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Determination of Piceid and Resveratrol in Spanish Wines Deriving from *Monastrell* (*Vitis vinifera L.*) Grape Variety

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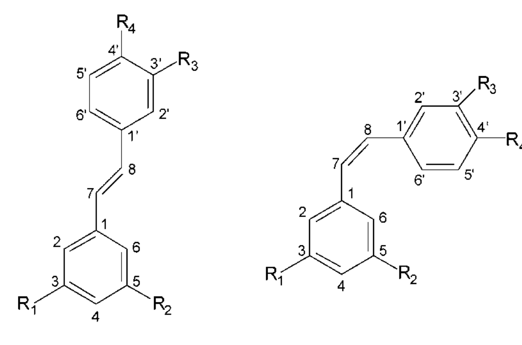
The presence of stilbenes in wine is becoming an important issue due to their claimed relation to a low incidence in coronary diseases and their increasing implication as cancer chemopreventive and neuroprotective agents. Total resveratrol content, quantified as glucoside and aglycone forms of resveratrol, has been determined in a survey of 45 *Monastrell* monovarietal Spanish red wine types (around 135 wine samples), belonging to *Alicante* and *Bullas* appellations. The average between ratio glucoside/aglycone forms of resveratrol in these wines was considerably high, ranging from 82 to 91% of resveratrol in its glycosidic form. This characteristic was observed in a high percentage of the studied wines, which were made under different winemaking procedures, and from different vintages (1995−2002). In addition, wines made using macerative fermentations with double amount of solid parts ("doble pasta") reached the highest levels of total stilbene content expressed as resveratrol equivalent, i.e., 30 mg/L (average of 18.8 mg/L). It can be concluded that high resveratrol glucoside concentration and low free isomer content can be considered characteristics of the *Monastrell* variety, as it happens to red wines deriving from other varieties grown at warm climates. This fact, also observed for other French and Portuguese red varieties, might play an important role in food habits involving these types of wines.

KEYWORDS: **Resveratrol; piceid; red wine; *Monastrell*; HPLC**

INTRODUCTION

Phytoalexines from the *Vitaceae* seem to be confined to a group of compounds belonging to the stilbene family (*1*), whose skeleton is based on the 1,2-diphenylethylene structure (**Figure 1**). Resveratrol (3,5,4′-trihydroxystilbene) and piceid (3,5,4′-trihydroxystilbene-3-*β*-D-glucoside) (**Figure 1**) are two of the major stilbene phytoalexins produced by *Vitaceae* (*1*−*4*). Mulberries, grapes, and wine are considered the most important human dietary sources of stilbenes (*5*). *trans*-Resveratrol is synthesized especially in skin and leaves (*1*, *3*, *6*, *7*), where it was first detected in 1976 (*5*). Another stilbene (**Figure 1**) such as piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene-3-*β*-D-glucoside), also named astringin, has been also found in *Vitis vinifera* cell cultures and wine (*8*, *9*). These compounds are present in wine in their cis and trans forms depending on factors such as UV radiation and winemaking process. There are other stilbene derivatives such as diglucosides, methylated, or polymeric forms, but these do not seem to be present in wine.

In the past decade, there has been a great interest in the presence of *trans*-resveratrol in wine due to its supposed protective effects against cardiovascular metabolism related diseases (*10*−*12*), as derived from several epidemiological studies (*13*, *14*). The mechanism of resveratrol's cardiovascular benefits has not been totally elucidated, but it has been attributed to its antioxidant (*9*, *15*) and anticoagulative properties (*16*). *trans*-Resveratrol has also exhibited chemopreventive activity against carcinogenesis (*17*, *18*). In addition, resveratrol has



	R1	R2	R3	R4
1	OH	OH	H	OH
2	GlcO	OH	H	OH
3	GlcO	OH	OH	OH

	R1	R2	R3	R4
4	OH	OH	H	OH
5	GlcO	OH	H	OH

Figure 1. Chemical structures of the stilbenes frequently found in wines. **1** and **4**, *trans*- and *cis*-resveratrol; **2** and **5**, *trans*- and *cis*-piceid; **3** *trans*-astringin.

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shown antioangiogenic activity (*19*, *20*). Recently, several phenolic compounds from plants, resveratrol among them, have been demonstrated to exert neuroprotection against the oxidative stress derived from chronic ethanol administration (*21*) or nitric oxide (NO) production in cultured neurons (*22*, *23*). Considerable interest has thus been focused on foodstuffs related to Mediterranean Aliment Culture lifestyle, where olive oil, grape, and wine consumptions seem to play an important role (*24*).

The presence of stilbenes in wine has been widely analyzed, to find profiles related to varieties, although further investigations are needed in this direction. Stilbenes concentration in wines vary depending on multiple factors such as grape variety, fungal infections, winemaking procedures, and climatological conditions (*25*, *26*) from values under 0.1 mg/L up to ∼20 mg/L (*8*, *26*). Moreover, the glucoside forms of resveratrol are supposed to be cleaved to its aglycon form, resveratrol, by hydrolysis during the winemaking process (*3*, *8*, *26*). Red wines usually contain higher stilbene concentrations than rose or white wines, probably due to a more prolonged skin contact during fermentation and their higher overall phenolic content (*27*−*29*). Resveratrol concentration was reported to be below or near 1 mg/L in studies done on several American wines (*27*, *28*) and wines from Burgundy (*29*). Low resveratrol or piceid concentrations were also found in a survey of Japanese wines (*30*), except for those deriving from Pinot noir or Merlot. Higher values for *trans*-resveratrol, up to 6−7 mg/L, were reported by Mattivi et al. in several Italian wines deriving from Cabernet-Sauvignon or Merlot (*25*). A larger survey was performed on commercial wines from different regions by Goldberg et al. (*31*−*33*). In the latter study, the highest levels of average resveratrol (trans plus cis isomers) and average piceid were found for Burgundy (5.4 and 5.1 mg/L, respectively), Bordeaux (7.9 and 2.2 mg/L) and Niagara, Canada (5.4 and 8.6 mg/L), although a considerable variability was also found. A survey on several Spanish wines done by Lamuela-Raventós et al. (*34*) reported total average resveratrol values of 9.3 and 9.1 for Pinot noir and Merlot, respectively. Furthermore, values of total resveratrol equiv up to 17.5 mg/L with a content of *trans*-resveratrol glucoside of 11 mg/L were found in red wines deriving from *Mourvèdre* variety (*35*).

Regarding red wines, certain cultivars such as Pinot Noir or Merlot seem to contain higher stilbene concentrations than Cabernet-Sauvignon, Muscat, Concord, Grenache, or Tempranillo (*30*, *34*, *36*, *37*). Moreover, the region of growth and its temperature may significantly influence the stilbene concentration (*2*, *38*). Most wines, such as Burgundy, Bordeaux, Pinot noir, or Merlot (*26*, *34*), exhibit profiles with higher amount of resveratrol in its free form than that of glycosidic derivatives. On the contrary, some wines from warm and dry regions (Spain, Italy, Portugal and South America) seem to have low concentration of free isomers and relatively high glucoside concentrations (*26*, *38*−*40*).

In this work, a total of 45 monovarietal wine types (around 135 wine bottles) deriving from *Monastrell* grapes and belonging to two different appellations of Spain, that is, Alicante and Bullas (Murcia), have been analyzed by HPLC, to contribute to the knowledge on stilbene content in relation to grape variety. This study has included regular wines and wines made under particular winemaking practices such as oak-aged, organic or "doble pasta" wines, from 1995 to 2002 vintages. The average content of the glycoside and aglycone forms of trans-resveratrol and its ratio was also determined. In addition, this is the first study in which *Monastrell* variety has been exhaustively studied.

MATERIALS AND METHODS

Chemicals. *trans*-Resveratrol and β-glucosidase (12.4 units/mg) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). *trans*-Piceid was purified by preparative HPLC, as described below, from a *Polygonum cuspidatum* root aqueous extract (Hu Zhang variety) obtained from DYNAFYT and distributed by NOVASAN S.A (Madrid, Spain). Methanol, acetonitrile, trifluoroacetic acid, methanol-d4, and acetone-d6, were purchased from Merck (Darmstadt, Germany). All solvents employed were liquid chromatography grade.

Wine Samples. *trans*-Piceid (*trans*-resveratrol glucoside), *cis*-piceid, *trans*-resveratrol, and *cis*-resveratrol were determined for 45 different red wine types, that is, 135 wine samples (750 mL bottles), many of them from commercial origin, belonging to two different appellations ("Denominaciones de Origen", D. O.) of Spain, Alicante and Bullas (Murcia). Red wine samples were monovarietal, pure monovarietal or blends (blended red wine contained 80% or higher *Monastrell* grapevine variety) from different vintages covering from 1995 to 2002.

Sample Preparation for HPLC Analysis. Samples were analyzed by direct HPLC injection, after filtration through 0.2-μm Whatman inorganic Anodisc 13 membrane filters from Whatman International LTD (Maidstone, England). When needed, wine samples were subjected to hydrolysis digestion with β-glucosidase, according to a previously published method (*2*, *38*). Briefly, 1 mL of wine was neutralized with NaOH until approximately pH 6.0, then 4 mg of β-glucosidase was added, and digestion was allowed for 18 h at 25 °C under darkness.

Standards. Isolation and purification of *trans*-piceid was done by using a preparative HPLC Knauer-Merck Wellchrom system equipped with two K-1800 pumps (250 mL/min) for binary elution, a dynamic blend camera, a manual injector, a K-2600 UV detector, and a fraction collector FC K-6 from Büchi (Flawil, Switzerland). A 12-mL sample of *Polygonum cuspidatum* root aqueous extract (110 g/100 mL) was centrifuged, filtered through a 0.45-mm nylon filter, and injected into preparative HPLC. Solvents and conditions used for preparative HPLC were the same as those described for the analytical one, as described later on this section, but using a flow adapted to a bigger size column (39 mL/min). UV detection was set at 280 and 306 nm. A LiChrospher RP-18 column (250 mm × 25 mm, 15 μm) from MERCK was employed for the chromatographic separation of the root aqueous extract of *Polygonum cuspidatum*. *trans*-Piceid was the most abundant compound at 306 nm (data not shown for briefness). Fractions deriving from preparative isolation and containing only pure compound, as checked by analytical HPLC, were combined and vacuum concentrated. A yield of approximately 50 mg of *trans*-piceid was obtained. Purified *trans*-piceid was further analyzed by analytical HPLC and showed a UV spectrum nearly identical to *trans*-resveratrol, as previously described by other authors (*32*, *41*), showing a broad absorption band between 290 and 330 nm with its maximum at 320 nm. To confirm its identity, *trans*-piceid was digested with β-glucosidase as described earlier, and pure *trans*-resveratrol was obtained. In addition, ^{1}H and ^{13}C NMR spectral data, which are shown later within this section, were identical to those previously described (*3*, *42*). *cis*-Resveratrol and *cis*-piceid were obtained by UV irradiation of diluted solutions of their respective trans isomers as described elsewhere (*32*, *41*) and were used to construct calibration curves obtained from data at 285 nm for quantitation of *cis*-isomers in wines. *cis*-Piceid and *cis*-resveratrol standards were handled in darkness due to their photosensitivity (*43*).

HPLC Analysis and Quantification. Separation and quantification of stilbenes were done by HPLC using a method modified from that one described by Dalluge et al. (*44*). *trans*-Piceid and *trans*-resveratrol were quantified using their respective pure standards. Concentrations of the trans isomers were determined using the external standard method and their response factors were deduced from their respective calibration curves. A Merck-Hitachi LaChrom system equipped with a D-7100 quaternary pump, D-7455 diode-array detector, D-7485 fluorescence detector, and L-7650 column oven was used. An analytical LiChrospher 100 RP-18 column (250 mm × 4 mm, 5 μm) from Merck was utilized and protected by a guard column of the same material. Separation was performed at a flow rate of 1 mL/min with a mobile phase composed of (A) H_2O + 0.05% TFA and (B) 60:40 MeOH-ACN + 0.05% TFA. Samples (25 μL) of each wine were injected into the HPLC system

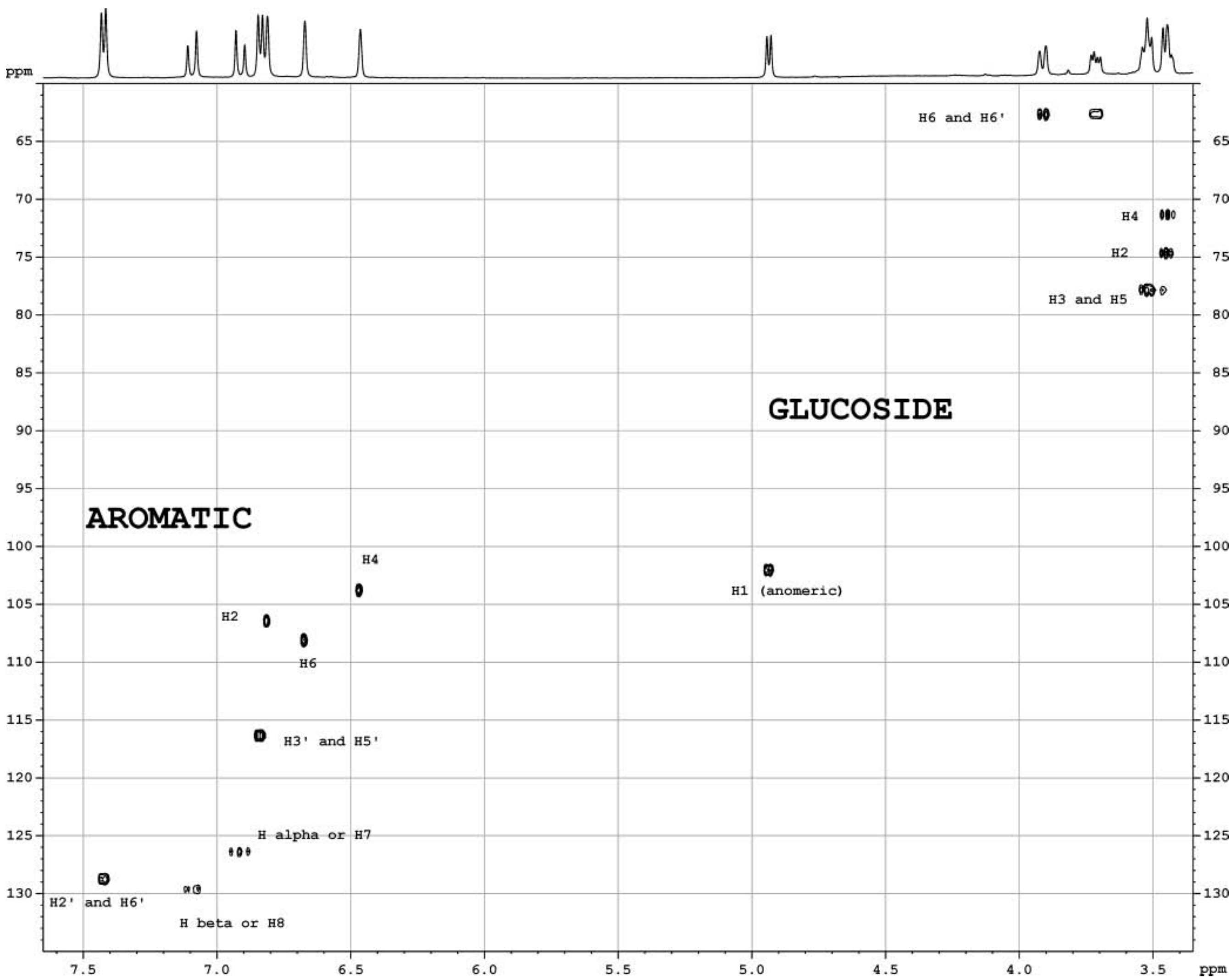

Figure 2. 2D Proton-Carbon HSQC NMR spectrum of trans-resveratrol-β-D-glucopyranoside in acetone-d6.

after filtration as described above. The multigradient solvent system was as follows: 0−5 min, from 10% B to 15% B; 5−40 min, from 15% B to 35% B; 40−55 min, from 35% B to 10% B. The column was equilibrated with starting conditions for 20 min after each analysis. Diode-array detection was used, and oven temperature was 35°C. Data were obtained from triplicate analyses on up to three wine bottles for each studied wine type. Relative standard deviation was always under 3% of the values. Cis isomer of resveratrol was found in negligible amounts (less than 0.05 mg/L) in all cases, therefore, data for this compound were not shown.

Nuclear Magnetic Resonance Spectroscopy. Nuclear magnetic resonance spectra for *trans*-resveratrol glucoside identification were collected on a Bruker AVANCE 500 spectrometer and probe HD 5 mm TXI 13C Z. The spectra were collected at 296 K in methanol-d4 or acetone-d6 as deuterated solvents, and the resonance of each methyl group of the solvents was used as reference for shift tabulated values (*45*). NMR experimental parameters corresponding to *trans*-resveratrol glucoside structure, which were confirmed with those previously published by other authors (*3*, *42*), were as follows:

^{1}H NMR (500 MHz, CD_6CO, ppm): δ 7.42 (d, 2H, J = 8.25 Hz, H2′ and H6′), 7.08 (d, 1H, J = 16.4 Hz, H7 or Hα), 6.91 (d, 1H, J = 16.2 Hz, H8 or Hβ), 6.84 (d, 2H, J = 8.2 Hz, H3′ and H5′), 6.81 (broad signal, 1H, H2), 6.67 (bs, 1H, H6), 6.46 (bs, 1H, H4), 4.93 (d, J = 7.6 Hz, H1′′), 3.91 (bs, 1H, H6′′), 3.72 (bs, 1H, H6′′), 3.54−3.43 (4H, m, H2′′, H3′′, H4′′, H5′′).

^{1}H NMR (500 MHz, CD_3OD, ppm): δ 7.37 (d, 2H, J = 8.6 Hz, H2′ and H6′), 7.02 (d, 1H, J = 16.2 Hz, H7 or Hα), 6.85 (d, 1H, J = 16.3 Hz, H8 or Hβ), 6.79 (t, 1H, J = 1.6 Hz, H2), 6.77 (d, 2H, J = 8.6 Hz, H3′ and H5′), 6.62 (t, 1H, J = 1.6 Hz, H6), 6.45 (t, 1H, J = 2.15 Hz, H4), 4.89 (bs + water signal, H1′′), 3.94−3.92, 3.73−3.69 (bs, 2H, 2 × H6′′), 3.48−3.45 (4H, m, H2′′, H3′′, H4′′, H5′′).

^{13}C NMR (125 MHz, CD_3OD, ppm): δ 160.47 (C3), 159.57 (C5), 158.46 (C4′), 141.42 (C1), 130.31 (C1′), 129.97 (C8 or Cβ), 128.91 (C2′ and C6′), 126.65 (C7 or Cα), 116.48 (C2′ and C6′), 108.33 (C6), 106.99 (C2), 104.07 (C4), 102.40 (C1′′ or C anomeric), 78.24 (C5′′), 78.04 (C3′′), 74.95 (C2′′), 71.47(C4′′), 62.58 (C6′′).

RESULTS AND DISCUSSION

Monastrell grape variety, also known as *Mourvèdre* in Southern France, or *Mataró* in California and *Cataluña*, is a native red variety from the Spanish Mediterranean coast. *Monastrell* grapes exhibit thick-skinned berries, which allow them to thrive vigorously in warm and arid climates. Wines produced from this variety tend to be high in alcohol and tannins and have distinctive balsamic, blackberries, and mineral flavors, especially when young. This is the major variety in several Spanish DOs (Appellations) such as Almansa, Valencia, Jumilla, Yecla, Alicante, and Bullas. In the last years *Monastrell*-based wines have considerably increased their value supposing an attractive high quality alternative to other well-established varieties in Spain.

The unequivocal structure of *trans*-resveratrol-β-D-glucopyranoside was elucidated by monodimensional (^{1}H and ^{13}C NMR) and bidimensional HSQC (^{1}H and ^{13}C). The monodimensional spectra in both deuterated solvents were coincident with those previously published by other authors (*3*, *42*). Assignation of NMR signals by HSQC in acetone-d_6 is shown in **Figure 2**.

Table 1. Stilbene Content (mg/L) for Young Red Wines Deriving from *Monastrell* Variety Grown in the Spanish Mediterranean Coast

wine type	vintage	apellation (D. O.)	*trans*-piceid (mg/L)	*cis*-piceid (mg/L)	*trans*-resveratrol (mg/L)	ratio[a] piceid/resveratrol	total resveratrol (mg/L)[b]
1	1999	Alicante	4.20	nd[c]	0.08	32.32	2.53
2	1999	Alicante	1.31	nd	1.14	0.67	1.91
3	2000	Alicante	15.38	nd	1.69	5.31	10.68
4	2000	Alicante	16.30	nd	1.53	6.23	11.06
5	2000	Alicante	7.19	nd	3.66	1.15	7.86
6	2000	Alicante	6.24	nd	1.83	1.99	5.47
7	2000	Alicante	1.19	nd	1.55	0.45	2.25
8	2000	Alicante	6.56	nd	traces[d]		1.92
9	2001	Alicante	17.09	nd	2.60	3.84	12.59
10	2001	Alicante	11.80	nd	2.13	3.23	9.03
11	2001	Alicante	13.04	nd	3.16	2.41	10.78
12	2001	Alicante	11.72	nd	traces		3.43
13	2001	Alicante	7.44	nd	1.05	4.15	5.40
14	2002	Alicante	7.34	7.24	2.34	3.65	10.86
15	1997	Bullas	6.08	nd	1.09	3.27	7.04
16	1998	Bullas	9.33	nd	1.59	3.43	6.91
17	1998	Bullas	9.59	nd	1.53	3.67	5.82
18	1999	Bullas	8.65	nd	1.86	2.73	4.64
19	1999	Bullas	6.48	5.34	1.75	3.95	7.14
20	2000	Bullas	7.52	nd	1.43	3.08	8.66
21	2000	Bullas	7.52	nd	1.75	2.51	6.15
		total avg	8.66	6.29	1.78	4.63	6.77

[a] Expressed as a molar ratio [b] Expressed as resveratrol equivalent (aglycone form) [c] nd, not detected [d] traces (<0.05 mg/L)

Table 2. Stilbene Content (mg/L) for Oak-Aged Red WinesDeriving from *Monastrell* Variety Grown in the Spanish Mediterranean Coast

wine type	vintage	apellation (D. O.)	*trans*-piceid (mg/L)	*cis*-piceid (mg/L)	*trans*-resveratrol (mg/L)	ratio[a] piceid/resveratro	total resveratrol (mg/L)[b]
22	1997, cr[c]	Alicante	7.18	4.56	2.56	2.68	9.42
23	1997, cr	Alicante	7.56	nd[d]	0.22	20.24	4.64
24	1998, cr	Alicante	7.78	nd	1.29	3.52	5.84
25	1998, cr	Alicante	6.95	nd	0.18	22.15	4.25
26	1999, cr	Alicante	6.03	2.23	traces[e]		2.42
27	2000, cr	Alicante	17.24	nd	2.20	4.59	12.28
28	2001, cr	Alicante	11.83	5.36	0.57	17.66	10.62
29	1997, re[c]	Alicante	10.21	nd	0.32	18.45	6.30
30	1995, cr	Bullas	9.06	nd	0.97	5.48	6.26
31	1996, re	Bullas	9.87	nd	1.44	4.00	7.21
32	1998, cr	Bullas	6.89	nd	0.83	4.88	4.85
33	1998, cr	Bullas	14.67	nd	1.80	4.75	10.38
		total avg	9.61	4.05	1.13	9.85	7.04

[a] Expressed as a molar ratio [b] Expressed as resveratrol equiv (aglycone form) [c] *crianza*, cr; *reserva*, re [d] nd, not detected [e] traces (<0.05 mg/L)

The trans configuration of the isolated compound was confirmed by the large coupling constant ($J = 16.3$ Hz). Data of relevant signals, in each deuterated solvent, such as the stilbene system and the anomeric carbon of the glucoside moiety were collected and confirmed (see bold assignments in NMR materials section).

To contribute to the characterization of the stilbenes profile in *Monastrell* wines, the levels of some of these compounds were determined in wines produced under different winemaking processes. **Table 1** shows the content of three stilbenes determined in 21 young *Monastrell* wines of vintages from 1997 to 2002 belonging to Alicante and Bullas appellations (Spain). The majority of wines analyzed showed higher levels of *trans*-piceid than of *trans*-resveratrol, reaching quite high concentrations of *trans*-piceid ranging from 15 to 17 mg/L (wines 3, 4, and 9), as compared to other red varieties such as Pinot noir or Merlot, which showed around 4 mg/L (*34*), or red wines from Spain, France or Canada, which showed levels around 3−7 mg/L (*32*). In most cases studied in this work, levels of cis isomers were much lower than their respective *trans*-isomers, with *cis*-resveratrol levels being almost negligible (<0.05 mg/L). The molar ratio glycoside/aglycone forms of resveratrol yielded an average value of 4.63, meaning that around 82% of resveratrol was in its glucosidic form. Vintages 2001 (wines 9−13), 2002 (wine 14) and some wines of vintage 2000 (wines 3, 4, and 20) showed the highest level of total resveratrol within these wine groups (defined as the sum of cis and trans forms of piceid and resveratrol) mainly due to their high content in *trans*-piceid. Although some young wines deriving from Alicante DO exhibited total resveratrol amount a little higher than those of Bullas appellation, their average total resveratrol values were quite similar, 6.84 and 6.62 mg/L, respectively.

Piceid and resveratrol levels for a group of oak-aged *Monastrell* wines of vintages from 1995 to 2001 are shown in **Table 2** ("crianza", 24 months oak barrel + bottle-aged with at least 12 months oak barrel-aged; "reserva", 36 months oak barrel + bottle-aged with at least 12 months oak barrel-aged). In this case, wines from vintages 2000 (wine 27) and 2001 (wine 28) also exhibited the highest content of total equivalent resveratrol, i.e., 10.6−12.3 mg/L. A previous study focused on the relationship between aging process and resveratrol content

Table 3. Stilbene Content (mg/L) for Organic, Sweet and "doble Pasta" Red Wines Deriving from *Monastrell* Variety Grown in the Spanish Mediterranean Coast

wine type	wine sample	vintage	apellation (D. O.)	*trans*-piceid (mg/L)	*cis*-piceid (mg/L)	*trans*-resveratrol (mg/L)	ratio[a] piceid/resveratrol	total resveratrol (mg/L)[b]
34	sweet Fondillón	1944	Alicante	4.51	nd	traces[e]		2.64
35	sweet Fondillón	1980	Alicante	6.00	nd	traces		3.51
36	sweet	2001	Alicante	nd[d]	nd	nd		nd
37	organic	2000	Alicante	5.83	4.55	traces		3.04
38	organic	2001	Alicante	11.89	3.72	1.36	6.69	10.49
39	organic	2002	Alicante	16.88	11.40	4.05	4.08	20.59
40	aged organic, cr[c]	2000	Alicante	4.15	3.49	traces		2.25
41	doble pasta	2001	Alicante	22.08	10.18	2.89	6.53	21.75
42	doble pasta	2001	Alicante	17.69	5.96	2.15	6.44	15.97
43	doble pasta	2001	Alicante	11.90	4.52	2.98	3.22	12.58
44	doble pasta	2001	Alicante	13.07	7.31	0.92	12.93	12.84
45	doble pasta	2002	Alicante	29.24	14.80	5.13	5.02	30.88
			total avg	13.02	7.33	2.78	6.41	12.41

[a] Expressed as a molar ratio. [b] Expressed as resveratrol equiv (aglycone form). [c] *crianza*: cr. [d] nd, not detected. [e] traces (<0.05 mg/L).

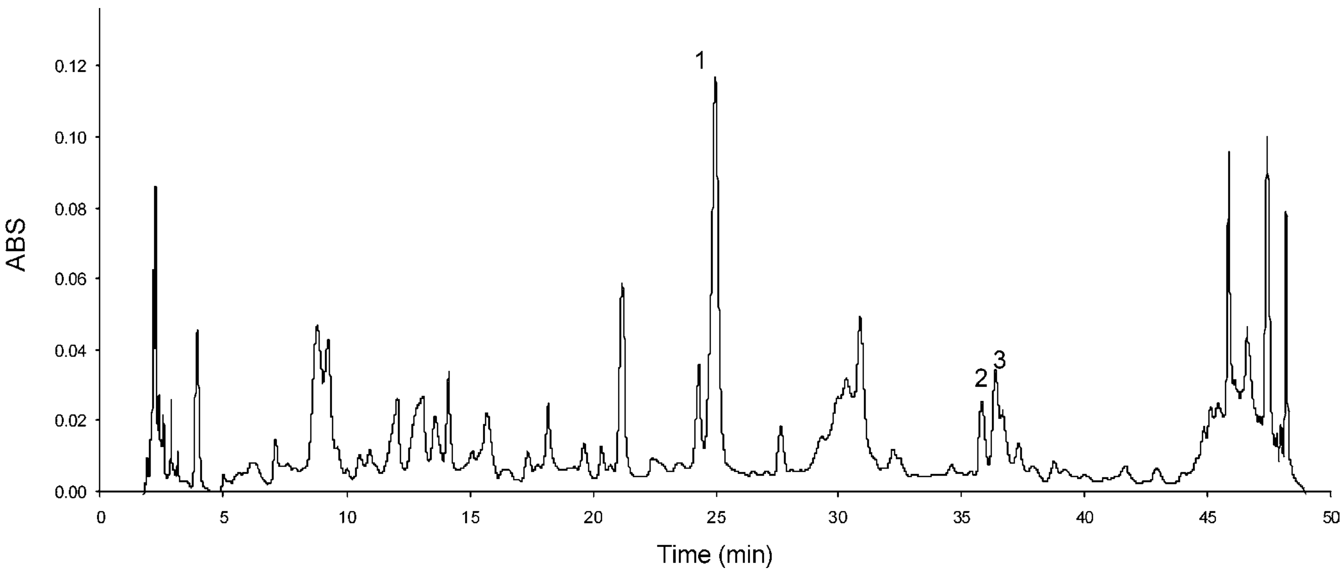

Figure 3. HPLC chromatogram corresponding to doble pasta *Monastrell* wine type 45 obtained at 306 nm, showing a high concentration of *trans*-piceid. Peak 1, *trans*-piceid; peak 2, *cis*-piceid; peak 3, *trans*-resveratrol.

performed on Ontario red wines showed important losses in both trans- and cis- isomers of resveratrol (*46*). However, the average of total resveratrol found in the analysis done in this work encompassing a total of twelve aged *Monastrell* wines was close to 7 mg/L, similar to the value observed for young wines in **Table 1**. Average ratio glucoside/aglycone for aged wines was a little bit higher, yielding more than 90% of resveratrol in its glucoside form. In general, wine-aging process for *Monastrell* wines did not seem to diminish the content of the glycosidic form of resveratrol in comparison to nonaged wines. According to our results, a previous study showed that wines made from Mourvédre variety conserved both resveratrol and piceid and presented similar total resveratrol concentration contents regardless of their age (*35*).

A group of unusual wines, such as sweet, organic, or doble pasta wines, all made from *Monastrell* grapes and deriving from Alicante DO, were also analyzed, and their resveratrol content is shown in **Table 3**. Sweet red *Monastrell* wines (wines 34−36) presented low or undetectable levels of the stilbenes analyzed in this work, and only sweet "Fondillón" showed significant levels of *trans*-piceid. Wines made from organic grapes (wines 37−40) showed similar or even higher levels of resveratrol to *Monastrell* wines made with regularly cultured grapes. A *Monastrell* wine from vintage 2002 and made from organic grapes (wine 39) yielded one of the highest contents in total resveratrol, that is, 20.59 mg/L, a value remarkably high considering that this wine was not subjected to special maceration process. In contrast, an aged organic wine (wine 40) showed very low levels of total resveratrol compared to nonaged ones, maybe due to the lower stability of these compounds under the low SO_2 concentrations and higher pH used in such wines (*47*). A group of particular wines called doble-pasta made from *Monastrell* grapes were also analyzed (wines 41−45). Doble pasta wines are obtained by the addition of solid parts left from rose winemaking process to a regular maceration-fermentation of a red wine, so maceration is made in the presence of double amount of solid material. This process yields wines with high color intensity and an alcoholic content as high as 16%. *Monastrell* doble pasta wines showed the highest level of stilbenes of all the wines studied. It is noteworthy that a doble pasta wine (wine 45) reached a considerably high content in *trans*-piceid (almost 30 mg/L), yielding a remarkable value of total resveratrol quantified as resveratrol equiv (i.e., 30.88 mg/L). **Figure 3** shows the HPLC chromatogram corresponding to this particular doble pasta *Monastrell* wine (2002) in which *trans*-piceid was the major compound observed at 306 nm. The average of total resveratrol for doble pasta *Monastrell* wines reached a value of 18.80 mg/L, higher than average values found for the majority of previously studied red wines, as reviewed in the Introduction (*30*, *34*, *35*, *37*, *39*, *48*, *49*). An increase of the stilbene content up to 10-fold has also been observed by other authors in wines made using long macerative fermentations, which allow a prolonged skin contact (*47*, *50*). Considering the results obtained from the 45 *Monastrell* wines studied, the average of total equiv resveratrol was around 8.25 mg/L, a value which is close to what it has been found also in Spanish wines for other *high stilbene level* varieties such as Pinot Noir or Merlot (i.e., 9.3 and 9.1 mg/L, respectively) (*34*).

A previous study on transfer and stability of stilbenes during fermentation and aging in a *Mourvèdre* variety (*35*), a variety identical to *Monastrell* but geographically adapted to South France, has also shown a higher content in piceid compared to resveratrol (67 vs 33%, respectively) but more similar values of cis and *trans*-piceid were obtained than in our case. These data are in agreement with our results and support the statement that a high piceid/resveratrol ratio is an intrinsic characteristic of *Monastrell* variety, which behaves independently from growth location and condition, according to which it had been suggested for other red varieties such as Pinot Noir (*8*, *34*).

Although other authors have previously found relatively high values of piceid in several red varieties, including Spanish wines (*32*, *34*), the values found in this study for *Monastrell* wines are superior to those found in most cases and are comparable to the maximum levels found in a study done on monovarietal Portuguese wines (*8*). The latter study found significant average values of *trans*-piceid (i.e., 11.8 and 13 mg/L in Portuguese and French wines, respectively). In our study, normal fermentation *Monastrell* wines yielded average values of *trans*-piceid of 8−10 mg/L (**Tables 1 and 2**). Furthermore, double macerative fermentation wines (doble pasta) *Monastrell* wines reached almost 30 mg/L of *trans*-piceid, with a mean value of 18.8 mg/L. Thus, high piceid content can be associated to this variety. It also has to be considered that a significant percentage of the wines studied (38%) showed values of *trans*-piceid over 10 mg/L and that 50% of the wines showed values of total stilbene higher than 7 mg/L. In addition, in all *Monastrell* wines analyzed, 82−91% of the stilbenes were in their glycosidic form. Considering that other stilbenoids such as astringin or viniferins were not determined in this study, it is assumed that total stilbene content of *Monastrell* wines could be even higher. The results presented in this work show that *Monastrell* wines exhibit markedly high levels of *trans*-piceid and low level of free *trans*-resveratrol. Since this fact was observed along several vintages and a diversity of winemaking processes, it can be postulated that this feature may suppose a phenotypical varietal character, as it has been postulated by other authors for other varieties grown at warm climates (*26*, *38*−*40*).

An estimation of the total stilbene intake from *Monastrell* wine (*8*), and considering a regular consumption of 200 mL/day of *Monastrell* wine, would mean a daily intake of stilbenes (calculated on the basis of the average value of total resveratrol) of approximately 1.65 mg/day/individual, and 3.8 mg/day/individual for people only drinking doble pasta wines. Considering that resveratrol bioavailability by oral administration in rats is around 40% (*51*), resveratrol peak plasma concentration achieved after ingestion of these wines would probably be within the low μM range, which might be increased after a prolonged consumption. These values are not too far from those concentrations at which resveratrol has demonstrated its biological activity (*52*, *53*). There are increasing evidences of the existence of β-glucosidase activity in human small intestine (*54*, *55*) so resveratrol glucoside may be deglycosylated and converted into bioavailable resveratrol during digestion. It has been also shown that *trans*-piceid shares similar biological activities with its aglycone form, *trans*-resveratrol (*56*−*58*). Nevertheless, it is still unclear if piceid's biological activity is exerted by itself or through resveratrol conversion; thus, further pharmacokinetic studies are needed.

ABBREVIATIONS USED

DO, appellation (denominación de origen); HPLC, high-performance liquid chromatography; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; UV, ultraviolet light.

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