

Terpene Compounds as Possible Precursors of 1,8-Cineole in Red Grapes and Wines

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While the contribution of 1,8-cineole to the aroma of wine has been reported, it is a matter of controversy that the vineyards producing such wines are surrounded by *Eucalyptus* trees, which may contribute their essence to the grapes. However, experimental information presented in this paper suggests that 1,8-cineole can be produced by chemical transformation of limonene and α -terpineol, and this process may be responsible for the occurrence of *Eucalyptus*-like aroma in Tannat wines from vines not grown in the vicinity of *Eucalyptus* trees. A mechanism for the chemical transformation of these aroma compounds is proposed.

KEYWORDS: *Eucalyptus* aroma; 1,8-cineole; limonene degradation; Tannat red wine

INTRODUCTION

Wine aroma depends on numerous factors, with special importance being given to the variety of grape, vinification, maturation, and aging (1–3). It is well-known that the secondary metabolites of grapes are responsible for the principal aroma compounds in grape must and provide the basis of varietal character (1, 2, 4). Numerous studies on the volatile compounds of *Vitis vinifera* wines, as reviewed by Strauss (5), Versini (2), and Rapp (3), helped to elucidate the basic flavor chemistry in this field of special interest. Fermentation increases the chemical and aroma complexity of wine by assisting in the extraction of compounds from solids present in the grape must, modifying some grape derived compounds, and producing a substantial amount of yeast metabolites (6).

Enormous efforts have been focused on the topic of varietal characterization (3), for which is necessary to understand the influence of specific compounds on the total flavor impression. A good example of this approach is the identification of monoterpenoids, compounds with strong sensory qualities and present in a diverse range of plants such as *Vitis vinifera* varieties. Monoterpenols, particularly linalool, geraniol, and nerol, are responsible for the characteristic floral aroma in grapes and wines of *V. vinifera* cultivars such as Muscat, Gewürztraminer, and Riesling (7). Acid-catalyzed rearrangements during wine processing and aging can also result in changes in concentration and formation of new compounds that were not present in the original grapes and young wines (8, 9). Moreover,

in grapes, terpenoids exist in both free and glycosidically bound forms (10), and some of the bound terpenoids may be released either chemically (11, 12) or by natural glycosidase activities of the grape or of yeast and bacteria during the vinification phases (13).

In wine tasting, the term “*eucalyptus*” describes a spicy, mintlike aroma of certain red wines. The typical eucalyptus odor (fresh, camphoraceous, cool) usually is related to the monoterpene compound 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo-[2.2.2]octane), commonly known as eucalyptol (14). It was recently reported that vineyards producing such wines are surrounded by *Eucalyptus* sp. trees, which may contribute their essence to the grapes (14). In this paper, we provide evidence suggesting that the presence of 1,8-cineole in wines can arise from precursors typical of the grape itself, like limonene. Thus, we may be able to explain the eucalyptus-like scent also in wines coming from vineyards far away from *Eucalyptus* tree cultivation as observed in the case of Tannat wines, described frequently as mint-like-flavored.

1,8-Cineole, and chemically related compounds, have been quantified by GC/MS/SIM in monovarietal Tannat wines and single varietal grape samples of this variety harvested from southern Uruguayan vineyards. We also report the results of deuterium-labeling experiments that identify the sequence of chemical rearrangements that convert limonene to 1,8-cineole through reaction steps which involve the cyclization of *trans*-1,8-terpine catalyzed by the temperature and acidic conditions that can be encountered by grapes and wines.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Limonene, α -terpineol, 1,8-terpine, and 1-heptanol were purchased from Aldrich (Milwaukee, WI).

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Table 1. Changes during Thermal Conditioning in Synthetic Wines Fortified with Limonene or α -Terpineol, and in Tannat Wine with No Added Limonene or α -Terpineol

	limonene $\mu\text{g/L}$ ($\pm\text{SE}$) ^a	α -terpineol $\mu\text{g/L}$ ($\pm\text{SE}$) ^a	<i>trans</i> -1,8-terpine $\mu\text{g/L}$ ($\pm\text{SE}$) ^a	<i>cis</i> -1,8-terpine $\mu\text{g/L}$ ($\pm\text{SE}$) ^a	1,8-cineole $\mu\text{g/L}$ ($\pm\text{SE}$) ^a
synthetic wine added with limonene (0.5 mg/L)	4.6 \pm 0.3	4.4 \pm 1.1	7.5	57.8 \pm 12.3	24.1 \pm 3.2
synthetic wine added with α -terpineol (0.5 mg/L)	3.4 \pm 0.9	23.5 \pm 2.2	50.8 \pm 2.9	245.8 \pm 52.3	8.4 \pm 0.3
wine	2.3 \pm 1.3	6.7 \pm 0.8	21.4 \pm 4.8	41.6 \pm 16.1	13.7 \pm 4.7

^a SE = standard error around the mean ($n = 2$).

1,8-Cineole (99.8%) was purchased from the Center of Agroindustrial Technology (Cochabamba, Bolivia). Analytical grade solvents, dichloromethane (HPLC quality) and deuterium oxide (D_2O), were purchased from Sigma-Aldrich (Milwaukee, WI).

Sample Preparation. Tannat grapes and wines, of different vintages, were sourced from experimental and estate vineyards, mostly located in the region of the southern part of Uruguay away from the influence of *Eucalyptus* trees.

Chemical Transformation Models. Samples of 60 mL of each Tannat and synthetic wine (12.9% v/v hydroalcoholic solution containing 3.5 g/L of tartaric acid, 2.5 g/L of malic acid, 60 mg/L of sodium metabisulfite, adjusted to pH 3.2 with sodium hydroxide, with 0.5 mg/L of limonene or α -terpineol), sealed under nitrogen in vials, were heated at 45 °C for 20 days. Afterward, each sample was extracted and analyzed by GC. All experiments were performed in duplicate. An oven at controlled temperature monitored by a standard thermometer was used.

Isolation of Volatiles. (a) *Volatiles in Grapes.* Fifty grams of grape berries was harvested, at different ripening stages, from several grapevines selected randomly in the vineyards studied. Grape seeds were carefully removed from the frozen berries, and the pulp and the skins were extracted by sonicating for 5 min an aqueous solution of CaCl_2 (4%), then adding with 0.10 mL of internal standard (1-heptanol at 274 ppm in a 95% v/v ethanolic solution) and extracting by hand-shaking with 3×15 mL of dichloromethane. The organic phases were then separated, dried over sodium sulfate, and concentrated as described below.

(b) *Volatiles in Wines and Synthetic Wines.* Samples of wine and synthetic wine (60 mL containing 0.12 mL of the internal standard solution) were extracted as previously described. The organic phases were dried over sodium sulfate and then concentrated to 1.5 mL on a Vigreux column, stored at -10 °C, and, immediately prior to GC-MS analysis, further concentrated to 100 μL under a gentle nitrogen stream.

Identification. GC-MS analyses were performed using a Shimadzu GC-17 gas chromatograph coupled with a Shimadzu QP 5050 mass spectrometer (70 eV; acquisition mass range: 40–400 amu) supported by reference libraries (15, 16) and equipped with a polar BP 20 (SGE, Australia) bonded fused-silica capillary column (25 m \times 0.25 mm i.d. \times 0.25 μm film thickness) with the following working parameters: injector temperature: 250 °C; interface temperature: 250 °C; carrier gas He: 92.6 kPa (55.9 cm/s); oven conditions: 8 min at 40 °C, 3 °C/min to 180 °C and 10 °C/min to 220 °C, 20 min at 220 °C; injection mode: split 1:40; injection volume, 1.0 μL . The identification of the compounds was confirmed by injection of pure standards and comparison of their retention index and relevant MS-spectra, while in SIM analysis by considering different typical fragments in a specific relevant ratio.

Quantitative Analysis. (a) *Thermal Conditioning Experiments in Wines and Synthetic Wines.* Volatile compounds (limonene, α -terpineol, *cis*- and *trans*-terpine, 1,8-cineole) were quantified by GC in the same experimental conditions as previously described for GC/MS, by the internal standard method using 1-heptanol (added as 2 mL/L of a 95% v/v ethanolic solution of 0.274 g/L) without consideration of calibration factors, that is, $F = 1.00$ for all compounds.

(b) *Grapes and Wines.* 1,8-Cineole was quantified by GC/MS/SIM comparing the area of the ion peak at m/z 81 with that of internal standard, m/z 70. A linear calibration curve ($r^2 > 0.96$; three replicate analyses at each concentration) was obtained when synthetic wine was

treated with a 1,8-cineole standard solution (final concentration between 0.2 and 6.0 $\mu\text{g/L}$) and submitted to the same extraction and analysis procedures.

Degradation Mechanism of Limonene and α -Terpineol. Samples of 20 mL of synthetic wines, prepared as previously described but with 10% (v/v) D_2O in place of water and with 0.5 mg/L of limonene or α -terpineol, were heated at 45 °C under nitrogen for 20 days. The percentage of deuterium incorporated into limonene and 1,8-cineole was determined by GC/MS/SIM following the ion peaks at m/z 121/122 and 136/137 for limonene/limonene-9-*d*, and m/z 154/155 for 1,8-cineole/1,8-cineole-2-*d* or 1,8-cineole-9-*d*. The analysis of 1,8-cineole-2,9-*d* (m/z 156) was not performed because of the low concentration of this species.

Determination of Olfaction Thresholds. The odor threshold value for 1,8-cineole was determined by triangle test (17) using a Tannat wine sample with added 1,8-cineole. The samples were presented in order of increasing concentrations (1.0, 2.0, 4.0, and 8.0 $\mu\text{g/L}$) to individual panel members (10 trained judges whose ages ranged from 23 to 40 years) for assessment on the basis of possible odor differences. The wines were presented in individual testing booths, and 60 mL of samples were served at 20 ± 1 °C in approximately 250 mL, clear, tulip-shaped wine glasses (ISO 3591, 1977) identified with two-digit random codes and covered with a watch glass. The lowest concentration corresponding to 50% correct identification by the panelists determined the threshold value (18, 19).

RESULTS AND DISCUSSION

Mechanism of 1,8-Cineole Formation. The volatile compounds identified in Tannat wine samples and their concentration ranges have been reported by Boido et al. (20). The levels of the monoterpene compounds, particularly mono-oxygenated monoterpenes, were all under their sensory thresholds, as is usually found for wines from neutral cultivars; however, rather high contents of limonene were found (up to 250 $\mu\text{g/L}$) (20).

Synthetic wines and a Tannat wine (1,8-cineole or terpene isomers not detected) were fortified with limonene or α -terpineol (0.5 mg/L), then heated to 45 °C for 20 days. The results, as reported in **Table 1**, show the presence of 1,8-cineole and both isomers of 1,8-terpine.

Previous studies have shown that α -terpineol can be formed from limonene under acidic conditions (21, 22). Furthermore α -terpineol can be transformed into 4-(2-hydroxypropyl)-1-methylcyclohexanol (terpin hydrate or 1,8-terpine) (23) and this latter compound to 1,8-cineole (24). The reactions promoted by the temperature and acidic conditions are summarized in **Figure 1**. Hydration of limonene to form α -terpineol is followed by cyclization of *trans*-1,8-terpine, but not *cis*-1,8-terpine, to 1,8-cineole which may explain the higher content of the *cis* form shown in **Table 1**. The data in **Table 1** also show that the synthetic wine with added α -terpineol contains limonene even after 20 days, consistent with the reversible character of the reaction steps involved. Furthermore, other pathways involving epoxidation of the double bonds of limonene and α -terpineol (25) could be hypothesized, leading to a complex mixture of

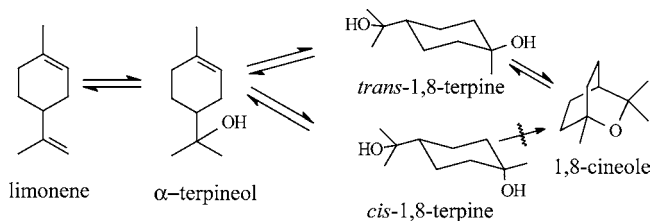


Figure 1. Mechanism proposed for 1,8-cineole synthesis from limonene in wines.

Table 2. Deuterium-Labeling Experiments for Limonene and α -Terpineol

synthetic wine		limonene added (0.5 mg/L)		α -terpineol added (0.5 mg/L)	
compound	<i>m/z</i>	% deuteration	\pm SE ^a	% deuteration	\pm SE
limonene	121/122	4.94	0.66	0.35	0.11
limonene	136/137	4.40	0.88	0.57	0.45
1,8-cineole	154/155	12.79	2.44	6.79	0.39

^a SE = standard error around the mean ($n = 2$).

minor compounds (α -terpinolene, 1,4-cineole, 1-terpineol) detected in the synthetic wine in our experimental conditions.

Deuteration Experiments. To verify the production of 1,8-cineole and the reversible nature of the reactions involved, 20 mL samples of synthetic wines, prepared with 10% (v/v) D₂O in place of water, and with 0.5 mg/L of limonene or α -terpineol, were heated at 45 °C under nitrogen during 20 days.

The results of the deuterium-labeling experiments are reported in **Table 2**, showing values which were lower than those expected, indicating that the isotopic equilibrium was not attained. The extent of deuterium labeling is consistent with a sequence of chemical rearrangements from limonene to 1,8-cineole, through reaction steps which involve the cyclization of *trans*-1,8-terpine promoted by the temperature and acidic conditions that can be reached by grapes and wines.

These reactions were accelerated by addition of acetic acid to synthetic wines (data not shown), thus indicating dehydration as the slowest reaction step (26). These results suggest the presence of two different kinetics, explaining, as reported in **Table 2**, the greater amount of deuterated limonene produced in synthetic wine added with limonene standard after 20 days, by comparison with the results obtained from α -terpineol in the same experimental conditions. As proposed in **Figure 2**, limonene-9-*d* (**9**) is produced from limonene (**1**) as a consequence of only one dehydration step, while limonene-2-*d* (**5**) and limonene-9-*d* (**9**) are both obtained from α -terpineol (**2**) through two slow dehydration steps.

Moreover, the obtained results also made it possible to explain the relationships between the deuterated forms of 1,8-cineole found in our experimental conditions (**Table 2**): a higher deuteration level, almost twice, for 1,8-cineole obtained from limonene (**1**) by the synthesis of 1,8-cineole-2-*d* (**8**) or 1,8-cineole-9-*d* (**12**), by comparison with the results obtained from α -terpineol (**2**), where only one molecule of 1,8-cineole-2-*d* (**8**) was produced through the faster step of 1,8-terpine formation (**Figure 2**).

Quantitative Analysis of 1,8-Cineole in Grape and Wine.

Tannat wines from vineyards growing out of the influence of *Eucalyptus sp.*, located in the Southern part of Uruguay and with particularly intense eucalyptus aroma, were analyzed. Wine volatiles were isolated and concentrated by extraction with dichloromethane, followed by GC/MS/SIM analysis. The 1,8-

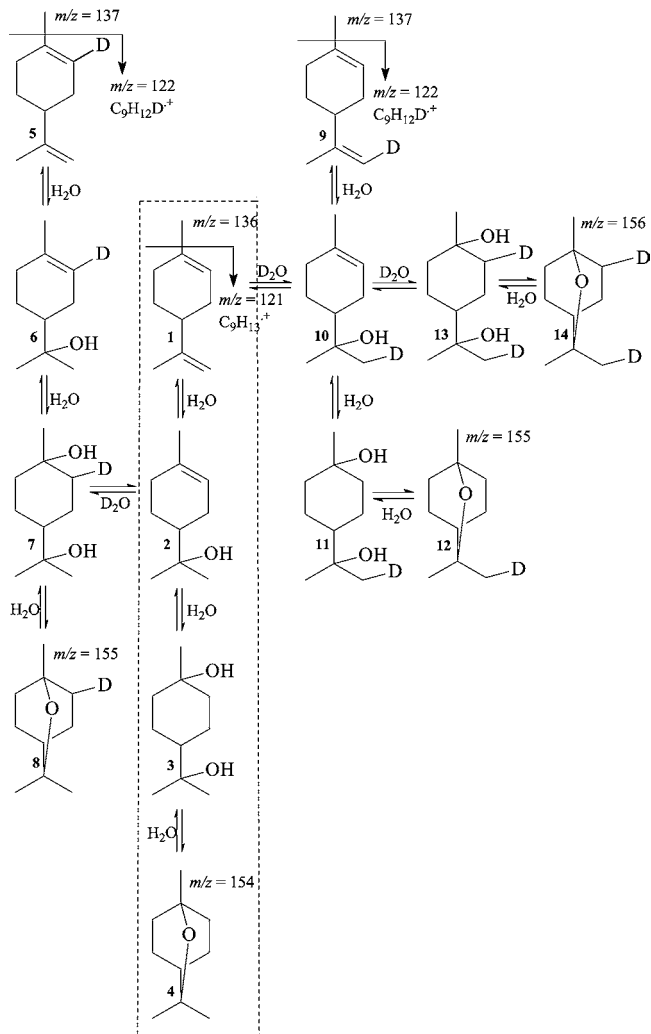


Figure 2. Equilibrium character of the reactions associated with the mechanism proposed for 1,8-cineole synthesis from limonene in wines. Deuterium labeling experiment.

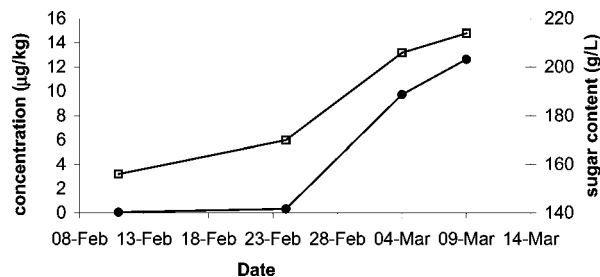


Figure 3. 1,8-Cineole contents in grape samples during ripening: (●-) 1,8-cineole content; (□-) sugar content.

cineole content in the samples of wine ranged from 1 to 5 μ g/L. The analyses performed on grapes collected at different ripening stages showed higher contents of 1,8-cineole for samples at the end of the vintage (overripe fruit) as reported in **Figure 3**.

Determination of Olfaction Thresholds. Odor threshold value for the 1,8-cineole was determined by the triangle test (17) by using a Tannat wine sample added with 1,8-cineole as described under Materials and Methods. The sensory threshold value found for 1,8-cineole in Tannat wine was 1.3 μ g/L, which is in agreement with literature data for a Merlot wine (14). This value was exceeded by the 1,8-cineole concentration found for many of the Tannat wines analyzed.

In conclusion, this study has identified a process by which "eucalyptus" aroma may develop in red wines of the Tannat variety, by formation of 1,8-cineole from limonene or α -terpineol. The explanation for these results can be found in the chemical rearrangements associated with limonene, which was shown to interconvert with 1,8-cineole at pH 3.2 and 45 °C. Furthermore, it was found that 1,8-cineole concentrations in grapes at the beginning of the ripening were very low, but showed an important increase throughout the ripeness. Finally, the studies with model wine showed that 1,8-cineole can be produced from limonene and α -terpineol under conditions related to those of red wine aging.

ABBREVIATIONS USED

GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; SIM, selected ion monitoring.

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