

## Dimer and trimer procyanidins in Carignan and Mourvèdre grapes and red wines

by

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### Procyanidines dimères et trimères des raisins et vins rouges de Carignan et Mourvèdre

**Résumé :** Des procyanidines dimères galloylées et non-galloylées, et trimères sont dosées dans deux cépages rouges: Carignan et Mourvèdre. Les procyanidines sont extraites des différentes parties de la grappe puis quantifiées par CLHP en phase inverse. Dans le raisin de Carignan et de Mourvèdre, la procyanidine B<sub>2</sub> est la plus abondante dans les pépins, tandis que la procyanidine B<sub>1</sub> est la plus abondante dans les rafles et pellicules. Les procyanidines trimères sont aussi présentes en quantités importantes. Différents vins rouges ont été élaborés à partir des cépages Carignan et Mourvèdre, en utilisant plusieurs techniques de vinification: vendange foulée; vendange éraflée, macération carbonique et le chauffage de la vendange. Les quantités les plus abondantes de procyanidines sont obtenues dans des vins issus d'une vendange entière et foulée.

**Key words :** procyanidin, polyphenol, analysis, bunch, berry skin, seed, pulp, rachis, red variety of vine, vinification, technology, red wine.

### Introduction

The importance of phenolic components in grapes and wines as constituents and as reactants is well known. Procyanidins are phenolic compounds and involved in quality aspects of wines (TIMBERLAKE and BRIDLE 1976), oxidation reactions (SIMPSON 1982; OSZMIANSKI *et al.* 1985; CHEYNIER *et al.* 1988; LEE and JAWORSKI 1988; CHEYNIER and RICARDO-DA-SILVA 1991), interactions with proteins (OH *et al.* 1985; YOKOTSUKA and SINGLETON 1987; POWERS *et al.* 1988; JOUVE *et al.* 1989; RICARDO-DA-SILVA *et al.* 1991 b), ageing behaviour of wines (HASLAM 1980), and certain physiological effects, namely oxygen-free radicals scavenger capacity (LAPARRA *et al.* 1978; MASQUELIER 1988; RICARDO-DA-SILVA *et al.* 1991 c).

Concerning red grapes, BOURZEIX *et al.* (1986) have studied the procyanidin composition of grape clusters for a group of red vine varieties grown in the South of France. Recently, OSZMIANSKI and LEE (1990) have quantified procyanidin B<sub>3</sub> in two red grapes (Concord and de Chaunac). With the maceration (skin contact) of grapes, procyanidins were extracted from the solid parts and released into musts and wines. BIDAUBAYLE *et al.* (1983) reported the evolution of must procyanidins with maceration time, and the effects of various conditions (SO<sub>2</sub>, ethanol levels, temperature) occurring during wine processing on the release of these compounds into the liquid medium have also been studied (OSZMIANSKI *et al.* 1986). SALAGOITY-AUGUSTE and BERTRAND (1984) have quantified four dimeric procyanidins in Cabernet-Sauvignon, Merlot and Malbec red wines. In view of a varietal and geographic classification of French red wines in terms of pigments and flavonoid compounds, ETIÉVANT *et al.* (1988) have also analysed four dimeric procyanidins in wines made from different red grapes. Finally, BOURZEIX *et al.* (1986) reported the effect of vinification technology on the procyanidin contents of red wines. However, only identified dimeric procyanidins were analysed. Consider-

ing the importance of procyanidins in enology and the fact that previous studies only reached the knowledge concerning the non-galloylated dimeric procyanidins, it was of great interest to evaluate the effect of winemaking procedure and vine variety on the major procyanidin contents and profiles of red wines.

We report here for the first time in red grapes and wines the quantification of procyanidins: B<sub>1</sub> 3-O-gallate, B<sub>2</sub> 3-O-gallate, B<sub>2</sub> 3'-O-gallate, trimer C<sub>1</sub> and trimer 2 (epicatechin-(4 $\beta$ →8)-epicatechin-(4 $\beta$ →8)-catechin).

## Materials and methods

### 1. Grapes

Carignan and Mourvèdre grapes were from the INRA experimental vineyard in Pech Rouge (Gruissan, Aude, France) and were hand-harvested in 1988 at commercial maturity (21.1, 22.6, 22.0 °Brix) for Carignan noir I, Carignan noir II and Mourvèdre, respectively. Carignan I and II mean two different plots of vines.

### 2. Wines

The wines were made at the experimental winery located at the station.

For Carignan, two assays were considered. In the first (I), three 3.5 t lots were randomly prepared from the same grapes and vinified separately. The winemaking procedures used were: entire crushed harvest (E.C.H.), destemmed harvest (D.H.) and carbonic maceration (C.M.). For E.C.H., grapes were crushed, SO<sub>2</sub> was added to the mash (50 mg/l), inoculated with 100 mg/l dried Fermivin yeast (*Saccharomyces cerevisiae*) and fermented to dryness (< 2 g/l residual sugar). The fermenting musts were punched down twice daily during maceration to provide a better extraction from grape solid parts. After 9 d of pomace contact at 22–28 °C (with skins, stems and seeds) we used a Vaslin horizontal press and assembled both press and free-run wines. The wine was then racked, filtered and SO<sub>2</sub>-adjusted prior to bottling.

For the D.H. lot, we applied the same technique as for E.C.H., except that the grapes were destemmed.

C.M. was performed in a CO<sub>2</sub> atmosphere at 32 °C for 9 d, then the lot was pressed via a Vaslin horizontal press. Both free-run and press musts were assembled, SO<sub>2</sub> added (50 mg/l), inoculated with 200 mg/l dried Fermivin yeast and at 20 °C fermented to dryness.

In the second lot of Carignan (II), three 3.5 t lots were randomly prepared from the same grapes and vinified separately. The winemaking procedures employed were: destemmed harvest, carbonic maceration and heating of the harvest (H.H.).

For D.H. II, the pomace-contact was 12 d.

C.M. was performed in the same way as for Carignan I. H.H. was used indirectly, with destemmed and crushed grapes. Pomace contact time was 30 min at 75 °C.

The lot was drained and pressed. SO<sub>2</sub> (50 mg/l) was added to the must, then inoculated with 100 mg/l dried Fermivin yeast and fermented to dryness. The wine was racked, filtered and SO<sub>2</sub>-adjusted prior to bottling.

For Mourvèdre, two 300 kg lots were randomly prepared from the same grapes and vinified separately. The winemaking procedures employed were entire crushed harvest and carbonic maceration.

For both techniques, we used the same procedures as with Carignan, except that pomace contact time was 7 d in E.C.H., and C.M. was maintained for 8 d.

Table 1  
Chemical composition of Mourvèdre and Carignan wines  
Composition chimique des vins de Mourvèdre et de Carignan

	Carignan I			Carignan II			Mourvèdre	
	ECH	DH	CM	DH	CM	HH	ECH	CM
Ethanol (% v/v)	11.55	11.80	11.55	12.50	12.35	12.90	12.05	12.45
Residual sugar (g/l)	2.0	2.0	1.8	1.8	1.7	1.3	1.8	1.5
Total extract (g/l)	28.1	26.8	26.3	25.0	22.7	22.3	26.6	25.3
Ash (g/l)	3.4	2.9	3.1	2.7	2.7	2.6	3.5	3.0
pH	3.68	3.60	3.75	3.56	3.62	3.60	3.88	3.93
Total acidity (g/l H <sub>2</sub> SO <sub>4</sub> )	3.45	3.45	3.05	3.90	3.25	3.30	2.95	3.05
Volatile acidity (g/l H <sub>2</sub> SO <sub>4</sub> )	0.23	0.23	0.25	0.39	0.23	0.34	0.36	0.42
Free SO <sub>2</sub> (mg/l)	35	30	35	10	20	10	10	10
Total SO <sub>2</sub> (mg/l)	80	85	65	35	65	40	20	20
K (g/l)	1.30	1.20	1.28	1.22	1.16	1.14	1.58	1.40
Absorbance 420 nm	0.250	0.240	0.277	0.282	0.244	0.323	0.195	0.168
Absorbance 520 nm	0.333	0.318	0.384	0.456	0.375	0.522	0.239	0.180
Absorbance 280 nm	0.876	0.744	0.923	0.632	0.721	0.647	0.729	0.589
Total anthocyanins (mg/l)	400	390	390	230	320	310	190	180

ECH: Entire crushed harvest.  
DH: Destemmed harvest.  
CM: Carbonic maceration.  
HH: Heating of the harvest.

### 3. Procyanidin standards

All procyanidins were obtained from a grape seed extract prepared as described in BOURZEIX *et al.* (1986) and isolated and identified following the procedure described in RICARDO-DA-SILVA *et al.* (1991 d) and RIGAUD *et al.* (1991).

### 4. Extraction

Details regarding extraction and preparation of methanol extracts of grape skins, seeds, pulps and stems were described in BOURZEIX *et al.* (1986).

### 5. Sample purification and HPLC analysis

The use of polyamide chromatography with three successive elutions allowed us to obtain a procyanidin fraction which was further analysed by reversed phase HPLC.

Details of the purification and HPLC analysis were described in a previous study (RICARDO-DA-SILVA *et al.* 1990).

Samples were filtered through 0.45  $\mu\text{m}$  membrane filters prior to injection (injection volume: 50  $\mu\text{l}$ ) onto the HPLC column, and the response factors were determined for each procyanidin by injection of known dilutions.

### 6. Chemical analysis in wines

Carignan and Mourvèdre were analysed for ethanol, residual sugar, total extract, ash, pH, total acidity, volatile acidity, free and total  $\text{SO}_2$ , K and total anthocyanins by the methods in standard use for wines as recommended by O.I.V. Absorbances were also measured in 1 mm cells at 420 and 520 nm (without dilution) and at 280 nm (on 1:50 dilution of the wine). In Table 1 the chemical composition of Carignan and Mourvèdre red wines is presented.

## Results and discussion

### Grapes

The procyanidin composition of the two Carignan and Mourvèdre grapes is presented in Tables 2, 3 and 4. In all red grapes studied, procyanidin  $\text{B}_2$  was the major component in seeds in accord with BOURZEIX *et al.* (1986) and ROMEYER *et al.* (1986), and procyanidin  $\text{B}_1$  in stems and skins in accord with BOURZEIX *et al.* (1986). These results are similar to those encountered in white grapes (BOURZEIX *et al.* 1986; KOVAC *et al.* 1990; RICARDO-DA-SILVA *et al.* 1991 a). As for white grapes (RICARDO-DA-SILVA *et al.* 1991 a), differences were observed in procyanidin profiles of grape seeds, stems or skins for all red grape varieties studied. Pulp of all vine varieties are devoid of procyanidins.

In all the different parts of the grape cluster galloylated dimeric procyanidins are present in much lower concentration than procyanidins without gallic acid esterified. The two trimeric procyanidins quantified in red grapes, in which only  $\text{C}_4$ — $\text{C}_8$  linkages between monomeric units are established, are also present in important levels compared to dimeric procyanidins; the same is true for white grapes (RICARDO-DA-SILVA *et al.* 1991 a). In stems and skins of Carignan and Mourvèdre grapes procyanidin trimer 2 was the second major important procyanidin analysed.

Mourvèdre grapes as a whole present much higher contents of procyanidins than those of Carignan. The two harvests of this variety show almost the same level. Also, the procyanidin contents of all red grape skins are nearly equal.

Table 2  
Procyanidin composition (on a fresh wt base) of Carignan I grapes  
Composition en procyanidines (référée au matériel frais) de la grappe de Carignan I

Procyanidins	Seeds		Stems		Skins		Total grape cluster (mg/kg)			
	(mg/kg clusters)	(mg/g seeds)	(mg/kg clusters)	(mg/g stems)	(mg/kg clusters)	(mg/g skins)				
Procyanidin B <sub>1</sub>	13.8	12 <sup>1)</sup>	0.53	63.5	53 <sup>1)</sup>	1.48	41.5	35 <sup>1)</sup>	0.20	118.8
Procyanidin B <sub>2</sub>	26.8	64	1.00	8.7	21	0.20	6.1	15	0.03	41.6
Procyanidin B <sub>3</sub>	12.6	43	0.49	11.9	41	0.28	4.7	16	0.02	29.2
Procyanidin B <sub>4</sub>	23.8	88	0.91	2.6	10	0.06	0.7	2	0.003	27.1
Procyanidin B <sub>1</sub> 3-O-gallate	0.7	18	0.03	1.8	46	0.04	1.4	36	0.006	3.9
Procyanidin B <sub>2</sub> 3-O-gallate	2.5	50	0.09	1.0	20	0.02	1.5	30	0.007	5.0
Procyanidin B <sub>2</sub> 3'-O-gallate	1.4	15	0.06	3.5	37	0.08	4.5	48	0.02	9.4
Procyanidin C <sub>1</sub>	20.6	46	0.79	15.2	34	0.35	9.3	20	0.04	45.1
Procyanidin trimer 2	15.3	23	0.59	29.6	44	0.69	22.0	33	0.10	66.9

<sup>1)</sup> Distribution within grape clusters in %.

Table 3  
Procyanidin composition (on a fresh wt base) of Carignan II grapes  
Composition en procyanidines (référée au matériel frais) de la grappe de Carignan II

Procyanidins	Seeds		Stems		Skins		Total grape cluster (mg/kg)			
	(mg/kg clusters)	(mg/g seeds)	(mg/kg clusters)	(mg/g stems)	(mg/kg clusters)	(mg/g skins)				
Procyanidin B <sub>1</sub>	10.2	10 <sup>1)</sup>	0.43	41.4	41 <sup>1)</sup>	1.46	50.1	49 <sup>1)</sup>	0.29	101.7
Procyanidin B <sub>2</sub>	24.9	71	1.06	2.9	8	0.10	7.4	21	0.04	35.2
Procyanidin B <sub>3</sub>	10.3	32	0.44	11.1	34	0.39	11.2	34	0.06	32.6
Procyanidin B <sub>4</sub>	15.6	72	0.66	5.8	26	0.21	0.4	2	0.002	21.8
Procyanidin B <sub>1</sub> 3-O-gallate	0.7	22	0.03	1.0	31	0.03	1.5	47	0.008	3.2
Procyanidin B <sub>2</sub> 3-O-gallate	2.5	51	0.10	0.5	10	0.02	1.9	39	0.01	4.9
Procyanidin B <sub>2</sub> 3'-O-gallate	2.5	44	0.10	1.0	18	0.03	2.2	38	0.01	5.7
Procyanidin C <sub>1</sub>	17.6	58	0.75	5.0	17	0.18	7.7	25	0.04	30.3
Procyanidin trimer 2	11.3	21	0.48	19.3	35	0.68	23.8	44	0.14	54.4

<sup>1)</sup> Distribution within grape clusters in %.

For the non-galloylated dimeric procyanidins of Carignan and Mourvèdre grapes, the results are very similar to those of BOURZEIX *et al.* (1986).

Both seeds and stems may be a very interesting material for the extraction of procyanidins, from an industrial point of view (pharmaceuticals, cosmetics, etc.).

However, Mauzac and Chardonnay grape seeds (RICARDO-DA-SILVA *et al.* 1991 a) are much richer in procyanidins than those of the red grapes mentioned here.

Table 4  
Procyanidin composition (on a fresh wt base) of Mourvèdre grapes  
Composition en procyanidines (référée au matériel frais) de la grappe de Mourvèdre

Procyanidins	Seeds			Stems			Skins			Total grape cluster (mg/kg)
	(mg/kg clusters)	(mg/g seeds)	(mg/g clusters)	(mg/g stems)	(mg/kg clusters)	(mg/g skins)	(mg/kg clusters)	(mg/g skins)		
Procyanidin B <sub>1</sub>	29.7	19 <sup>1)</sup>	0.73	97.5	60 <sup>1)</sup>	2.33	34.0	21 <sup>1)</sup>	0.17	161.2
Procyanidin B <sub>2</sub>	61.2	84	1.52	6.4	9	0.15	5.4	7	0.03	73.0
Procyanidin B <sub>3</sub>	41.3	46	1.02	38.4	43	0.92	9.3	11	0.05	89.0
Procyanidin B <sub>4</sub>	53.3	89	1.32	4.0	7	0.10	2.3	4	0.01	59.6
Procyanidin B <sub>1</sub> 3-O-gallate	2.7	34	0.08	4.2	53	0.10	1.0	13	0.01	7.9
Procyanidin B <sub>2</sub> 3-O-gallate	9.3	50	0.29	4.0	22	0.10	5.3	28	0.03	18.6
Procyanidin B <sub>2</sub> 3'-O-gallate	13.6	68	0.43	5.0	25	0.12	1.3	7	0.01	19.9
Procyanidin C <sub>1</sub>	47.0	56	0.16	31.5	37	0.75	5.6	7	0.003	84.1
Procyanidin trimer 2	34.2	26	0.85	77.2	59	1.84	20.1	15	0.10	131.5

1) Distribution within grape clusters in %.

## Wines

The procyanidin composition of wines made by different procedures is presented in Table 5.

The entire crushed harvest (E.C.H.) produced the highest levels of the non-galloylated procyanidins, both for Carignan and Mourvèdre grapes.

On the contrary, the winemaking of destemmed harvested (D.H.) lots gave lower contents of the non-galloylated procyanidins in the wines.

Winemaking by carbonic maceration (M.C.) gave wines which were also relatively rich in procyanidins and quite similar to those made with heating of the harvest (H.H.).

These results confirmed those of BOURZEIX *et al.* (1986), concerning the four dimeric procyanidins, B<sub>1</sub>—B<sub>4</sub>.

For the galloylated dimeric procyanidins, which were also present in red wines, no significant differences were encountered between their levels in the wines made with the various winemaking procedures employed, both for Carignan and Mourvèdre grapes.

Procyanidin B<sub>1</sub> is the major dimer of grape skins and stems (but not of seeds) and also in all red wines produced in this study; this indicates that, with pomace contact, a

release of flavans and procyanidins from skins and stems is very important, whereas that from seeds seems to be poor, in accord with others' (DU PLESSIS and DE WET 1968; CHEYNIER *et al.* 1989).

Finally, all Carignan and Mourvèdre wines presented almost the same procyanidin profiles, not depending on the winemaking procedures used.

Table 5

Procyanidin composition (mg/l) in relation to the wine making procedure

Le rapport entre la composition en procyanidines des vins (mg/l) et la vinification employée

Variety	Specific wine making procedure	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>2</sub>	C <sub>1</sub>	T.2
		3-O-g.	3-O-g.	3'-O-g.						
Carignan I	ECH	121.9	47.6	16.2	10.9	1.9	3.1	2.5	21.1	31.2
	DH	75.3	38.4	11.9	11.8	1.9	3.6	2.5	16.5	24.7
	CM	112.6	26.9	14.6	5.6	2.0	2.8	1.4	14.5	27.5
Carignan II	DH	30.8	12.0	4.3	3.4	0.5	1.2	0.5	7.6	6.6
	CM	65.4	13.5	8.1	4.2	1.4	1.8	0.9	9.6	18.2
	HH	51.9	27.3	9.2	8.7	1.3	1.8	1.5	11.8	12.4
Mourvèdre	ECH	148.4	28.5	22.2	10.2	2.6	6.9	1.3	14.5	48.5
	CM	90.9	16.2	12.3	2.8	2.1	6.5	0.5	8.7	27.3

B<sub>1</sub> 3-O-g.: Procyanidin B<sub>1</sub> 3-O-gallate.B<sub>2</sub> 3-O-g.: Procyanidin B<sub>2</sub> 3-O-gallate.B<sub>2</sub> 3'-O-g.: Procyanidin B<sub>2</sub> 3'-O-gallate.C<sub>1</sub>: Procyanidin trimer C<sub>1</sub>.

T.2.: Procyanidin trimer 2.

ECH: Entire crushed harvest.

DH: Destemmed harvest.

CM: Carbonic maceration.

HH: Heating of the harvest.

### Conclusions

In all red grapes studied, procyanidin B<sub>2</sub> was the major component in seeds, while procyanidin B<sub>1</sub> was the major component in stems and skins.

Trimeric procyanidins are also present in grapes in important levels, compared to those of the dimeric procyanidins.

Entire crushed harvest produced the highest levels of procyanidins in red wines. The profile and concentration of procyanidins in wines, compared to those of the seeds, stems and skins, indicates that an important release of flavans and procyanidins from skins and also from stems occurred.

Galloylated procyanidins which were present in lower concentrations compared to those of the non-galloylated ones were quantified in red wines for all grape varieties and technologies used.

The winemaking procedure employed seems to influence largely the procyanidin contents of wines, but no consistent change in procyanidin profiles was encountered.

### Summary

Dimer procyanidins, galloylated or not, and trimers were analysed in the red grapes Carignan and Mourvèdre. Procyanidins were extracted from the various parts

of the grape bunch and then quantified by HPLC. In Carignan and Mourvèdre grapes, procyanidin B<sub>2</sub> was the major component in seeds, whereas procyanidin B<sub>1</sub> was the major component in stems and skins. Trimeric procyanidins were also present in grapes in larger amounts. Various red wines were produced from Carignan and Mourvèdre grapes, using different winemaking procedures: entire crushed harvest, destemmed harvest, carbonic maceration and heating of the harvest. Entire crushed harvest gave red wines with the highest levels of procyanidins. An important release of procyanidins, especially procyanidin B<sub>1</sub> from skins and stems, when present, occurred.

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