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SHORT COMMUNICATIONS

Occurrence of Resveratrol in Selected California Wines by a New HPLC Method

INTRODUCTION

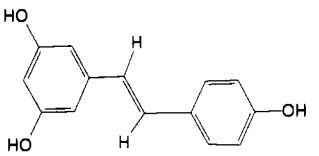
Resveratrol is a stilbene phytoalexin produced by grape vines in response to fungal infection (Langcake, 1981). It is known to occur in grapes (Jeandet et al., 1991) as well as in wine (Siemann and Creasy, 1992). Importantly, there are studies which demonstrate resveratrol to have biological effects that suggest a potential cardiovascular health benefit. These include studies on lipid metabolism (Arichi et al., 1982), arachidonic acid metabolism (Kimura et al., 1985), and platelet aggregation (Shan, 1988). These papers are particularly interesting in light of the "French paradox", where Toulouse residents, who consume their alcohol largely in the form of red wine, have a very low mortality rate from cardiac diseases, despite a fat consumption rate similar to that in the United States (Seigneur et al., 1990). Another recent study also points to reduced mortality, largely from a reduction in cardiac disease, in those who consume moderate amounts of alcohol, with even less cardiovascular disease mortality among wine drinkers (Klatsky, 1992).

In light of this importance we set out to develop an analytical method that was both quick and capable of detecting reasonably low levels of resveratrol. Siemann and Creasy's (1992) method appears to have very high sensitivity, near 1 μg/L, but sample analysis time is several days. On the other hand, the gas chromatography methods report a limit of detection of approximately 1 mg/L and have been developed for grape analysis, not wine (Jeandet et al., 1992; Langcake and Pryce, 1976).

MATERIALS AND METHODS

The standard, *trans*-resveratrol, was synthesized in this laboratory using a method that will be described elsewhere. Its UV (Siemann and Creasy, 1992) and ^{1}H NMR (Moreno-Manas and Pleixats, 1985) spectra were identical to those described in the literature.

The samples that were analyzed were wines from the north coast of California, from the 1989 harvest, the year that is now commercially available. Three different red varieties were studied: Cabernet sauvignon, Zinfandel, and Pinot noir. In addition, a white Bordeaux, several Sauternes, and a late harvest Chardonnay (1990) were selected because they were made with grapes that were described as suffering a *Botrytis cinerea* infestation.



trans-resveratrol

Liquid–Liquid Extraction. An initial volume of 500 mL of wine is reduced in volume to 450 mL by rotary evaporation to reduce the ethanol content. The aqueous sample is extracted twice with 100 mL of chloroform and the chloroform discarded. Then the wine is extracted with 3 × 50 mL of ethyl acetate, and the combined extracts are washed twice with approximately 100 mL of saturated sodium chloride. The extract is evaporated in vacuo to dryness and then dissolved in 10 mL of a (1:1) water/acetonitrile solution that is filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE).

HPLC Procedure. A Hewlett-Packard (Santa Clara, CA) Model 1090 with a diode array UV–visible detector coupled to an HP Chem Station was used for solvent delivery system and detection. A Novapack C_{18} column, 3.9 × 150 mm, 4-μm particle size, from Waters/Millipore was used for the stationary phase with a flow of 0.5 mL/min. The solvents used for the separation were as follows: solvent A, 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5; solvent B, 20% A with 80% acetonitrile.

The separation is carried out with 23.5% B, at a flow of 0.5 mL/min, for 18 min, and then the column is flushed with a 5-min gradient to 100% B; the initial conditions are then reestablished for 5 min before another sample is injected.

The eluant is monitored at 306, 316, 280, and 250 nm. In addition, UV spectra, from 200 to 400 nm, were taken over the elution time for resveratrol (14–17 min, see Figure 1). The observed peak was checked for the presence of significant interferences by comparing its spectrum with that for pure resveratrol, in particular by comparing the ratio of absorbances at 306 and 250 nm (where *trans*-resveratrol has absorbance maximum and minimum, respectively).

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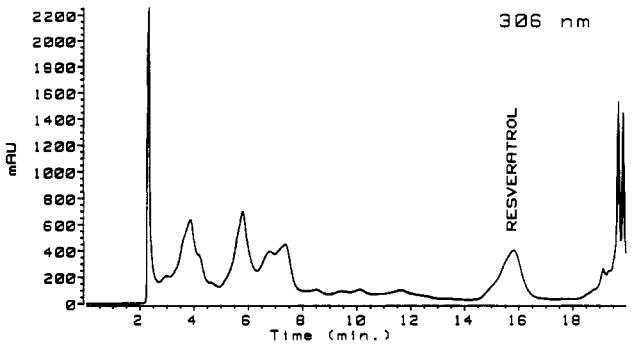

Figure 1. HPLC chromatogram of a red wine extract obtained by separation on a 4-μm Novapack C_{18} column using 76.5% solvent A and 23.5% solvent B, with a flow of 0.5 mL/min, monitoring the eluent at 306 nm.

RESULTS AND DISCUSSION

The primary difficulty in the analysis of resveratrol in wine at levels below 1 mg/L is the presence of interferences. With pure resveratrol solutions, the compound could be detected when 25 μL of a 10 μg/L solution was injected onto our HPLC system, monitoring absorbance at 306 nm. The challenge was to find a simple prechromatography cleanup that would reduce the presence of interferences.

A number of different extraction methods were compared: a dry column of PVPP–Celite (2:1), a column of Celite only, a quaternary amine ion-exchange resin (Bio-Rad AG 1-X10), and various liquid–liquid extraction methods. Both the PVPP–Celite column and the quaternary amine column had very low recoveries when the columns were extracted with ethyl acetate. The Celite extract had a good recovery of resveratrol, but important interferences were not eliminated from the HPLC analysis. We finally chose a two-step liquid–liquid extraction for its ability to minimize the interferences in the HPLC chromatogram despite a modest recovery (45%) due to inefficient extraction by ethyl acetate. In this procedure we washed the wine sample with chloroform to reduce interferences before extracting the resveratrol from the aqueous solution with ethyl acetate. However, these interferences were not completely removed, and it is their presence that limits the sensitivity of the method to 0.05 mg/L, not the reduced recovery.

The resulting procedure provides a relatively rapid analysis of resveratrol in wines, using equipment widely available in wine analysis laboratories. It also has the advantage that no derivatives need be prepared, as in several gas chromatography based methods.

To establish the method's reproducibility, the extraction was repeated several times by spiking known amounts of *trans*-resveratrol into a wine that had none present. In each case, a linear correlation of integration response with concentration was obtained ($r \geq 0.999$). The limit of detection in wines was determined to be 0.05 mg/L. At lower concentrations an interference became apparent by its UV absorbance, which alters the absorbance ratios of 306 and 250 nm. In four wine samples tested we could see the characteristic UV chromophore of resveratrol, but it was altered by the presence of an interference; in those cases, we could only say that the amount was less than 0.05 mg/L. In two other cases we could not detect any amount of resveratrol.

Figure 1 shows the chromatogram obtained of one red wine after the liquid–liquid extraction. The HPLC conditions were selected after buffers were tried at both pH 2.6 (50 mM phosphate) and that described above, pH 1.5, varying the concentration of the organic eluant to optimize the separation of resveratrol from its adjacent compounds in the chromatogram.

Table I. Resveratrol Content of the Tested Varietal Wines

variety	appellation	resveratrol, mg/L
Cabernet sauvignon	Napa Valley	<0.05[a]
Cabernet sauvignon	Napa Valley	0.09
Cabernet sauvignon	St. Helena (Napa)	<0.05[a]
Cabernet sauvignon	Napa Valley	<0.05[a]
Zinfandel	Napa Valley	0.06
Zinfandel	Napa Valley	0.11
Zinfandel	Napa Valley	nd[b]
Pinot noir	Carneros	0.68
Pinot noir	Carneros	0.23
Pinot noir	Russian River	0.21
white Bordeaux	Sauternes	<0.05[a]
Chardonnay (1990)	Santa Cruz	nd[b]

[a] Resveratrol detected but amount was not quantified due to interferences (see text). [b] Not detected.

To demonstrate the utility of the method, 12 California wines from the most widely available vintage year, 1989, were obtained. Rather than selecting just one or two wines from each region of the state, we decided to focus on a specific region and selected the north coast. The results from each wine are listed in Table I. Since resveratrol is found in the grape skins (Jeandet et al., 1991), we chose to look at red wines, where the must typically is left in contact with the grape skins during fermentation to extract aroma, taste, and color components. Thus, one would not expect to find much resveratrol in white wines (which generally have little to no skin contact after pressing). One possible exception could be late harvest white wines where the grapes had been infected with *B. cinera*, a mold infection (desired in the case of these sweet wines). *Botrytis* infections are known to stimulate resveratrol production in vines, and it is possible that the same effect would be seen in grapes. Also, the winemaker of wine 12 stated that the grapes needed 3 days of pressing, which might be adequate juice/skin contact time to extract resveratrol into the juice.

In Table I, the most obvious results is that the Pinot noir wines had much higher levels of resveratrol than the other red varieties, with Cabernet sauvignon and Zinfandel having similar ranges of levels. This is notable in light of the observation that Pinot noir vines are known to produce less resveratrol than Cabernet sauvignon (Jeandet et al., 1992) and that, in a study of resveratrol in grape berries, Cabernet sauvignon was noted to be a high resveratrol producer, although Pinot noir was not studied (Creasy and Coffee, 1988). Importantly, the California wines in this study have resveratrol levels as high those from France or New York (Siemann and Creasy, 1992), although a comprehensive survey will be necessary to determine a significant average content.

For the two late harvest wines from *Botrytis*-infected grapes, the content of resveratrol was very low in Sauternes and nonexistent in the Chardonnay, contrary to what we expected. However, grapes are known to lose their capacity to produce resveratrol during ripening (Jeandet et al., 1991), and it is possible that at such a late point in the growing season there is negligible capacity for the grape to produce resveratrol, even in the face of a fungal infection. The fact that the grapes sustain such a fungal infection may result from their inability to produce resveratrol. Alternatively, resveratrol may not be extracted from the skins by grape juice.

CONCLUSIONS

The analysis of resveratrol in wines is facilitated by a simple extraction procedure combined with chromatographic separation. This will make it possible to survey a large number of wines for resveratrol content, and such an effort will make it possible to uncover many important factors that control resveratrol levels in wine. It should also be possible to adapt this method to the analysis of resveratrol in grapes and other plant tissue. In our small sample of wines, north coast Pinot noir had high levels of resveratrol, up to 0.6 mg/L, while Zinfandel or Cabernet sauvignon had moderate levels, 0.1 mg/L and below.

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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*, 1766–1770.

Creasy, L. L.; Coffee, M. Phytoalexin Production Potential of Grape Berries. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 230–234.

Jeandet, P.; Bessis, R.; Gautheron, B. The Production of Resveratrol [(3,5,4″)-trihydroxystilbene] by Grape Berries in Different Development Stages. *Am. J. Enol. Vitic.* **1992**, *42*, 41–46.

Jeandet, P.; Sbaghi, M.; Bessis, R. The production of resveratrol (3,4,5′-trihydroxystilbene) by grapevine *in vitro* cultures, and its application to screening for grey mould resistance. *J. Wine Res.* **1992**, *3*, 47–57.

Kimura, Y.; Okuda, H.; Arichi, S. Effects of stilbenes on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta* **1985**, *834*, 275–278.

Klatsky, A. L.; Armstrong, M. A. Alcoholic Beverage Choice and Coronary Artery Disease: Do Red Wine Drinkers Fare Best? *Circulation* **1992**, *86* (Suppl. I) 464.

Langcake, P. Disease resistance of *vitis* spp. and the production of the stress metabolites resveratrol, γ-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18*, 213–226.

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*, 77–86.

Moreno-Manas, M.; Pleixats, R. Dehydroacetic acid chemistry. A new synthesis of resveratrol, a phytoalexine of *vitis vinfera*. *Anal. Quim.* **1985**, *81*, 157–161.

Seigneur, M.; Bonnet, J.; Dorian, B.; Benchimol, D.; Drouillet, F.; Gouverneur, G.; Larrue, J.; Crockett, R.; Boisseau, M.; Ribereau-Gayon, P.; Bricaud, H. Effect of the consumption of alcohol, white wine, and red wine on platelet function and serum lipids. *J. Appl. Cardiol.* **1990**, *5*, 215–222.

Shan, C. W. Effects of polydatin on platelet aggregation of rabbits. *Yaoxue Xuebao* **1988**, *23*, 394–396.

Siemann, E. H.; Creasy, L. L. Concentration of the Phytoalexin Resveratrol in wine. *Am. J. Enol. Vitic.* **1992**, *43*, 49–52.

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