mitoyl chloride, 112-67-4; *all-trans*-lutein dipalmitate, 547-17-1; β-ε-carotene-3-monol monomyristate-3′-monol monopalmitate, 115116-89-7; β-ε-carotene-3-monol monopalmitate-3′-monol monomyristate, 86826-05-3; *all-trans*-lutein didecanoate, 115095-06-2; *all-trans*-lutein dilaurate, 23852-66-6; *cis*-lutein monomyristate, 115182-30-4; *cis*-lutein monopalmitate, 115182-29-1; neolutein dimyristate A, 115223-32-0; neolutein dimyristate A′, 115223-56-8; neolutein myristate/palmitate A, 115266-14-3; neolutein myristate/palmitate A′, 115266-15-4; neolutein dipalmitate A, 115223-33-1; neolutein dipalmitate A′, 115223-34-2; *all-trans*-α-carotene, 432-70-2; 9-*cis*-violaxanthin, 26927-07-1; di-*cis*-violaxanthin, 115182-31-5; *all-trans*-luteoxanthin, 1912-50-1; 9-*cis*-luteoxanthin, 115182-32-6; *all-trans*-violaxanthin dilaurate, 49795-35-9; *all-trans*-violaxanthin dimyristate, 113531-83-2; *all-trans*-violaxanthin dipalmitate, 113531-84-3; *all-trans*-violaxanthin myristate/palmitate, 115095-03-9; *all-trans*-zeaxanthin myristate/palmitate, 115095-08-4; 13-*cis*-violaxanthin, 75715-58-1; 15,15′-*cis*-violaxanthin, 24620-97-1; 9-*cis*-violaxanthin monomyristate, 115182-24-6; 9′-*cis*-violaxanthin monomyristate, 115182-27-9.

LITERATURE CITED

Cholnoky, L.; Szabolcs, J.; Nagy, E. "Untersuchungen über die Carotinoidfarbstoffe IV: α-Kryptoxanthin". *Justus Liebigs Ann. Chem.* **1958**, *616*, 207–218.

Englert, G. "Carotenoids Chemistry and Biochemistry". In *N.M.R. of Carotenoids*; Britton, G., Goodwin, T. W., Eds.; Pergamon: Oxford, 1982; pp 107–134.

Enzell, C. R.; Wahlberg, I. "Biochemical Application of Mass Spectrometry". In *Carotenoids*; Waller, G. R., Dermer, O. C., Eds.; Wiley-Interscience: New York, 1980; Chapter 13B, p 423.

Eugster, C. H.; Karrer, P. "Taraxanthin und Tarachrom, sowie Beobachtungen über Stereoisomere Trollixanthine". *Helv. Chim. Acta* **1957**, *40*, 69–79.

Goodwin, T. W. "The Biochemistry of Carotenoids". In *Carotenoids in Seed-Bearing Plants-Photosynthetic Tissues*; Chapman and Hall: New York, 1980; Vol. 1, Chapter 4, p 96.

Khachik, F.; Beecher, G. R.; Whittaker, N. F. "Separation, Identification, and Quantification of the Major Carotenoid and Chlorophyll Constituents in Extracts of Several Green Vegetables by Liquid Chromatography". *J. Agric. Food Chem.* **1986**, *34*, 603–616.

Khachik, F.; Beecher, G. R. "Separation and Identification of Carotenoids and Carotenol Fatty Acid Esters in Some Squash Products by Liquid Chromatography. 1. Quantification of Carotenoids and Related Esters by HPLC". *J. Agric. Food. Chem.* **1988**, preceding paper in this issue.

Liaaen-Jensen, S. "Marine Natural Products". In *Marine Carotenoids*; Academic: New York, 1978; Vol. 2, Chapter 1, pp 1–73.

Marty, C.; Berset, C. "Contribution de la Spectrometrie de masse en desorption/Ionisation Chimique (D/IC) dans l'Analyse des Pigments Carotenoides: Cas du β-Carotene et de Quelques Derives Oxydes". *Analusis* **1986**, *14*, 32–39.

Matsuno, T.; Maoka, T.; Katsuyama, M.; Hirono, T.; Ikuno, Y.; Shimizu, M.; Komori, T. "Comparative Biochemical Studies of Carotenoids in Fishes-XXIX: Isolation of New Luteins, Lutein F and Lutein G from Marine Fishes". *Comp. Biochem. Physiol., B: Comp. Biochem.* **1986**, *85B*(1), 77–80.

Mayer, H. "Carotenoid Chemistry and Biochemistry". In *Synthesis of Optically Active Carotenoids with ε-End Group*; Britton, G., Goodwin, T. W., Eds.; Pergamon: Oxford, 1982; pp 55–70.

Petracek, F. J.; Zechmeister, L. "Reactions of β-Carotene with *N*-Bromosuccinimide: The Formation and Conversions of Some Polyene Ketones". *J. Am. Chem. Soc.* **1956**, *78*, 1427–1434.

Ritter, E. De.; Purcell, A. E. "Carotenoids as Colorants and Vitamin A Precursors". In *Carotenoid Analytical Methods*; Bauernfeind, J. C., Ed.; Academic: New York, 1981; Chapter 10, p 903.

Strain, H. H. "Leaf Xanthophylls: The Action of Acids on Violaxanthin, Violeoxanthin, Taraxanthin and Tareoxanthin". *Arch. Biochem. Biophys.* **1954**, *48*, 458–468.

Tsukida, K.; Zechmeister, L. "The Stereoisomerization of β-Carotene Epoxides and the Simultaneous Formation of Furanoid Oxides". *Arch. Biochem. Biophys.* **1958**, *74*, 408–426.

Vetter, W.; Englert, G.; Rigassi, N.; Schwieter, U. "Carotenoids". In *Spectroscopic Methods*; Isler, O., Ed.; Birkhauser Verlag: Basel, 1971; Chapter 4, pp 243–266.

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HPLC Identification of Phenols in Vidal Blanc Wine Using Electrochemical Detection

Susan Mahler, Paul A. Edwards,* and Mary G. Chisholm

High-performance liquid chromatography with electrochemical detection was used to identify eight nonflavanoid phenols in Vidal blanc wines. Identifications were made by comparing both the capacity factor (k') and electrochemical behavior of wine phenols to those exhibited by pure phenols in standard solutions. More components were identified by electrochemical detection than by photodiode array techniques.

The chemistry of wines made from grapes indigeneous to the northeastern United States has yet to be studied to the extent of those made from *Vitis vinifera* cultivars. Recently, several studies of the cultivar Vidal blanc have been initiated as part of an effort to improve the understanding of these wines and, ultimately, the ability of small, regional vintners to produce high-quality products. Vidal blanc is a French-American hybrid derived from the *V. vinifera* variety Ugni blanc (also known as St. Emilion in France and Trebbiano in Italy) and the *Vitis riparia* variety Seibel 4986 (Rayon d'Or). One early goal of this research was to find a method (or methods) of determining the phenolic composition of regional, French–American hybrid white wines. Because high-performance liquid chromatography (HPLC) has been used increasingly in recent years to both qualitatively and quantitatively study

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wines, this study was undertaken to determine the applicability of certain HPLC techniques to the identification of phenols in Vidal blanc wines.

A review by Evans (1983) describes how HPLC techniques have been used to study several features of wine chemistry. Recent work on the phenolic content of wines describes (1) the analysis of constituent organic acids, including phenolic acids, by derivatization with phenacyl bromide (Mentasti et al., 1985); (2) the identification of phenolic compounds in musts and wines (Singleton and Trousdale, 1983; Barroso et al., 1983; Salagoïty-Auguste and Bertrand, 1984; Diez et al., 1984; Jaworski and Lee, 1987; Lee and Jaworski, 1987; Lunte, 1987); (3) the distribution of constituent compounds in grapes and wines (Bakker and Timberlake, 1985a,b; Singleton et al., 1986); (4) the variation of constituent compounds as a function of aging (Bakker et al., 1986); and (5) the variation of phenolic composition as a function of the barrel used for aging (Jindra and Gallander, 1987). Because the primary focus of the research has been on wines made from *vinifera* cultivars, the HPLC literature on wines made from hybrid and *labrusca* cultivars is rather sparse; only three of the studies cited above have non-*vinifera* wines as their primary focus (Jindra and Gallander, 1987; Jaworski and Lee, 1987; Lee and Jaworski, 1987).

We report here a procedure for identifying certain nonflavanoid phenols found in Vidal blanc wine. Based on work by Roston and Kissinger (1981), this procedure involves the extraction of the acidic phenols into ethyl acetate and the use of electrochemical detection in the HPLC analysis of the extract. Electrochemical detection is attractive because sufficient information to make identity assignments is obtained from a comparison of the capacity factors (k') and hydrodynamic voltammograms for sample constituents and standards (Roston et al., 1982), without the need for additional evidence such as that provided by thin-layer chromatography. The requisite hydrodynamic voltammograms are derived from the electrochemical behavior observed in a series of chromatography experiments. Electrochemical detection is specifically suitable for the study of wine phenols (Lunte, 1987; Sontag and Friedrich, 1988), and Nagels and Creten (1985) have shown electrochemical to be more selective than UV detection in the analysis of phenolic acids in plant materials.

Our use of electrochemical detection as a means of identification was stimulated by previous work in this laboratory in which procedures for studying the phenolic composition of musts and wines made from *vinifera* cultivars were applied to wines made from hybrid cultivars indigenous to the northeastern region of the United States. With use of the procedure reported by Singleton and Trousdale (1983), which employs ultraviolet (UV) detection methods, it was possible to identify a characteristic "fingerprint" for Vidal blanc wine. Unfortunately, no constituent phenols were identified because of poor agreement between the capacity factor (k') of wine constituents and pure phenolic compounds in standard solutions. In addition, satisfactory separation of the neutral and acidic phenols following the procedure of Salagoïty-Auguste and Bertand (1984) was never accomplished (Mahler and Edwards, 1986) because the separations were inefficient at the recommended pH and several phenols were observed in both fractions. Jaworski and Lee (1987) also found this procedure to be inefficient for procyanidins in their study of the *labrusca* cultivar Niagara.

EXPERIMENTAL SECTION

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 used as received. Eleven Vidal blanc wines were obtained from local, commercial wineries. Standard solutions were made by dissolving the desired mass of pure phenol as shown in Table I in 500 mL of aqueous solvent containing 12% (v/v) ethyl alcohol and 5% (w/v) dextrose. The first standard solutions contained vanillic acid as component 5. The need to replace vanillic acid with *m*-hydroxybenzoic acid in later standard solutions will be explained in the discussion section.

Table I. Standard Solutions

component number	compound (all acids)	k' (k')[a]	concn, mg/L
1	gallic	0.6 (0.4)	107
2	gentisic	1.1 (0.9)	101
3	3,4-dihydroxyphenylacetic (DHP)	1.4	100
4	4-hydroxyphenylacetic (HP)	2.8 (2.7)	100
5	vanillic or	3.6 (3.8)	100
	m-hydroxybenzoic (MHB)	3.6	50
6	caffeic	4.4 (4.3)	100
7	salicylic	5.5 (6.1)	50
8	*p*-coumaric	8.7 (8.9)	47
9	ferulic	11.9 (11.3)	104

[a] k' values in parentheses are from Roston and Kissinger (1981).

Sample Preparation. Twenty milliliters of standard solution or 50 mL of wine was acidified with 2 M HCl to pH 2 and saturated with NaCl. The solution was then extracted with three 20-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 10 mL of ethyl acetate, and the ethyl acetate solutions were again combined. For each sample, 7 mL of this solution was pipetted into a vial, the ethyl acetate evaporated under a stream of nitrogen at ambient temperature, and the solid residue frozen until needed. Before use, 1.0 mL of mobile phase was injected into each vial 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 332 gradient liquid chromatography system with a 20-μL sample loop, a Beckman ULTRASPHERE ODS 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. 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 87.3% (v/v) HPLC-grade water, was pumped isocratically at a flow rate of 1 mL/min.

All precautions suggested by Roston and Kissinger (1981) were taken to avoid electrode passivity. Sample and standard were injected alternatively, and mobile phase was passed through the detection cell for at least 0.5 h between samples. To avoid any bias or hysteresis in the electronics, the variation in electrode potential over the range +500 to +1000 mV required to determine electrochemical behavior was not done in constantly increasing or decreasing steps. Rather, after an initial chromatogram was obtained with the electrode potential at +1000 mV, the second might have been obtained at +700 mV, the third at +800 mV, etc., until the desired range of electrode potentials had been covered. One other precaution that could be taken

is to thermostat the column in order to minimize variations in capacity factor caused by fluctuations in temperature.

Five of the eleven wine samples and the standard solutions were analyzed a second time on the Beckman LC system described above with a Varian Polychrom 9060 diode array detector in place of the electrochemical detector. The peak sense wavelength was 282 nm, and the ultraviolet spectrum over the range 220–367 nm of each component was obtained as it was eluted from the column. All other column, mobile phase, and pumping conditions were as previously described.

Characterization. In all cases, the identification of sample components was based on two criteria. Tentative identifications were made by comparing the capacity factors ($k' = (t_R - t_M)/t_M$, where t_R is the retention time of the component and t_M is the retention time of nonretained solute) of unknown sample components to those exhibited by the pure phenolic acids in standard solutions. Identifications were confirmed by comparing the voltammetric behavior of unknown sample components again with that exhibited by the pure phenolic acids in standard solutions (Roston and Kissinger, 1981; Ward et al., 1985). Standards and wine samples were chromatographed with the electrode potential of the detector at +1000, +900, +800, +700, +650, +600, +550, and +500 mV in order to determine the characteristic potential curve (hydrodynamic voltammogram) for each individual component. The hydrodynamic voltammogram for a compound was obtained by plotting the electrode potential versus the relative recorder response. In this case, the relative recorder response at a given electrode potential was defined to be the ratio of the integrated area of the peak associated with that component to the integrated area of the peak for that same component when the electrode potential was +1000 mV. A smooth polynomial curve was then drawn through the data for each compound, to assist in displaying the general shape of that compound's hydrodynamic voltammogram. These curves should not be misinterpreted where they imply unacceptable, negative values for the relative response; no negative responses were observed.

The evidence provided by the electrochemical information proved invaluable as a criterion for making identifications. Several identifications that were made would have been impossible without the information provided by the electrochemical behavior, because of variations in retention time and k', possibly resulting from interactions between the phenolic solutes themselves. Indeed Boessenkool et al. (1986) have demonstrated that identifications should not be based on capacity factor match alone because k' can vary as a function of the composition of the eluent, and Shoup and Mayer (1982), using electrochemical detection in their study of phenols in environmental samples, observed that the capacity factor for particular phenols could be modified by the presence of other organic species. The significance of intra- and intermolecular interactions, including hydrogen bonding, in retention behavior has been explored for substituted benzenes (Yashan, 1982) and substituted phenols (Chang and Tan, 1985). Because the phenolic acids studied here contain both phenol and carboxylic acid functional groups, they could also be expected to exhibit significant intermolecular interactions. The extent to which those interactions occur will be a function of the composition of the sample, which will vary between standard and sample solutions and from sample to sample. The response of the detector was also not constant, with variations up to 20% being observed between runs. This occasionally made determination of electrochemical behavior difficult and may have prevented additional identifications. These latter variations were manifestations of electrode passivation and aging of the electrode (Roston and Kissinger, 1981).

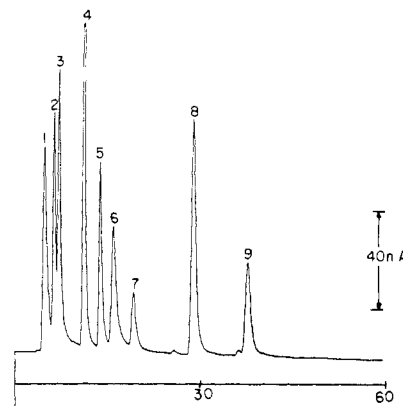

Figure 1. Chromatogram of a standard solution containing vanillic acid as component 5. Conditions: 4.6 × 250 mm ULTRASPHERE ODS column; flow rate, 1.0 mL/min; detector potential, +1000 mV. The numbering corresponds to that in Table I.

The identification of wine constituents using photodiode array detection was also based upon two criteria. The initial criterion was again the agreement between the capacity factors (k') associated with wine components and those associated with the pure phenols in standard solutions. The confirming criterion was the agreement between the Purity Parameter calculated within the Varian Polychrom 9060 diode array detector (Alfredson and Sheehan, 1985) over the range 254–367 nm of the UV spectra of the wine components and of the pure phenols.

RESULTS AND DISCUSSION

Figure 1 represents the chromatogram of the ethyl acetate extract of a standard solution after the extracting solvent has been removed and the residue reconstituted in mobile phase. Capacity factors (k') for each of the numbered components in Figure 1 are collected in Table I along with those reported by Roston and Kissinger (1981). The standard solution, without extraction, was also studied. As predicted by Levin and Grushka (1986), the capacity factors associated with compounds in extracts of standard solutions that had been redissolved in mobile phase as shown in Table I were less than those associated with the same compounds in the pure standard solutions. This difference in capacity factors arises because solute must be redistributed between solvent and mobile phase when the sample solvent is different from the mobile phase, in addition to being partitioned between the mobile and stationary phases. However, when the sample solvent is the same as the mobile phase, solute is only partitioned between mobile and stationary phases.

The hydrodynamic voltammograms of compounds with one oxidizable phenolic group (4-hydroxyphenylacetic, vanillic, *p*-coumaric, salicylic, ferulic acids), displayed in Figure 2, showed sharp decreases in current response, and only ferulic acid exhibited a significant relative response when the electrode potential was +500 mV. In contrast, as shown in Figure 3, compounds with two oxidizable phenolic groups (gentisic, 3,4-dihydroxyphenylacetic, caffeic acids) exhibited very gradual decreases in current response and showed a relative response greater than 0.5 when the electrode potential was +500 mV. The hydrodynamic voltammogram for gallic acid, a compound with three oxidizable phenolic groups, also shown in Figure 3, is similar to that of the other compounds shown, except that the relative response is a little lower at +500 mV. The

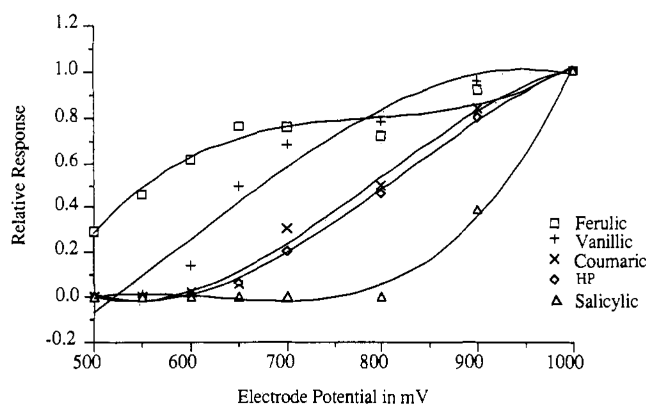

Figure 2. Hydrodynamic voltammograms of standard compounds with one oxidizable phenolic group.

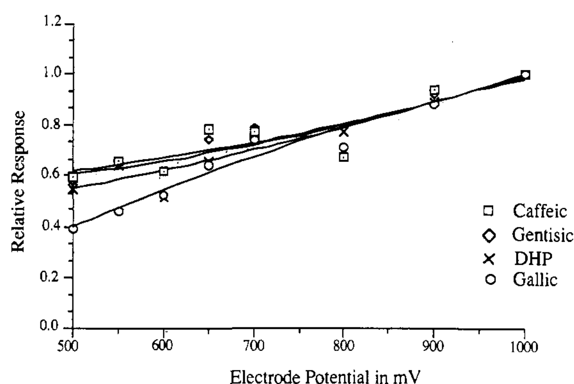

Figure 3. Hydrodynamic voltammograms of standard compounds with two and three oxidizable phenolic groups.

Table II. Vidal Blanc Components

peak number	compound (all acids)	k'	no. of wines showing peak
1	gallic	0.4	10/11
2	unassigned	0.6	6/11
3	unassigned	0.7	11/11
4	gentisic	1.0	4/11
5	3,4-dihydroxyphenylacetic (DHP)	1.2	10/11
6	4-hydroxyphenylacetic (HP)	2.3	11/11
7	*m*-hydroxybenzoic (MHB)	3.6	4/11
8	caffeic	4.4	9/11
9	salicylic	5.8	3/11
10	*p*-coumaric	7.9	6/11
11	unassigned	9.2	5/11

hydrodynamic voltammogram exhibited by a specific compound did not appear to depend upon whether the compound was in a standard solution or in an extracted and reconstituted sample of the standard.

A typical chromatogram of Vidal blanc wine is shown in Figure 4. The peak assignments are collected in Table II. One of the unassigned peaks (2) was not observed in the sample shown in Figure 4. After both the capacity factor and electrochemical behavior of wine components are compared to those of the phenolic compounds in standard solutions, the following compounds have been identified in Vidal blanc wine: gallic, gentisic, 3,4-dihydrophenylacetic, 4-hydroxyphenylacetic, *m*-hydroxybenzoic, caffeic, salicylic, *p*-coumaric acids. The frequency of occurrence of each phenol in the wine samples, also shown in Table II, provides evidence for the large variation of phenolic content between samples from different sources. Those phenols whose frequency of occurrence is low may not be characteristic of Vidal blanc wine. A further explanation follows as to how these assignments were made.

Four compounds, gentisic, 3,4-dihydroxyphenylacetic,

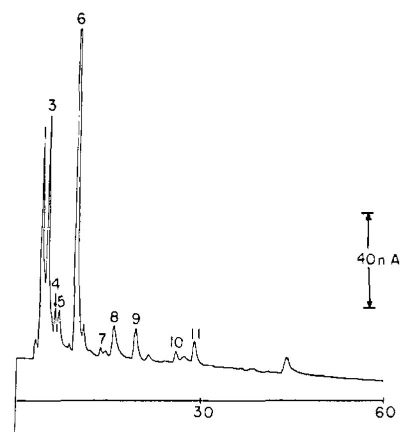

Figure 4. Typical chromatogram of the ethyl acetate extract of Vidal blanc wine. Conditions: 4.6 × 250 mm ULTRASPHERE ODS column; flow rate, 1.0 mL/min; detector potential, +1000 mV. The numbering corresponds to that in Table II.

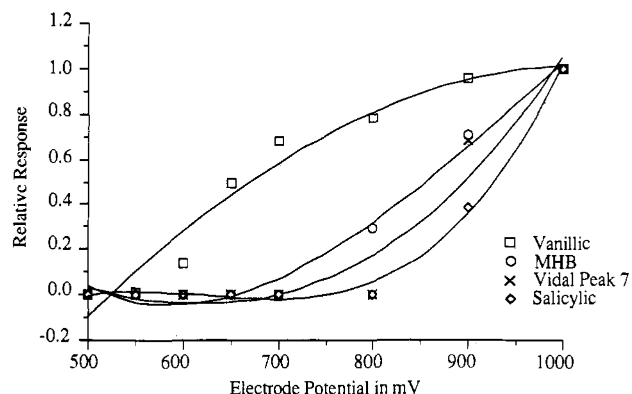

Figure 5. Hydrodynamic voltammograms of Vidal blanc peak 7 and standard phenolic compounds.

caffeic, and salicylic acids, exhibited similar capacity factors in both standard and wine samples, as shown in Tables I and II. For these compounds, the similar electrochemical behavior of corresponding peaks in sample and standard solutions served as a confirmation of the assignments suggested by the similar capacity factors.

As can also be seen in Tables I and II, the agreement between the capacity factors exhibited by 4-hydroxyphenylacetic and *p*-coumaric acids in standard and wine solutions is not good enough to permit unambiguous assignments. In these cases, the observation of similar electrochemical behavior of corresponding peaks was critical in making the assignment. As can be seen in Figure 2, the electrochemical behavior of these two compounds in the standard solutions was remarkably similar and distinguishable from that of other phenols with one oxidizable phenolic group. Because wine peaks 6 and 10 exhibited electrochemical behavior similar to that of 4-hydroxyphenylacetic and *p*-coumaric acids, respectively, the assignment has been made as shown in Table II.

In the case of Vidal blanc peak 7, the use of capacity factor alone would lead to an incorrect assignment. The average capacity factor for Vidal blanc peak 7 is very close to that exhibited by vanillic acid in the original standard solutions, 3.62 versus 3.64, respectively. However, as can be seen in Figure 5, the electrochemical behavior of Vidal blanc peak 7 is very different from that exhibited by vanillic acid, with the hydrodynamic voltammogram for peak 7 dropping sharply at a higher potential than that of vanillic acid. A sharp break in the hydrodynamic voltammogram is indicative of a phenol containing a single oxidizable phenolic group. Indeed the hydrodynamic

Table III. Comparisons of Identifications as a Function of Method[a]

compound (all acids)	wine A	wine B	wine C	wine D	wine E
gallic	ECD		ECD	ECD	ECD
gentisic				ECD	
3,4-dihydroxyphenylacetic (DHP)		BOTH	BOTH	ECD+	ECD
4-hydroxyphenylacetic (HP)	BOTH	BOTH	BOTH	BOTH	BOTH
vanillic		DAD	DAD		
m-hydroxybenzoic (MHB)				ECD	
caffeic	BOTH	BOTH	DAD+	BOTH	BOTH
salicylic		ECD		ECD	ECD
p-coumaric	ECD	ECD	BOTH	BOTH	BOTH

[a] Key: DAD, strong evidence for assignment obtained by photodiode array detection; ECD, strong evidence for assignment obtained by electrochemical detection; BOTH, strong evidence for assignment obtained by both detection techniques; +, suggestive but not definitive evidence obtained by the unspecified technique.

voltammogram for peak 7 is strikingly similar to that exhibited by salicylic acid; however, the capacity factors are too different to permit such an assignment. Additionally, Vidal blanc peak 9 has already been assigned as salicylic acid on the basis of both similar capacity factors and electrochemical behavior. Barroso et al. (1983) observed very similar capacity factors for vanillic and *m*-hydroxybenzoic acids. Since *m*-hydroxybenzoic and *p*-hydroxybenzoic acids are isomers of salicylic acid (*o*-hydroxybenzoic acid), they might be expected to exhibit electrochemical behaviors similar to that of salicylic acid. When a standard solution was prepared with *m*-hydroxybenzoic acid in place of vanillic acid, a peak was observed exhibiting a capacity factor similar to that of vanillic acid and wine component 7. However, the electrochemical behavior of *m*-hydroxybenzoic acid was very different from that of vanillic acid and very similar to that of salicylic acid and wine peak 7. Thus, on the basis of the similarity of BOTH capacity factor and electrochemical behavior, we believe Vidal blanc peak 7 to be *m*-hydroxybenzoic acid.

The assignment of Vidal blanc peak 1 as gallic acid further illustrates the advantage of having more than capacity factor as a criterion for making identifications. Peaks 1–3 in the typical Vidal blanc wine chromatogram (Figure 4) have capacity factors close to that exhibited by gallic acid in the standard solutions. Fortunately, the hydrodynamic voltammogram for one of the peaks more closely paralleled that exhibited by gallic acid that did the behavior of the other two peaks. As shown in Figure 3, gallic acid exhibits a relative response greater than zero all the way down to an electrode potential of +500 mV, and, in nine of the eleven wine samples, Vidal blanc peak 1 exhibited that same behavior. Vidal blanc peaks 2 and 3 on the other hand generally exhibited lower values for the relative response, usually zero by +500 mV. However, in two wine samples, this was not the case. In one wine sample, peak 2 exhibited the high response at +500 mV characteristic of gallic acid, while peak 1 exhibited a response very close to zero. In a second sample, peak 1 exhibited a response too low to be indicative of gallic acid but peak 2 was not observed. Thus, it would appear that gallic acid was not observed in this latter sample.

The advantage of using electrochemical detection over photodiode array techniques in the identification of phenols in wines can be seen in the data collected in Table III. Gentisic, *m*-hydroxybenzoic, and salicylic acids were all detected by electrochemical but not diode array means. Vanillic acid is the only compound detected by diode array but not electrochemical methods. Sontag and Friedrich (1988), using electrochemical detection, also did not find vanillic acid in the two wine samples they studied; however, they did find it in other samples of distilled alcoholic beverages. There are at least six other examples in Table III in which a component was identified in a wine by electrochemical but not diode array detection. There is only one case in which the reverse may be true, specifically caffeic acid in wine B.

The fact that we were able to identify eight phenolic acids in Vidal blanc wine demonstrates that electrochemical detection is one technique that may be used in conjunction with high-performance liquid chromatographic studies of non-*vinifera* white wines. Simply matching the capacity factors exhibited by standard and wine components to make assignments as done by Barroso et al. (1983) and Jindra and Gallander (1987) is inconclusive because the capacity factors of these compounds reflect intermolecular interactions that vary from sample to sample. Photodiode array detection is another technique that may be used, but it is not as sensitive as electrochemical detection (Nagels and Creten, 1985).

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Registry No. DHP, 102-32-9; HP, 156-38-7; MHP, 99-06-9; gallic acid, 149-91-7; gentisic acid, 490-79-9; caffeic acid, 331-39-5; salicyclic acid, 69-72-7; *p*-coumaric acid, 7400-08-0.

LITERATURE CITED

Alfredson, T.; Sheehan, T. "Advances in Multichannel Photodiode Array-based LC Detection". *Am. Lab.* **1985**, *17*(8), 40–54.

Bakker, J.; Timberlake, C. F. "The Distribution of Anthocyanins in Grape Skin Extracts of Port Wine Cultivars as Determined by High Performance Liquid Chromatography". *J. Sci. Food Agric.* **1985a**, *36*, 1315–1324.

Bakker, J.; Timberlake, C. F. "The Distribution of Anthocyanins in Young Port Wines as Determined by High Performance Liquid Chromatography". *J. Sci. Food Agric.* **1985b**, *36*, 1325–1333.

Bakker, J.; Preston, N. W.; Timberlake, C. F. "The Determination of Anthocyanins in Aging Red Wines: Comparison of HPLC and Spectral Methods". *Am. J. Enol. Vitic.* **1986**, *37*, 121–126.

Barroso, G. C.; Torrijos, R. C.; Pérez-Bustamante, J. A. "HPLC Separation of Benzoic and Hydroxycinnamic Acids in Wines". *Chromatographia* **1983**, *17*, 249–252.

Boessenkool, H.-J.; Cleij, P.; Goewie, C. E.; van den Broeck, H. H.; van't Klooster, H. A. "Computer-Aided Library Search of Combined LC-Retention and Diode-Array-UV-Spectral Data". *Mikrochim. Acta* **1986**, *II*, 75–92.

Chang, C. A.; Tan, L. J. "Effects of Solvent Composition and Temperature on the Normal Phase Liquid Chromatographic

Separation of Substituted Phenols". J. Liq. Chromatogr. 1985, 8, 995–1007.
Diez, C.; Gômez-Cordovês, C.; Hernândez, T.; Santa Maria, G. "Bedeutung von phenolischen Verbindungen mit niederem Molekulargeweicht zur Untersuchung des Ursprungs der Weine". Dtsch. Lebensm. Rundsch. 1984, 80, 13–17.
Evans, M. E. "High Performance Liquid Chromatography in Oenology". J. Liq. Chromatogr. 1983, 6(s-2), 153–178.
Jaworski, A. W.; Lee, C. Y. "Fractionation and HPLC Determination of Grape Phenolics". J. Agric. Food Chem. 1987, 35, 257–259.
Jindra, J. A.; Gallander, J. F. "Effect of American and French Oak Barrels on the Phenolic Composition and Sensory Quality of Seyval blanc Wines". Am. J. Enol. Vitic. 1987, 38, 133–138.
Lee, C. Y.; Jaworski, A. "Phenolic Compounds in White Grapes Grown in New York". Am. J. Enol. Vitic. 1987, 38, 277–281.
Levin, S.; Grushka, E. "System Peaks in Liquid Chromatography: Their Origin, Formation, and Importance". Anal. Chem. 1986, 58, 1602–1607.
Lunte, S. M. "Structural Classification of Flavonoids in Beverages by Liquid Chromatography with Ultraviolet-Visible and Electrochemical Detection". J. Chromatogr. 1987, 384, 371–382.
Mahler, S.; Edwards, P. A. "Studies of the Chemical and Sensory Analysis of Wines: High Performance Liquid Chromatography". Proceedings of the 40th Annual Eastern Colleges Science Conference, Duquesne University, Pittsburgh, PA, 1986; p 23.
Mentasti, E.; Gennaro, M.; Sarzanini, C.; Baiocchi, C.; Savigliano, M. "Derivatization, Identification and Separation of Carboxylic Acids in Wines and Beverages by High-Performance Liquid Chromatography". J. Chromatogr. 1985, 322, 177–189.
Nagels, L. J.; Creten, W. L. "Evaluation of the Glassy Carbon Electrochemical Detector Selectivity in High Performance Liquid Chromatographic Analysis of Plant Materials". Anal. Chem. 1985, 57, 2706–2711.
Roston, D. A.; Kissinger, P. T. "Identification of Phenolic Constituents in Commercial Beverages by Liquid Chromatography with Electrochemical Detection". Anal. Chem. 1981, 53, 1695–1699.
Roston, D. A.; Shoup, R. E.; Kissinger, P. T. "Liquid Chromatography/Electrochemistry: Thin Layer Multiple Electrode Detection". Anal. Chem. 1982, 54, 1417A–1434A.
Salagoïty-Auguste, M.-H.; Bertrand, A. "Wine Phenolics—Analysis of Low Molecular Weight Components by High Performance Liquid Chromatography". J. Sci. Food Agric. 1984, 35, 1241–1247.
Shoup, R. E.; Mayer, G. S. "Determination of Environmental Phenols by Liquid Chromatography". Anal. Chem. 1982, 54, 1164–1169.
Singleton, V. L.; Trousdale, E.; "White Wine Phenolics: Varietal and Processing Differences as Shown by HPLC". Am. J. Enol. Vitic. 1983, 34, 27–34.
Singleton, V. L.; Zaya, J.; Trousdale, E. "Compositional Changes in Ripening Grapes: Caftaric and Coutaric Acids". Vitis 1986, 25, 107–117.
Sontag, G.; Friedrich, O. "Bestimmung phenolischer Verbindungen in alkoholischen Getränken durch HPLC mit elecktrochemischen Detektor". Z. Lebensm. Unters. Forsch. 1988, 186, 130–133.
Ward, C. J. P.; Radzik, D. M.; Kissinger, P. T. "Detection of Toxic Substances in Polyurethane Food Bags by Liquid Chromatography/Electrochemistry". J. Liq. Chromatogr. 1985, 8, 677–690.
Yashin, Y. I. "Selectivity in Lquid Adsorption Chromatography". J. Chromatogr. 1982, 251, 269–279.

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Total Phenolics, Condensed Tannins, and Protein-Precipitable Phenolics in Young and Mature Leaves of Oak Species

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Total phenolics, condensed tannins, proanthocyanidins, leucoanthocyanins, and protein-precipitable phenolics were determined in young and mature leaves of *Quercus incana*, *Quercus semecarpifolia*, and *Quercus ilex*. Total phenols in young leaves of both *Q. semecarpifolia* and *Q. incana* were higher, but no difference was observed for *Q. ilex*. Condensed tannins increased with maturity except in the case of *Q. ilex* where no change was observed; the same trend was observed for proanthocyanidins and leucoanthocyanins. Protein-precipitable phenolics and total phenols were highest in *Q. semecarpifolia* and lowest in *Q. ilex*. The protein-precipitable phenolics had an excellent correlation ($r = 0.995$) with total phenols.

The utilization of tree foilage as feed for ruminants is a common practice during periods of fodder shortages (Negi et al., 1979). Oak leaves are used as fodder for ruminants in India and various other developing countries (Neser et al., 1982; Makkar et al., 1986). Incorporation of oak (*Quercus incana*) leaves into mixed rations up to a level of 14% has been reported to be safe (Negi et al., 1979; Lohan et al., 1983). Feeding at higher levels or given as the sole feed results in oak poisoning (Makkar et al., 1986). Adverse effects of oak leaves to ruminants have been attributed to the presence of tannins in the leaves (Lohan et al., 1983; Makkar et al., 1986). The deleterious effects of tannins depend on the quantity, types, and their protein-precipitating capacity (Feeny and Bostock 1968; Feeny, 1970; Makkar et al., 1987). In the present study, total tannin content as total phenolics, condensed tannins, proanthocyanidins, leucoanthocyanins, and protein-precipitable phenolics of young and mature leaves of three oak species, *Q. incana*, *Quercus semecarpifolia* and *Quercus ilex*, is presented. The implication of the results in relation to adverse effects of oak leaves in livestock is discussed.

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