Oak Lactone Isomer Ratio Distinguishes between Wines Fermented in American and French Oak Barrels

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A procedure that involves solvent extraction, evaporation, and gas chromatographic analysis can be used to distinguish between wines fermented in Amercan oak and wines fermented in French/European oak. The parameter that makes the distinction is the ratio of *cis*- to *trans*-β-methyl-γ-octalactone (oak lactone). Other studies of oak have reported oak lactone ratios different from ours, which we attribute to the complex lactone/acid/ester equilibria in aqueous ethanol solutions, reactions that are not allowed to equilibrate in typical laboratory analyses. However, by taking the equilibration kinetics into account, it should be possible to adapt this procedure to determine the origin of oak wood samples as well as wine and spirit samples that have been aged in oak.

Keywords: *Wine; oak lactone; barrel; gas chromatography*

INTRODUCTION

Oak barrels have been used to store and age wine and other beverages for centuries. White oak is used because it can produce a barrel that does not leak. At present, oak barrels are used in the production of wines including sherries as well as in distilled spirits—principally whiskeys, bourbon, and scotch. Where standard-sized oak barrels are used (approximately 55–60 gal or 210–228 L), current practice is directed toward enhancing the flavor of the beverage by incorporating flavor components from the oak into the beverage.

At present there are two principal market sources of oak for barrels, the United States and France, though other countries do produce some oak barrels. An important market factor is the price differential: oak barrels produced from French oak cost from 2–4 times those of U.S. oak. The differential is partially caused by the greater losses in coopering French oak, due to a more irregular grain structure and higher wood porosity. Thus, the wood must be split instead of sawn.

There is also a growing movement of unfinished oak across the Atlantic: U.S. cooperage houses are purchasing French oak and vice versa for barrel production. Given the new market fluidity, there is a need for the means to distinguish between the two types of wood, but no such procedures have been described to date.

In France, and across Europe, there are two commercial white oak species used for cooperage, *Quercus pedunculata* (or *Q. robur*) and *Q. sessilis* (or *Q. petraea* or *Q. sessiliflora*). In the United States, there are several commercial white oak species, the most important being *Q. alba*, which is found throughout the United States east of the 100th meridian. It is used for nearly all commercial barrel production, but some other species in overlapping areas and hybrids of these with *Q. alba* are also used (Singleton, 1974). *Q. garryana* is geographically separate from the other American species and is found in the northwestern United States (it is called Oregon oak) (Miller and Lamb, 1985), and there is a small supply of barrels made from this wood.

The β-methyl-γ-octalactones, commonly known as the oak or whiskey lactones, are components found in white

Table 1. Barrel Lots Used in the Chardonnay Fermentation

lot	wood source[a]	pH	TA	alcohol %
1	U.S., Missouri/Arkansas	3.34	6.85	13.2
2	U.S., Missouri/Arkansas	3.34	6.70	13.3
3	U.S.	3.32	6.51	13.2
4	U.S., Oregon	3.35	6.68	13.3
5	U.S., Missouri	3.34	6.65	13.1
6	U.S., Kentucky	3.35	6.39	13.1
7	U.S., Kentucky	3.34	6.65	13.2
8	France, Troncais	3.33	6.41	13.2
9	France, Nevers	3.34	6.41	13.3
10	France, Allier	3.32	6.35	13.3
11	France, center of France	3.34	6.65	13.1
12	France, center of France	3.34	6.74	13.3
13	France, Allier	3.32	6.61	13.3
14	Hungary	3.33	7.20	13.3
15	Hungary	3.34	6.45	13.3
	mean	3.33	6.61	13.2
	SD	0.02	0.22	0.1

[a] All American oak was coopered in the United States; all European oak was coopered in Europe.

oak which contribute to the flavor impact of the oak. The aroma threshold for the *cis* isomer is 92 ppb and for the *trans* isomer 460 ppb in white wine (Chatonnet, 1991). Since there is generally more *cis* isomer, the sensory importance of the *trans* isomer is low. The aroma of both *cis* and *trans* oak lactones is variously described as woody or oaky at low concentration and coconut or varnish at high concentrations (Chatonnet et al., 1990). In this study we demonstrate that the ratio of oak lactones, under controlled conditions, can be used to determine the source of wood used to barrel ferment white wine. The lactone ratio may be useful for oak wood analysis with some additional development.

EXPERIMENTAL PROCEDURES

Wine Making. The barrels were obtained from sources in the United States and Europe. Nine barrels were used for each fermentation lot; 15 different barrel types were obtained, and these are listed in Table 1. While the toasting levels requested from the coopers was always dark/heavy, the interior stave darkness was extremely variable, ranging from very dark to just perceptible darkening, indicating that toasting was quite variable.

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Chardonnay grapes were harvested at 23.5° Brix from the Forest Lake vineyard in the Lodi, CA, area. The grapes were immediately crushed in a bladder press and acidified with tartaric acid to 8.5 g/L titratable acid, pH 3.45, and the juice was stored at 11 °C for 48 h. An inoculum of *Saccharomyces cerevisiae* strain cerevisiae (Montrachet, Red Star) yeast was added on September 5, 1992, and thoroughly mixed, and the juice was transferred to barrel after 3 days. The barrels were stored at 16 °C, and fermentation commenced in 12 h. Most sugar fermentations were complete after 5 days. Malolactic fermentation was induced at dryness with *Leuconostoc oenos* MCW (Vinquery) and finished in all wines within 2 weeks. The wines were left on the yeast lees and stirred up and topped off biweekly until March. The wines were bottled over several days during the first week of April after the 9 barrels of each lot were blended into a tank, bringing free sulfite levels to 30 mg/L and passing through a 1 μm membrane filter. One set of nine samples from the individual barrels of one lot was collected before bottling.

Standard wine analyses were carried out by direct measurement of pH using a Fisher Accumat 925 pH meter with a Hach One combination electrode. The titratable acidity (TA) was measured by titrating to a phenolphthalein endpoint and expressing the amount of hydroxide used as the equivalent amount of tartaric acid that would have been consumed for the same titration. The alcohol level (v/v) was measured by observing boiling point depression in an ebulliometer.

Extraction Procedure. Fifty milliliters of a wine sample was transferred to an Erlenmeyer flask, to which 0.5 mL of 100 ppm 3,4-dimethylphenol (internal standard) and 15 g of ammonium sulfate were added, and the solution was mixed until dissolution. This wine solution was extracted three times with dichloromethane (10, 5, and 3 mL) in a separatory funnel (2 min of mixing per extraction). The solutions were centrifuged (3 min at 2000 rpm) to break the emulsion that formed. The combined organic extracts were then washed twice with 25 mL of 5% sodium bicarbonate (45 s of mixing) to remove carboxylic acids. The organic phase was concentrated by evaporation under nitrogen gas to approximately 1 mL. The concentrate was then transferred to a vial, sealed, and stored in a freezer until GC analysis was carried out.

Gas Chromatographic (GC) Analysis. A Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (FID), a Hewlett-Packard 7673A autosampler, and a Hewlett-Packard 3392A integrator was used. The column was a DB-Wax (stabilized Carbowax, J&W Scientific, 30 m long, 0.25 mm i.d., 0.25 μm film thickness), and helium (0.4 bar of column head pressure) was used for the carrier gas. The analysis used a temperature program with an initial temperature of 70 °C for 2 min, a temperature gradient of 5 °C/min to a final temperature of 220 °C, and a final time of 20 min (total run time = 52 min). The injector was set to 200 °C and the detector at 220 °C. The injected sample was 1 μL. Relative retention time was 0.824 for the *cis* isomer and 0.772 for *trans* isomer with respect to 3,4-dimethylphenol. The two peaks were identified as isomers of β-methyl-γ-octalactone by GC–MS, and the identities of the two isomers were assigned according to the work of Chatonnet (1991), which shows that the *trans* isomer elutes first on a Carbowax column. Equal FID detector response was assumed for the two isomers.

RESULTS AND DISCUSSION

The standard wine analyses on pH, TA, and alcohol all showed very little variance with the exception of the high TA measurement for lot 14; we have no explanation for that variation (Table 1), but variance of this parameter will have a negligible effect on our results.

As seen in Figure 1, the amounts of the *cis* and *trans* oak lactones found in our French- and U.S.-sourced wood are quite variable, the range of the variation being a factor of 8. While the American oak has higher levels of oak lactones, on average, as other studies have noted (Guymon and Crowell, 1972), in our sample there are American oak samples with levels comparable to the
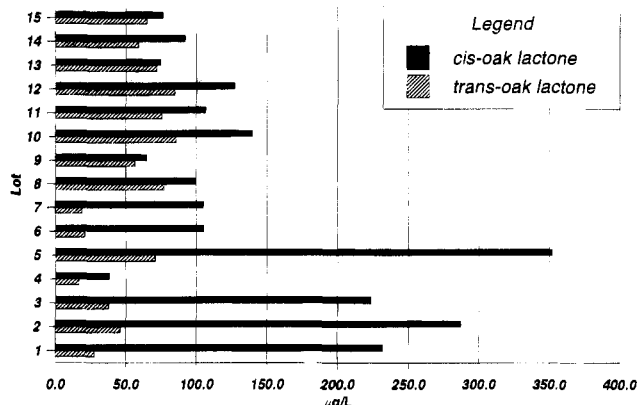
Figure 1. *cis* and *trans* oak lactone levels in wines produced by fermentation in different barrel lots.
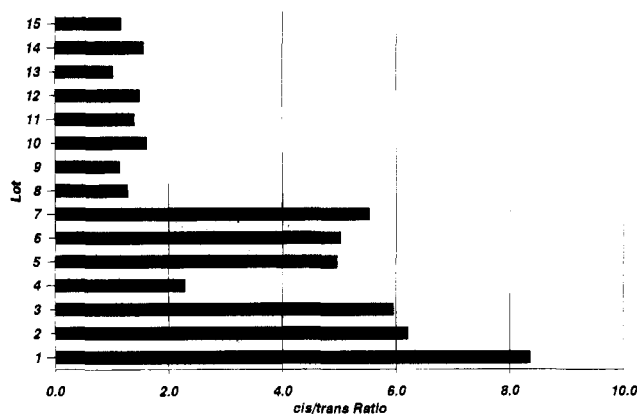

Figure 2. *cis* to *trans* oak lactone ratios in wines produced by fermentation in different barrel lots.

average French oak levels. In the finished wine it is clear that lots 6 and 7 had levels comparable to the average for the European barrels as shown in Figure 1. It is also notable that the lowest levels are found in wine from the Oregon oak barrels. Thus, the level of oak lactones is a poor indicator of wood source.

However, it is clear that the ratios of the *cis* and *trans* oak lactones in fermented wine are characteristic of the wood's genetic source. For all of the European wood, the ratio is between 1 and 1.5, with an average of 1.3 ± 0.2, and for the American wood (except the Oregon oak), the average is 6 ± 1.3, with a range from 5 to 8 (see Figure 2). Thus, by analyzing the ratio of *cis* to *trans* oak lactone by gas chromatography, it is possible to determine the source of wood used to ferment these wines. Statistically, the likelihood that these results are significant is much greater than 99.99%.

Results from the individual barrels of lot 10 showed a wide range of values, the ratio varying from 0.84 to 6.1. Averaging the levels of each lactone isomer over the nine barrels and then calculating the ratio from these amounts gave a ratio of 1.7, the same observed for the composite wine. These results reiterate that the variance in natural products is substantial and that large sample numbers are required to eliminate this variance.

Previous studies have pointed out at least two factors that are supposed to affect both the levels of oak lactones and the ratio of the isomers. Our results strongly suggest that the basis of the differences observed in these previous experiments was analysis of nonequilibrium mixtures, in which the equilibrium between the lactone and open forms had not been attained under standard conditions.
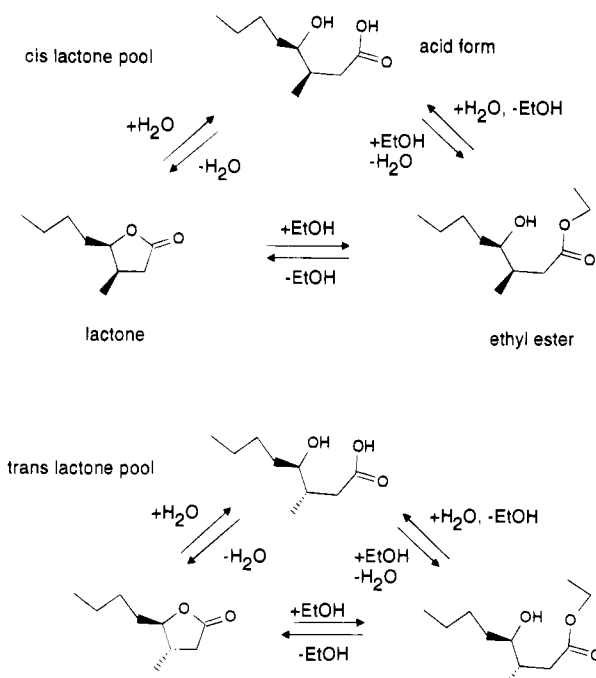
Figure 3. Chemical equilibria of *cis* and *trans* oak lactones in wine.

The oak lactones occur as the lactones themselves, as the open acid forms, and as the ethyl esters in ethanol-containing solutions such as wine and distilled spirits (Figure 3). We will refer to the sum of all three forms for each isomer as the pool of *cis* isomers and the pool of *trans* isomers. The analysis procedure used here as well as in other studies detects only the volatile lactone form.

As can be seen in Figure 3, there are a number of different equilibria that will determine the amount of the lactone forms observed under different circumstances. First, there is the simple lactone hydrolysis equilibria. In addition, there are lactone ethanolysis equilibria, as well as ethyl ester hydrolysis equilibria. The amount of each lactone form, the form detected by GC, will depend on a number of factors. These are (1) the amounts of the total pools of *cis* and *trans* forms in the wood, (2) the concentration of ethanol (significantly different between wine and whiskeys) in an extracting solution, (3) the pH of an extracting solution, (4) any treatment of the wood that could cause hydration or dehydration of the wood, and (5) the equilibration time of any solution after extraction has occurred. The pH and alcohol levels in the finished wines are not substantially different, as seen in Table 1, and thus these factors will not alter the equilibria between lots.

Here, the extraction from the wood was carried out by wine that was the same for all barrel lots; thus, the pH and ethanol concentrations were nearly identical. Also, the extraction times were on the order of months, so that there was adequate time to reach equilibrium. Thus, of the above factors, the only significant differences were those due to the wood itself and barrel manufacture.

It is very important to note that because the *cis* and *trans* isomers are chemically different, the equilibria and reaction rates descibed in Figure 3 will be different. Consequently, with the same ratio of pools of the *cis* and *trans* isomers, different proportions of each lactone form will be present under specific conditions, where the effective amount of water changes, such as in wood

Table 2. Comparison Data from Chatonnet (1991)

	calculated *cis/trans* oak lactone ratios			
source	untoasted	light toast	medium toast	heavy toast
lab extracts of wood[a]	4	6	12	11
wines, after 8 months in barrel[b]		1.8	1.3	2.1

[a] Averages of three values from Table 25 (Chatonnet, 1991).
[b] Averages of two barrels from Table 35 (Chatonnet, 1991).

vs in wine. Importantly, the isomer ratio should change as wood is dried by heating, as in toasting (Chatonnet, 1991).

However, our results suggest a hypothesis that white oak woods contain a fixed ratio of pools of *cis* to *trans* oak lactones and that this ratio is dependent on genetic traits of the wood. Heating or other changes that alter the hydration state of the wood can alter the proportions of the lactone form in each pool, and these changes are not identical for each isomer. Thus, toasting wood will lead to an increase in the proportion of the amount of the lactone form, and thus rapid extraction and GC analysis will reveal an "increase" in oak lactone levels—until the heating causes destruction or evaporation. Apparently the *cis* lactone, for kinetic or thermodynamic reasons, dehydrates to a greater extent under these conditions, and the ratio of *cis* to *trans* isomers increases as well.

However, these changes will be reversed when two samples toasted to different levels are then allowed to equilibrate under the same conditions with an aqueous solution, as during storage of wine in an oak barrel for 6 months. Under these conditions, the lactone pools are extracted into the wine and each form attains relative levels determined by the acidity and ethanol content of the wine. Our data show that the same ratios are found for samples of similar genetic extraction and that variations in barrel manufacture appear to have negligible effects.

This hypothesis is supported by Chatonnet's (1991) data on French wood, although the small number of barrels in his experiments leads to some variance in the data (Table 2). He shows that the *cis/trans* ratio increases dramatically in wood as a result of toasting for samples that were extracted in laboratory experiments. The *cis/trans* ratios increased from 4 to 12, going from untoasted to medium toast, but when the ratio is determined from wine stored in barrels for 8 months, the ratio is always near the range 1−2 regardless of toasting levels, as we observe in our European barrels.

Another barrel production process, the seasoning of the green wood by storage of the wood outdoors for 1−3 years before coopering, has been reported to affect the levels of oak lactones (Sefton et al., 1993). In this paper, it was revealed that the levels increased on seasoning under dry conditions (in Australia), while the levels decreased on seasoning under humid conditions (France). These observations confirm our hypothesis that dehydrating conditions will increase the levels of the oak lactone. In wood, the pH is not nearly as acidic as wine, so the acid forms will be stabilized by deprotonation, and under high water conditions, one might therefore expect to find very little of the lactone form present, as was observed in the seasoning study.

Analytical procedures to determine the source of wood samples on the basis of the ratio of oak lactones will have to be designed with an equilibration time under specific conditions. To facilitate these procedures, future studies will focus on the determination of the

equilibria constants of hydrolysis and esterification, as well as the reaction rates.

In conclusion, we have discovered a relatively simple means to discriminate between the sources of wood, either American or European oak, used to ferment white wine. It appears likely that this method could be adapted, with careful consideration of equilibria and equilibration kinetics, to distinguish between wood samples as well as the wood source for barrels used to store other beverages.

ACKNOWLEDGMENT

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