Direct Gas Chromatographic–Mass Spectrometric Method To Assay cis-Resveratrol in Wines: Preliminary Survey of Its Concentration in Commercial Wines

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A method to measure *cis*-resveratrol in wine, as well as *trans*-resveratrol simultaneously, has been developed. One milliliter of wine is subjected to solid phase extraction on a C_{18} column followed by elution with ethyl acetate, and 1 µL of eluate is directly injected into a gas chromatographic–mass spectrometer. Quantitation uses selective ion monitoring at mass 228 with qualifier ions at masses 227 and 229. The method gives excellent linearity and recovery, with a detection limit of 10 µg/L and a coefficient of variation in the range of 5–6%. In a survey of >450 commercial red wines, the highest concentrations of *cis*-resveratrol were found in wines from Burgundy (France), Oregon (United States), Switzerland, and Bordeaux (France). Wines from New World (other than Oregon) and Mediterranean countries had much lower concentrations. Climate and fungal pressure appear to be among the determinants of the *cis*-resveratrol as well as the *trans*-resveratrol content of commercial red wines.

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin which is present in a number of plant species (Hillis *et al.*, 1974; Ingham, 1976; Kumar *et al.*, 1988; Hanawa *et al.*, 1992), but it has evoked the widest interest as a constituent of grape berries and other components of the Vitaceae. In *Vitis vinifera*, its synthesis is enhanced by UV light, injury, and fungal infection (Langcake and Pryce, 1976, 1977; Jeandet *et al.*, 1994), and it may confer protection against the latter eventuality. Even more intriguing has been its relatively high concentration in oriental folk medicine (Nonomura *et al.*, 1963). These studies were followed by investigations of its biological activity, which suggested that it might have properties conducive to protection against atherosclerosis and coronary heart disease (Arichi *et al.*, 1982; Kimura *et al.*, 1985; Frankel *et al.*, 1993). In a landmark publication, Siemann and Creasy (1992) described its presence in commercial table wines and proposed that it may be responsible for the decreased mortality from coronary heart disease associated with wine-drinking populations (St. Leger and Cochrane, 1979; Hegsted and Austad, 1988; Renaud and De Lorgeril, 1982).

Although resveratrol exists in two isomeric forms, all of the above investigations focused only upon the *trans*-isomer, since the *cis*-isomer has never been identified in grapes. Further publications described the concentrations of *trans*-resveratrol in California (Lamuela-Raventos and Waterhouse, 1993), Italian (Mattivi, 1993), Burgundy (Jeandet *et al.*, 1993), and Swiss wines

(Pezet *et al.*, 1993). Using a newly developed direct-injection gas chromatographic–mass spectrometric (GC–MS) method (Goldberg *et al.*, 1993a), we reported our results with a large range of commercial wines and noted marked regional and varietal dependent differences in their *trans*-resveratrol concentrations (Goldberg *et al.*, 1993b). Very recently, Jeandet *et al.* (1993), using a method based upon gas chromatography (GC), reported that *cis*-resveratrol was the predominant isomer in the red Burgundy wines that they analyzed and confirmed these findings in a longitudinal study utilizing high-performance liquid chromatography (Jeandet *et al.*, 1995). We found relatively high concentrations of this isomer in red wines from Ontario using a GC–MS method requiring prior derivatization with bis[trimethylsilyl]trifluoroacetamide (BSTFA) (Soleas *et al.*, 1994).

Because the potential biological significance of the *cis*-isomer has been suggested by a report that its potency as an inhibitor of protein kinases is equal to that of *trans*-resveratrol (Jayatilake *et al.*, 1993), we have developed a direct GC–MS assay for both isomers which circumvents the need for derivatization, and we have compared their concentrations in a wide range of commercial wines from the major wine-producing countries.

MATERIALS AND METHODS

Sample Preparation. C_{18} SPE bonded porous silica cartridges from Supelco Inc., Bellefonte, PA (500 mg, 3 mL volume), were preconditioned with 3 mL of ethyl acetate followed by 3 mL of 96% (v/v) ethanol and twice with 3 mL of 10% (v/v) ethanol. One milliliter of sample was applied, and the cartridge was dried for 45 min under vacuum with nitrogen gas. The adsorbed resveratrol was eluted with 2 mL of ethyl acetate, and the first 1 mL was collected for analysis. Gravity flow was used in the above procedures. The cartridge can be

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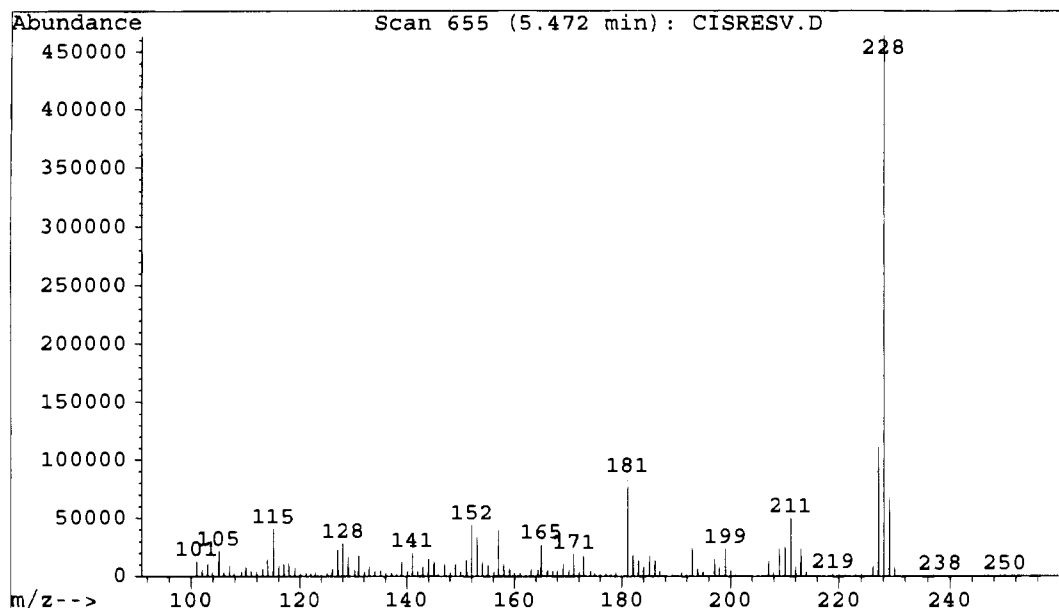**1246** *J. Agric. Food Chem.,* Vol. 43, No. 5, 1995

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Figure 1. Mass ion spectrum of *cis*-resveratrol eluting at retention time of 5.47 min demonstrating characteristic ion cluster of mass 228 (molecular ion, used for quantitation) together with qualifier ions of mass 229 (C-isotope) and 227 (M − H). The other ions present in this scan correspond in relative abundance to those of pure *trans*-resveratrol, which elutes at 6.44 min in this system.

reconditioned with zero carryover up to 10 times with 60% (v/v) ethanol and storage in 96% (v/v) ethanol.

GC−MS Analysis. One microliter of ethyl acetate eluent was introduced by an automatic sample injector (Hewlett-Packard Model 76733 GC/SFC) into a Hewlett-Packard GC Model 5890 with quadrupole MS Detector (Model 5970) coupled through a DB-17 ht column (J&W Scientific, Folsom, CA) 15 m long, 0.25 mm internal diameter, and 0.15 μm film thickness. The following temperature program was used: initial 150−160 °C held for 6 min; raised at 20 °C/min to 290 °C, held for 2 min; 25 °C/min to 305 °C, held for 5 min. The peaks for *cis*- and *trans*-resveratrol had retention times of approximately 4.3 and 5.7 min, respectively, and were quantitated by selective ion monitoring (SIM) using the molecular ion at mass 228, with M − H (mass 227) and the C-isotope (mass 229) employed as qualifiers.

Calibration. *trans*-Resveratrol (catalog no. R 5010) was purchased from Sigma, St. Louis, MO, and a stock solution of 1142 mg/L in 96% (v/v) ethanol was maintained at 4 °C in a dark container for up to 4 months. Five standards covering the range 0.1−10.0 mg/L were made up in the ethyl acetate eluate of a red wine sample from the C_{18} cartridge and analyzed in duplicate. Calibration curves were constructed after the matrix signal due to the unspiked wine eluate was subtracted.

For *cis*-resveratrol calibration, we irradiated standards of *trans*-resveratrol for 10 min in a UV box at 254 nm and intensity 990 μW/cm^2 (Chromato-Vue, Ultraviolet Products, San Gabriel, CA). This converted approximately 70% of the *trans*-resveratrol to an earlier-eluting peak which yielded a mass ion spectrum (Figure 1) identical to that which we have already determined for *trans*-resveratrol (Goldberg et al., 1993a). Moreover, the ion abundance of this peak was exactly equal to the difference in ion abundance of the authentic *trans*-resveratrol peak before and after irradiation. Assuming the reduction in *trans*-resveratrol by irradiation to equal the generation of *cis*-resveratrol, we constructed a calibration curve for the latter at six concentrations over the range 0.1−12.5 mg/L. Linearity was excellent ($r = 0.999$), and the slope of the line relating abundance to concentration was identical to that for *trans*-resveratrol, yielding the following regression equation: y (abundance) $= m$ (slope, abundance mg^{-1} L) × (concentration, mg L^{-1}) $+ C$ (intercept, abundance), where $m = 49927$ (SE, 8650; $P < 0.0001$) and $C = -29610$ (SE, 58058; $P = 0.64$). The calibration curve constructed with the latter was therefore used to quantitate both isomers.

RESULTS

Characteristics of the GC−MS Technique. Figure 2A demonstrates the total ion chromatogram of a red wine eluate from a C_{18} SPE column directly injected into the GC−MS. This wine was devoid of any peaks corresponding to *cis*- or *trans*-resveratrol. Figure 2B is a total ion chromatogram of the same wine spiked with *trans*-resveratrol irradiated for 30 min to achieve almost complete conversion to the *cis*-isomer at a retention time of 5.47 min. The small peak at 6.44 min represents residual *trans*-resveratrol. With time, increased baseline and some degradation of peak contours occur, but the original resolution can be restored by removing the first 50 cm of the column.

Since the analytical features of the *trans*-resveratrol assay in the present work were very similar to those we have already reported (Goldberg et al., 1993a), emphasis will be given to our experience with the assay of *cis*-resveratrol.

Recovery. A *trans*-resveratrol standard was irradiated for various intervals of time to generate four concentrations of the *cis*-isomer. These concentrations were quantitated by GC−MS, and a portion of each solution was added to four different wines which had been previously analyzed. After mixing, 1 mL was taken for solid phase extraction and direct injection. The calculated recoveries (Table 1) averaged 95.2% overall, with a range of 92.2−97.5%. Care must be taken to ensure that the cartridges are perfectly dry before elution with ethyl acetate. With this precaution, we concluded that recoveries were satisfactory and did not require correction.

Detection Limit. On the basis of three SD of replicate analyses of wines with low *cis*-resveratrol concentration and zero signal with pure ethyl acetate, the lowest concentration significantly different from zero was 10 μg/L.

Precision. This was determined by performing 10 replicate analyses on two wines, with each replicate eluted from a C_{18} cartridge independently. The results as mean ± SD (CV %) were as follows: 0.33 ± 0.02 (6.1) and 4.34 ± 0.23 (5.3) mg/L.

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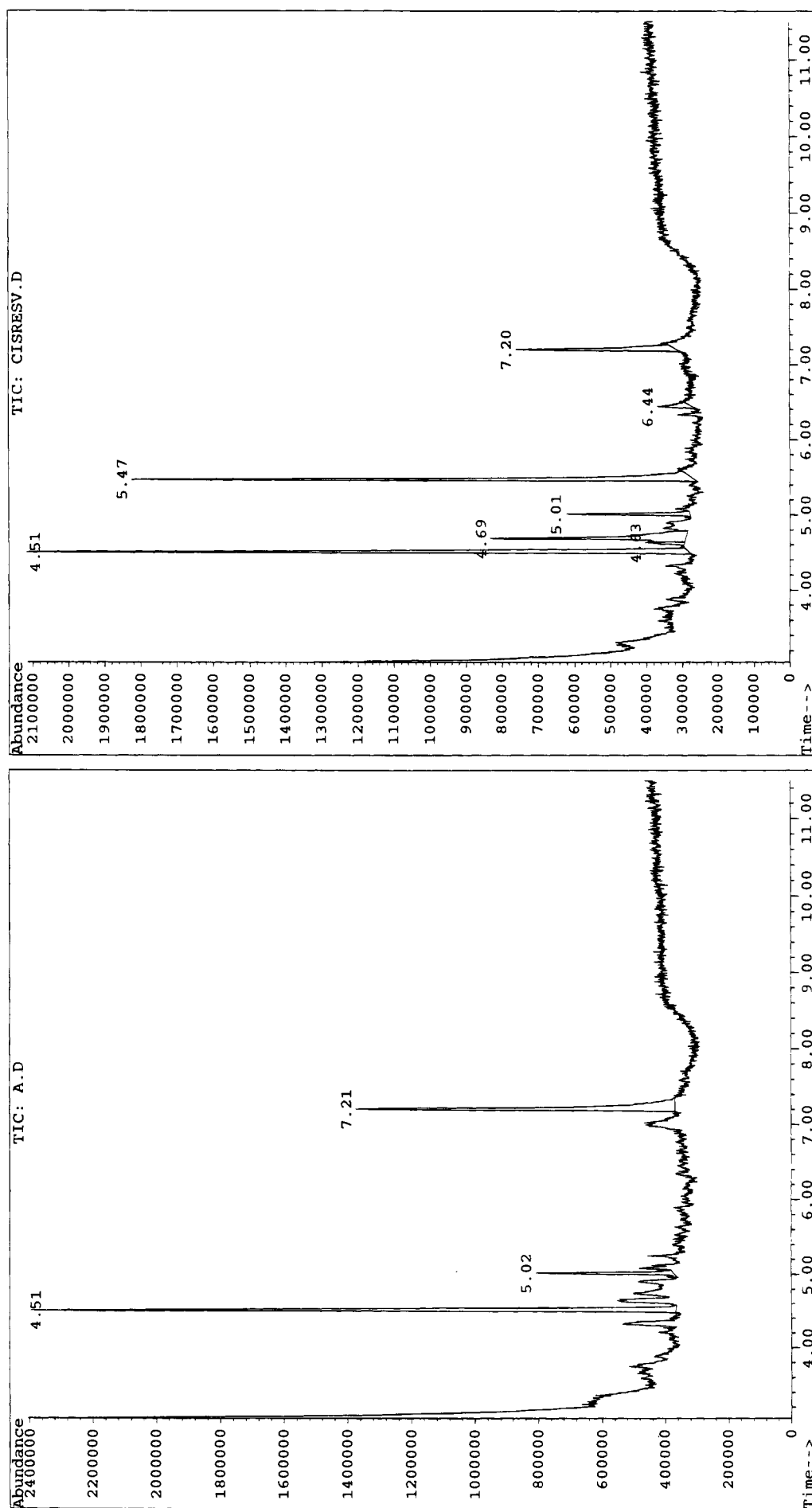*J. Agric. Food Chem.*, Vol. 43, No. 5, 1995 **1247**

Figure 2. (A, Left) Total ion spectrum of a red wine eluate with no detectable *cis*- or *trans*-resveratrol (Californian Red Burgundy, nonvintage). (B, Right) Total ion spectrum of eluate of same wine to which *cis*-resveratrol (prepared by irradiation of a solution of pure *trans*-resveratrol for 30 min) was added to a final concentration of 10 mg/L. The peak at 5.47 min is that of *cis*-resveratrol, and that at 6.44 min is due to a trace of residual *trans*-resveratrol.
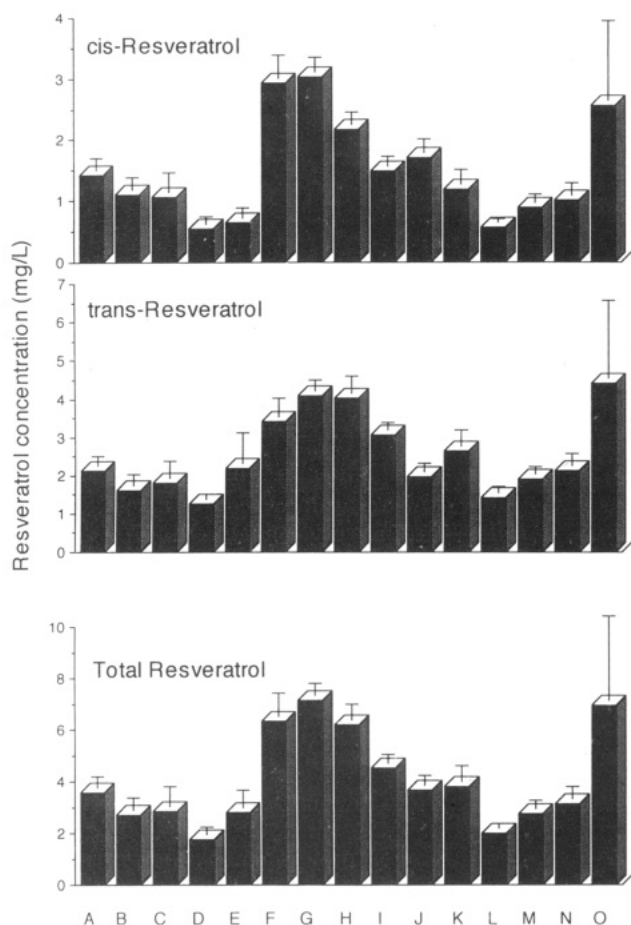
Figure 3. Concentrations of *cis*-resveratrol (top), *trans*-resveratrol (middle), and total resveratrol (bottom) in commercial red wines from various countries and regions as follows: (A) Canada, Ontario (*n* = 38); (B) United States, California (*n* = 62); (C) Australia (*n* = 39); (D) South America (*n* = 25); (E) South Africa (*n* = 11); (F) United States, Oregon (*n* = 14); (G) France, Burgundy (*n* = 44); (H) France, Bordeaux (*n* = 40); (I) France, Rhone Valley (*n* = 40); (J) France, Beaujolais (*n* = 18); (K) France, Midi (*n* = 17); (L) Italy (*n* = 69); (M) Spain and Portugal (*n* = 29); (N) central Europe (*n* = 16); (O) Switzerland (*n* = 6). Columns and vertical bars represent mean and SE, respectively.

Table 1. Recovery of *cis*-Resveratrol Added to Four Different Wine Samples at Four Different Concentrations after the Endogenous Wine Concentrations Are Subtracted

added (mg/L)	found (mg/L)	recovery (%)
3.1	3.0 ± 0.08	97.5 ± 2.5
5.7	5.5 ± 0.09	97.0 ± 1.6
10.5	9.7 ± 0.38	92.2 ± 3.7
13.3	12.6 ± 0.41	94.4 ± 3.1

Stability. Twenty wines, after the initial analyses, were capped and kept in the dark for up to 6 weeks at room temperature and at 4 °C. The *cis*-resveratrol concentrations were stable at the latter temperature and at room temperature for 7 days, but a mean decline to 67% of the initial value occurred after 6 weeks at room temperature ($P < 0.001$, paired *t*-test). Even when stored in the dark uncapped, *cis*-resveratrol remained stable for up to 48 h at both temperatures.

Resveratrol Concentrations of Commercial Red Wines. In an 8-month period commencing January 1, 1994, more than 450 red wines submitted to the Liquor Control Board of Ontario were analyzed within 2 weeks of arrival from the vineyard or negotiant. The data are presented in Figure 3.

cis-Resveratrol Concentrations. Among New World wines, the concentrations in Canadian red wines (mean

1.43 mg/L) were higher than those from California (mean 1.10 mg/L), Australia (mean 1.07 mg/L), South America (mean 0.54 mg/L), and South Africa (mean 0.64 mg/L), but the highest concentrations in wines of this category were found in those of Oregon (mean 2.94 mg/L).

Of French wines, Burgundy reds had much the highest concentrations (mean 3.04 mg/L), followed by the wines of Bordeaux (mean 2.16 mg/L), Beaujolais (mean 1.70 mg/L), and the Rhone Valley (mean 1.49 mg/L). Only those of the Midi (mean 1.19 mg/L) fell within the range of means encountered in New World wines.

Wines from the remaining countries of Europe had lower concentrations than those of France, apart from Switzerland (mean 2.55 mg/L), with Italian red wines having especially low concentrations (mean 0.56 mg/L).

trans-Resveratrol Concentrations. The data presented here support the general conclusions of our earlier study in which we surveyed the concentrations of this constituent in >300 commercial wines (Goldberg *et al.*, 1993b) and will therefore not be described further.

Total Resveratrol Concentrations. These followed a similar pattern to the *cis*-resveratrol concentrations, with Canadian red wines having higher concentrations than those of wines from other New World countries apart from those of Oregon, for which the mean concentration was 6.33 mg/L, while the wines from South America were the lowest in the entire survey (mean 1.78 mg/L). For French wines, the mean total resveratrol concentrations increased in the order Beaujolais (3.66 mg/L) < Midi (3.81 mg/L) < Rhone (4.53 mg/L) < Bordeaux (6.18 mg/L) < Burgundy (7.13 mg/L), wines from the last-named region having the highest concentration of any region in this survey. Of the remaining European wines, those from Italy (mean 1.98 mg/L), Spain and Portugal (mean 2.77 mg/L), and central Europe (mean 3.14 mg/L) were rather low, but those from Switzerland (mean 6.94 mg/L) had the second highest concentrations in this survey.

DISCUSSION

Up to now, few methods to measure *cis*-resveratrol concentrations have been published. Jeandet *et al.* (1993) described a GC method and subsequently a high-performance liquid chromatographic (HPLC) method (Jeandet *et al.*, 1995) for this purpose. Both require large starting volumes (100 mL) followed by multiple solvent extractions, whereas our method requires only 1 mL of sample. The solid phase extraction that we utilize yields excellent recovery for both resveratrol isomers; this recovery is likely to be higher than that of Jeandet *et al.* (1993, 1995), who did not report on this characteristic in either of their papers. In line with this notion, our *trans*-resveratrol concentrations in Burgundy wines are approximately 800% and our *cis*-resveratrol concentrations 300% higher than those reported by these authors. We have described a GC–MS method for both resveratrol isomers utilizing BSTFA derivatization prior to injection (Soleas *et al.*, 1994), but the present direct injection method circumvents this step and is consequently faster, cheaper, and technically more simple to perform.

The method that we have developed represents a modification of our previously published method for *trans*-resveratrol, which also uses a direct injection GC–MS procedure, but the new method has been facilitated by a different stationary phase, a shorter column, and a faster temperature program. GC and HPLC methods require pure *cis* standards for calibration since the absorbance spectra of the isomers are very different

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(Siemann and Creasey, 1992) and equivalence of their response in flame ionization or electron capture detection systems cannot be assumed. We have found it extremely difficult to synthesize cis-resveratrol by the Wittig condensation technique suitable for synthesis of the trans-isomer (Moreno-Manas and Pleixats, 1985). Under the conditions employed, most of the cis product undergoes rapid isomerization and cannot readily be stabilized to allow its purification in adequate yield (Goldberg et al., 1993a). Although Jeandet et al. (1993, 1995) have claimed to have synthesized cis-resveratrol for use as standards in their methods, they have not presented details of their procedure or proof of purity of the isolated material. In our method, the absence of a pure standard is not a handicap. It can be generated by UV irradiation of trans-resveratrol, with which it shares an identical mass spectrum and ion abundance. The calibration curve for both isomers may therefore be prepared with pure synthetic trans-resveratrol, which has recently become available commercially from Sigma. Its universal use as a calibrant could remove one major source of variability between laboratories which are currently utilizing different preparations that are either synthesized or purified from natural sources.

In general, the characteristics of the cis-resveratrol assay match those that we have already reported by direct injection GC−MS for the trans-isomer (Goldberg et al., 1993a). Both isomers can be quantitated with good sensitivity and precision and linearity over a wide dynamic range of concentrations that encompasses those encountered in >98% of commercial red wines. Because recovery of the trans-isomer diminishes at concentrations >10 mg/L, we recommend dilution of samples exceeding this value. Recovery of the cis-isomer averages around 95% at all concentrations. Both isomers are also stable when protected from light for at least 6 weeks at 4 °C and are not prone to oxidation within at least 48 h of exposure to air.

The survey that we have conducted on commercial red wines is by far the largest yet reported and the only one to examine wines outside a narrow geographical area such as the United States (Siemann and Creasey, 1992; Lamuela-Raventos and Waterhouse, 1993), Burgundy (Jeandet et al., 1993), Italy (Mattivi, 1993), and Ontario (Soleas et al., 1994). This survey has generated many interesting observations for which conclusive explanations cannot be provided. Rather, they form a basis for speculation and the formulation of hypotheses that can be tested prospectively in a rigorous and objective manner.

Despite the fact that cis-resveratrol has not been detected in grapes or other vine products (Jeandet et al., 1993; Soleas et al., 1994), it was nevertheless present in all wines that we analyzed. Its concentration was usually lower than that of the trans-isomer, but on occasions it was the predominant form. This suggests that it is produced during fermentation, but it is not known how this occurs. UV irradiation is unlikely to be a factor. The glucoside of trans-resveratrol, polydatin, is present in grape berries (Waterhouse and Lamuela-Raventos, 1994) and it is also found in wines from the Rhone Valley (Roggero and Archier, 1994). We have preliminary evidence (unpublished) that a β-glucoside of cis-resveratrol can be extracted from grape skins but await definitive identification. If this can be confirmed, hydrolysis of cis-polydatin could account, at least in part, for the appearance of cis-resveratrol in wine. It is possible that yeast enzymes catalyze isomerization of trans-resveratrol or release of the cis-isomer through the degradation of viniferins, polymers of resveratrol which

are found quite extensively in berry skins (Langcake, 1981). Wine-making techniques, especially those that determine the time and temperature of fermentation, duration of skin contact, strain of yeast, barrel-aging, and use of clarification procedures, may influence these processes. Some of these variables have been tested for their contribution to trans-resveratrol concentration of wines from northern Italy (Mattivi and Nicolini, 1993), and we are currently examining the influence of these factors in Ontario wines; however, more extensive investigations in other wine-growing regions would be advantageous to secure definitive answers to these questions.

The highest concentrations of cis-resveratrol were present in red wines of Burgundy (mean 3.04 mg/L) and Oregon (mean 2.94 mg/L), which are produced from var. Pinot Noir. Next highest concentrations were found in Swiss red wines (mean 2.55 mg/L), which are predominantly produced from Pinot Noir with or without var. Gamay. This latter is the varietal from which Beaujolais is vinted, and apart from the wines of Bordeaux, these wines had the next highest cis-resveratrol concentration (mean 1.70 mg/L). This suggests that certain grape varietals, at least Pinot Noir and Gamay, are characteristic in producing wines enriched in cis-resveratrol.

On the other hand, the wines of Canada, California, South America, and Australia were predominantly from var. Cabernet Sauvignon, and yet their mean cis-resveratrol concentrations varied over a nearly 3-fold range from 0.54 (South America) to 1.43 (Canada), suggesting that climate (temperature and humidity) may have an important role as a determinant of cis-resveratrol concentrations in the wines produced from this cultivar, possibly because of its influence upon fungal pressure as proposed by Jeandet et al. (1994, 1995). The wines of Bordeaux, which, especially in the Medoc, are vinted from a mixture which includes >50% var. Cabernet Sauvignon and are grown in cooler damper conditions than those prevailing in California, Australia, and South America, had higher cis-resveratrol concentrations (means 2.16 mg/L) than did New World wines. It is unlikely that the blending of cultivars such as Merlot, Cabernet Franc, and Malbec in the Bordeaux wines could account for these higher concentrations since similar blends as well as wines produced predominantly or exclusively from these cultivars were included among the New World wines analyzed and they showed no trend toward values higher than those of wines exclusively from var. Cabernet Sauvignon in these regions.

The cis-resveratrol concentrations of red wines from the Rhone Valley and the Midi were lower than those of Burgundy and Bordeaux wines. While the warmer, drier climate in these regions may be a factor in generating lower fungal pressure, resulting in reduced cis-resveratrol concentrations, it is possible that the cultivars from which these wines are produced (which include var. Shiraz, Mourvedre, Carignane, and Cinsault) have intrinsic properties leading to these lower concentrations. However, these wines are blends of the above and other cultivars in widely varying proportions, and this question can only be resolved by studies at the level of the individual vineyard where the relevant factors can be controlled and precise information about grape cepage (the proportion of different cultivars included in the grape blend) obtained. Similar considerations apply to the other European wines, and it cannot be ascertained from the present data whether the rather low mean cis-resveratrol concentrations in Italian, Iberian, and central European wines reflect

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climate, grape cepage, or both. Many of the cultivars used (e.g., Nebbiolo and Sangiovese in Italy) are grown only in specific countries or regions, but we favor the view that climate is the predominant factor because in all of these countries var. Cabernet Sauvignon is grown and the wines from this cultivar were much lower in *cis*-resveratrol than Bordeaux wines or Canadian Cabernet Sauvignon wines.

When the total resveratrol concentrations of wines in this survey were critically scrutinized, they seemed to bear the same relationship to climate and cultivar as the *cis*-resveratrol concentrations. Thus, Canadian red wines had higher total resveratrol concentrations (mean 3.55 mg/L) than other New World wines except those of Oregon, whose mean value (6.33 mg/L) was at a level exceeded only by the wines of Burgundy (mean 7.13 mg/L) and Switzerland (mean 6.94 mg/L). Much higher total resveratrol concentrations were present in Bordeaux wines (mean 6.18 mg/L) than in those from the Rhone Valley (mean 4.53 mg/L) and the Midi (mean 3.83 mg/L). Italian red wines had low total resveratrol concentrations (mean 1.98 mg/L). It therefore seems that wines from var. Pinot Noir have higher total resveratrol concentrations than those from other cultivars irrespective of origin and that wines from var. Cabernet Sauvignon have variable concentrations depending upon the country of origin and possibly reflecting climatic conditions or fungal pressure. These latter considerations may also explain, at least in part, the lower concentrations in wines from more southern countries of Europe.

In conclusion, *cis*-resveratrol has been shown in this survey to be a significant component of commercial wines from every wine-producing region of the world. Wines that are high in *trans*-resveratrol tend to be also high in *cis*-resveratrol, and their concentrations may be subject to the same variables such as cultivar, climate, soil composition and drainage characteristics, fungal pressure, and wine-making techniques. Nevertheless, individual wines from the same grape and region show considerable variations in the ratio of the two isomers. It is important to establish the origin of *cis*-resveratrol in wine and the factors responsible for its generation, since its biological properties are likely to add significantly to the health benefits for red wine consumption.

LITERATURE CITED

Arichi, H.; Kimura, Y.; Okuda, H.; Baba, K.; Kozawa, M.; Arichi, S. Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb. et Zucc. on lipid metabolism. *Chem. Pharm. Bull.* **1982**, *30,* 1766.

Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341,* 1103.

Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E. P.; Karumanchiri, A.; Waterhouse, A. L. A definitive gas-chromatographic mass-spectrometric assay for resveratrol suitable for industrial and biological samples. *Clin. Biochem.* **1993a**, *26,* 126.

Goldberg, D. M.; Karumanchiri, A.; Ng, E.; Diamandis, E. P.; Yan, J.; Soleas, G. J.; Waterhouse, A. L. Resveratrol content of wines assayed by a direct gas chromatographic-mass spectrometric technique. *Am. J. Enol. Vitic.* **1993b**, *44,* 344.

Hanawa, F.; Tahara, S.; Mizutani, J. Antifungal stress compounds from *Veratrum grandiflorum* leaves treated with cupric chloride. *Phytochemistry* **1992**, *31,* 3005.

Hegsted, D. M.; Ausman, L. M. Diet, alcohol and coronary heart disease in men. *J. Nutr.* **1988**, *118,* 1184.

Hillis, W. E.; Hart, J. H.; Yazaki, Y. Polyphenols of *Eucalyptus sideroxylon* wood. *Phytochemistry* **1974**, *13,* 1591.

Ingham, I. L. 3,5,4′-Trihydroxystilbene as a phytoalexin from groundnuts (*Arachis hypogaea*). *Phytochemistry* **1976**, *15,* 1791.

Jayatilake, G. S.; Jayasuriya, H.; Lee, E-S.; Koonchanok, N. M.; Geahlen, R. L.; Ashendel, C. L.; McLaughlin, J. L.; Chang, C-J. Kinase inhibitors from *polygonum cuspidatum*. *J. Nat. Prod.* **1993**, *56,* 1805.

Jeandet, P.; Bessis, R.; Maume, B. F.; Sbaghi, M. Analysis of resveratrol in Burgundy wines. *J. Wine Res.* **1993**, *4,* 79.

Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P. Production of the phytoalexin resveratrol by grape berries, as a response to *botrytis* attack under natural conditions. *J. Phytopathol.* **1994**, in press.

Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. Resveratrol content of wines of different ages: Relationship with fungal disease pressure in the vineyard. *Am. J. Enol. Vitic.* **1995**, *46,* 1.

Kimura, Y.; Okuda, H.; Arichi, S. Effects of stilbenes on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta* **1985**, *834,* 275.

Kumar, R. J.; Jyostna, D.; Krupadanam, G. L.; Srimannarayana, G. Phenanthrene and stilbenes from *Pterolobium hexapetallum*. *Phytochemistry* **1988**, *27,* 3625.

Lamuela-Raventos, R. M.; Waterhouse, A. L. Occurrence of resveratrol in selected California wines by a new HPLC method. *J. Agric. Food Chem.* **1993**, *41,* 521.

Langcake, P. Disease resistance to *Vitis* spp. and the production of the stress metabolites resveratrol, ξ-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18,* 213.

Langcake, P.; Pryce, R. J. The production of resveratrol by *Vitis vinifera* and other members of the *Vitaceae* as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, *9,* 77.

Langcake, P.; Pryce, R. J. The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation. *Phytochemistry* **1977**, *16,* 1193.

Mattivi, F. Solid phase extraction of *trans*-resveratrol from wines for HPLC analysis. *Z. Lebensm. Unters. Forsch.* **1993**, *196,* 522.

Mattivi, F.; Nicolini, G. Influence of vinification technique on the resveratrol content of wines. *L'Enotecnico* **1993**, *81* (July–Aug).

Moreno-Manas, M.; Pleixats, R. Dehydroacetic acid chemistry. A new synthesis of resveratrole, a phytoalexine of *Vitis vinifera*. *Anal. Quim.* **1985**, *81,* 157.

Nonomura, A.; Kanagawa, H.; Makimoto, A. Chemical constituents of polygonaceous plants. I. Studies on the components of Ko-jo-kon (*Polygonum cuspidatum* SIEB et ZUCC). *Yakugaku Zasshi* **1963**, *83,* 988.

Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography and highly sensitive fluorimetric detection. *J. Chromatogr.* **1994**, *663,* 191.

Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339,* 1523.

Roggero, J-P.; Archier, P. Measurement of resveratrol and one of its glycosides in wine. *Sci. Aliments* **1994**, *14,* 99.

Siemann, E. H.; Creasy, L. L. Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, *43,* 49.

Soleas, G. J.; Goldberg, D. M.; Diamandis, E. P.; Karumanchiri, A. *Cis*-Resveratrol and its concentration relative of that of *trans*-resveratrol in Ontario wines analyzed by a GC/MSD derivatization method. *Am. J. Enol. Vitic.* **1994**, *45,* 364.

St. Leger, A. S.; Cochrane, A. L.; Moore, F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **1979**, *1,* 1017.

Waterhouse, A. L.; Lamuela-Raventos, R. M. The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry* **1994**, *37,* 571.