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Structure of a new dimeric acetaldehyde malvidin 3-glucoside condensation product

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Abstract—A new pigment was detected in wine-like model solution containing malvidin 3-*O*-glucoside and acetaldehyde. This compound was isolated, and its structure was investigated by UV, MS and NMR spectroscopies. The analytical data allowed identification of it to 8,8 methyl methine-linked malvidin 3-*O*-glucoside dimer. The occurrence of this pigment was demonstrated in red wine. A polymeric fraction was also isolated from the model solution and analysed by LC/MS. Detection of methyl methine-linked malvidin 3-*O*-glucoside oligomers proved that acetaldehyde mediated self-condensation of anthocyanins is possible, and that the C6 position of anthocyanins seems reactive as the C8 although to a lesser extent.

Red wine is a very complex medium, which evolves during its conservation and ageing. With storage time, the colour of young red wine changes from red-bluish, towards the reddish-brown tint of matured wine and the astringency decreases.[1] Reactions of phenolic compounds, particularly anthocyanins and condensed tannins, play a major role in organoleptic changes of wines.[2] Various mechanisms involving acetaldehyde or not were proposed to explain these transformations.[2–5] The most well-known reaction corresponds to acetaldehyde-mediated condensation between either flavanols only or anthocyanins and flavanols. The latter yields purple pigments with characteristic UV–visible spectra showing two maxima at 280 and 540 nm and a shoulder at 450 nm.

Acetaldehyde is a natural compound occurring in wines, produced either by yeast metabolism during fermentation[6] or by ethanol oxidation in the presence of phenolic compounds.[7] Actually, micro-oxygenation of wine was proven to favour acetaldehyde production and subsequent reactions involving acetaldehyde among which the formation of methyl methine-linked compounds, usually referred to as ethyl-linked compounds was shown to be prominent in the first months.[8]

In this connection, reactions between malvidin 3-*O*-glucoside and acetaldehyde were studied in a wine-like model solution at pH 3.2. A new pigment with two absorbance maxima in the visible range, at 450 and 528 nm was detected by LC/DAD analysis after 1 week of storage (Fig. 1).

Mass spectrometry data obtained by LC-ESI MS in the positive ion mode showed a major mass signal at m/z 1029, and two other signals at m/z 1011 and 506. On the basis of the UV–visible spectrum and the mass spectrometry data, the mass signals were attributed to the malvidin 3-*O*-glucoside-ethyl-malvidin 3-*O*-glucoside under different forms. Anthocyanins exist under several forms in equilibrium, the two major ones at wine pH value being the red flavylium cation (20–25%) and the colourless hydrated hemiketal form (75–80%). Minor forms include quinoidal bases which are only prevalent at higher pH values.

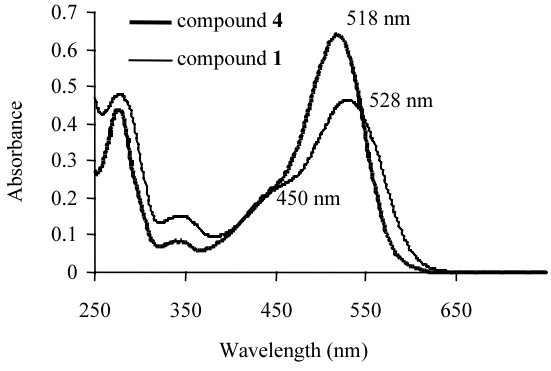

Figure 1. UV–visible spectra of compounds **1** and **4**.

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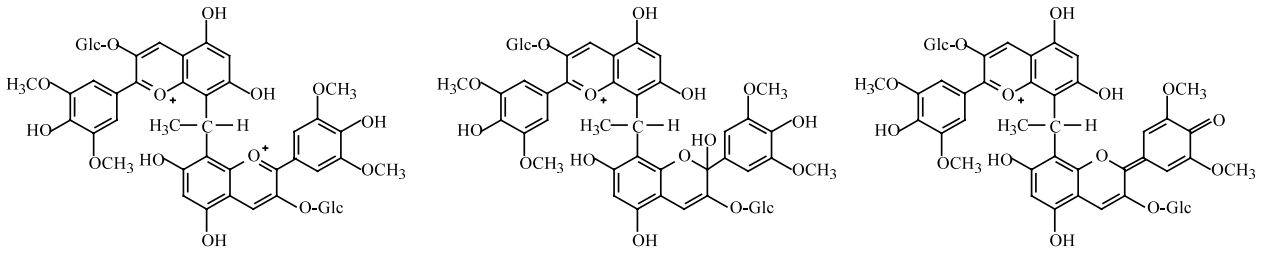

Figure 2. Structure of methyl methine-linked malvidin 3-*O*-glucoside dimer under different forms.

The ion peak at 1029 corresponds to one hydrated form and one cationic moiety in the dimeric pigment (Fig. 2, **2**). The mass signal at 1011 corresponds to the other form of the same pigment containing one neutral quinoidal base and one flavylium cation (Fig. 2, **3**), and that at 506 to the di-flavylium cation detected as a doubly charged ion (Fig. 2, **1**).

In order to confirm this hypothesis and to determine the position of the ethyl linkage, the compound was isolated by HPLC at the semi-preparative scale, and investigated by NMR spectroscopy.

1D and 2D NMR experiments were performed for both the dimer and malvidin 3-*O*-glucoside in DMSO-d_6/TFA (9:1) to ensure that they were only under (di) flavylium forms in the solutions (Fig. 2, **1**, Fig. 3, **4**). ^{1}H and ^{13}C chemical shifts are shown in Table 1. Chemical shifts obtained for malvidin 3-*O*-glucoside are consistent with those already reported.[9,10] Compared with malvidin 3-*O*-glucoside, ^{1}H NMR spectrum of the dimeric compound presented two additional peaks, a doublet at 2.09 ppm and a quadruplet at 5.55 ppm which were attributed to methyl and methine groups of the ethyl-bridge, respectively. In contrast, only one peak at 6.79 ppm corresponding to A ring protons was observed. The relative intensity of two protons of this resonance is consistent with an ethyl linkage between the two malvidin moieties implying the positions C6 and/or C8. However, the existence of only one set of proton signals corresponding to one malvidin spin system indicated a symmetry in the dimeric structure, although it still contains chiral centres in the glucosyl residues. Consequently, the dimer can be either the C8-ethyl-C8 or the C6-ethyl-C6 regio-isomer. The other protons of the malvidin moieties were easily attributed from the ^{1}H spectrum. The signal at 8.03 ppm with a relative intensity of four protons could be readily assigned to the equivalent protons H2′ and H6′ of the B rings. The signals of methoxy protons of the B rings were observed at 3.88 ppm whereas the protons H4 are easily identified at 9.04 ppm.

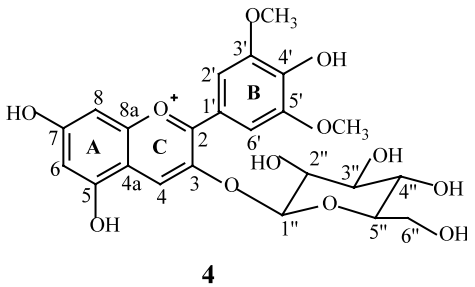

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Figure 3. Numbering of malvidin 3-*O*-glucoside under flavylium form.

The position of the ethyl linkage was elucidated by NOESY experiment. Through-space couplings were observed between B rings protons (H2′, H6′, OCH_3) and the set of protons of the ethyl-linkage. This indicated a spatial proximity between the B rings and the ethyl bridge which is only fulfilled in the case of a C8 linkage. Thus, the signal at 6.79 ppm was attributed to the H6 protons. Assignment of glucoside protons was performed using 2D ^{1}H COSY experiments. After the proton resonances had been assigned, all the corresponding carbons were attributed from short-range HSQC experiment. The attribution of quaternary carbons was easily obtained from a long-range correlation HMBC experiment.

The signals of the anomeric proton H1″ and of H2′, H6′ appear like a triplet and a broad doublet, respectively. In fact, the signal of the anomeric proton is actually composed of two overlapping doublets, belonging to each glucosyl moiety. Concerning the signal of H2′, H6′, it is constituted of two very close singlets, corresponding to the B ring protons (H2′, H6′) of each malvidin 3-*O*-glucoside unit. The protons of B rings and of glucosyl residues are diastereotopes meaning that they are not fully in the same environment and show in slightly different chemical shifts.

Molecular modelling was used to clarify spatial conformation. The results indicated that the compact structure where the B rings are nearly parallel and overlap is not possible. Only extended structures (like in Figs. 2 and 1) showing anti-parallel B rings position are probable.

A polymeric fraction was also isolated from the wine-like model solution after 1 week of storage and analysed by LC/MS and by ESI MS with continuous flow injection in the positive ion mode. Aside from the signals corresponding to the ethyl-linked dimer and the monomer, mass spectrometry data showed mass signals at m/z 1529, 765, 1024 and 1042. The first two mass signals were attributed to ethyl-linked malvidin 3-*O*-glucoside trimer under different forms. The signal m/z 1529 corresponds to a trimer containing one flavylium cation and two quinoidal bases, and m/z 765 is the doubly charged ions of the trimeric form showing two flavylium cations and one quinoidal base. The signals at 1024 and 1042 were

Table 1. ^{1}H (500 MHz) and ^{13}C (125.75 MHz) assignments of compounds **4** and **1** in DMSO/TFA (9:1) at 25°C

Position	Malvidin 3-*O*-glucoside		Methyl methine-linked malvidin 3-*O*-glucoside dimer	
	δ ^{1}H (ppm), m, *J* (Hz)	δ ^{13}C	δ ^{1}H (ppm), m, *J* (Hz)	δ ^{13}C
CH			5.55; q; J 7.40	26.62
CH_3			2.09; d; J 7.42	18.59
2	–	162.48	–	162.15
3	–	145.06	–	144.06
4	8.94; s	136.55	9.04; s	136.42
4a	–	113.26	–	113.24
5	–	158.77	–	156.55
6	6.72; d; *J* 2.02	103.00	6.79; s	102.19
7	–	170.10	–	167.53
8	7.02; d; *J* 1.98	94.89	–	109.62
8a	–	157.13	–	153.97
1′	–	119.23	–	118.87
2′, 6′	7.93; s	109.96	8.02; s; 8.03; s[a]	109.39
3′	–	149.51	–	148.80
4′	–	145.87	–	144.91
5′	–	149.51	–	148.80
OCH_3	3.90; s	56.50	3.88; s	56.00
1″	5.35; d; *J* 7.84	103.25	5.43; d; *J* 8.09; 5.46; d; *J* 8.09[a]	102.64
2″	3.45; m	74.52	3.46; m	73.69
3″	3.40; m	76.82	3.41; m	76.43
4″	3.25; m	69.76	3.23; m	69.89
5″	3.48; m	78.12	3.49; m	77.89
6″ A	3.73; m	61.54	3.72; m	60.91
6″ B	3.52; m	61.54	3.50; m	60.91

[a] H1″ and H2′, H6′ of both malvidin 3-*O*-glucoside units are not fully equivalent, as discussed in text.

attributed to doubly charged ions of tetrameric structures, with two flavylium cations and two quinoidal bases, and two flavylium cations and two hydrated moieties, respectively. These results showed, contrarily to our previous findings,[11] that C6 position of anthocyanins seems reactive as the C8 although to a lesser extent.

This study showed the formation of a new type of pigment involving anthocyanins and acetaldehyde. The occurrence of the dimeric pigment with one hydrated and one cationic moieties, which appeared the most favoured form at wine pH, was demonstrated in red wine. The formation of such compounds in wine-like model solution and in wine indicate their probable contribution to colour change observed during wine ageing. These results finally open perspectives for further investigations. Studies on physical–chemical properties such as hydration kinetics and copigmentation of this dimer are underway.

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