Piceid, the Major Resveratrol Derivative in Grape Juices

Ana I. Romero-Pérez, Maite Ibern-Gómez, Rosa M. Lamuela-Raventós,* and M. Carmen de la Torre-Boronat

Nutrició i Bromatologia, CèRTA, Facultat de Farmàcia, Universitat de Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Spain

The levels of *trans*-piceid, *cis*-piceid, *trans*-resveratrol, and *cis*-resveratrol have been measured in 36 grape juices using an HPLC system with spectral analysis of eluting peaks. The piceid (glucosides) were the major component in the grape juices. In red grape juices the average concentrations were 3.38 mg/L for *trans*-piceid, 0.79 mg/L for *cis*-piceid, 0.50 mg/L for *trans*-resveratrol, and 0.06 mg/L for *cis*-resveratrol. In white grape juices the levels were, on average, 0.18 mg/L for *trans*-piceid, 0.26 mg/L for *cis*-piceid, and 0.05 mg/L for *trans*-resveratrol, and *cis*-resveratrol was not detected in any sample. Levels of total resveratrol (*trans*- and *cis*-resveratrol and -piceid) found in red and in white grape juices are similar to those described in Spanish red and white wines. Due to their resveratrol content, as well as other phenolics, grape juices may have a beneficial health effect of interest to those who cannot drink wine.

Keywords: *Piceid; resveratrol; grape juices*

INTRODUCTION

Over the past few years, many epidemiological studies have shown a negative correlation between moderate wine consumption and the risk of coronary heart disease (Gronbaek et al., 1995; Hertog et al., 1993; Klatsky and Armstrong, 1993; Renaud and de Lorgeril, 1992; Renaud et al., 1998). Recently, moderate wine consumption has also been related to preventing cancer (Renaud et al., 1998) and other degenerative disorders such as Alzheimer's disease or dementia (Dorozynski, 1997; Orgogozo et al., 1997).

Wine may not be the only beverage that exerts these beneficial effects. The daily consumption of grape juice may reduce the incidence of coronary artery disease and acute platelet thrombus formation by decreasing the platelet contribution to coronary artery disease, in a manner similar to wine (Folts, 1997; Folts et al., 1997a,b). Moreover, grape juice has an antioxidant capacity similar to that measured in wine (Day et al., 1997; Frankel et al., 1998; Simonetti et al., 1997; Wang et al., 1996).

Resveratrol, a phenolic compound found in grapes and wine, has been linked to these effects. Resveratrol can inhibit cellular events associated with tumor initiation, promotion, and progression (Fontecave et al., 1998; Jang et al., 1997; Mgbonyebi et al., 1998), reduce cell death from oxidative stress (Chantavitayapongs et al., 1997), inhibit the oxidation of human low-density lipoprotein (LDL) (Frankel et al., 1993, 1995; Mérillon et al., 1996; Vinson et al., 1995), inhibit platelet aggregation and eicosanoid synthesis (Chung et al., 1992; Kimura et al., 1985, 1995; Pace-Asciak et al., 1995), and prevent anti-inflammatory activity (Ferrero et al., 1997), and it is an agonist for the estrogen receptor (Gehm et al., 1997).

Resveratrol 3-*O*-β-D-glucoside (piceid) is the main component of the *Polygonum cuspidatum* root, used in Japanese and Chinese folk medicine for the treatment of some cardiac ailments, including atherosclerosis and inflammation.

trans-Resveratrol, *trans*-piceid, and their *cis* isomers (Figure 1) have been found in wines (Lamuela-Raventós et al., 1995; Romero-Pérez et al., 1996). *cis*-Resveratrol, *trans*-piceid, and *cis*-piceid are physiologically as important as *trans*-resveratrol. *cis*-Resveratrol possesses activity comparable to that of *trans*-resveratrol in inhibiting protein−tyrosine kinase (Jayatilake et al., 1993) and platelet aggregation (Bertelli et al., 1996; Chung et al., 1992; Varache-Lembège et al., 1996). Piceid isomers have properties similar to those of resveratrol in inhibiting platelet aggregation (Chung et al., 1992; Orsini et al., 1997; Shan et al., 1990; Varache-Lembège et al., 1996) and in inhibiting oxidation of human LDL (Mérillon et al., 1996). On the other hand, in a manner less active than *trans*-resveratrol, *trans*-piceid reduces the elevations of lipid levels (Arichi et al., 1982) and inhibits eicosanoid synthesis (Kimura et al., 1985).

Some authors have found that vinification techniques have marked effects on resveratrol and piceid levels in wine, particularly maceration with skins (Mattivi et al., 1995; Jeandet et al., 1995). Clarifying agents and filters can decrease resveratrol and piceid levels (Lamuela-Raventós et al., 1995; Soleas et al., 1995a; Tobella and Waterhouse, 1996; Vrhovsek et al., 1997). Some of these agents are also employed in commercial grape juice-making.

To date, only two studies have measured resveratrol in grape juices. Yasui et al. (1997) have found concentrations of *trans*-resveratrol in Japanese grape juices ranging from 0.04 to 0.44 mg/L, and Soleas et al. (1995b) reported levels of *trans*-resveratrol between 3 and 15 μg/L and did not detect *cis*-resveratrol in varietal grape juices. However, they did not analyze for the glycosides *trans*-piceid and *cis*-piceid, and these are the major compounds in white wines (Romero-Pérez et al., 1996).

Here, the levels of *trans*-piceid, *cis*-piceid, *trans*-

* Author to whom correspondence should be addressed (telephone 00.34.93 4024508; fax 00.34.93.4035931; e-mail lamuela@farmacia.far.ub.es).

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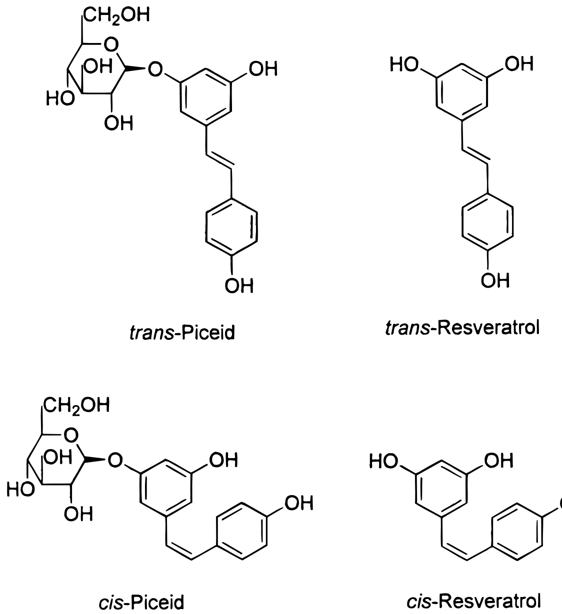

Figure 1. Chemical structures of resveratrol and piceid isomers.

resveratrol, and *cis*-resveratrol in commercial and winery grape juices are described.

MATERIALS AND METHODS

Standards. *trans*-Resveratrol was purchased from Sigma Chemical Co. (St. Louis, MO). *trans*-Piceid was extracted from the roots of *Polygonum cuspidatum* as described by Waterhouse and Lamuela-Raventós (1994). *cis*-Resveratrol and *cis*-piceid were obtained under sunlight exposure of the respective standard solutions containing the *trans* isomer. Within 10 min, ~90% isomerization was achieved. The compounds were characterized as *cis* isomers by their spectra using a diode array detector and by comparison with literature data (Jeandet et al., 1997; Trela and Waterhouse, 1996).

Samples. A total of 36 grape juices were analyzed, 27 of which were white juices and 9 were red grape juices. White samples were from different sources. Twelve were varietal grape juices obtained from the free run juices before the start of the vinification process of different wineries in different vintages. All of the others, red and white, were commercial juices that were available in Barcelona markets but could not be identified as to their varietal source.

HPLC Analysis. The samples were filtered through Whatman inorganic Anopore membrane filters (Anotop 10 plus, 0.2 μm) and directly injected in duplicate onto a Hewlett-Packard (HP) 1050 instrument equipped with an HP autoinjector, ChemStation, and an HP 1050 diode array UV−visible detector. The system was equipped with a Tracer Nucleosil column C_{18} 120 (25 × 0.4 cm), 5 μm particle size, with a precolumn of the same material, maintained at 40 °C.

The HPLC conditions were similar to those described by Lamuela-Raventós et al. (1995); however, due to differences in the system pressure when the apparatus was changed, the elution profile had to be adjusted as follows: 0 min, 83.5% A, 16.5% B; 13 min, 82.0% A, 18.0% B; 15 min, 82.0% A, 18.0% B; 17 min, 77.0% A, 23.0% B; 21 min, 75.0% A, 25.0% B; 27 min, 68.5% A, 31.5% B; 30 min, 100% A, 0% B, where solvent A was glacial acetic acid in water (52.6:900) (v/v) and solvent B was 20% phase A and 80% acetonitrile.

The modified HPLC method was validated according to the United States Pharmacopoeia (USP 23). The values of selectivity, precision, limit of detection (0.003 mg/L), and limit of quantitation (0.01 mg/L) were similar to those obtained in our previous study (Lamuela-Raventós et al., 1995).

Table 1. Resveratrol and Piceid Levels in White and Red Grape Juices

grape juice	*trans*-piceid[a] (mg/L)	*cis*-piceid[b] (mg/L)	*trans*-resveratrol (mg/L)	*cis*-resveratrol (mg/L)	total amount
reds					
1	4.31	0.38	0.79	0.08	5.56
2	4.74	0.11	0.60	0.12	5.57
3	2.15	0.08	0.47	0.01	2.71
4	0.77	0.27	0.15	nd[c]	1.19
5	4.94	0.23	0.41	nd	5.58
6	0.53	0.16	nq[d]	nd	0.69
7	7.34	0.17	1.09	0.09	8.69
8	4.65	5.66	0.93	0.23	11.47
9	0.96	0.09	0.09	nd	1.14
av	3.38	0.79	0.50	0.06	4.73
whites					
10	0.13	0.34	0.03	nq	0.50
11	0.03	nq	nd	nd	0.03
12	0.05	nq	nd	nd	0.05
13	0.48	0.03	0.07	nq	0.58
14	0.15	0.03	0.05	nq	0.23
15	0.07	0.05	nd	nd	0.12
16	0.08	nd	nd	nd	0.08
17	0.04	nq	0.03	nq	0.07
18	0.14	0.01	0.03	nq	0.18
19	0.18	0.10	0.04	nd	0.32
20	nd	nd	nd	nd	nd
21	nd	nd	nd	nd	nd
22	0.30	nd	0.08	nd	0.38
23	0.06	nd	nd	nd	0.06
24	0.83	0.11	0.19	nq	1.13
Macabeo 1993	0.08	0.19	0.03	nq	0.30
Xarel.lo 1993	0.17	0.92	0.05	nq	1.14
Parellada 1993	0.28	0.86	0.05	nq	1.19
Chardonnay 1993	0.10	0.12	0.06	nq	0.28
Macabeo 1994	0.08	0.11	0.04	nd	0.23
Xarel.lo 1994	0.15	0.77	0.04	nq	0.96
Parellada 1994	0.15	0.50	0.05	nq	0.70
Chardonnay 1994	0.07	0.21	0.04	nd	0.32
Macabeo 1995	0.13	0.29	0.04	nq	0.46
Xarel.lo 1995	0.30	1.09	0.05	nq	1.44
Parellada 1995	0.25	0.59	0.06	nq	0.90
Chardonnay 1995	0.27	0.28	0.13	nq	0.68
av	0.18	0.26	0.05		0.49

[a] Quantified as *trans*-resveratrol. [b] Quantified as *cis*-resveratrol. [c] nd < 0.003 mg/L. [d] nq < 0.01 mg/L.

RESULTS AND DISCUSSION

The quantitation of *cis* isomers was performed through a calibration curve obtained at 285 nm after sunlight exposure of known amounts of *trans*-resveratrol under the conditions described by Romero-Pérez et al. (1996). Table 1 shows the results obtained for all red and white grape juices analyzed.

The levels of the four compounds in the red samples averaged 10-fold higher than in the white juices and showed greater variability. The total resveratrol content in red samples was between 0.69 and 14.47 mg/L (mean = 4.73 mg/L) and in white samples was between not detected and 1.44 mg/L (mean = 0.49 mg/L). Levels of total resveratrol found in red and white grape juices are similar to those described in Spanish red and white wines (5.65 and 0.48 mg/L, respectively) by Lamuela-Raventós et al. (1995) and Romero Pérez et al. (1996). It is interesting to note that the fresh juices from the wine-making grape varieties had a total average resveratrol content of 0.72 mg/L, more than twice higher than the commercial juices at 0.29 mg/L. It is not possible to tell whether this difference is due to the use of different varieties, changes during storage, or differences in juice production.

The different contents of resveratrol between white and red samples were described previously in wines (Romero-Pérez et al., 1996). The lower levels found in white wines are due to the absence of skin contact during the fermentation process. However, here the differences in juice resveratrol content between white and red grape juices have to be attributed to the juice processing technology (i.e., pressing and skin color extraction procedures) because the levels of *trans*-resveratrol in grape skins are similar for red and white grape berries (Okuda and Yokotsuka, 1996).

Red Grape Juices. *trans*-Piceid was present at the highest concentrations in the red samples, followed by *cis*-piceid and *trans*-resveratrol. *cis*-Resveratrol, with the lowest levels, was not detected in four of nine red samples analyzed. The aglycons account for only 10% of the total resveratrol content.

White Grape Juices. In the varietal grape juices, the highest levels of total resveratrol were found in the Xarel.lo variety, followed by Parellada. These levels are similar to those found previously in white wines made with the same varieties (Romero-Pérez et al., 1996).

The compounds found in white grape juices at the higher levels were the piceid isomers, whereas *cis*-resveratrol could not be quantified or was not detected in any of the samples and it has never been reported in grape berry skins (Jeandet et al., 1991; Langcake and Pryce, 1976).

trans-Piceid had the highest levels in the 15 commercial samples (mean = 0.17 mg/L), and the levels of *cis*-piceid (mean = 0.04 mg/L) and *trans*-resveratrol (mean = 0.03 mg/L) were similar. In two of these white grape juices, no resveratrol derivatives could be detected. In the noncommercial samples, *cis*-piceid was the highest compound (mean = 0.49 mg/L), followed by *trans*-piceid (mean = 0.17 mg/L) and *trans*-resveratrol (mean = 0.05 mg/L).

According to Hollman et al. (1995) and Paganga and Rice-Evans (1997), the absorption of some phenols from the diet is enhanced by conjugation with glucose. If the glucoside facilitates absorption, then the piceids could be more efficiently absorbed than the aglycons.Thus, grape juice, in particular red grape juice, may be an alternative dietary source to wine to achieve the beneficial effect of resveratrol. Grape juice lacks alcohol, which in moderation has some beneficial properties (Rimm et al., 1996), but it cannot be present in foods for particular populations such as for children or patients with hepatic diseases.

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