

Identification and Quantification of Geosmin, an Earthy Odorant Contaminating Wines

Philippe Darriet,* Monique Pons, Sophie Lamy, and Denis Dubourdieu

Faculté d'œnologie, Université Victor Segalen Bordeaux 2, 351 cours de la libération,
33405 Talence Cedex, France

Musty, earthy odors are highly detrimental to the aromatic quality of wines. A characteristic aroma of freshly tilled earth, damp cellar was studied in some red and white wines of different origins. The extraction and purification of the wines marked by this odor have shown after analysis by gas chromatography–olfactometry a unique strong odorous zone having the same odor as the one perceived at tasting. The compound responsible for this odorous zone was identified by gas chromatography–mass spectrometry as geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), which possesses a distinctive earthy odor. Geosmin may be present in wines at levels higher than the racemic geosmin olfactory perception threshold, thus suggesting its contribution to their off-aroma. Moreover, the presence of this compound in juice taken from freshly crushed grapes suggests that microorganisms that develop on the grapes may contribute to the presence of this compound in wines.

Keywords: *Geosmin; earthy odor; wine aroma; grape contamination; microorganism*

INTRODUCTION

The production of quality wines requires great attention to possible sources of chemical or microbiological contamination during the ripening of the grapes, as well as during vinification and aging. Among such defects, musty, earthy odors are particularly detrimental. These odors may be due to contamination of must or wine during storage in the winery or bottle-aging. The sources of this type of contamination may be contaminated materials, such as cement-lined vats or barrels, or products for treating wines, corks, and musty cellar atmospheres, that is, factors usually associated with development of microorganisms on these materials (Marais et al., 1975; Lefebvre et al., 1983; Amon et al., 1987). These odors may also result from the development of undesirable fungal or bacterial microflora leading to grape rot under unfavorable climatic conditions (Ribéreau-Gayon et al., 1964; Pucheu Planté and Seguin, 1978).

The compounds responsible include trichloroanisole and tetrachloroanisole (2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole), which possess a strong musty odor. 2,4,6-Trichloroanisole may be vectored in wines by contaminated corks (Tanner et al., 1981; Dubois and Rigaud, 1981; Buser et al., 1982; Amon et al., 1989), whereas 2,3,4,6-tetrachloroanisole can originate from the air of cellars where wooden beams have been treated with insecticides containing polychlorophenols (Dubois and Rigaud, 1981; Chatonnet et al., 1994).

With regard to earthy odors in wines, they have been rarely cited (Dubois et al., 1984). Some compounds have been described as having this odor such as geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), 2-methylisoborneol, and 2-methoxy-3-isopropylpyrazine, which are all found in nature (Maga, 1987), or synthetic substances such

as 2-ethylfenchol and some tertiary alcohols (Polak et al., 1978; Napolitano et al., 1996). Geosmin and 2-methylisoborneol have been tentatively identified in wines contaminated by corks or barrel taints (Amon et al., 1987, 1989).

In this paper, we set out to understand the nature of a characteristic earthy odor found in wines in the early stage of their aging. We report the identification and assay of a strong earthy odorant, geosmin, in red and white wines and its identification in grape juice prior to alcoholic fermentation.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Pentane (Pestipur quality) from SDS (Peypin, France) was distilled over a column (82 × 2.8 cm) to improve its purity. Geosmin, degree of purity = 98.9%, was provided by Wako Chemicals Industries (Osaka, Japan) as a mixture of (+) and (–) enantiomers. 2-Methylisoborneol was from the same company, 2,4,6-trichloroanisole was from Aldrich, and 2,3,4,6-tetrachloroanisole was from Interchim (Montluçon, France).

Wine Samples. Wine samples were collected from different wineries a few days or weeks and up to 7 months after fermentation. The wines were analyzed a few days after receipt of the samples or kept at 10 °C, protected from light and corked with metallic stoppers.

Extraction of Geosmin from Wine and Grape Juice. Wine (750 mL) was extracted in 1-L flasks by three successive extractions of distilled pentane (30, 20, and 20 mL, respectively) with magnetic stirring for 10 min each time. The combined organic phases were then dried with anhydrous sodium sulfate and concentrated to 500 μ L under nitrogen flow (~100 mL/min). For the identification of geosmin in grape juice, the extract concentrated to 100 μ L was directly injected by gas chromatography–mass spectrometry (GC-MS).

Partial Purification of the Wine Extract by Adsorption Chromatography. The pentane extracts obtained from 1.5 L of wine were pooled and vacuum-concentrated to 10 mL with a rotary evaporating apparatus; the water bath was at a temperature below 4 °C, and there was no odor in the condensate distillate. The extract was then concentrated to

* Author to whom correspondence should be addressed (e-mail philippe.darriet@oenologie.u-bordeaux2.fr; telephone 00 33 5 56 84 64 88; fax 00 33 5 56 84 64 68).

500 μ L under nitrogen flow and further purified by liquid chromatography on silica gel (Aldrich, 70–230 mesh, 60 Å). The silica was activated 12 h beforehand at 120 °C. After being cooled in a dehumidifier, the silica was deactivated by adding 5 mL of ultrapure water (Milli-Q, Millipore) to 15 g of silica. Pentane was added to the silica before the flash chromatography water-cooled column (10 mm \times 100 mm) was filled. The wine extract was then applied on the top of the column, and the purification of geosmin was obtained by an elutrope series of solvents (40 mL), using 0.5 bar pressure of nitrogen [pentane (I), pentane/dichloromethane (80:20) (II), pentane/dichloromethane (60:40) (III), and pentane/dichloromethane (50:50) (IV)]. Each fraction was concentrated to 500 μ L and then analyzed by GC.

Capillary GC—Olfactometry (GC-O). The analysis was carried out on a Hewlett-Packard 5890-II (Hewlett-Packard, Avondale, PA) gas chromatograph coupled with an olfactory detector using the ODO1 installation [Scientific Glass Engineering (SGE), Ringwood, Australia]. The carrier gas was hydrogen U (Air Liquide, France) with a column head pressure of 140 kPa. One microliter of the extract was injected in splitless mode (injector port temperature, 230 °C; purge time, 1 min; purge flow, 50 mL/min) onto a fused silica capillary column type BP20 [SGE, 50 m, 0.25 mm i.d., 0.25 μ m film thickness (f.t.)] and onto a BPX5 capillary column (SGE, 50 m, 0.25 mm i.d., 0.25 μ m f.t.). For on-column injection, an HP on-column injector was used with the same column head-pressure; 1 μ L of extract was directly injected using a fused silica needle. For all analysis, the temperature program was as follows: 45 °C, 1 min, then 3 °C/min to 230 °C, 10 min isothermal.

GC-MS. The analysis was performed on an HP 5890-I coupled with an HP 5970 mass spectrometer [electronic impact (EI), 70 eV; transfer line temperature at 250 °C] and on a Varian 3400CX gas chromatograph coupled with a Saturn 2000 mass spectrometer (EI, 70 eV) (Varian, Harbor City, CA). For both chromatographs, the carrier gas was helium N60 (Air Liquide). The sample (1 μ L) was injected in the splitless mode and analyzed on the BP20 and the BPX5 capillary columns using the same conditions as for the GC-O analysis.

Quantification of Geosmin in Grape Juice and Wines. Fifty microliters of 2-undecanone (100 mg/L in ethanol) was added to 500 mL of juice or wine. This was then extracted in a 1-L flask with purified pentane (20, 10, and 10 mL, successively) and 10 min of magnetic stirring each time. The combined organic phases were dried and concentrated to 500 μ L. They were then analyzed by GC-MS as previously described using the HP5890-I–HP5970 mass selective detector, in the selected ion monitoring mode (SIM). Geosmin was quantified with the m/z 112 ion, after evidence of the presence and normal abundance of other characteristic m/z ions, 111, 168, and 182, at the retention time of this compound. m/z 58 ion was used as internal standard (2-undecanone).

Measurements under these conditions were linear between 70, 140, 280, and 560 ng/L ($R^2 = 99.98$). The repeatability factor for the geosmin assay was 5.25% with the BP20 column (determined using five samples of red wine supplemented with 170 ng/L geosmin). The percent recovery of geosmin from the wine was determined at all of the concentrations studied for the calibration curve by comparing the ratio of the peak surface of geosmin (112 ion) with the peak surface of the internal standard (58 ion) in the chromatogram of the wine extract and the corresponding chromatogram of the standard solution of geosmin supplemented with the internal standard. The coefficient of extraction of geosmin from wine varied between 79 and 88% according to the different concentrations.

Olfactory Perception Threshold Measurement. The perception threshold of (\pm)-geosmin (minimum concentration below which 50% of the tasters failed to taste the difference with the control) was established by a triangular test at five increasing concentrations in distilled water and neutral white and red wines. The solutions, presented in glasses corresponding to AFNOR (Association Française des Normes) standards, were tasted by a 35-person jury.

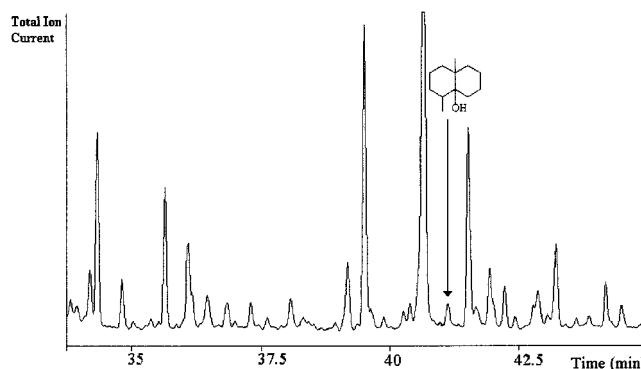


Figure 1. Gas chromatogram obtained with mass spectrometry detection of a purified Cabernet Sauvignon wine extract (FII) injected onto a BP20 column.

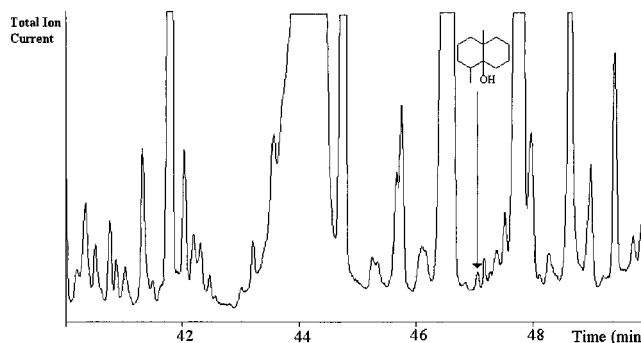


Figure 2. Gas chromatogram obtained with mass spectrometry detection of a purified Cabernet Sauvignon wine extract (FII) injected onto a BPX5 column.

RESULTS AND DISCUSSION

Identification and Assay of Geosmin in Wine.

During a first step, red and white wines having a strong earthy odor were extracted with pentane and then analyzed by GC-O. The analysis performed on capillary columns with different polarities (BP20, BPX5) using splitless and on-column injection revealed the presence of only one strong odorous zone, reminiscent of the earthy odor perceived during tasting. Different compounds presenting an earthy or musty odor (geosmin, 2-methylisborneol, 2,4,6-trichloroanisole, and 2,3,4,6-tetrachloroanisole) were injected using the same chromatographic conditions to verify their retention time with that of the odorous zone. Geosmin was found as the only compound having the same retention time as the odorous zone.

To identify geosmin, a pentane extract obtained from 1.5 L of Cabernet Sauvignon wine very marked by this odor was purified by adsorption chromatography on silica gel. Fraction II, which smelled very earthy, was analyzed by GC-MS on the Varian system. On two different capillary columns, at the retention times of 40.6 min (BP20) and 47 min (BPX5), a mass spectrum was obtained having ions and abundances similar to those of geosmin (Figure 1, 2, and 3).

The content of geosmin in various Bordeaux wines [sweet Bordeaux wines, Bordeaux reds (Bordeaux, Haut-Medoc, Listrac, and Moulis)] and Algerian wines fermented and aged in stainless steel vats was determined (Table 1). The levels varied between 20 and 300 ng/L in the wines analyzed, thus indicating that the concentrations were sometimes clearly higher than the perception threshold of the racemic mixture of geosmin as described in the literature [\sim 20 ng/L in water (Maga,

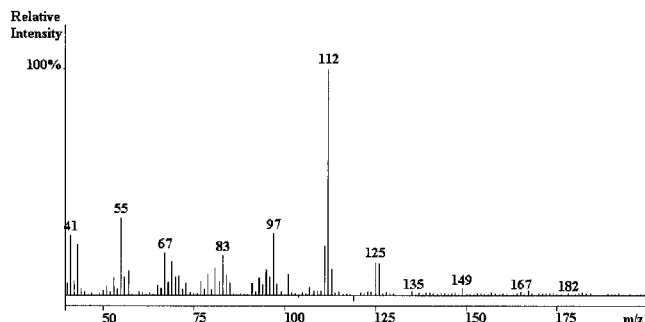


Figure 3. Mass spectrum of geosmin obtained from a purified Cabernet Sauvignon wine extract (FII) injected onto a BP20 column.

1987)], and according to our findings with racemic (\pm)-geosmin (10 ng/L in water, 80–90 ng/L in a neutral red wine, and 60–65 ng/L in neutral white wine). These results suggest the importance of geosmin in the earthy aroma of the wines analyzed. Moreover, enantiomeric ($-$)-geosmin has been shown to be the natural form of this compound (Gerber et al., 1965; Ayer et al., 1976; Wherkoff et al., 1991), which in water possesses a perception threshold 11 times lower than that of ($+$)-geosmin (Polak et al., 1992). Consequently, ($-$)-geosmin could be the main enantiomer form present in wines and the olfactory perception threshold should be lower than that indicated. These observations are in good agreement with the fact that earthiness was clearly evidenced in red wines possessing >80 ng/L of geosmin. GC analysis of a wine extract on a chiral capillary would make it possible to clarify this point.

Geosmin could be quantified in the wines analyzed at the end of alcoholic and malolactic fermentation (red wines analyzed in November 1998). Bordeaux reds analyzed 7 months after fermentation were also found to have concentrations ranging from 80 to 110 ng/L (1998 Bordeaux reds analyzed in April and May 1999).

Geosmin could still be measured in red and rosé wine after several years of aging. Concentrations of 200 ng/L were found in a 1997 Algerian rosé wine and 60 ng/L in a 1994 Medoc sampled in a vat in October 1997 and analyzed two years later (Table 1). Contrary to initial findings in experiments using acidified aqueous solutions, which indicated its rapid degradation (Gerber et al., 1965), we found geosmin to be relatively stable in acidic wines (pH 3.2–3.7).

In sweet wines fermented in the same cellar and produced from grapes infected with noble rot (*Botrytis cinerea*) and picked by selective harvesting, geosmin was not detected in wines produced with grapes picked in the first wave but was detected in wines made from grapes picked later (Sauternes LP, Table 1). In some wines from the same appellation, geosmin reached very high levels (SA1, Table 1).

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) has long been known as a contaminant of water and many stored foods and fruits (Gerber et al., 1965; Acree et al., 1976; Buttery et al., 1976; Maga, 1987). Its presence in wine reported by Australian authors (Amon et al., 1987, 1989) using the GC-O technique has been linked to wine contamination due to cork taint and barrel taint. The formal identification of geosmin in the wines we have analyzed cannot be attributed to contamination due to corks or barrels as the wines were stored in stainless steel vats or bottles with metal stoppers. Moreover, in all cellars where wine was sampled, only some vats contained geosmin.

Geosmin was also identified during the 1999 vintage at high concentrations (250 ng/L) in a red must obtained from crushed Cabernet Sauvignon grapes before the onset of alcoholic fermentation (Bordeaux 1999) (Table 1). This identification suggests that microorganisms that develop on grapes may be responsible for its presence in wines. Besides *Botrytis cinerea*, a wide range of fungal, yeast, and bacterial flora may develop on grapes

Table 1. Concentration of Geosmin in Wines and Grape Juice

type of sample	origin ^a	grape variety	geosmin concn ^b (ng/L)	analysis performed (month/year)	
1998 sweet wines	Sauternes SA1	Semillon	250	Nov 1998	
	Sauternes SA2	Semillon	82	Nov 1998	
	Sauternes LP, first selective picking second selective picking third selective picking fourth selective picking	Semillon			Nov 1998
		Semillon			Nov 1998
		Semillon	5	Nov 1998	
	Semillon	12	Nov 1998		
1999 red wines	Bordeaux P1	Cabernet Sauvignon	120	Nov 1999	
	Bordeaux P2	Cabernet Sauvignon	45	Nov 1999	
1998 red wines	Haut-Medoc CI	Cabernet Sauvignon	300	Nov 1998	
	Haut-Medoc Q1	Cabernet Sauvignon	80	Nov 1998	
	Haut-Medoc Q2	Cabernet Sauvignon	80	May 1999	
	Haut-Medoc OC3	Cabernet Sauvignon	85	May 1999	
	Listrac BO	Cabernet Sauvignon	110	May 1999	
	Moulis MA	Cabernet Sauvignon	20	Dec 1998	
	Moulis DU	Cabernet Sauvignon	4	Dec 1998	
	Moulis PE	Cabernet Sauvignon	10	Dec 1998	
	Listrac G1	Cabernet Sauvignon	35	Dec 1998	
	Listrac G2	Cabernet Sauvignon	40	Dec 1998	
Listrac G3	Cabernet Sauvignon	50	Dec 1998		
1994 red wine	Haut-Medoc GI	Cabernet Sauvignon	60	April 1999	
1997 rosé	Algeriy	Cabernet Sauvignon	200	Dec 1998	
1999 grape juice	Bordeaux	Cabernet Sauvignon	250	Oct 1999	

^a The origin indicated does not mean that the wines have been validated for the label of guaranteed vintage (AOC) as Bordeaux, Sauternes, Haut-Medoc, Listrac, and Moulis ^b (\pm) geosmin olfactory perception threshold was determined at 10 ng/L in water, 60–65 ng/L in neutral white wine, 80–90 ng/L in neutral red wine (see Materials and Methods).

picked in unfavorable weather conditions (Ribéreau-Gayon et al., 1964; Pucheu-Planté et al., 1978; Grape Pest Management, 1992). The impact of *B. cinerea* and other microorganisms on organoleptic defects in wine has been reported on many occasions (Ribéreau-Gayon et al., 1964, 1982). Geosmin may be produced by several species of microorganisms, including Actinomycetes bacteria (*Streptomyces* sp.) (Gerber et al., 1965) and blue algae (Safferman et al., 1967). More recently, geosmin was also incriminated in the development of a number of *Penicillium* sp. fungi (Pisarnitskii et al., 1988; Mattheis et al., 1992; Börjesson et al., 1993; Larsen et al., 1994, 1995). An etiological field study should now be performed to investigate the role of grape microbial parasite species in the development of geosmin in grapes.

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