HPLC Determination of Non-Flavonoid Phenols in Vidal Blanc Wine Using Electrochemical Detection

Pamela J. Woodring,[†] Paul A. Edwards,*[,‡] and Mary G. Chisholm[§]

Department of Chemistry and Physics, Mercyhurst College, 501 East 38th Street, Erie, Pennsylvania 16546, Cooper Hall, Department of Chemistry, Edinboro University of Pennsylvania, Edinboro, Pennsylvania 16444, and The Pennsylvania State University at Erie, The Behrend College, Station Road, Erie, Pennsylvania 16563

High-performance liquid chromatography with electrochemical detection was used to identify and quantitate eight acidic phenols in seven Vidal blanc wines. Positive identifications were obtained by comparing the capacity factor (k') and electrochemical behavior of wine phenols to those of standard solutions containing pure phenols. Quantitation was achieved by comparing the peak areas associated with wine components to those of standard solutions of known phenolic composition and concentration. The average concentrations of the eight phenols identified were as follows: gallic acid, 1.45 mg/L; gentisic acid, 2.25 mg/L; 3,4-dihydroxyphenylacetic acid, 0.96 mg/L; *p*-hydroxybenzoic acid, 0.17 mg/L; *m*-hydroxybenzoic acid, 0.84 mg/L; caffeic acid, 12.97 mg/L; salicylic acid, 0.66 mg/L; *p*-coumaric acid, 1.94 mg/L.

Phenolic compounds in wine are important because they contribute to the color, taste, and texture of the wine. The use of high-performance liquid chromatography (HPLC) to examine the phenolic composition of *vinifera* wines has been reviewed by Evans (1983). The technique has been used extensively with wine made from grapes grown in California (Singleton and Trousdale, 1983), the Pacific Northwest (Ong and Nagel, 1978), France (Salagoïty-Auguste and Bertrand, 1984), and Australia (Somers et al., 1987). Investigation of the phenolic composition of white non-*vinifera* grapes and wines indigenous to the northeastern United States is an increasingly active field of research (Jaworski and Lee, 1987; Jindra and Gallander, 1987; Lee and Jaworski, 1987, 1989; Mahler et al., 1988). Work in this laboratory has focused on the application of HPLC with electrochemical detection (Lunte, 1987; Roston and Kissinger, 1981; Roston et al., 1982) to examine the phenolic composition of the French–American cultivar Vidal blanc. Mahler et al. (1988) discussed the advantages of using HPLC with electrochemical detection in their report of the identification of acidic phenols in Vidal blanc wines. That technique was modified in this study to quantitate non-flavonoid phenols found in Vidal blanc wine.

EXPERIMENTAL SECTION

The procedures used in this study were as described by Mahler et al. (1988) except for minor modifications described below. The non-flavonoid phenols were extracted with ethyl acetate, and electrochemical detection was used in the HPLC analysis of the extract. The method used in this work was derived from that first reported by Roston and Kissinger (1981).

Materials. Seven Vidal blanc wines were provided by wineries located in Pennsylvania. Three wines were made from grapes grown in York county, the Piedmont region, which is hotter, and four wines were made from grapes grown in Erie county, where the climate is modified by the lake and is cooler. The wines were obtained in bottles, contained 10–11% alcohol, and were analyzed 2 years after harvest.

Reagents. Samples of phenols to be used as standards were obtained from Aldrich Chemical Co. and were used without further purification. Reagent grade ethyl acetate was obtained from Fisher Scientific Co. and also was used as received. Standard solutions were made by dissolving the required mass of pure phenol in 2 L of aqueous solvent containing 12% (v/v) ethyl alcohol and 5% (w/v) dextrose to simulate a wine environment. The concentration of gallic, gentisic, 3,4-dihydroxyphenylacetic (DHP), and caffeic acids in the standard solutions was 50 mg/L, and that of *p*-hydroxybenzoic (PHB), *m*-hydroxybenzoic (MHB), salicylic, and *p*-coumaric acids was 25 mg/L.

Sample Preparation. Parallel extractions of a standard solution (5 mL) and a wine (15 mL) were performed in triplicate for each wine tested. Wine samples and standard solutions were acidified with 1 M HCl to pH 2 and saturated with NaCl. The solutions were then extracted with three 5-mL portions of ethyl acetate for 10 min on a magnetic stirrer to prevent the formation of an emulsion. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and decanted. The residual sodium sulfate was washed with 1 mL of ethyl acetate, and the ethyl acetate solutions were again combined. The ethyl acetate was evaporated under a stream of nitrogen at ambient temperature, and the solid residue was frozen until needed. Before use, the residue was dissolved in 1.0 mL of mobile phase in the case of a wine sample or 2.0 mL in the case of a standard.

Instrumental Procedures. Samples were analyzed on a Beckman Model 322 gradient liquid chromatography system with a 20-μL sample loop, a Keystone Scientific Spherisorb ODS2 column (4.6 mm × 250 mm), and an LDC/Milton Roy e.c. Monitor amperometric detector containing Ag/AgCl reference, carbon polyethylene working, and 316 stainless steel auxiliary electrodes. The column was enclosed in an Aura Industries, Inc., Model CJB column jacket to minimize variations in capacity factors caused by fluctuations in temperature. The temperature was maintained at 20 °C using an ethylene glycol solution pumped from a Neslab Instruments, Inc., Endocal refrigerated circulating bath. Chromatograms were recorded on an Altex C-R1A integrator. The mobile phase, composed of 2% (v/v) 2-propanol, 2% (v/v) acetic acid, 8.7% (v/v) HPLC grade methanol, 0.018 M ammonium acetate, and 87.35% (v/v) HPLC grade water, was pumped isocratically at a flow rate of 1 mL/min. As reported by Mahler et al. (1988), all precautions suggested by Roston and Kissinger (1981) were taken to avoid electrode passivity.

Characterization. Identification of wine phenolics was made based on the same two criteria reported by Mahler et al. (1988).

[†] Mercyhurst College.
[‡] Edinboro University of Pennsylvania.
[§] The Pennsylvania State University at Erie.

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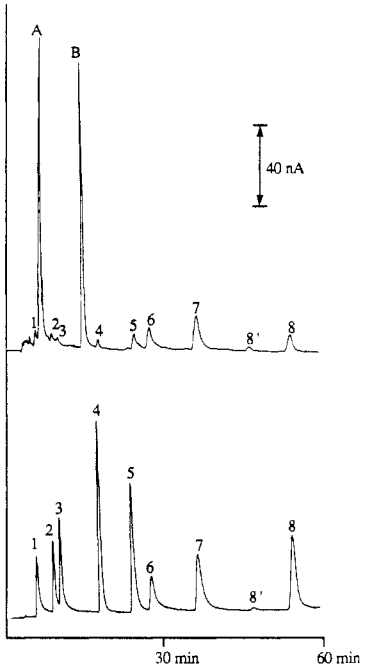

Figure 1. Typical chromatograms of ethyl acetate extracts of wine samples (upper) and standard solutions (lower). Conditions: 4.6 × 250 mm Spherisorb ODS2 column; flow rate, 1.0 mL/min; detector potential, +1000 mV. The numbering corresponds to that used in Tables I and II.

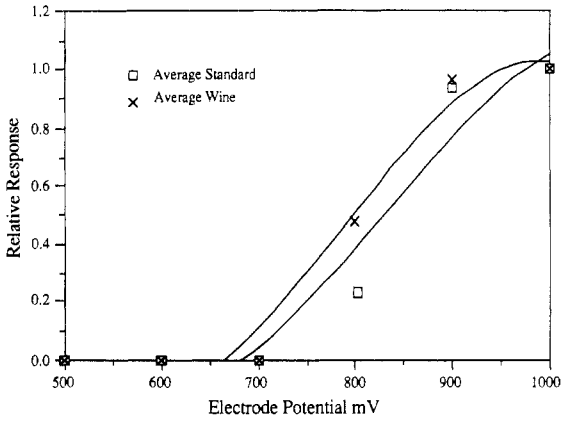

Figure 2. Average hydrodynamic voltammograms of *p*-coumaric acid in standard and wine samples.

Preliminary assignments were made by comparing the capacity factor (k') of unknown sample components to those of pure phenolic acids in standard solutions. Assignments were then confirmed by comparing the corresponding electrochemical behaviors, a criterion that proved invaluable for making identifications. This was especially true for components having similar capacity factors because it provided a further means of identifying sample components with confidence. However, on occasion the determination of the electrochemical behavior was difficult due to the inconsistent response of the detector, with variations up to 20% being observed between runs. This occasionally made determination of electrochemical behavior difficult and may have prevented additional identifications. These variations were manifestations of electrode passivity and aging of the electrode (Roston and Kissinger, 1981).

Quantitation. After positive identifications were made, the data were then quantified to determine the concentration of individual phenols in the wine. Concentrations were calculated by using the following ratio:

$$\frac{\text{wine peak area}}{\text{wine phenol concn}} = \frac{\text{standard peak area}}{\text{standard phenol concn}}$$

The wine phenol concentration was calculated by taking into account the different sample volumes extracted (5 mL of standard and 15 mL of wine) and different volumes of mobile phase used to dissolve the solid residue (2.0 mL for the standard and 1.0 mL for the wine). Concentrations were calculated in units of mg/L to facilitate comparison with other data, particularly the literature relevant to wines, which is presented in this form, and converted to gallic acid equivalents (GAE) with the conversion equations of Singleton (1974). A general equation for this conversion is

$$\text{GAE (mg/L)} = C_X\ \text{(mg/L)} \times \frac{\text{no. reactive OH}_X}{\text{MW}_X} \times \frac{\text{MW}_{GA}}{\text{no. reactive OH}_{GA}}$$

where C_X is the concentration of phenol X, MW_X is the molecular weight of phenol X, and MW_{GA} is the molecular weight of gallic acid.

Table I. Identification of Phenols

component no.	compd (all acids)	standard k'	wine k'
1	gallic (3,4,5-trihydroxybenzoic)	0.93	0.91
A	unassigned		1.18
2	gentisic (2,5-dihydroxybenzoic)	1.99	1.93
3	DHP (3,4-dihydroxyphenylacetic)	2.41	2.36
B	unassigned		3.89
4	PHB (*p*-hydroxybenzoic)	5.06	5.04
5	MHB (*m*-hydroxybenzoic)	7.06	7.11
6	caffeic (3,4-dihydroxycinnamic)	8.11	8.10
7	salicylic (*o*-hydroxybenzoic)	11.17	10.96
8	*p*-coumaric (*p*-hydroxycinnamic)	17.41	17.25

RESULTS AND DISCUSSION

Figure 1 presents the chromatograms of the ethyl acetate extracts of a standard solution and a typical Vidal blanc wine after the extracting solvent had been removed and the residue reconstituted in the mobile phase. A comparison of the capacity factors (k') for each of the numbered components in the wine and the standard of Figure 1 is shown in Table I. There was good agreement between capacity factors exhibited by wine and standard components on chromatograms obtained on the same day. However, capacity factors varied between runs carried out more than a few days apart. As previously discussed by Mahler et al. (1988), this variation in retention times and, in turn, capacity factors may have resulted from interactions between the phenols themselves. A further complication was that the composition of the extracts was observed to change with time. For example, peak 8′ in Figure 1 was not detected in chromatograms of wine or standard extracts immediately after the residue had been dissolved in the mobile phase. However, this peak was observed in subsequent chromatograms of the same solution and is believed to be the cis isomer of *p*-coumaric acid from its electrochemical behavior, which was identical with that of the trans isomer.

Additional information for peak identification was provided by determining the electrochemical behavior of each phenol in both the standard and sample solutions. The hydrodynamic voltammogram of a typical compound with one oxidizable phenolic group such as *p*-coumaric acid is shown in Figure 2. The electrochemical behavior shows a sharp decrease in current response as the electrode potential is decreased from +900 to +700 mV and also exhibits no response when the electrode potential is +500 mV. In contrast, as shown in Figure 3, compounds with two or more oxidizable phenolic groups such as gallic acid exhibit smaller decreases in current response with electrode potential and show a relative response >0.5 when the electrode potential is +500 mV. These characteristics did not vary with time for either the standard or the

Table II. Concentration of Vidal Blanc Phenols[a]

compound (all acids)	wine A	B	C	D	E	F	G	av	av GAE
gallic	2.25	0.67	1.26	1.26	1.25	0.79	2.64	1.45	1.45
gentisic	2.13	2.51	0.89	2.72	1.98	2.99	2.50	2.25	1.24
DHP	0.90	0.50	0.52	1.12	1.01	1.01	1.62	0.96	0.97
PBH	NA	NA	0.15	0.23	0.16	0.07	0.21	0.17	0.10
MHB	3.13	1.21	0.27	0.27	0.17	T	NA	0.84	0.52
caffeic	39.59	18.37	4.37	7.01	5.97	4.85	10.62	12.97	12.25
salicylic	1.70	0.0	T	0.0	0.0	0.0	0.38	1.04	0.64
p-coumaric	2.69	4.56	0.77	1.33	1.45	1.14	1.63	1.94	0.85
av total phenolic content								21.60	18.16

[a] Concentrations are expressed in mg/L unless otherwise noted. NA: data not determined because compound was not included in reference solution. T = trace (peak area too small to integrate).

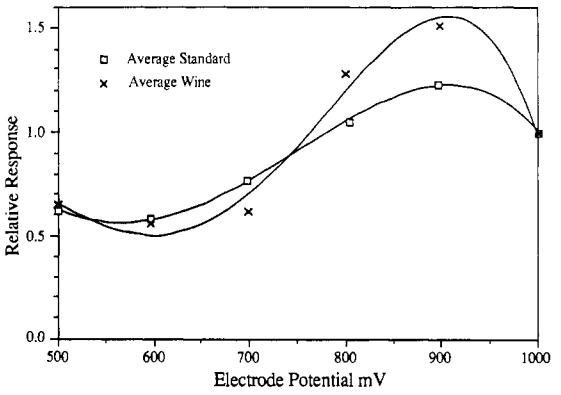

Figure 3. Average hydrodynamic voltammograms of gallic acid in standard and wine samples.

wine sample. The data displayed in Figures 2 and 3 are the average of the responses of *p*-coumaric acid and gallic acids in all standard and wine solutions analyzed.

Some of the peak identifications required both a good standard–wine match of capacity factors and electrochemical behavior. Because of stabilization of capacity factors achieved by column jacketing, it was determined that the peak labeled B in Figure 1, previously identified as *p*-hydroxyphenylacetic acid (HP) by Mahler et al. (1988), had a capacity factor different from that exhibited by HP in a standard solution. Thus it was first suspected that peak 4, instead of peak B, was HP from the capacity factor match. However, even though the capacity factor of peak 4 matched that of HP, the electrochemical behavior did not. The phenol *p*-hydroxybenzoic acid (PHB) exhibits a capacity factor very similar to that of HP. PHB also demonstrated the same electrochemical behavior as wine peak 4. Therefore, we believe peak 4 to be PHB and not HP as originally identified. The match of electrochemical behavior was critical in making this identification. This illustrates that matching capacity factors exhibited by standard and wine components to make assignments as carried out by Barroso et al. (1983) and Jindra and Gallander (1987) can be unreliable. We have found it necessary to use evidence from a second method to make identifications with confidence.

By comparing both the capacity factor and electrochemical behavior of wine components to those of the phenols in standard solutions, we have identified and quantitated the following compounds in Vidal blanc wine as shown in Tables I and II: gallic, gentisic, 3,4-dihydroxyphenylacetic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, caffeic, salicylic, and *p*-coumaric acids. All of these acidic phenols were identified and quantitated in all seven of the wine samples studied except for salicylic acid, which was found in three samples. The concentration of each phenol in the wine samples is shown in Table II. PHB and MHB were not quantified in all samples because the corresponding standard compound was not contained in the reference solution being analyzed in parallel with those wines. All phenols were quantitated by using data collected from wine and reference samples analyzed on the same day.

The data in Table II show that caffeic acid, with an average concentration of 12.97 mg/L, was the phenol consistently found in the highest quantity in the samples studied, while PHB, averaging 0.17 mg/L, was found in the lowest concentrations. The individual phenol concentrations that varied the most were MHB (0.17–3.13 mg/L) and caffeic acid (4.37–39.57 mg/L). The average total concentration of phenols identified in this study of Vidal blanc wine was 21.60 mg/L or 18.16 GAE. Since two major components labeled peaks A and B have yet to be identified and quantitated, the value of 18.16 GAE obtained for the average total concentration of phenols compares favorably with other results. Tryon et al. (1988) obtained an average of 60 GAE for the total nonflavonoid content of some French–American hybrid white wines using ultraviolet spectroscopy. However, the data for amounts of individual phenols should be interpreted with some caution. The wines of greater total phenolic content gave poor peak area reproducibility for samples of the same wine. The reproducibility of the electrochemical response is a problem that persists despite the fact that precautions were taken to avoid electrode passivity (Roston and Kissinger, 1981; Mahler et al., 1988). Also, certain phenols were not well resolved. For example, caffeic acid (3,4-dihydroxycinnamic acid) occurs as cis and trans isomers, and the cis isomer coelutes with MHB. Thus some fraction of the phenol reported as MHB may actually be the cis isomer of caffeic acid.

There are few quantitative data available for the phenolic content of French–American hybrid wines, making comparisons difficult because of differences in the experimental procedures used. Jindra and Gallander (1987) identified six acidic phenols in Seyval blanc wine, including gallic (1.8 mg/L), caffeic (1.1 mg/L), and *p*-coumaric (0.6 mg/L) acids. In their study of white grapes grown in New York, Lee and Jaworski (1987) found that *trans*-caffeoyl tartrate was the predominant acidic phenol and was present in significantly higher quantities in native American grapes. The results for hybrid grapes were mixed, with Seyval showing a very high concentration and Ravat 34 one of the lowest. Since the tartrate esters hydrolyze to mostly free hydroxycinnamic acids during vinification (Somers et al., 1987), the wines made from grapes high in *trans*-caffeoyl tartrate should show a high caffeic acid content. This was found to be the case for Vidal blanc, where caffeic acid was found to be the most abundant acidic phenol, with the most variation between wines. The caffeic acid content of Seyval blanc wine was

not found to be significantly higher than that of other phenols (Jindra and Gallander, 1987), although Seyval grapes were found to have one of the highest *trans*-caffeoyl tartrate contents (Lee and Jaworski, 1987).

At this stage there is no clear picture of how the phenolic content of hybrid grapes and wines varies. Native American grapes (*Vitis labruscana*) show a higher phenolic content than *Vitis vinifera* cultivars (Lee and Jaworski, 1987) but the hybrid cultivars show a wide range of phenolic content. This is not surprising since it is already known that the phenolic content varies with cultivar (Singleton and Trousdale, 1983; Nagel et al., 1979; Lee and Jaworski, 1987), climate (Lee and Jaworski, 1987), growing season (Singleton and Trousdale, 1983), and location, both regional and local (Nagel et al., 1979). Future work using this technique will focus on identifying the compounds associated with peaks A and B. Work done by Lee and Jaworski (1987) and Oszmianski et al. (1988) suggests that these peaks could be tartaric acid esters of caffeic and *p*-coumaric acids. The work will be expanded to include the use of HPLC with electrochemical detection to quantitate the flavonoid composition of non-*vinifera* wines. Recent work by Vérette et al. (1988) suggests that it may be the flavonoid rather than the nonflavonoid composition that is important in determining the bitterness and astringency of white wines.

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Registry No. Gallic acid, 149-91-7; gentisic acid, 490-79-9; 3,4-dihydroxyphenylacetic acid, 102-32-9; *p*-hydroxybenzoic acid, 99-96-7; *m*-hydroxybenzoic acid, 99-06-9; caffeic acid, 331-39-5; salicylic acid, 69-72-7; *p*-coumaric acid, 7400-08-0.