in agreement with earlier studies reporting a drop in anthocyanin concentration after the initial increase (Nagel and Wulf 1979; Gao et al. 1997). Monitoring of flavonoid composition in wines made by pressing immediately after flash release and fermentation in the liquid phase demonstrated that anthocyanins, flavonols and proanthocyanidins undergo rapid changes (about 50% loss for flavonols and anthocyanins, 40% loss for proanthocyanidins after five days of fermentation), while flavanol monomers are not affected (Morel-Salmi et al. 2006). This provides good evidence that anthocyanin and tannin reactions that have been reported to take place slowly during aging actually start very early in the wine-making process.

9B.3 Reactions in Wine

Changes in flavonoid composition taking place during wine making and aging involve both enzymatic and chemical processes. The former processes, due to grape, yeast or fungi and exogenous enzymes occur mostly in to the early stages while chemical reactions continue during aging.

9B.3.1 Flavonoid Reactivity

Reactions of flavonoids are primarily due to the reactivity of the phenolic rings. On one hand, the resonance between the free electron pair of a phenolic oxygen and the benzene ring enhances electron delocalisation and confers the position adjacent to the hydroxyl a partial negative charge and thus a nucleophilic character (showing an excess of electrons and thus prone to react with electrophiles, showing an electron deficiency). Such nucleophilic sites are encountered on the phloroglucinol A-ring of flavonoids, in C6 and C8 (for the numbering, see Fig. 9B.1), due to their meta hydroxyl substitution pattern. On the other hand, the acidity of the phenolic hydroxyl groups leads to formation of phenate ions and subsequent oxidation to a semiquinone radical, or in the case of *o*-diphenolic groups as often encountered in the B-ring, to an *o*-quinone. The latter is an electrophilic species and thus prone to suffer nucleophilic addition. Other examples of electrophilic species include the anthocyanin flavylium cations and the carbocations resulting from acid-catalysed cleavage of proanthocyanidin interflavanic linkages.

9B.3.2 Enzymatic Processes

9B.3.2.1 Enzymatic Oxidation

The major grape enzymes involved in flavonoid degradation is polyphenoloxidase (PPO) that catalyses *o*-hydroxylation of monophenols (Fig. 9B.4(1)) and oxidation of *o*-diphenols to the corresponding *o*-quinones (Fig. 9B.4(2)). Its action is particularly important in white wine technology as extensive decompartmentation of the enzyme and its substrates (phenolic compounds and oxygen) takes place at pressing. Catechins and flavonol aglycones are rather poor substrates for grape PPO compared to caffeoyltartaric acid. Glycosylated flavonoids, epicatechin gallate and proanthocyanidins cannot be oxidized directly by grape PPO, presumably due to steric hindrance. However, they can react with the enzymically generated caffeoyltartaric acid quinone through coupled oxidation (Fig. 9B.3) and nucleophilic addition reactions (Fig. 9B.4) (Cheynier and Van Hulst 1988; Cheynier et al. 1988, 1989a, 1995; Cheynier and Ricardo Da Silva 1991), as described in Chapter II.2.3 for anthocyanins. In addition, disproportionation of an *o*-quinone and an *o*-diphenol can yield two semiquinone radicals (Fig. 9B.5) which can then undergo radical coupling (Fig. 9B.6). Enzymatic oxidation can also be catalysed by other enzymes such as laccase arising from *Botrytis cine*rea (grey mold or noble rot) and peroxidases which accept a wider range of substrates.

9B.3.2.2 Enzymatic Hydrolysis

Other enzymes degrading flavonoids include various hydrolases, originating from microorganisms or from pectolytic preparations added during wine-making. Some yeast strains show glycosidase activities, including β-glucosidase which is active on flavono and anthocyanin 3-glucosides. Tannase activity (tannin acyl hydrolase, EC 3.1.1.20) catalysing hydrolysis of galloyl esters has been observed in numerous fungi, including *B. cinerea*, and in lactic acid bacteria (Matthews et al. 2006). The use of exogenous enzymes is becoming more and more popular to help clarification, increase press yield, extraction, and release of aroma compounds from their glucosidic precursors. These enzyme preparations are pectinases and α-glucanases. They may show side activities such as cinnamate esterase, tannase and β-glucosidase. Hydrolysis of flavonol glycosides has been reported to result in haze development as the insoluble flavonol aglycones precipitated out (Somers and Ziemelis 1985).

9B.3.3 Chemical Reactions

As stated above, flavonoids react as nucleophiles through their C8 and C6 positions. Acid-catalysed cleavage of the interflavanic linkages of proanthocyanidins (cf. Fig. 9B.3) also takes place spontaneously in wine, yielding an intermediate electrophile. A third group of reactions involves the *o*-diphenolic B-ring which can be oxidized to electrophilic quinones. Several mechanisms arise from these reactivities.

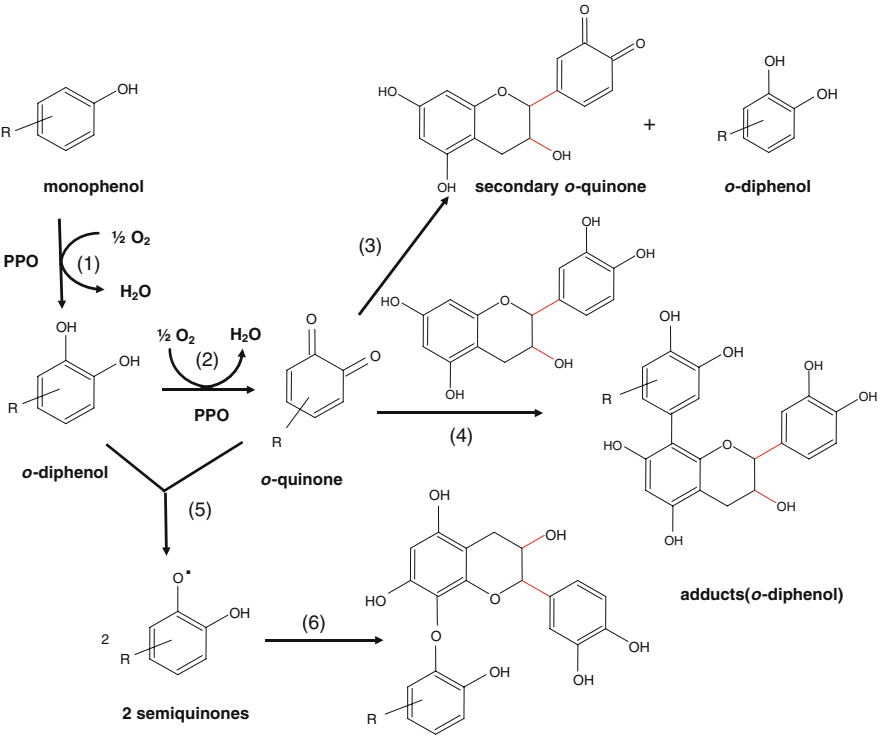

Fig. 9B.4 Enzymatic formation and reactions of quinones

Those involving both flavonol and anthocyanins have been described in Chapter 9A and will not be detailed here.

9B.3.3.1 Reactions Based on Acid-Catalysed Cleavage of Proanthocyanidins

Precursors

The precursors of these reactions are, on one hand, proanthocyanidins and, on the other hand, any kind of flavonoid that can act as a nucleophile. The latter include flavonols, dihydroflavonols, flavanol monomers, proanthocyanidins, and anthocyanins under their hemiketal form (for anthocyanin reactivity, see Chapter 9A).

Reaction Mechanism

The reaction starts with acid catalysed cleavage of a proanthocyanidin interflavanic linkage (Figs. 9B.3 and 9B.5(1)). The intermediate carbocation thus generated then undergoes nucleophilic addition. When the nucleophile is another flavanol (Fig. 9B.5(2a)), the product is a new proanthocyanidin molecule. As a result of this

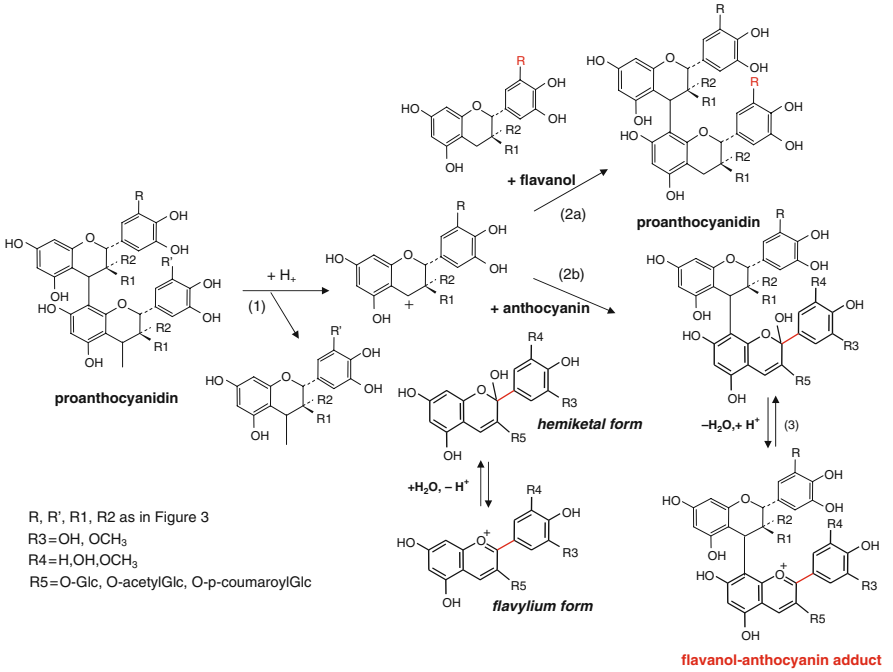

Fig. 9B.5 Polymerisation reactions based on acid-catalysed cleavage of proanthocyanidins

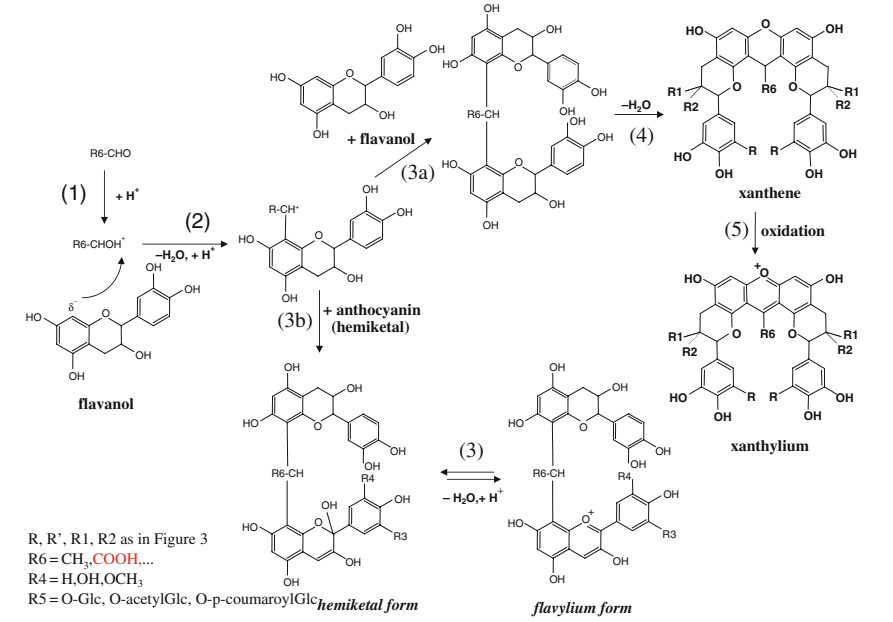

Fig. 9B.6 Condensation reactions with aldehydes

process, first described by Haslam (1980), the average DP of proanthocyanidins can be modified, either increased if the nucleophilic flavanols are polymers, or decreased if they are low molecular weight compounds. In the presence of excess amounts of monomers, side reactions of the carbocations leading to unknown species are much reduced and polymers are gradually replaced by oligomers (Vidal et al. 2002). Anthocyanins (under their hemiketal form) and presumably flavonols can replace the flavanol as a nucleophile. In the case of anthocyanins, the product is a colorless flavanol-anthocyanin hemiketal adduct (Fig. 9B.5(2b)) which then can give rise to the corresponding flavylium adduct, through protonation and dehydration (Fig. 9B.5(3)) (Ribéreau-Gayon 1982). Another mechanism involving the flavene form of the anthocyanin and subsequent oxidation of the resulting adduct to the flavylium pigment has also been proposed (Jurd 1969). Recent mass spectrometry studies have confirmed the former pathway (Salas et al. 2003) and established the presence of flavanol-anthocyanin adducts in wine (Salas et al. 2004, 2005).

Evidence in Wine

Evidence of such adducts in wine fractions has been provided, as detailed in Chapter 9A. These include F-A^+ (Alcalde-Eon et al. 2006; Boido et al. 2006) and F-A-A^+ (Alcalde-Eon et al. 2006) adducts based on different flavanol and anthocyanin units and $(F)_n$-A^+ adducts deriving from different flavanols monomers and oligomers (Hayasaka and Kennedy 2003). Proanthocyanidins arising from these reactions cannot be distinguished from those extracted from grapes. However, detection of F-A^+ adducts without prior fractionation (Morel-Salmi et al. 2006) confirmed the occurrence of the acid-catalyzed interflavanic bond breaking process in wines.

Factors Affecting the Reaction

The first step of the reaction is acid-catalyzed and thus largely determined by pH. When used for analytical purposes in reactions such as thiolysis or phloroglucinolysis, the reaction is performed under strongly acidic conditions. However, it takes place spontaneously at pH values such as encountered in wines. For instance, conversion of proanthocyanidin polymers to dimers was demonstrated at pH 3.2 (Vidal et al. 2002). When procyanidin dimer B2-3′-*O*-gallate (Ec-EcG) was incubated at pH 2, $(Ec)_n$-EcG oligomers (with $n = 1, 2, 3$) were formed. These oligomers that result from successive cleavage of the interflavanic bond and addition of B2-3′-*O*-gallate onto epicatechin carbocation were not detected at pH 3.8. In model systems containing B2-3′-*O*-gallate and malvidin 3-glucoside, flavanol anthocyanin adducts were formed at pH 2 but not at pH 3.8 (Salas et al. 2003), meaning that the acid catalyzed cleavage rather than the proportion of anthocyanin in the hemiketal form (decreasing with pH) was the limiting factor. However, such adducts were detected in a series of red wines (Morel-Salmi et al. 2006) showing pH values in the range 3.59–3.82 (Doco et al. 2007).

9B.3.3.2 Reactions Involving Aldehydes

Precursors

Various aldehydes are encountered in wine. The most abundant is acetaldehyde which is both a product of yeast metabolism and an oxidation product of ethanol. Glyoxylic acid, resulting from oxidation of tartaric acid, especially catalyzed by metal ions (Fe, Cu) or ascorbic acid, can also be present. Other aldehydes reported to participate in these reactions include furfural and 5-hydroxymethylfurfural that are degradation products of sugar and can be extracted from barrels (Es-Safi et al. 2000), vanillin which also results from oak toasting, isovaleraldehyde, benzaldehyde, propionaldehyde, isobutyraldehyde, formaldehyde and 2-methylbutyraldehyde which are present in the spirits used to produce fortified wines (Pissara et al. 2003).

Reaction Mechanism

The reaction mechanisms, first proposed by Timberlake and co-workers (Timberlake and Bridle 1976), was recently established by mass spectrometry (Fulcrand et al. 1996). It starts with protonation of the aldehyde (Fig. 9B.6(1)), yielding an intermediate carbocation which then suffers nucleophilic addition from the A-ring of the flavonoid. The resulting adduct, through protonation and dehydration steps, gives rise to another carbocation (Fig. 9B.6(2)) which reacts with a second flavonoid molecule (Fig. 9B.6(3a,b)). As both the C6 and C8 positions are reactive, the reaction can continue through the remaining free nucleophilic sites, leading to polymerisation. When several nucleophiles are present, they combine randomly to form a large variety of oligomers and polymers (Es-Safi et al. 1999a). Polymerisation was first established in the case of flavanol monomers and acetaldehyde. When both anthocyanins and flavanols are present, the latter react more readily with acetaldehyde and anthocyanins were initially thought to terminate the polymerisation chain. However, the detection of methylmethine-linked anthocyanin oligomers resulting from this process ruled out this hypothesis (Atanasova et al 2002). The hydration constants of methylmethine-linked malvidin 3-glucoside dimer (Atanasova et al 2002) and of flavanol-methylmethine-malvidin 3-glucoside (Duenas et al. 2006b) were determined. This indicated that one of the units of the anthocyanin dimer is under the hemiketal form and can be involved in further polymerisation while the anthocyanin in the flavanol adduct is predominantly under the flavylium form and thus less prone to react as a nucleophile.

Similar processes have been observed with other aldehydes, such as glyoxylic acid. However, the carboxymethine-linked oligomers resulting from reaction with glyoxylic acid proceeded to xanthylium salts rather than to larger polymers (Es-Safi et al. 1999b). The postulated pathway involves dehydration and cyclisation of the carboxymethine dimer (Fig. 9B.6(4)) followed by oxidation of the resulting xanthene (Fig. 9B.6(5)) that was also detected in the medium. Formation of xanthylium salts was also shown in the case of furfural and hydroxymethylfurfural (Es-Safi et al. 2000).

Evidence in Wine

A number of flavanol-methylmethine-anthocyanins have been detected in wine (see Chapter 9A for extensive list) as well as methylmethine-linked flavanol dimers (Cheynier et al. 1997a; Saucier et al. 1997b). More recently, a method to determine methyl-methine linkages has been proposed (Drinkine et al. 2007a), showing that they represented less than 4% of bonds between flavanol units in wine although this proportion increased with aging (Drinkine et al. 2007b). The other aldehyde derivatives have not yet been detected in wine.

Factors Affecting the Reaction

The rate of condensation reactions depends on the concentration of the precursors involved, and especially on that of the aldehyde, and on its protonation rate, which is primarily determined by pH. On the other hand, acidic conditions also favour cleavage of the products. The methylmethine bridges are particularly susceptible to acid-catalysed cleavage which leads to rearrangement and further polymerisation (Es-Safi et al. 1999a; Escribano-Bailon et al. 2001). This also yields vinylflavanol structures which add to anthocyanins to form flavanyl-pyranoanthocyanins (Cheynier et al. 1999a; Mateus et al. 2002) and flavanylvinylpyranoanthocyanins (portisins) (Mateus et al. 2003) as described in Chapter 9A.

Acetaldehyde is generated during alcoholic fermentation but its concentration can be much increased as a result of oxygen exposure in the wine-making or aging process. Metal ions such as iron (Oszmianski et al. 1996) or copper (Clark and Scollary 2002) much enhance browning of catechin in wine-like medium containing tartaric acid due to the formation of xanthylium pigments (Es-Safi et al. 1999b). Metals were first postulated to catalyse oxidation of tartaric acid to glyoxylic acid but it was latter demonstrated that copper(II) also accelerated the bridging of two (+)-catechin units by glyoxylic acid (Clark et al. 2003). Ascorbic acid, after an initial antioxidant phase, also increased formation of xanthylium salts, as it crossed-over to pro-oxidant (Bradshaw et al. 2003). Catechin reaction was faster with glyoxylic acid than with acetaldehyde and even faster when both aldehydes were present (Drinkine et al. 2005). Under the conditions used in this experiment, methylmethine and carboxymethine bridged dimers as well as mixed polymers were observed but no xanthylium salt could be detected, either because of the rather short incubation time used or because no metal catalyst was present. However, products arising from glyoxylic acid condensation have never been detected in wine, possibly because its formation rate from tartaric acid is the limiting factor.

9B.3.3.3 Reactions Involving Quinones

Precursors

These reactions involve, on one hand, the nucleophilic A-rings of flavonoids such as flavanols, and on the other hand, electrophilic quinones arising from enzymatic or chemical oxidation of phenolic compounds.

Reaction Mechanism

Quinones are electrophilic species which can thus add various nucleophiles, including flavonoids. They are also powerful oxidants which can oxidize *o*-diphenolic compounds such as *o*-diphenolic flavonoids to secondary quinones as described above. Addition of catechin to its quinone generated by enzymatic oxidation with polyphenoloxidase (Guyot et al. 1996b) or peroxidase (Weinges and Muller 1972) and by autoxidation (Hathway and Seakins 1957; Young et al. 1987) yield B-type dehydrodicatechins in which the catechin moieties are linked through a biphenyl bond between the A-ring of one (initially acting as the nucleophile) and the B-ring of the other (initially present as the quinone). These colorless species further oxidize to yellow A-type dehydrodicatechins. Additional dehydrodicatechins linked through biphenylether bonds and thus presumably resulting from radical coupling of two catechin semiquinones were also formed after enzymatic oxidation, especially at lower pH values which stabilise the semiquinones radicals (Guyot et al. 1996b). When other oxidizable phenolics such as phenolic acids are present, formation of codimers is also observed (Fulcrand et al. 2006; Richard-Forget et al. 1992). Depending on the relative redox potentials of the phenol/quinone couples, the flavanol can act as the nucleophile or as the electrophile in these reactions. In particular, in the presence of malvidin 3-glucoside which cannot be oxidized to an *o*-quinone, oxidation products were formed by addition of the anthocyanin onto the catechin quinones (Duenas et al. 2006a).

Evidence in Wine

To our knowledge, no oxidation product of flavanols or flavonols has been yet detected in wine, the only oxidation products evidenced so far being Grape Reaction Product (GRP, i.e. 2, *S*-glutathionyl caftaric acid) and anthocyanin caftaric acid adducts arising from addition of glutathione and of anthocyanins, respectively, onto caftaric acid quinone. However, the role of flavanols in non-enzymic browning of white wines is well documented (Simpson 1982; Cheynier et al. 1989b). Whether this results from autoxidation reactions, from formation of xanthylium salts through condensation reactions with aldehydes, or from other unknown processes remains to be established.

Factors Affecting the Reaction

Enzymatic reactions occur early in the wine making process unless laccase is present as a result of grape fungal contamination. They depend primarily on the available oxygen, on the presence of reductants such as ascorbic and sulfites, and on the ratio of glutathione to caftaric acid. Caftaric acid quinone is the major product of enzymatic oxidation in grape musts. Reductants reduce the quinone back to caftaric acid while glutathione traps it as GRP. When both are depleted, the excess quinones, if any, react with flavonoids through coupled oxidation or nucleophilic addition

reactions. Indeed, aeration of musts induced losses of flavanols and compensated for their increased extraction following skin contact in white wine making (Cheynier et al. 1989b).

Flavonoid autoxidation in wine is a slow process but its rate increases with pH. For instance, products arising from addition of malvidin 3-glucoside onto epicatechin quinone were observed only at pH 4 and above (Duenas et al. 2006a). Oxidation of flavanols and formation of B-type and A-type dehydrodicatechins was observed in the wine pH range (Oszmianski et al. 1996). When catalysts such as metal ions are present, oxidation of tartaric acid and subsequent formation of xanthylium pigments compete with autoxidation reactions.

9B.3.3.4 Reactions with Other Electrophiles

Precursors

Other electrophiles include anthocyanin flavylium cations. The intermediate cation generated, in mildly acidic conditions, from vescalagin, an ellagitannin present in wines after barrel aging or addition of oak chips and tannin extracts, has also been shown to participate to such reactions (Quideau et al. 2003).

Reaction Mechanism

Again this involves nucleophilic substitution of a flavonoid at its nucleophilic C8 or C6 centre on an electrophilic center, which may be the C4 of an anthocyanin flavylium or the carbocation generated by protonation and dehydration of vescalagin.

As described in Chapter 9A, nucleophilic addition of the flavanol onto the C4 position of the flavylium ion generates an anthocyanin flavanol (A-F) flavene adduct which can either oxidize to the flavylium pigment (Jurd 1967, 1969) or rearrange to another colorless structure in which the anthocyanin and flavanol units are linked through an additional 2-O-7 ether bond as found in A-type proanthocyanidins (Jurd and Waiss 1965; Bishop and Nagel 1984; Remy-Tanneau et al. 2003). Further reactions of the flavylium salt have also been reported to yield yellow xanthylium salts (Jurd 1967, 1969; Jurd and Somers 1970; Somers 1971; Timberlake and Bridle 1976; Baranowski and Nagel 1983; Liao et al. 1992; Santos-Buelga et al. 1995). However, the postulated structures have never been confirmed. Detection of other xanthylium salts resulting from degradation of the intermediate flavylium adduct suggests that the latter is rather unstable (Duenas et al. 2006a).

Addition of catechin or epicatechin onto vescalagin yield complex tannin structures called acutissimin and epiacutissimin (Quideau et al. 2003) that have been isolated earlier from *Quercus acutissima.* It is worth noting that the isomer of vescalagin (i.e. castalagin) fails to undergo this reaction.

Evidence in Wine

A-type bound anthocyanin-flavanols were detected in wine as dimers and as larger oligomers (anthocyanin-(epi)cat$_n$, with n > 1) (Remy et al. 2000; Salas et al. 2005). The presence of such oligomers (n = 1 through 7) was confirmed by mass spectrometry (Hayasaka and Kennedy 2003).

Factors Affecting the Reaction

Nucleophilic addition of flavanols onto malvidin 3-glucoside was observed in a wide range of pH values (2–6). However, in the case of proanthocyanidins, flavanol-anthocyanin adducts resulting from acid catalysed cleavage of the interflavanic bonds were the predominant species formed at pH 2. The intermediate A-F flavene proceeded to different products according to the pH value and substrates. At pH 2, in the case of epicatechin it yielded the A-type A-F dimer while at pH 3 and above, it oxidized to the flavylium (Duenas et al. 2006a). Such flavylium adducts formed from proanthocyanidins appeared rather stable (Malien-Aubert et al. 2002; Salas et al. 2003). That formed from epicatechin could not be detected but was converted to xanthylium salts through a mechanism involving opening and cleavage of the anthocyanin C-ring (Duenas et al. 2006a).

9B.4 Interactions with Other Grape and Wine Constituents

Flavonoids are prone to interact between them and with other wine constituents such as proteins or polysaccharides. Associations of anthocyanins (i.e. self association and copigmentation) modify tint and enhance the intensity and stability of colour. Aggregation of flavanols as well as their interactions with proteins is responsible for haze development in wines and other beverages such as beer. Moreover, astringency perception results from their interaction with salivary proteins, as detailed in Chapter 9B. Interactions of flavonoids with proteins or polysaccharides lead to colloidal phenomena that may impede the efficiency of clarification and stabilization treatments but are also taken advantage of in processes such as fining and addition of protecting colloids. In addition, various technological problems are related to adsorption of flavonoids on surfaces. In particular, adsorption on plant cell walls limits extraction of flavonoids into the must and wine while adsorption on tank surfaces and filtration membranes result in difficult cleaning and/or clogging of the equipment.

9B.4.1 Interaction Processes

Interactions involving flavonoids are based on several phenomena, all deriving from electrostatic interactions:

- hydrogen bonding, which is a non covalent bond arising mainly from permanent dipole – permanent dipole interactions (a particular type of Keesom interactions).
- london interactions, also called dispersion forces, occurring between two induced dipoles. These interactions are further strengthened by the partial desolvation of the hydrophobic surfaces coming into contact and release of some water molecules from the solvation shell into the bulk phase (hydrophobic effect).

These forces are rather weak when compared to covalent binding, but their range of action is larger.

In addition to these non-covalent and reversible bonds, covalent binding through nucleophilic addition or radical coupling can occur, in particular as a result of oxidation reactions. Copigmentation has thus been proposed to be the first step leading to formation of anthocyanin-flavanol adducts in red wine (Brouillard and Dangles 1994). Besides, quinones arising from oxidation of phenolic compounds can suffer nucleophilic addition from the SH or NH2 groups of proteins (Pierpoint 1969).

9B.4.2 Flavonoid Interactions

9B.4.2.1 Copigmentation

Among flavonoid interaction processes, copigmentation has been extensively studied; for a review see Brouillard and Dangles (1993).

Actors

It involves, on one hand an anthocyanin under its flavylium or quinonoidal base form, and on the other hand another planar hydrophobic structure that can be another anthocyanin unit (self-association), or another colorless species (copigment), covalently bound (intramolecular copigmentation) or not (intermolecular copigmentation) to the anthocyanin pigment.

Interaction Mechanisms

Copigmentation is driven by hydrophobic vertical stacking between the anthocyanin and the copigment to form π-π complexes from which water is excluded. The flavylium cation as well as the quinonoidal base are planar hydrophobic structures and can be involved in such complexes whereas the hemiketal form cannot. The association thus results in displacement of the anthocyanin hydration equilibrium from the colorless hemiketal to the red flavylium form that can be easily measured by spectrophotometry.

Evidence in Wine

Copigmentation in wine is estimated from the effect of dilution on the absorbance values in the visible range, at a given pH and ethanol content (Boulton R 2001). Indeed, decreases in the absorbance values (reflecting the concentration of flavylium ions) higher than the dilution factor are attributed to disruption of the copigmentation complexes. On this basis, copigmentation has been reported to contribute 30–50% of the color of young red wines.

Factors Affecting the Interaction

Copigmentation, involving a shift from the colorless hemiketal to the flavylium, is especially important in mildly acidic conditions such as the wine pH range where hemiketal forms are prevalent. Association constants determined by spectrophotometry (89M-1; for epicatechin (Malien-Aubert et al. 2002), 101M-1 for catechin (Mirabel et al. 1999b) indicate that flavanol monomers are poor copigments of malvidin 3-glucoside compared to flavonols ($>10^3$/M; Malien-Aubert et al. 2002). Association constants could not be accurately determined in the case of proanthocyanidins which were not available as pure compounds, but appeared even lower (4/M for a fraction of procyanidin oligomers (Mirabel et al. 1999b).

Surprisingly, copigmentation was enhanced in the presence of 12% ethanol (Mirabel et al. 1999b), reaching 157/M for epicatechin and 68/M for the procyanidin fraction.

9B.4.2.2 Self Association and Aggregation

Flavanol self-association has been demonstrated by means of NMR (Dufour and Bayonove 1999; Mirabel et al. 1999a) and mass spectrometry (Sarni-Manchado and Cheynier 2002) and their aggregation has been studied by means of dynamic light scattering (Poncet-Legrand et al. 2003; Riou et al. 2002; Saucier et al. 1997a) and cryo-transmission electron microscopy (Poncet-Legrand et al. 2003).

Actors

Self association of catechin and epicatechin was revealed by NMR (Dufour and Bayonove 1999) but did not lead to aggregation (Poncet-Legrand et al. 2003). In contrast, epicatechin gallate and, to a lesser extent, epigallocatechin gallate (Poncet-Legrand et al. 2003), as well as procyanidins (Riou et al. 2002) and methyl-methine linked catechin oligomers resulting from acetaldehyde condensation (Saucier et al. 1997a) aggregate into metastable colloidal particles in wine-like ethanolic solutions. Aggregation and precipitation of flavonols is restricted to aglycones which exhibit lower solubility than their glycosides.

Interaction Mechanisms

Van der Waals interactions between similar entities in a polar solvant are attractive. The formation of hydrogen bonds between the solvant and the solute ensures its solubility. As flavanol aggregates are not charged and ionic interactions are not significantly involved, the large incidence of ionic strength indicates that hydrophobic effect is the major driving force (Poncet-Legrand et al. 2003).

Wine often exhibits turbidity due to the presence of micro-organisms, cell debris, potassium hydrogen tartrate crystals and other insoluble material. Flavonol aglycones have been shown to be responsible for the formation of haze and deposits in white wines (Somers and Ziemelis 1985). In red wines, the presence of colloidal size-range particles was shown by light scattering experiments after centrifugation (Vernhet et al. 2003). Phenolic compounds and especially proanthocyanidins are involved in the formation of protein haze (Waters et al. 1995) and are major components of precipitates and aggregates adsorbed on tank material (Vernhet et al. 1999a, 1999b) or filtration membranes (Vernhet and Moutounet 2002). However, these particles also contain other material such as proteins, polysaccharides or potassium hydrogen tartrate so that self-aggregation of phenolic compounds in wine and its role in the aggregation processes cannot be easily determined.

Factors Affecting the Interaction

The structure of the molecule itself affects the interaction mechanisms. In addition to the molecular formula, external parameters such as flavonoid concentration and medium composition play an important part. Self-aggregation was observed with galloylated monomers and proanthocyanidin fractions, but not with catechin or epicatechin. Thus, flavanol aggregation seems to require the presence of at least three phenolic rings (or two *o*-diphenolic rings) in the molecule as this enables it to establish bridges with other polyphenols (Baxter et al. 1997a). Aggregation of procyanidins first increased with mDP up to 5 for non-galloylated procyanidin fractions and to DP 10 for galloylated procyanidins from grape seeds and then decreased for larger polymers, suggesting that higher molecular weight procyanidins can adopt a conformation that increases their solubility. The gallic acid ring favours self-association, as evidenced by NMR (Baxter et al. 1997a), but this was not confirmed in the case of oligomeric fractions. Scattered intensity, aggregate size and polydispersity indexes increased with the flavanol concentration. Size and polydispersity indexes also increased with ionic strength and decreased when the ethanol content was raised. No aggregation was observed at 20% ethanol for any of the fractions up to 5g/L (Poncet-Legrand et al. 2003). Self association constants recorded for epicatechin were also five times weaker in 10% ethanol (Dufour and Bayonove 1999) than in water (Baxter et al. 1997a), which is in agreement with the proposed hydrophobic interaction mechanism.

9B.4.3 Flavonoid Interactions with Other Macromolecules

9B.4.3.1 Interactions with Proteins

Interactions between tannins and proteins have been extensively studied (Hagerman 1989; Haslam and Lilley 1988; Haslam et al. 1992), owing to their role in haze formation, astringency perception, and nutritional and anti-nutritional effects resulting from inhibition of various enzymes and reduction of dietary protein digestion. Other effects include reduced adsorption of β-casein at the air-liquid interface in the presence of epigallocatechin gallate with potential consequences on foam properties (Sausse et al. 2003).

Actors

Flavonoid protein complexation shows little specificity. However, lower molecular weight flavonoids (i.e. flavonols, non galloylated flavanol monomers) display moderate affinity for proteins and do not form aggregates. Similarly, although all proteins interact with tannins, proline rich structures such as encountered in proteins most commonly used as fining agents (e.g. gelatin, casein) or in salivary proline rich proteins (PRP) involved in astringency perception, are particularly prone to interact with tannins. Binding of flavonols and flavones to some proteins such as serumalbumine which is involved in their transport in plasma is well documented (Boulton et al. 1998; Dufour and Dangles 2005). Flavonols have also been shown to adsorb on polyvinylpolypyrrolidone (PVPP) (Laborde et al. 2006) but they have not been reported to interact with wine proteins.

Interaction Mechanisms

Interactions between flavonoids and proteins rely upon both Van der Waals-London interactions and hydrogen bonding (Oh et al. 1980; Luck et al. 1994; Murray et al. 1994; Charlton et al. 1996). A study performed by using NMR indicated stacking of the phenolic rings with the proline residues in proline sequences and stabilisation of the complexes through hydrogen bonding between the H acceptor site of the adjacent peptide bond and the hydrogen atom of the phenolic hydroxyl (Murray et al. 1994). More recently, an isothermal titration calorimetry (ITC) experiment showed that the interaction of flavanols with poly-L-proline involves both entropic (associated to hydrophobic effect and conformational changes) and enthalpic (attributed to hydrogen bonding) phenomena (Poncet-Legrand et al. 2007). The latter is prevalent in the case of flavanol monomers and the former in that of polymers. Interaction does not necessarily lead to precipitation. Flavonoids can form soluble complexes with peptides and proteins, as shown by NMR (Baxter et al. 1997b; Charlton et al. 2002b; Hemingway et al. 1999), mass spectrometry (Sarni-Manchado and Cheynier 2002) or fluorimetry (Dufour and Dangles 2005). Aggregation of flavanols with casein (Jobstl et al. 2006), poly-l-proline

(Poncet-Legrand et al. 2006) and human salivary PRP (Pascal et al. 2007) occurs in three stages:

- ligand binding and saturation of binding sites together with folding of the protein, in the case of intrinsically unstructured proteins such as salivary PRP (Pascal et al. 2007) or casein (Jobstl et al. 2006)
- formation of rather small and homogenous protein-flavonoid aggregates
- further aggregation and precipitation

Adsorption of flavonols on PVPP also involves Van der Waals interactions with associated hydrophobic effect and hydrogen bonding (Laborde et al. 2006).

Evidence in Wine

Protein haze due to interactions of flavanols with proteins and peptides is well documented in beer (Outtrup 1989) but also takes place in wine. Pathogenesis related proteins which are synthesized by vine following pathogen attacks seem to be particularly involved in these processes (Waters et al. 1996). Protein precipitation also occurs as a result of fining treatments that consist in adding exogenous proteins to precipitate tannins out in order to stabilise the wine and reduce its astringency. The proteins most commonly used for red wine fining are gelatins, albumins and caseins. Plant proteins from lupine or wheat have recently been tested as alternatives to gelatin (Maury et al. 2003). All of them, as well as salivary proteins (Sarni-Manchado and Cheynier 2002) selectively precipitate higher molecular weight flavanols (Ricardo da Silva et al. 1991b; Maury et al. 2001; Sarni-Manchado et al. 1999), which also exhibit higher astringency (Vidal et al. 2003). However, only a small proportion of flavonoids was recovered in the pellet and the wine composition was not significantly modified by the treatment (Maury et al. 2003). The loss of astringency observed after fining may thus be partly due to the inclusion of flavanols in soluble complexes.

Factors Affecting the Interaction

Interactions and formation of insoluble complexes with proteins increase with the number of phenolic rings, and especially of *o*-diphenolic rings and thus with polymerisation and galloylation (Haslam and Lilley 1988; McManus et al. 1985). The intensity of mass spectrometry signals corresponding to soluble peptide flavanol complexes increased from the monomers to the dimers and with galloylation (Sarni-Manchado and Cheynier 2002). ITC experiments failed to detect any interaction of poly-L-proline with catechin or epicatechin while association constants of 3.7×10^4/M and 8.1×10^4/M were determined for epigallocatechin gallate and epicatechin gallate, respectively. That of an oligomeric procyanidin fraction from grape seeds was even higher (3.4×10^5/M) confirming that the affinity of flavanols for proteins increases with their chain length. Furthermore, interaction of

flavanol monomers with proteins follows the same order as their partition coefficients between octanol and water, meaning that it increases with hydrophobicity of the phenolic compound (Poncet-Legrand et al. 2007). Phenolic oxidation, generating polymeric species, resulted in enhanced protein interactions as evidenced by higher inhibition of enzymes (Guyot et al. 1996a), or changes in casein adsorption properties at the air/liquid interface (Sausse et al. 2003). As mentioned above, higher molecular weight flavanols are also selectively precipitated out by proteins. Moreover, within gelatins (Maury et al. 2001) or glutens (Maury et al. 2003), smaller molecular weight proteins appeared more selective than larger ones. The interaction mechanism also depends on protein concentration. At low concentration, it occurs in three stages as the polyphenol/protein ratio increases, as described above: saturation of the interaction sites, formation of metastable colloids, and aggregation leading to haze. At high protein concentration, direct bridging occurs, resulting in lower aggregation and turbidity thresholds. Interactions of flavonols with proteins (Dufour and Dangles 2005) as well as their adsorption on PVPP is much more efficient with aglycones as the sugar residue on the glycosides weakens the driving forces (Laborde et al. 2006). Finally, other parameters such as the solvent characteristics, the presence of other solutes and the temperature influence protein/flavanol association and the properties of resulting complexes. Thus the affinity between tannins and PRPs is lower at higher temperatures (Charlton et al. 2002a). The presence of polysaccharides prevents coprecipitation of tannins and proteins (Luck et al. 1994; Cheynier et al. 2006). Ionic strength and pH affect proteins solubility. Precipitation of tannin protein complexes is highest at the protein pHi as electrostatic repulsions are minimal (Calderon et al. 1968; Perez-Maldonado et al. 1995; Charlton et al. 2002a; Kawamoto and Nakatsubo 1997). The effect of ionic strength and ethanol content on the interactions of epigallocatechin gallate with a salivary PRP was investigated (Pascal et al. 2006). Increasing the ionic strength with sodium chloride or tartrate ions resulted in an increased stability of the aggregates, meaning that aggregation was not driven by repulsive electrostatic interactions. In 12% ethanol, the protein was not fully dissolved and aggregation with epigallocatechin gallate required much higher concentrations of the latter, confirming the role of hydrophobic interactions.

9B.4.3.2 Interactions with Polysaccharides

Actors

Major wine polysaccharides, including mannoproteins originating from yeasts and plant cell wall constituents (e.g. arabinogalactan proteins (AGP) and rhamnogalacturonan II (RGII)), have been shown to interact with flavanols (Riou et al. 2002). Besides, arabic gum (a mixture of arabinogalactans and arabinogalactan proteins) can be added as a protecting colloid to limit or prevent aggregation, flocculation and precipitation of tannins and tannin-protein complexes (Pellerin and Cabanis 1998).

However, at higher doses, it can lead to instability and haze development (Saucier et al. 1996; Siebert et al. 1996).

Interaction Mechanisms

Light scattering studies have shown that interactions of mannoproteins and of some arabinogalactan proteins with procyanidins prevent aggregation of the latter and result in small and stable particles. Adding other polysaccharides such as RGII monomer had no effect while RGII dimer increased aggregation and led to precipitation (Riou et al. 2002). The efficiency of mannoproteins as particle stabilizers decreased as their molecular weight increased, suggesting that the mechanism involved is steric stabilisation (Poncet-Legrand et al. 2007). The stabilising effect increased with ionic strength, ruling out an electrostatic stabilisation mechanism. Polysaccharides were also shown to limit precipitation of tannin protein complexes (Cheynier et al. 2006; Haslam et al. 1992; Luck et al. 1994). This was attributed to formation of soluble ternary protein-polysaccharide-flavonoid complexes, again mediated by hydrogen bonding and hydrophobic effect (McManus et al. 1985).

Evidence in Wine

Among wine polysaccharides, mannoproteins play an important role in protein haze stabilisation (Waters et al. 1994; Dupin et al. 2000). Gelatin fining of a wine phenolic extract in wine-like solution resulted in a much higher precipitation rate than when the same treatment was applied on the original wine. After addition of wine polysaccharides at the concentration normally encountered in wines, precipitation was reduced back to the level measured in wine, confirming the stabilizing effect of polysaccharides (Cheynier et al. 2006).

Factors Affecting the Interaction

The size and conformation of both the polyphenol and the polysaccharide are important. The presence of hydrophobic cavities such as encountered in cyclodextrins favours the interactions with phenolic molecules of appropriate shape or mobility (Smith et al. 1994). Polyphenols bind to dextran gels such as Sephadex used in chromatography, with affinity increasing with their molecular weight (Lea and Timberlake 1974), but do not interact with dextran oligomers (Williamson et al. 1995). The effect of polysaccharides also depends on the medium composition. Thus the effect of mannoproteins on procyanidin aggregation was stronger at lower ethanol concentration, i.e. under conditions in which procyanidins were in poor solvent and polysaccharides in good solvent. At high ionic strength, all mannoprotein fractions, including those of higher molecular weight, efficiently stabilized polyphenols.

9B.4.4 Flavonoid Adsorption on Solid Material

9B.4.4.1 Adsorption on Plant and Yeast Cell Walls

Actors

Plant and yeast cell walls consist mostly of polysaccharides along with smaller amounts of proteic material. Adsorption of flavanols on isolated plant cell walls (Renard et al. 2001) and on yeast lees (Mazauric and Salmon 2005, 2006) has been demonstrated. The latter also retained anthocyanins, as stated in Chapter 9A.

Mechanisms

Adsorption of proanthocyanidins on cell walls is driven by low energy bonds as described above for polysaccharides. The apparent affinity constants were higher with polysaccharides showing hydrophobic domains such as cross-linked pectins and xyloglucans than for cellulose (Le Bourvellec et al. 2005). Adsorption increased greatly with concentration, suggesting that stacking of polyphenols takes place once they are bound with the cell wall material (Renard et al. 2001). Cooperative mechanisms were also postulated to explain why lower molecular weight procyanidins were more easily desorbed (Renard et al. 2001).

Evidence in Wine

Adsorption of flavonoids on plant and yeast cell walls has been reported to contribute to poor retention of flavanols in wine (Sun et al. 1999) as well as losses of colouring matter (Vasserot et al. 1997). However, all these studies have been performed using model systems which might not reproduce the complexity of real wines. Moreover, adsorption on solid plant material may also impede extraction into the wine. In red wine-making, large proportions of flavonoids and especially of proanthocyanidins are not retained in the wine but recovered in the pomace after pressing (Morel-Salmi et al. 2006). Various technologies, including physical treatments such as thermovinification, must freezing or flash release and treatment with pectolytic enzymes have become of common practice in red wine making. They are reported to enhance the release of phenolic compounds into the must, as a result of cell or vacuole membrane damage or enhanced desorption of polyphenol from the cell wall material. Higher extraction rates of all phenolics were observed after flash release or thermotreatment (Morel-Salmi et al. 2006). The efficiency of pectolytic enzymes appears more variable: the amount of total phenolic compounds, and especially of derived pigments in red wines was enhanced in some cases (Pardo et al. 1999; Bautista-Ortin et al. 2005) and not modified in others (Bautista-Ortin et al. 2005; Doco et al. 2007). More recent studies have shown that the increase in colour is associated to enhanced proanthocyanidin content and higher conversion rate of anthocyanins to derived pigments (Ducasse et al. 2007).

Factors Affecting the Interaction

Phenolic acids and flavanol monomers do not absorb on plant cell walls while adsorption of procyanidins increases with their degree of polymerisation (Renard et al. 2001). Selective adsorption of molecules showing higher proportions of galloylated units and of catechin was also demonstrated (Le Bourvellec et al. 2004). Similarly, higher molecular weight proanthocyanidins were preferentially adsorbed on yeast lees (Mazauric and Salmon 2006). Adsorption of procyanidins on plant cell walls was not affected by pH. It increased with ionic strength and decreased as the temperature increased and in the presence of ethanol (Le Bourvellec et al. 2004), like procyanidin self-aggregation (Poncet-Legrand et al. 2003). Partial desorption from isolated plant cell wall material could be achieved by rinsing with buffer and was more efficient in the presence of dissolved polysaccharides (Renard et al. 2001). In contrast, desorption from yeast lees was extremely difficult, indicating stronger adsorption (Mazauric and Salmon 2006). This is possibly due to the presence of larger amounts of non polysaccharidic components such as proteins. Indeed proanthocyanidins were also show to be difficult to release from the pellets recovered after protein fining (Maury et al. 2003).

9B.4.4.2 Adsorption on Winery Equipment

Actors

Formation of tartrate crystals is a major source of instability in wines and treatments such as cold stabilisation, electrodialysis and ion-exchange are usually performed to prevent it from occurring in the bottled wine (Vernhet et al. 1999a). Polysaccharides and polyphenols, as well as yeast cells, were shown to be associated to tartrate crystals in the deposits formed after cold stabilisation both in white wines (Vernhet et al. 1999a) and in red wines (Vernhet et al. 1999b). Wine polysaccharides and polyphenols are also involved in the fouling of organic membranes during microfiltration of red wine (Vernhet and Moutounet 2002).

Mechanisms

Yeast cells represented at least 20% of the tartrate deposits in red wines. Scanning electron microscopy observations revealed that they adhere first on the stainless steel and suggest that they act as primary nucleation germs for crystallization (Vernhet and Moutounet 2002). The differences in shapes and composition of crystals formed in white and red wines may be attributed to higher adsorption of organic material in the latter (Vernhet and Moutounet 2002) as this is known to block the crystal growth (Rodriguez-Clemente and Correa-Gorospe 1988). Arabinogalactan protein and mannoproteins are the major polysaccharides in crystals and seem to take part in the reduced growth rate (Vernhet and Moutounet 2002). Since they had almost no effect on tartrate crystallization in model ethanolic solutions (Gerbaud and Gabas 1997), this may be related to their interaction with procyanidins (Riou et al. 2002).

The mechanisms of microfiltration membrane fouling were investigated in wine and model solutions (Vernhet and Moutounet 2002). The sharp decline observed in microfiltration fluxes within the first minutes of the process could not be attributed to adsorption alone. They can be explained by a two step mechanisms involving first interaction of the wine constituents with the membrane, quickly followed by their aggregation at the pore entrance (Vernhet and Moutounet 2002).

Factors Affecting the Interaction

Crystal appearance and growth are slower in red wines than in white wines and also differ within red wines. Arabinogalactan-proteins and mannoproteins were the major polysaccharides in the precipitates while rhamnogalaturonan II could not be detected. The average degree of polymerisation of proanthocyanidins in the deposit was higher that that of wine proanthocyanidins, indicating that polymers were selectively associated with the tartrate crystals. A preferential association of apolar flavonols was similarly observed, presumably as their lower solubility favours adsorption on surfaces.

Polyphenol adsorption under static conditions increased with the polarity of the membrane and the ability of its surface to act as hydrogen acceptor in hydrogen bonding which strengthens the interaction (Vernhet and Moutounet 2002). Polysaccharide adsorption was negligible in static conditions and decreased as the polarity of the membrane surface increased (Vernhet et al. 1997). However, polysaccharides played a major role in the fouling process, presumably due to the formation of colloidal aggregates with procyanidins. Indeed, fouling appeared largely determined by the pore size distribution of the membranes (Vernhet and Moutounet 2002). Moreover, the performance of the membranes and of back-pulsing operations in restoring the flux is related to the ratio of fine (colloidal) particles to large particles (e.g. yeast cells) as the former can penetrate into the membrane pores and produce irreversible internal fouling while the latter develop external fouling which is mostly reversible (Boissier et al. 2008; Vernhet et al. 2003).

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