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Castavinol, a New Series of Polyphenols from Bordeaux Red Wines

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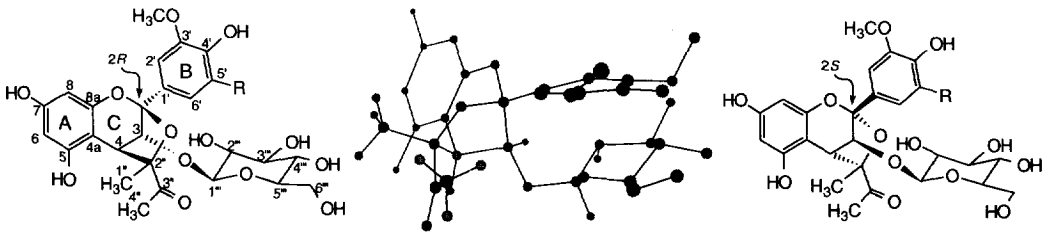
Abstract : A series of polyphenols with a new skeleton, named castavinol, has been isolated from Bordeaux red wines. The structural elucidation of these three natural products was achieved by UV, MS, IR, NMR ^{1}H and ^{13}C spectroscopies. The most probable pathway for their biogenesis and chemical properties related to the colour modification upon ageing of wines are given.

Polyphenols, such as tannins, have been shown to possess important *in vitro* biological properties like radical scavenging[1] or antioxidant[2] but also, these compounds, brought about by red wine, have recently been mentioned to be responsible in the protection against cardiovascular disorders[3] as an explanation to the « French Paradox »[4-6]. In an earlier study[7] we have demonstrated that the genuine concerned components were not only proanthocyanidins and anthocyanins but also polyphenol heterosides with a genine part different from the anthocyanidin. In this letter, we describe the chemical structure analysis and the chemical reactivity of three polyphenol glucosides, isolated from Bordeaux red wines[cépage castavinol, 1989 vintage, Pessac-Léognan Graves made from Cabernet franc and Cabernet-Sauvignon vines. #8490], bearing a new skeleton, named « castavinol » (Figure 1).



1 R = OCH_3
2 R = H
3 R = OH

3D Model of **1** (R = OCH_3)
(only the interesting H's are shown)

1'
2'
3'

Figure 1

Structural elucidation :

As these glucosides differ only by the nature of one substituent of ring B of the flavan 3-ol derived framework, only the structure of the major compound **1** will be thoroughly described[8] here and differences for **2** and **3** pointed out. The ^{1}H NMR spectrum of **1**, showed only the signals of two equivalent (H2' and H6' of the B-ring (δ 7.33 ppm) and two non equivalent (meta coupled) aromatic protons (H6 and H8 of the A-ring, which resonated as broad singlets at δ 6.37 and δ 6.26 ppm and easily exchangeable in presence of D_2O), those of a glucosyl residue in addition to two equivalent arylmethoxyl groups (δ 4.12 ppm) and two isolated methyl singlets (δ 2.68 and 1.48 ppm). The J-modulated ^{13}C NMR spectrum[9] revealed 24 signals accounting for 27 carbon atoms due to 3 overlappings, including a carbonyl of a ketone function at δ 217.1 ppm, confirmed by the infrared

band at 1694 cm^{-1}. This is consistent with the negative FAB-mass spectrum (triethanolamine) quasimolecular ion peak at m/z 579.5 $[M-H]^{-\cdot}$ ($C_{27}H_{32}O_{14}$) and the UV spectrum with a maximum absorption at λ 269 nm. Assignments of the ^{1}H and ^{13}C signals and connectivities between protons and carbons, were made using ^{1}H-^{1}H COSY[10,11], ^{1}H-^{13}C HMQC[12] and HMBC[13] (Table 1).

	^{13}C	^{1}H	COSY	HMBC	*J* or signal width (Hz)
2	109.7				
3	82.0	5.04 *bs*	4.39	103.5, 102.9, 98.6	4.12
4	45.7	4.39 *bs*	5.04	217.1, 155.3, 153.7, 109.7, 103.5, 98.6, 82.0	3.88
4a	103.5				
5	155.3				
6	97.3	6.37 *bs*	6.26	157.9, 155.3, 103.5, 96.1, 45.7	3.51
7	157.9				
8	96.1	6.26 *bs*	6.37	157.9, 153.7, 103.5, 97.3	3.63
8a	153.7				
1'	127.0				
2'/6'	106.2	7.33 *s*		148.4, 136.1, 127.0, 109.7, 106.2, 82.0	
3'/5'	148.4				
4'	136.1				
1''	20.6	1.48 s		217.1, 98.6, 45.7	
2''	98.6				8.5
3''	217.1				
4''	26.1	2.68 *s*		217.1, 98.6	
OMe	57.4	4.12 *s*		148.4, 106.2	
1'''	102.9	4.21 *d*	3.18	82.0, 76.64, 76.56	7.8
2'''	74.0	3.18 *dd*	4.21, 3.40	102.9, 76.64	
3'''	76.64	3.40 *m*	3.49, 3.18	102.9, 74.0, 70.3	
4'''	70.3	3.49 *m*	3.40	76.64, 76.56, 61.6	
5'''	76.56	3.40 *m*	4.05, 3.89, 3.49	102.9, 76.64, 70.3, 61.6	
6'''	61.6	3.89 *dd*	4.05, 3.40	76.56, 70.3	5.5, 12.3
		4.05 *d*	3.89, 3.40		12.3

Table 1 : ^{1}H and ^{13}C NMR data of 5'-OMe-castavinol **1** (D_2O, 500 MHz)

Cross-peaks between sugar protons in the COSY were in favour of a β-*D*-glucopyranose residue, also confirmed by the ^{13}C chemical shifts (see table) and by the signal of the anomeric proton at δ 4.21 ppm with a coupling constant $J_{H1'''-H2'''}$ = 8 Hz. The linkage with the aglycone part is deduced by the ^{3}J correlation observed between proton H1''' and carbon at δ 82 ppm attributed to the C-3 (HMBC), bearing the proton at δ 5.04 ppm (HMQC). The singlet signal at δ 4.12 ppm, integrating for six protons, was characteristic of the two aromatic methoxyl groups of the B-ring in symmetrical positions (3' and 5') separated by an hydroxyl group in position 4'. Clearly, all these signals could be interpreted as resulting of the presence of the gross skeleton of malvidin 3-*O*-glucoside with untouched A and B rings but modified on its C-ring.

Assignments of chemical shifts of carbons C-6 and C-8 could not be made, as previously reported in the proanthocyanidin case[14], unambiguously by a long range correlation between a H-2 proton and the C-8a in the HMBC spectrum because the C-2 carbon atom is quaternary. However, the argument to distinguish proton H-6 from proton H-8 came from a ROESY[15] cross-peak between the proton at δ 6.26 ppm and H-2', H-6' protons of B-ring, that is only possible for H-8. From these proton assignments, chemical shifts at δ 155.3 and δ 153.7 ppm could have been assigned to carbon C-5 and to C-8a, respectively, which both showed a cross-peak with the H-4 proton signal at δ 4.39 ppm. Besides the expected correlations of H-4 with C-3 and C-4a, most important were those with two quaternary carbons which resonated at δ 109.7 (C-2) and δ 98.6 ppm (C-2") and with the

carbonyl group at δ 217.1 ppm (C-3"). These two latter oxygenated carbons along with the two methyl groups (δ 2.68 and 1.48 ppm), correlated to them in the HMBC spectrum, clearly were part of the same "4 carbon atom unit" supplementary to the starting malvidin skeleton.

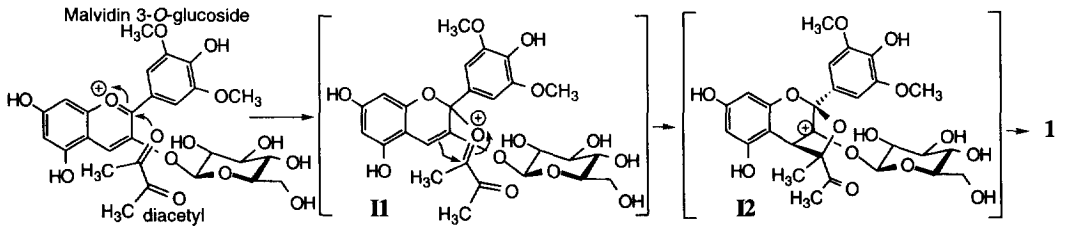


Figure 2 : Proposed biogenesis of castavinol derivatives.

A hypothesis on the biogenesis was helpful to derive the structure : since these compounds were extracted from wines, they could result from a mixed origin. A C_4 yeast metabolite, such as diacetyl, could react through a nucleophilic addition on common grape metabolites like anthocyanins to lead to the new molecules of the castavinol series. Figure 2 shows a likely mechanism that forms a new cationic intermediate **I1** in which the vinylic double bond, no longer conjugated, adds on the electrophilic ketoxonium to form the C-4→C-2" bond. The last step to **1** corresponds to the stabilizing reduction, under enzymatic control from the yeast or by any chemical (SO_2, polyphenols, ...), of the resulting cation on C-3 (**I2**). Two ways have to be envisioned for the carbonyl addition onto the pyrilium ring : either on the top face (Fig. 2) leading to the 2*R* series (**1** to **3**), or on the bottom face leading to the 2*S* series (**1'** to **3'**) that cannot be excluded from the recorded data. Relative configurations of C-3 and C-4 was deduced from the weak COSY cross-peak between H-3 proton signal and signal at δ 4.39 ppm (H-4). These two signals appeared in the proton spectrum as broad singlets typical of a small coupling constant only compatible with the dihedral angle H(3)-C(3)-C(4)-H(4) of 81° measured on the stereoisomer of **1** shown in 3D-model[16] (Fig. 1). The opposite configuration at C-3, would give a dihedral angle of 39° and a larger coupling constant.

Therefore, castavinol **2** and 5'-OH-castavinol **3**, showing the same NMR spectra as 5'-OMe-castavinol **1** but for the B-ring system, would result from the condensation of 2,3-butanedione with peonidin 3-glucoside and with petunidin-3-glucoside, respectively.

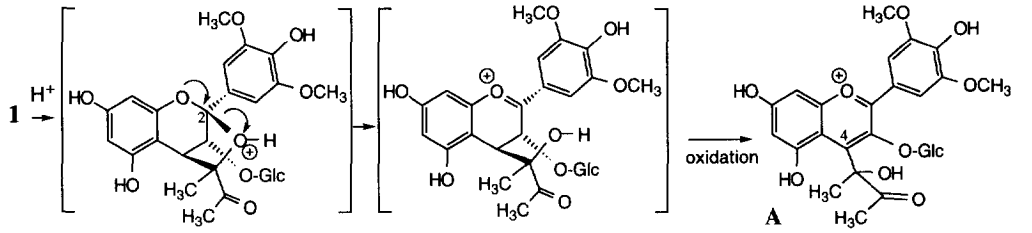


Figure 3 : "Bate-Smith" reaction in the castavinol series.

The presence of two oxygen atoms on C-2 was further proved by a chemical test usually used in the field of proanthocyanidin series, *i.e.* the Bate-Smith reaction. Upon heating a small amount of compound **1** in *n*-BuOH/HCl (4/1), an intense red colouration revealed the formation of the pyrilium salt **A** (Figure 3). This newly formed pigment was compared to the malvidin 3-*O*-glucoside : a bathochromic shift of 10 nm was observed

(λmax =544 nm), due to the C-4 alkyl residue whose linkage was further proved by a quasimolecular ion peak at m/z 580.5 $[M+H]^+$ positive FAB-MS.

Conclusion : A new series of compounds isolated from red wines has been characterized and its potentiality to give a positive Bate-Smith test, demonstrated. Till now, this test was considered as specific of proanthocyanidins, but it must be enlarged to the new family of castavinol derivatives. These compounds are structurally related to the condensation products premonitorily prepared by Jurd[17], with this difference that in our case, they result from a [1,2]-addition on a carbonyl leading to a tetrahydrofuran ring rather than to a tetrahydropyran. The role of these new polyphenols in the colour changes during ageing of red wines and their physiological properties will be published elsewhere.

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References

1. Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Riceevans, C. *Arch. Biochem. Biophys.* **1995**, *322*, 339-346.

2. Kanner, J.; Frankel, E.; Granit, R.; German, B.; Kinsella, J. E. *J. Agr. Food Chem.* **1994**, *42*, 64-69.

3. Fuhrman, B.; Lavy, A.; Aviram, M. *Am. J. Clin. Nutr.* **1995**, *61*, 549-554.

4. Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. *J. Agr. Food Chem.* **1995**, *43*, 890-894.

5. Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. *Lancet* **1993**, *341*, 454-457.

6. Ruf, J. C.; Berger, J. L.; Renaud, S. *Arterioscler. Thromb.Vasc. Biol.* **1995**, *15*, 140-144.

7. Balas, L. *Tanins catéchiques : isolement, hémisynthèse et analyse structurale par RMN 2D homo- et hétéro-nucléaire. Contribution à l'étude des tanins du raisin et des vins de Bordeaux*, PhD Thesis, Univeristé de Bordeaux 2, 1992.

8. Other data for compound **1** = 46° (c = 0.217, H_2O). IR ν cm^{-1} : 3363, 1694, 1681, 1651, 1633, 1614, 1518, 1465, 1455, 1424, 1354, 1207, 1119, 1013, 903, 833, 774. TLC (Si gel F-254, $CHCl_3$:MeOH:HOAc 70:30:3) *Rf* 0.69.

9. Le Coq, C.; Lallemand, J. Y. *J. Chem. Soc., Chem. Commun.* **1981**, *4*, 150-152.

10. Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229-2246.

11. Nagayama, K.; Kumar, A.; Wuthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321-334.

12. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565-569.

13. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.

14. Balas, L.; Vercauteren, J. *Magn Reson Chem* **1994**, *32*, 386-393.

15. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207-213.

16. Molecular modeling and energy minimization calculations were carried out using the "Chem Office" Software package, CSC Inc., Cambridge, Massachusetts, USA.

17. Jurd, L. *Amer. J. Enol. Viticult.* **1969**, *20*, 191-195.

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