Journal of Agricultural and Food Chemistry

OCTOBER 1994
VOLUME 42, NUMBER 10

SHORT COMMUNICATIONS

Analysis of Wines for Resveratrol Using Direct Injection High-Pressure Liquid Chromatography with Electrochemical Detection

Keywords: *Resveratrol; wine; HPLC; electrochemical detectors; phenolic stilbenes; quercetin*

INTRODUCTION

Resveratrol [(*E*)-3,4′,5-trihydroxystilbene] is produced by grapevines (Langcake, 1981) and occurs in grapes (Jeandet *et al.*, 1992) and wines (Siemann and Creasy, 1992; Lamuela-Raventos and Waterhouse, 1993; Pezet *et al.*, 1994) produced from these grapes. It has been speculated that this substance may have positive effects on human cardiovascular health, acting to lower serum lipid levels (Siemann and Creasy, 1992).

Recently, details on the development and application of a method for analysis of resveratrol in wines using HPLC with monitoring at several wavelengths of UV light were published (Lamuela-Raventos and Waterhouse, 1993). The method involved reduction of volume of 500 mL of wine to approximately 450 mL to remove ethanol, extraction with chloroform to remove interfering substances, extraction with ethyl acetate to isolate the resveratrol, evaporation of the ethyl acetate, and reconstitution of the residue in 10 mL of aqueous acetonitrile followed by filtration of the sample through 0.45 μm Teflon filters prior to HPLC analysis using an octadecyl reversed-phase column. The authors noted that recovery of resveratrol was somewhat modest (45%) but that the sensitivity of the method was limited to about 0.05 mg/L by the presence of interferences rather than recovery. The method proved to be successful, especially in decreasing the time needed for analysis compared to one previous method (Siemann and Creasy, 1992) and in sensitivity compared to another (Jeandet *et al.*, 1992; Langcake and Pryce, 1976).

Very recently, the application of HPLC with fluorescence detection to the analysis of wines and grape berries for resveratrol and pterostilbene has been described (Pezet *et al.*, 1994). Fluorescence detection allowed rapid analysis of wines for the two target compounds. The method possesses sufficient selectivity and sensitivity to allow direct analysis of wines without prior sample preparation.

We have been interested in resveratrol and other phenolic stilbenes elucidated in various plant species because of their possible role in fungus resistance of certain species of woody plants (Schultz *et al.*, 1992). We have found that reversed-phase HPLC with electrochemical detection (EC) is very useful for determining phenolic substances such as resveratrol and other hydroxylated stilbenes in biological systems including plants and products deriving from plants. Electrochemical HPLC detectors are relatively well-known in the field of neurochemistry but are used less often in other areas. EC detectors are selective detectors. When operated in the oxidative mode, they respond well to phenols and are capable of parts-per-billion or even lower detection levels for these substances.

This paper demonstrates that reversed-phase HPLC with electrochemical detection allows analysis of wines for resveratrol by direct injection with sensitivities at least as good as those based on fluorescence detection. This level of selectivity and sensitivity was obtained using a simple isocratic HPLC system.

MATERIALS AND METHODS

Resveratrol (Figure 1) was synthesized in this laboratory using a method developed for synthesis of the tetrahydroxylated stilbene piceatannol (Bajaj *et al.*, 1987). Quercetin was purchased from Sigma Chemical Co. (St. Louis, MO), and 2,6-di-*tert*-butyl-4-methylphenol (BHT) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Two liquid chromatographs were used in this study. One chromatograph has gradient pumps, a variable-wavelength UV detector with provision for stopped-flow scanning (200–800

0021-8561/94/1442-2077$04.50/0

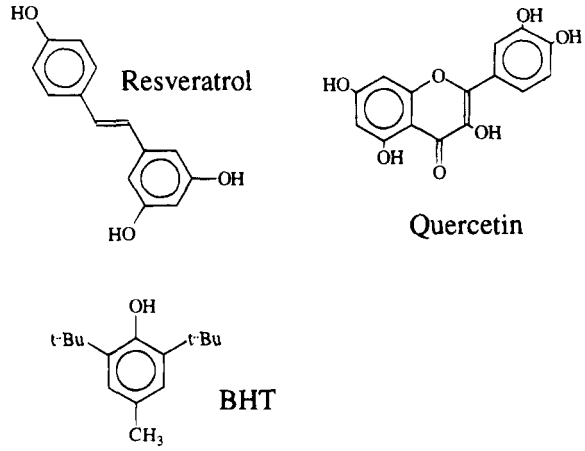

Figure 1. Structures of BHT, quercetin, and resveratrol.

nm) of the UV spectra of components being analyzed, and a variable fluorescence detector (Perkin-Elmer, Norwalk, CT). This chromatograph was used to confirm quantitation of resveratrol in some of the red wine samples and to obtain UV spectra of synthetic resveratrol and the component of the Pinot noir wine which eluted with the retention time of resveratrol. All quantitative determinations were obtained using a very simple modular isocratic liquid chromatograph assembled from various components. A Simplex micrometering pump (LDC/Milton Roy, Riviera Beach, FL), a coiled stainless-steel tubular pulse dampener, a pressure gauge (Alltech Associates, Deerfield, IL), a six-port injection valve (Valco Instruments, Houston, TX), an electrochemical detector (Model LC-3, Bioanalytical Systems, Lafayette, IN), and a strip-chart recorder (Linear Instruments, Irvine, CA) comprised this chromatograph. Samples were quantitated by measuring peak heights manually.

The electrochemical detector included a glassy carbon working electrode and a silver/silver chloride reference electrode (Bioanalytical Systems). Columns used were all 150 mm long, 4.6 mm i.d., and were packed in our laboratory. Two columns of different chemistry were used. These were an octadecyl column (Nucleosil C_{18}, 5 μm, Macherey-Nagel, Duren, Germany) and a benzyldimethylsilyl bonded phase. The benzyldimethyl packing was prepared in this laboratory following procedures previously described using irregular silica gel with a particle size of 7 ± 2 μm (McMurtrey, 1988). The Nucleosil C_{18} column was used for all quantitative determinations. Mobile phases were 0.05 mol/L $NH_4H_2PO_4$ (E. Merck, Darmstadt, Germany) in 25% (v/v) aqueous acetonitrile (Optima grade, Fisher Chemical, Fair Lawn, NJ). Mobile phases were prepared using deionized water.

Extraction cartridges used were from Worldwide Monitoring (Horsham, PA). These consisted of C_{18} groups bonded to silica gel and were endcapped: size, 500 mg; adsorbent, sample volume, 6 mL.

Wines were purchased from commercial suppliers in the area. Wines selected include the following: (1) Classic Red, Deer Valley Vineyards, Gonzalez, CA (nonvintage blend of Barbera, Pinot noir, Zinfandel, and Ruby Cabernet); (2) 1991 California Zinfandel, Sutter Home Winery, St. Helena, Napa County, CA; (3) 1991 Concha y Toro, 75% Cabernet Sauvignon/25% Merlot, Rapel, Vina Concha y Toro, SA, Chile; (4) 1990 Eye of the Swan Cellars California Pinot noir, Sebastiani Vineyards, Sonoma, CA; (5) 1986 Northern Sonoma Cabernet Sauvignon of California, The Reserve Cellars of Ernest and Julio Gallo, Northern Sonoma County, CA; (6) Blossom Hill Reserve Collection Chardonnay, Madera, CA; (7) 1992 Beaujolais-Villages Jadot, Beaujolais-Villages Controlee, Louis Jadot, Beaune, Cote-D'Or, France; (8) 1992 Saint-Louis Beaujolais, appellation Beaujolais Controlee, Barton & Guestier, Blanquefort, France (burgundy red wine); (9) Classic White, premium California table wine, Deer Valley Vineyards, (nonvintage blend of 60% French Colombard, 15% Chenin blanc, 7% Muscat, and 18% white Riesling); (10) 1991 Sauvignon Blanc of California, Ernest and Julio Gallo, Modesto, CA; and

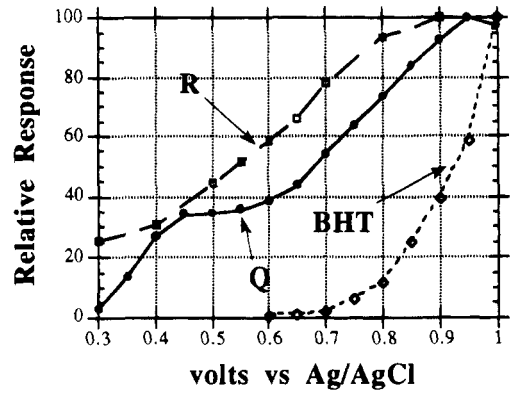

Figure 2. Relative response (peak height) of resveratrol (R), quercetin (Q), and butylated hydroxytoluene (BHT) at various voltages of the working electrode relative to a silver/silver chloride reference electrode. Responses were normalized to the signal at 1.00 V applied potential.

(11) Gallo Cocktail Pale Dry Sherry of California, Ernest and Julio Gallo, Modesto, CA.

RESULTS AND DISCUSSION

Electrochemical detectors are operated with the working electrode at a potential at which analyte molecules undergo electrochemical reactions. The specific potential that is used depends on the structures of the molecules being analyzed. Resveratrol; quercetin, a natural product; and BHT, a synthetic phenol antioxidant often added to various foods, (see Figure 1 for structures) were analyzed at applied voltages from +0.300 to +1.000 V vs Ag/AgCl. The results are presented in Figure 2. Both resveratrol and quercetin give rather broad responses, apparently because of the presence in these molecules of more than one phenolic hydroxyl. In contrast, the applied voltage–response curve from BHT is much more narrow. The response of resveratrol at relatively low applied voltages is an advantage since a low voltage will give a relatively low background signal and reduces the response of interfering substances. In effect, the use of a low working voltage increases the selectivity of the detector. For example, analysis of a mixture of resveratrol and BHT at 0.5 V would give a strong signal for resveratrol but no signal for BHT. If 0.9 V or higher were used, both compounds could be monitored. We chose 0.500 V vs Ag/AgCl for analysis of resveratrol.

Detector response to standard samples of resveratrol (peak height) was linear over the range tested (0.10–8.00 mg/L) with a correlation coefficient of 0.999. The region of linearity extends to concentrations less than 0.10 mg/L. Quantitation could be extended to at least 0.01 mg/L and perhaps lower by simply using a more sensitive attenuation of the EC detector. The limit of detection of resveratrol in wine appears to be approximately 1 ppb (μg/L) for the method as described. Even greater sensitivities might be obtained by concentrating the analyte molecules. In this context, we evaluated a C_{18}-based extraction cartridge and found that it allowed extraction of 1.00 mg/L resveratrol from wine with a recovery of 89 ± 1%. Extraction cartridges can be employed to concentrate resveratrol in wine samples (Mattivi, 1993) without recourse to rotary evaporation, which has been reported to lead to decomposition of hydroxylated stilbenes (Pezet *et al.*, 1994). Cartridge isolation also could be used to isolate other components of wine that have lipophilicities similar to that of resveratrol. The extraction efficiencies of various

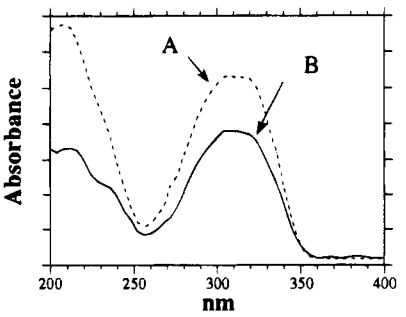

Figure 3. UV spectra of (A) resveratrol in Pinot noir wine and (B) synthetic resveratrol. Spectra were obtained by stopped-flow scanning of the HPLC eluent.

wine components eluting after about 10 min were approximately equivalent to that found for resveratrol.

We were somewhat surprised by the apparent instability of resveratrol during concentration by rotary evaporation at 40 °C as described by Pezet *et al.* (1994). We have isolated resveratrol from the heartwood of decay-resistant trees. We have found that the resveratrol present in heartwood is stable in dried, ground heartwood samples for at least 2 years. In addition, we have found that hydroxystilbenes are usually thermally stable. Part of the instability of resveratrol during isolation by rotary evaporation may arise from catalysis by the glass surface or by light-mediated reactions. Certainly, analyte loss during concentration of parts-per-million or parts-per-billion samples is always a possible source of analytical inaccuracy and must be guarded against.

There appeared to be little if any interfering materials in the chromatograms with electrochemical detection. This conclusion was reached as a result of several factors. First, the electrochemical detector is a selective detector. Second, the resveratrol peaks were very symmetrical. They show no evidence of overlapping or coeluting substances. Third, the stopped-flow UV spectra for synthetic resveratrol and for the resveratrol peak obtained from analysis of 100 μL of the Pinot noir wine were essentially identical (Figure 3). Fourth, we isolated resveratrol from the Pinot noir wine with the same C_{18} column used during quantitative determinations. This material was then analyzed using the EC detector connected to a benzyldimethylsilyl column. Only one peak eluted with a retention time which matched exactly that of resveratrol. Fifth, the three wines with the highest resveratrol levels (the two Beaujolais wines and the Pinot noir) were analyzed using a fluorescence detector. Resveratrol levels by these two techniques were equivalent within experimental error.

Eleven wines were analyzed for resveratrol content. The results are given in Table 1, with representative chromatograms displayed in Figure 4. As expected, white wines contained very little resveratrol. In contrast, the Pinot noir tested contained resveratrol at a level of 5.01 ± 0.25 mg/L. In general, red wines from vines originating in the Burgundy region of France (Gamay, Pinot noir) appear to contain relatively high levels of resveratrol. While absolute amounts are different from the values reported in this paper, others have reported similar trends. Lamuela-Raventos and Waterhouse (1993) found higher levels in Pinot noir wine (from 0.21 to 0.68 mg/L) than in Zinfandel wines (0.06 and 0.11 mg/L) and Cabernet Sauvignon (<0.05–0.09 mg/L). All wines in this study were from the same area of California and were the same vintage (1989). Pezet *et al.* (1994) analyzed wines produced in Switzerland. These authors found the highest resveratrol levels in a Gamay wine (1.48 mg/L) with levels in a Pinot and a Gamaret wine of 0.67 and 0.43 mg/L, respectively.

Table 1. Concentration of Resveratrol in Selected Wines

wine	origin	concn[a] (mg/L)
(11) dry sherry	California	≤0.01
(6) Chardonnay	California	≤0.02
(9) generic white	California	≤0.02
(10) Sauvignon blanc	California	≤0.02
(5) Cabernet Sauvignon	California	0.99 ± 0.08
(2) Zinfandel	California	1.38 ± 0.18
(3) Cabernet Sauvignon/Merlot	Chile	1.56 ± 0.08
(1) generic red	California	2.74 ± 0.08
(8) Beaujolais (Gamay)	France	3.27 ± 0.14
(7) Beaujolais (Gamay)	France	3.55 ± 0.06
(4) Pinot noir	California	5.01 ± 0.25

[a] Mean ± sample standard deviation, from three or more determinations.

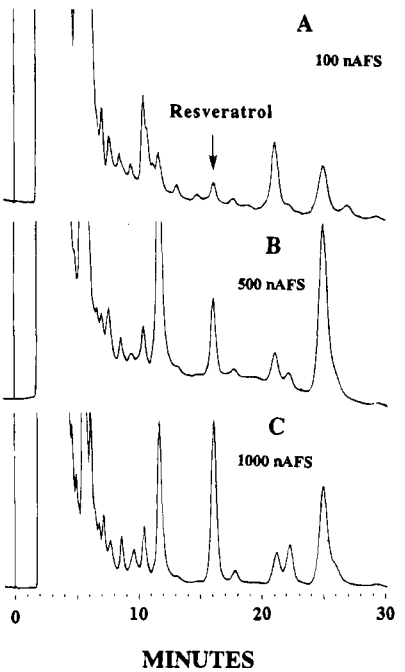

Figure 4. Representative chromatograms of analysis of wines: (A) Chardonnay, wine 6, resveratrol content ≤0.02 mg/L; (B) Cabernet Sauvignon, wine 5, resveratrol content 0.99 ± 0.08 mg/L; (C) Pinot noir, wine 4, resveratrol content 5.01 ± 0.25 mg/L. Sensitivity in (A) is 5 times greater than in (B) and 10 times greater than in (C).

Data available at this time are insufficient to allow complete assessment of the levels of resveratrol in commercial wines to be made. Knowledge of year-to-year variation of resveratrol levels in wines produced in one area, as well as variation of resveratrol content as a result of geographical area, would allow better estimates to be made of the amounts of resveratrol that average wine drinkers ingest. When such data become available, more meaningful assessment of the possible role that resveratrol in the diet may have on human health may be possible.

The electrochemical detector can also be used to monitor wines for the presence of other phenolic substances. For example, the substance eluting with a retention time of 25 min in the chromatograms displayed in Figure 4 is primarily quercetin. We did not quantitate this material because there are substances which overlap. Relatively minor modification of the analytical methodology should allow quercetin (and other hydroxyflavanoids) to be quantitated.

The analytical techniques described here allow analysis of wines for resveratrol without sample pretreatment. Method sensitivity is more than adequate to monitor levels of resveratrol at considerably less than 1 mg/L (1 part per million). The method was developed using the most simple liquid chromatograph and therefore is relatively inexpensive. The method appears to compete successfully with methods based on other means of detection in terms of convenience, sensitivity, and cost.

ACKNOWLEDGMENT

We gratefully acknowledge the support of the Department of Chemistry and Biochemistry of the University of Southern Mississippi, the Forest Products Laboratory of Mississippi State University, and the U.S. Department of Agriculture (Grant WUR3556726) for support of this research.

LITERATURE CITED

Bajaj, R.; Gill, M. T.; McLaughlin, J. L. Improved preparative synthesis of piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene). *Rev. Latinoam. Quim.* **1987**, *18*, 79–80.

Jeandet, P.; Sbaghi, M.; Bessis, R. The production of resveratrol (3,4′,5-trihydroxystilbene) by grape berries in different development stages. *Am. J. Enol. Vitic.* **1992**, *42*, 41–46.

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*, 521–523.

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.

Mattivi, F. Solid-phase extraction of trans-resveratrol from wines for HPLC analysis. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 522–525.

McMurtrey, K. D. Reaction of silica gel with trimethylsilyl donors under conditions useful for end-capping HPLC bonded phase packings. *J. Liq. Chromatogr.* **1988**, *11*, 3375–3384.

Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography with highly sensitive fluorometric detection. *J. Chromatogr. A* **1994**, *663*, 191–197.

Schultz, T. P.; Boldin, W. D.; Fisher, T. H.; Nicholas, D. D.; McMurtrey, K. D.; Pobanz, K. Structure-fungicidal properties of some 3- and 4-hydroxylated stilbenes and bibenzyl analogues. *Phytochemistry* **1992**, *31*, 3801–3806.

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

Received for review May 18, 1994. Accepted August 2, 1994.

Kenneth D. McMurtrey,*,† James Minn,† Kirk Pobanz,† and Tor P. Schultz‡

Department of Chemistry and Biochemistry, University of Southern Mississippi, Box 5043, Hattiesburg, Mississippi 39406, and Mississippi Forest Products Laboratory, P.O. Drawer FP, Mississippi State, Mississippi 39762-5724

† University of Southern Mississippi.

‡ Mississippi Forest Products Laboratory.