A Global Survey of *Trans*-Resveratrol Concentrations in Commercial Wines

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Using a solid-phase extraction followed by direct-injection gas chromatography-mass spectrometry, the concentration of *trans*-resveratrol was measured in a representative selection of wines from most of the prominent wine-producing countries and regions of the world. With the exception of Swiss and German wines, virtually all of the white wines tested had *trans*-resveratrol concentrations <0.1 mg/L. Consistently high concentrations were measured in wines from Pinot noir, irrespective of origin. On the other hand, Cabernet Sauvignon wines covered a wide range of concentrations, with relatively high values in those from cool-climate countries such as Ontario and the Bordeaux region of France, whereas such wines from warmer climates such as California, South America, and Australia tended to have much lower concentrations. Differences between the communes of Bordeaux and Burgundy were noted, with Cotes de Nuits wines having higher concentrations than those of Cotes de Beaune, and in Bordeaux, wines of the Medoc, St. Emilion, and Pomerol had lower concentrations than those found in wines from other communes. Among the other prominent wine-producing regions, wines from Italy, Spain and Portugal tended to have low concentrations in line with their relatively warm and dry climatic conditions, but those of the Rhone Valley where the climate is similar had relatively high *trans*-resveratrol concentrations. The higher concentrations reported in this study compared with three earlier reports could be due to the quantitative recovery inherent in the solid-phase extraction used in the present assay, and/or losses occurring during organic phase extraction in the latter.

KEY WORDS: stilbenes, gas chromatography, mass spectrometry, Pinot noir, Cabernet Sauvignon, polydatin.

Trans-resveratrol (3,5,4'-trihydroxystilbene) has been identified as a constituent of various plant species including groundnuts (15,35), eucalyptus (13,14), *Pterolobium hexapetallum* (22), *Bauhinia racemosa* (1), and *Veratrum grandiflorum* (11). Other sources described in earlier literature up to 1980 are included in the review by Gorham (10). Much interest has focused upon its presence in vines and grapes because of its potent antifungal activity and its correlation with resistance to fungal infection (5,25,26,33,38). In vines and peanuts, its synthesis is stimulated by stress such as infection (18,27), UV light (8,28) and traumatic damage (3,28). Treatment with antifungal agents or chemicals may also enhance *trans*-resveratrol synthesis in susceptible species (6,11). The potential production of *trans*-resveratrol in response to UV irradiation varies with grape maturity and season, declining after veraison according to the species tested (4,16).

Because of the antifungal properties of *trans*-resveratrol, which have been demonstrated not only in vines but also in peanuts (39), cDNA clones for resveratrol synthase have been isolated from peanuts (36) and grapevines (30). These have been inserted into vectors and expressed in *E. coli* (30) and tobacco plants (12), emphasizing the potential agricultural applications of *trans*-resveratrol. Another field of biological interest was stimulated by reports that resveratrol is present in polygonaceous plants which have been widely used in Japan as a source of herbal medications for the treatment of fungal, inflammatory and lipid disorders (32). It was subsequently shown in a range of experiments that preparations of *trans*-resveratrol could modulate hepatic synthesis of cholesterol and triglyceride in the rat (2), inhibit 5-lipoxygenase activity and production of several arachidonate metabolites in rat polymorphonuclear leukocytes (21), antagonize antigen-induced contractions of guinea-pig trachea (34), and block the oxidation of human low-density lipoprotein which is believed to be a major mechanism in atherosclerosis (7).

Few studies have been carried out on the content of *trans*-resveratrol in wine, despite interest in its biological properties described above. Siemann and Creasy (37) reported very low concentrations in white wine, most having <0.03 mg/L, although three Chardonnays from New York State contained 0.05 to 0.10 mg/L. Most US red wines had low *trans*-resveratrol concentrations, but values up to 0.65 mg/L were found in red Bordeaux wines. The relatively low *trans*-resveratrol concentrations of red Californian wines were subsequently confirmed (23) although wines from Pinot noir had significantly higher levels (0.21 to 0.68 mg/L) than those from Cabernet Sauvignon and Zinfandel. Using solid phase extraction and high performance liquid chromatogra-

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phy (HPLC), Mattivi (29) measured *trans*-resveratrol concentrations up to 7.17 mg/L in red wines from the Trentino region of Italy. Highest concentrations were found in wines from Cabernet Sauvignon (1.33 to 7.17 mg/L), with those from Merlot (3.14 to 6.03 mg/L) and Pinot noir (3.22 to 5.93 mg/L) being next highest. The author did not specify whether the wines were finished (from bottle or from cask) and whether or not they had been filtered. Analyses carried out on Burgundy wines (Pinot noir) revealed total resveratrol concentrations (*cis* as well as *trans*) ranging from 0.4 to 2.0 mg/L (17). These authors also reported total resveratrol concentrations in white Burgundy wines from Chardonnay and Aligote ranging from 0.001 to 0.082 mg/L, values that are somewhat higher than the *trans*-resveratrol values found in Chardonnay wines from New York State (37). The paucity of data on the *trans*-resveratrol content of wines stems from the technical difficulties in its assay and the lack of availability of a representative selection of wines for analysis. We have overcome the first problem by developing a direct-injection Gas Chromatography-Mass Spectrometric (GC-MS) assay that allows samples to be analyzed at a rate of three per hour (9). Through the Liquor Control Board of Ontario, we have had access to all wines entering this province, since they must first be submitted to a Quality Assurance protocol before being released for public consumption. We have therefore taken the opportunity to measure the *trans*-resveratrol concentration in over 300 wines sampled in approximately 12 months, and we present our findings on the variation of this constituent in a wide range of wines classified as to country, region, and cultivar of origin.

Materials and Methods

Wines were randomly selected from approximately 1200 submitted for analysis to the Quality Assurance Laboratory of the Liquor Control Board of Ontario directly from the producer or negociant during calendar year 1993 and were analyzed within two weeks of delivery. They were kept at 4°C tightly stoppered in the dark for up to five days prior to *trans*-resveratrol assay in those instances where other constituents had first been analyzed. Under these conditions, *trans*-resveratrol is 100% stable for up to at least six weeks (unpublished observations). The GC-MS procedure for *trans*-resveratrol assay was performed as previously described (9). In brief, 1 mL of wine was passed through a preconditioned C-18 SPE cartridge (Supelco), and the retained *trans*-resveratrol was eluted with 1 mL of ethyl acetate, 1 μL of which was injected directly into a Hewlett Packard GC Model 5890 with quadrupole MS Detector (Model 5970) coupled through a DB-5 column, 30 m long, 0.25 mm internal diameter, and 0.25 μm thickness at initial and final oven temperatures of 190°C and 315°C, respectively. The molecular ion was detected and quantitated at a mass of 228, with qualifier ions at 229 and 227. The sensitivity of the assay as routinely employed was down to 0.05 mg/L. The linearity extended to 10 mg/L (it is recommended that samples be diluted and reanalyzed if this concentration is exceeded), and analytical precision (coefficient of variation) was 2.5% to 6.2%.

As standard, a preparation of *trans*-resveratrol was synthesized from appropriately substituted phenols by means of a Wittig reaction modified by Waterhouse (submitted to Anal. Chem.) from the method of Moreno-Manas and Pleixats (31). The final product was >95% pure by NMR and UV spectroscopy.

All data are given as mean ± SD (mg/L), and differences between the various wine categories were analyzed for statistical significance using Student's t-test. The validity of this procedure was confirmed by estimating the skewness and kurtosis of all data sets; moreover, the sets compared did not differ widely in numbers of data points or variance.

Results

White wines: Over 100 white wines were analyzed. Very few had *trans*-resveratrol concentrations >0.1 mg/L. All six Swiss wines tested had higher concentrations ranging from 0.11 to 0.62 mg/L. One of 22 French wines, a Sauternes (botrytized Sauvignon blanc and Semillon), exceeded this limit, having 0.27 mg/L. Of 24 wines from USA, all were <0.1 mg/L except for a Napa Sauvignon blanc (1.3 mg/L). All of 18 white wines from Hungary and Central Europe, 20 from Italy, seven from Canada, two from South America, and eight from Australia had <0.1 mg/L. This limit was exceeded in only three of 10 German wines, all from the Riesling grape, which ranged from 0.75 to 1.22 mg/L.

Fortified wines: Of six ports analyzed, only one (0.16) exceeded 0.1 mg/L. Similarly low *trans*-resveratrol concentrations were found in all of 4 sherries tested.

Red wines. *(a) Canadian:* Thirty-six Ontario wines of the 1990 and 1991 vintages were analyzed (Fig. 1). The mean ± SD *trans*-resveratrol concentration was 3.16 ± 1.34 mg/L. Non-VQA wines, *i.e.* those not made from locally grown grapes, had low concentrations.

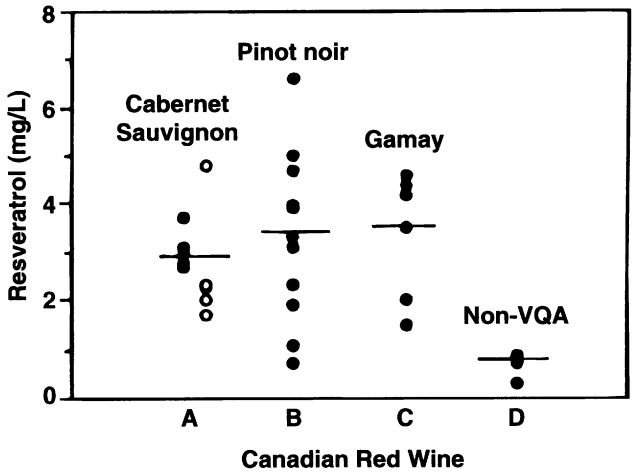

Fig. 1. *Trans*-resveratrol concentrations of Canadian (Ontario) red wines. Cabernet Sauvignon exclusively (●, n = 5) and predominantly (❑, n = 5); Pinot noir (n = 16); Gamay (n = 6); non-VQA (n = 4).

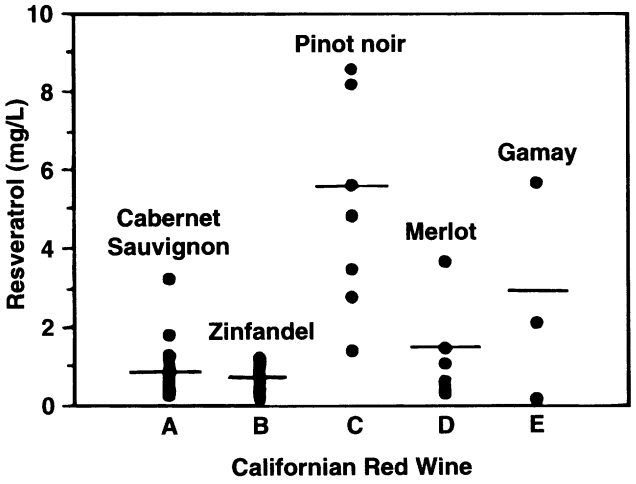

Fig. 2. *Trans*-resveratrol concentrations of Californian red wines. Cabernet Sauvignon (n = 30); Zinfandel (n = 14); Pinot noir (n = 7); Merlot (n = 7); Gamay (n = 3).

Among the VQA wines, those from Gamay noir, Pinot noir, Cabernet Sauvignon, and blends all had comparable mean values.

(b) Californian: Sixty-one wines were analyzed and are presented in Figure 2 according to grape varietal. Wines from Pinot noir were significantly higher than those from other varietals ($t = 8.12$; $p < 0.001$). The overall mean *trans*-resveratrol concentration for all these Californian wines was 1.47 ± 1.76 mg/L, significantly lower than the Canadian red wines ($t = 3.63$; $p < 0.001$).

(c) Australian: As shown in Figure 3, most wines had *trans*-resveratrol concentrations <3 mg/L. Wines that were entirely from Shiraz had higher concentrations than those from Cabernet Sauvignon or blends ($t = 2.55$; $p < 0.05$). The overall mean for all 41 was 1.47 ± 1.26, significantly lower than that of Canadian wines ($t = 5.30$; $p < 0.001$). Not shown in Figure 4 is the only Australian Pinot noir analyzed, which had a *trans*-resveratrol concentration of 13.4 mg/L.

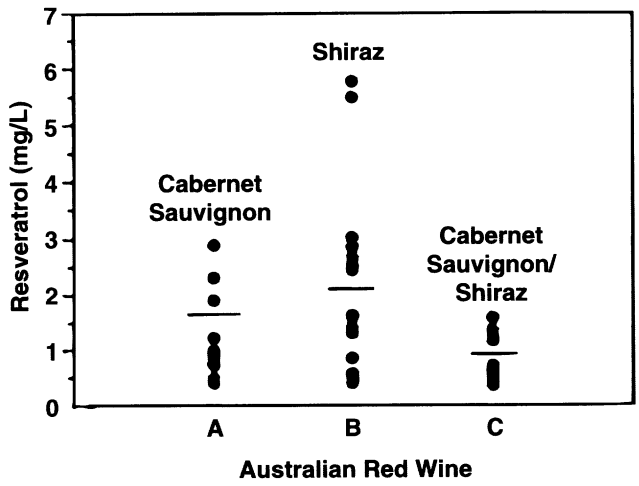

Fig. 3. *Trans*-resveratrol concentrations of Australian red wines. Cabernet Sauvignon (n = 13); Shiraz (n = 17); Cabernet-Shiraz blend (n = 11).

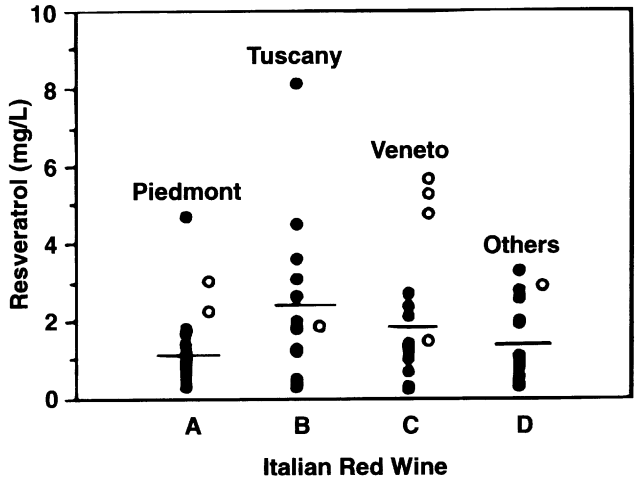

Fig. 4. *Trans*-resveratrol concentrations of Italian red wines. Piedmont (n = 19); Tuscany (n = 16); Veneto (n = 17); Others (n = 15). Open circles represent wines exclusively or predominantly from Cabernet Sauvignon.

(d) Italian: Sixty-seven wines were classified into three major areas: Tuscany where the Sangiovese grape predominates, Piedmont where Nebbiolo is the principal grape, and Veneto whose wines are made from a fairly wide range of cultivars (Fig. 4). In each region, certain wines were from Cabernet Sauvignon, and these tended to have higher *trans*-resveratrol concentrations than those from other sources. The differences between regions were not significant. Overall, the mean ± SD was 1.76 ± 1.51, significantly lower than the *trans*-resveratrol concentration of Canadian wines ($t = 4.23$; $p < 0.001$).

(e) French: These were subdivided into specific regions as follows:

(i) Rhone Valley: Quite high *trans*-resveratrol concentrations occurred in all wines from this region (Fig.

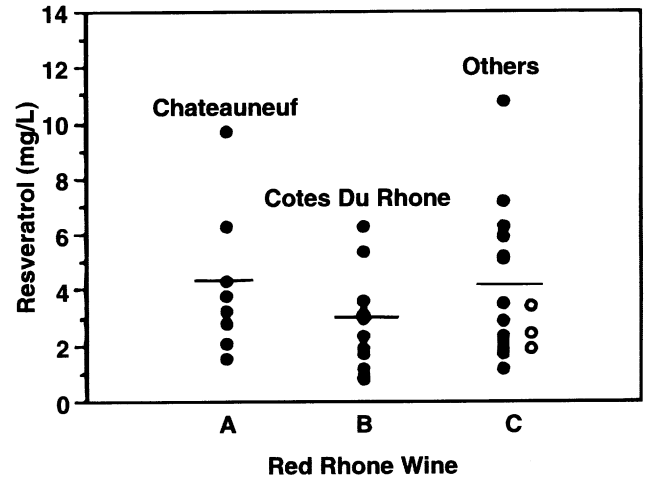

Fig. 5. *Trans*-resveratrol concentrations of Rhone Valley red wines. Chateauneuf-du-Pape (n = 8); Cotes du Rhone (n = 11); Others (n = 17, open circles represent the 3 Hermitage wines).

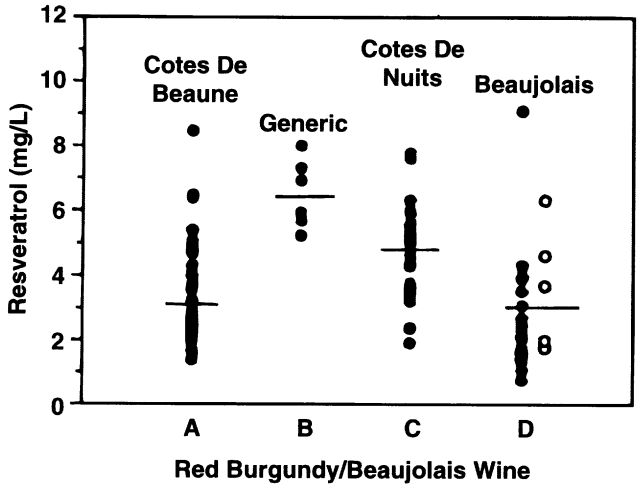

Fig. 6. *Trans*-resveratrol concentrations of red Burgundy (var. Pinot noir) and Beaujolais (var. Gamay) wines. Cotes de Beaune (n = 33); Cotes de Nuits (n = 22); Generic (n = 6); Beaujolais (n = 30, with 6 Beaujolais Nouveau indicated by open circles).

5). There was little difference among those of Chateau neuf, Cotes de Rhone, and other areas (including Gigondas, Cote Rotie, and Hermitage, all three wines in the last category being identified as open circles in Fig. 5). Overall, the mean for all 36 wines was 3.60 ± 2.38. This was significantly higher than those of Italy ($t = 4.79$; $p < 0.001$), California ($t = 5.05$; $p < 0.001$) and Australia ($t = 5.00$; $p < 0.001$).

(ii) Beaujolais (var. Gamay): The mean of 30 samples was 2.88 ± 1.72 mg/L (Fig. 6). They were all from the 1990 and 1991 vintages except for six "Beaujolais nouveaux" indicated as open circles in Figure 6. These *trans*-resveratrol values were higher than those of Italian ($t = 3.23$; $p < 0.005$), Californian ($t = 3.63$; $p < 0.001$), and Australian wines ($t = 3.99$; $p < 0.001$). Two rosé wines not included in Figure 6 had concentrations of 0.23 and 0.10 mg/L.

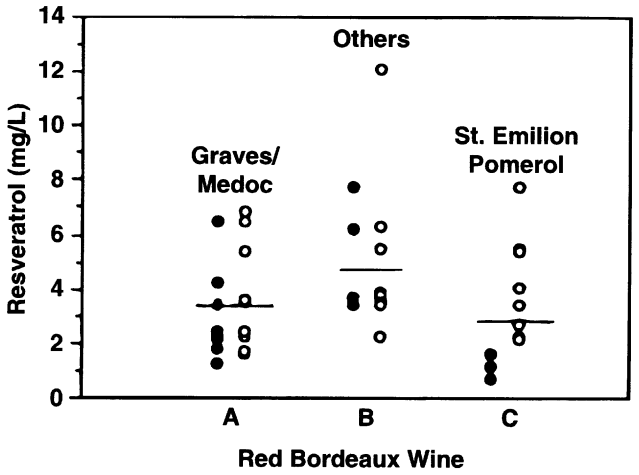

Fig. 7. *Trans*-resveratrol concentration of red Bordeaux wines. Open circles represent wines from the 1990 vintage, all others being 1989 or older. Graves/Medoc (n = 18); St. Emilion/Pomerol (n = 12); Others (n = 14).

(iii) Bordeaux: Figure 7 displays the *trans*-resveratrol concentrations of 44 wines from this region. Wines from the Medoc and St. Emilion-Pomerol had lower concentrations than those from the other Bordeaux communes ($t = 2.25$; $p < 0.05$). Most of these wines were from the 1989 and 1990 vintages, although about 20% were from older vintages. The values for wines of the 1990 vintage were in the same range as those of older vintages except in St. Emilion-Pomerol, where they were significantly higher (see open circles, Fig. 7). The overall mean (3.89 ± 2.20 mg/L) was higher than that of Italian ($t = 6.02$; $p < 0.001$), Californian ($t = 6.25$; $p < 0.001$), and Australian wines ($t = 6.14$; $p < 0.001$).

(iv) Burgundy: These wines had the highest resveratrol concentration among all the French wines analyzed in the present study (Fig. 6). Wines (var. Pinot noir) from the Cotes de Nuits (4.72 ± 1.52 mg/L) had higher concentrations than those from the Cotes de Beaune (3.79 ± 1.62 mg/L; $t = 2.14$; $p < 0.05$), but surprisingly, generic wines bearing the appellation "Bourgogne Rouge" (6.51 ± 1.08 mg/L) had higher values than those of the previous two appellations ($t = 3.43$; $p < 0.01$). All but 12 of the 61 wines were from the 1990 vintage, considered enologically to be the 'best' of the decade, but the *trans*-resveratrol content of the older wines, mostly from the 1989 and 1988 vintages, was surprisingly high (5.25 ± 1.84 mg/L), suggesting that it remained stable for at least the first few years after bottling in agreement with the findings of Jeandet *et al.* (19). Taken together, the overall mean for these Burgundy wines, 4.39 ± 1.73 mg/L, was significantly higher than that of Italian ($t = 9.16$; $p < 0.001$), Californian ($t = 6.25$; $p < 0.001$), Australian ($t = 9.26$; $p < 0.001$), Beaujolais ($t = 3.92$; $p < 0.001$), and Canadian wines ($t = 3.32$; $p < 0.005$).

(v) Others: Seven wines of the Midi from vintages between 1989 and 1991 had *trans*-resveratrol concentrations ranging from 0.88 to 12.1 mg/L (mean, 3.90). Two Alsatian Pinot noirs had 2.96 and 7.94 mg/L, and a Pinot noir from the Loire Valley had 10.8 mg/L.

(f) Central Europe: Ten red wines from Hungary, Bulgaria, and Slovakia had a mean *trans*-resveratrol concentration of 3.26 ± 1.18 mg/L. They were derived from many different cultivars including Merlot, Cabernet Sauvignon, Cinsault, and Zweigelt.

(g) Spain and Portugal (Iberian Peninsula): The 26 wines from this region could not be classified as to grape of origin, but their mean *trans*-resveratrol concentration was 1.64 ± 0.95 mg/L (Fig. 8).

(h) Other European: Five red wines from Greece had *trans*-resveratrol concentrations ranging from 0.6 to 4.3 mg/L (mean 1.95). Six wines from Switzerland had concentrations ranging from 5.0 to 12.3 mg/L and, like the white wines from this country, had the most consistently high concentrations recorded for any country or region.

(i) South America: The 23 wines from Chile and Argentina comprised 13 Cabernet Sauvignon, and one each of Merlot, Malbec, and Syrah. The remaining

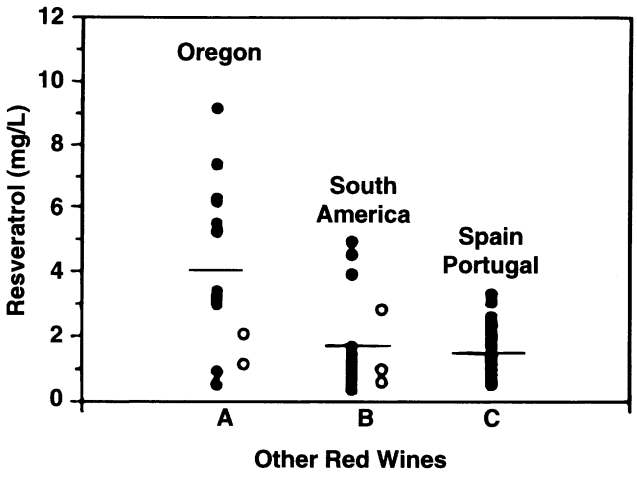

Fig. 8. *Trans*-resveratrol concentrations of red wines from Oregon (n = 16), South America (n = 23) and the Iberian Peninsula (n = 26). Open circles represent wines from Cabernet Sauvignon grapes (Oregon) and wines other than Cabernet Sauvignon (South America).

seven were blends of Cabernet Sauvignon with other grapes. Their *trans*-resveratrol concentrations ranged from 0.54 to 4.95 mg/L (1.21 ± 0.95), with no striking difference between the Cabernet Sauvignons and the other cultivars (Fig. 8).

(j) Oregon: All but two of the 16 red wines from this region were from Pinot noir. The latter ranged from 0.9 to 9.1 mg/L in *trans*-resveratrol concentration (mean 4.30 ± 2.27), with the one wine each of Merlot and Limburger being towards the lower end of this range (Fig. 8).

Discussion

From this survey, two main conclusions can be drawn. First, wines from Pinot noir grapes seem to have high *trans*-resveratrol concentrations relative to those from other cultivars irrespective of the country or region of origin. This holds true for Burgundy, California, and Oregon. High *trans*-resveratrol concentrations were uniformly found in the very few Pinot noir wines from other origins such as Australia, Alsace, and the Loire Valley. Thus, it seems that climate and terroir do not influence the *trans*-resveratrol concentrations which this particular grape yields on vinification. Since the highest concentrations in the grape skin occur at veraison and decline thereafter (4,16), the high concentrations in these wines may be due to the earlier harvesting of this grape which is notoriously susceptible to inclement weather conditions towards the time of harvesting. Alternatively (or conjointly), the characteristically thin skin of this cultivar renders it especially prone to fungal infection and hence induction of *trans*-resveratrol synthesis (18,19).

It is somewhat paradoxical that wines from Pinot noir grapes have higher *trans*-resveratrol concentrations than those from Cabernet Sauvignon and Chardonnay since the content in the leaves of these grape varietals is in the order Pinot noir < Chardonnay < Cabernet Sauvignon (20). It seems that the *trans*-resveratrol content of leaves does not necessarily reflect that of the berry skins (4) or of the wine which is vinted from the latter (37).

The second conclusion is that the *trans*-resveratrol concentration of wines from the Cabernet Sauvignon grape show marked fluctuations which appear to be temperature-dependent. Thus, Cabernet Sauvignon wines from California, Australia, and South America have much lower *trans*-resveratrol concentrations than those from Bordeaux and Ontario. The cooler and more humid conditions in the latter two regions may partly explain these differences.

Wines from Italy and the Iberian peninsula which again are subject to warmer and drier conditions tend to have low *trans*-resveratrol concentrations. However, those from the Rhone Valley, which can also be described as relatively warm and dry, have high *trans*-resveratrol concentrations. It is therefore probable that these differences between three regions of climatic similarity are due to the intrinsic resveratrol-synthesizing capacity of the different cultivars employed. For example, the majority of the Italian wines we sampled were from the Nebbiolo or Sangiovese grapes; those from the Rhone Valley were vinted from Grenache, Mourvedre, Syrah, and Carignan grapes; those of Spain were from Tempranillo, Garnacha, and Graciano; those from Portugal tended to be blends of Bastardo, Alvarelhao, and Touigo. The Ontario wines which we sampled had relatively high concentrations irrespective of the cultivar when they were of certified origin (Vintners Quality Alliance, VQA). The non-VQA wines which derive wholly or partly from imported juices originating from California, Australia, or South America had lower *trans*-resveratrol concentrations in line with those of wines from the respective countries of origin.

We did find some differences between various communes within a single region: thus, among Burgundy wines, those from the Cotes de Nuits had higher concentrations than those from the Cotes de Beaune, and wines from the Medoc and St. Emilion-Pomerol had lower concentrations than those from other regions of Bordeaux.

Like Jeandet *et al.* (19), we did not detect reduction of *trans*-resveratrol concentration according to age, as would be expected if it diminished with storage. In this regard, it is interesting that Mattivi (29) reported values for Cabernet Sauvignon wines of three vintages, the oldest of which had the highest and the youngest the lowest *trans*-resveratrol concentrations. In this respect, the differences may equally reflect the climatic and harvest-time conditions of the various vintages such as fungal disease pressure (18). We have found the concentration of *trans*-resveratrol to be very stable when wine samples have been stored in dark glass ampoules (similar in color but less thick than commercial wine bottles) at both room temperature and 4°C without exposure to direct sunlight (Goldberg *et al.*, submitted to Anal. Chem.).

The *trans*-resveratrol concentrations reported in

our work are very much higher than those in the publications by Siemann and Creasy (37), Lamuela-Raventos and Waterhouse (23), and Jeandet *et al.* (17). The highest total resveratrol (*cis* plus *trans*) concentration of any Burgundy wine recorded by Jeandet *et al.* (17) was around 2 mg/L. The highest *trans*-resveratrol concentration of any wine reported by Siemann and Creasy (37), a red Bordeaux, was <1 mg/L, and the values assayed by Lamuela-Raventos and Waterhouse (23), all wines being from Californian grapes, were much lower. In agreement with all these authors, we found that white wines had very low *trans*-resveratrol concentrations despite high skin concentrations (4,16), presumably due to minimal skin contact associated with white wine production (35). It is, however, remarkable that the white and red wines of Switzerland were the highest in their respective categories. While these uniformly high levels could be due to grape varietal alone, it is possible that the rigorous climatic conditions which these vines have to survive stimulate their production of *trans*-resveratrol, or that the differences can be explained by vinification procedures such as carbonic maceration, filtration, and barrel-aging. These variables are currently under investigation by our group for Niagara wines.

The methods developed by Siemann and Creasy (37), Lamuela-Raventos and Waterhouse (23), and Jeandet *et al.* (17) all employed organic-phase extraction prior to the subsequent analysis which was by HPLC in the first two and GC in the last. Our data are more compatible with those of Mattivi (29), who like ourselves, used a solid phase extraction prior to analysis by HPLC and GC-MS, respectively. It should also be noted that whereas Jeandet *et al.* (17) used GC as the final step in their analysis, derivitization was first carried out prior to injection on to the column, whereas in our method 1 μL of the ethyl acetate extract eluting from the C18 column was injected directly. Thus, the lower results reported in previous papers could have arisen from significant losses during the organic solvent phase extractions. It is also worth noting that Mattivi (29) employed *trans*-4-hydroxystilbene as an internal standard to correct for recovery in his analytical procedure. It is not known as yet whether the 3-ß-glucoside of *trans*-resveratrol (piceid or polydatin), which is present in a number of grape species (24), is hydrolyzed and/or volatilized, at least in part, after injection into the GC-MS and thereby contributes to the abundance at mass 228 *etc.*, augmenting the peak (and qualifiers) attributed to free *trans*-resveratrol. This glycoside seems to be present in high amounts in certain French red wines (P. Jeandet, personal communication, 1993), Finally, although the data presented and cited in this paper all relate to *trans*-resveratrol, we have, in line with Jeandet *et al.* (17), noted the occurrence of *cis*-resveratrol in wines, and have begun a systematic study of the relationship between the two isomers of resveratrol in wines of different origins.

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