Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties

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Forty-four odor-active compounds were quantified in Scheurebe and Gewürztraminer wines, respectively. Calculation of odor activity values (OAVs) of odorants showed that differences in odor profiles of both varieties were mainly caused by *cis*-rose oxide in Gewürztraminer and by 4-mercapto-4-methylpentan-2-one in Scheurebe. On the basis of their high OAVs, ethyl octanoate, ethyl hexanoate, 3-methylbutyl acetate, ethyl isobutyrate, (*E*)-*β*-damascenone, and 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one (wine lactone) were further potent odorants in both varieties. The compounds were dissolved in a water/ethanol mixture in various combinations and in concentration levels equal to those in wine. The results indicated that the aromas of Gewürztraminer and Scheurebe models were in good agreement with the original wines.

Keywords: *Gas chromatography; stable isotope dilution assay; quantitation; white wine; Gewürztraminer; Scheurebe*

INTRODUCTION

Recently it has been shown by application of gas chromatography/olfactometry (GC/O) methods, such as aroma extract dilution analysis (AEDA) and static headspace analysis/olfactometry (SHA/O), that 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one (wine lactone), ethyl 2-methylbutyrate, 3-methylbutanol, 2-phenylethanol, 3-ethylphenol, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone belong to the potent odorants of Gewürztraminer and Scheurebe wines (Guth, 1997). 4-Mercapto-4-methylpentan-2-one was detected as potent odorant only in the variety Scheurebe, whereas *cis*-rose oxide was a key substance for the overall flavor of Gewürztraminer wine.

AEDA and SHA are suitable tools for recognition of odor-active compounds (Ullrich and Grosch, 1987; Guth and Grosch, 1993a), but the methods are afflicted with some simplifications; for example, no corrections were made for the losses of odorants during isolation procedure. To establish exactly the flavor differences between Scheurebe and Gewürztraminer wines, it is therefore necessary to quantify the levels of recognized odorants and to calculate the odor activity values (OAVs; ratio of concentration to odor threshold value of the compound). According to the results the compounds were added in various combinations to a water/ethanol mixture, and then the aroma of each model was compared with that of the original wine. The results are reported in the present paper.

EXPERIMENTAL PROCEDURES

Wine. Gewürztraminer, vintage 1992, and Scheurebe wine, vintage 1993, were purchased from a winery in Ballrechten-Dottingen, Germany.

Chemicals. 4-Methyl-3-penten-2-one, [1,3-$^{13}C_2$]acetone, [$^{13}C_2$]acetic acid (**c-16**), [$^{13}C_2$]acetaldehyde (**c-40**), [$^{2}H_6$]dimethyl sulfide (**d-41**), 3-hydroxyacetophenon, tetrahydrolinalool, sodium hydrogen sulfide, anhydrous $AlCl_3$, and lithium aluminum deuteride were from Aldrich (Steinheim, Germany). Compounds **1**−**10**, **14**−**21**, **24**−**33**, **35**−**37**, **41**, and **43** (Table 1) were also from Aldrich; nerol oxide and compound **11** were from Roth (Karlsruhe, Germany); **13**, **23**, **37**, **42**, and **44**−**46** were from Merck (Darmstadt, Germany); and **24** was a gift from Haarmann and Reimer (Holzminden, Germany).

Synthesis. *1,1-Diethoxy-[$^{13}C_2$]ethane (**c-1**).* A mixture of [$^{13}C_2$]acetaldehyde (2 mmol, 88 mg) and triethyl orthoformate (2 mmol, 296 mg) was added to a solution of NH_4NO_3 (10 mg) in ethanol (2 mmol, 92 mg) and stirred for 8 h at 25 °C. After addition of aqueous saturated NaCl solution (5 mL), the title compound was isolated by extraction with pentane (2 × 5 mL).

MS(EI) of **c-1**: *m*/*z* (%) 47 (100), 75 (50), 104 (M^+, 15).

*cis-[$^{2}H_2$]Rose Oxide [**d-11**; (2SR,4RS)-4-Methyl-2-(2-methylprop-1-enyl)-[4,5-$^{2}H_2$]tetrahydropyran].* A mixture of nerol oxide (1 mmol, 150 mg) and platin(IV) oxide (10 mg) in [^{2}H]-methanol (5 mL) was deuterated in a laboratory autoclave (Roth, Karlsruhe, Germany) at 5 × 10^5 mPa for 5 min. After filtration and addition of water (5 mL), the title compound was isolated by extraction with pentane (2 × 5 mL). The organic layer was washed with aqueous HCl (1 mol/L, 5 mL) and water (5 mL) and then dried over anhydrous Na_2SO_4. After concentration to a volume of 1 mL by distilling off the solvent on a Vigreux column (50 × 1 cm), the solution containing crude **d-11** (30%, GC) was purified by preparative GC (3 m × 2 mm stainless steel column packed with SE-54 (10%, w/w) on Chromosorb W, 80−100 mesh) as described earlier (Guth and Grosch, 1989).

MS(EI) of **d-11**: *m*/*z* (%) 141 (100), 69 (72), 142 (45), 83 (24), 70 (20), 156 (M^+, 15).

*4-Mercapto-4-[^{13}C]methyl [1,3,5-$^{13}C_3$]pentan-2-one (**c-13**).* Concentrated H_2SO_4 (2 *μ*L) was added to [1,3-$^{13}C_2$]acetone (2 mmol, 12 mg), and the mixture was stirred at 25 °C for 24 h. After addition of sodium hydrogen sulfide (1 mmol, 56 mg), the reaction vessel was sealed with a septum and stirred for further 12 h at 25 °C. After addition of water (5 mL), the 4-mercapto-4-[^{13}C]methyl[1,3,5-$^{13}C_3$]pentan-2-one (**c-13**) formed was extracted with diethyl ether (2 × 5 mL) and then re-extracted with NaOH (0.1 mol/L, 2 × 10 mL). Acidification of the aqueous layer with HCl (0.1 mol/L) to pH 3, followed by extraction with pentane (2 × 10 mL) and drying over Na_2SO_4, yielded 4-mercapto-4-[^{13}C]methyl[1,3,5-$^{13}C_3$]pentan-2-one (**c-13**, 10 mg).

MS(EI) of **c-13**: *m*/*z* (%) 44 (100), 58 (25), 86 (20), 102 (15), 136 (M^+, 10).

*2-Phenylethyl[1,2-$^{13}C_2$]acetate (**c-24**).* The mixture consisting of [$^{13}C_2$]acetic acid (5 mmol, 310 mg), 2-phenylethanol (0.5 mmol, 61 mg), and concentrated H_2SO_4 (5 mg) was refluxed for 4 h. After addition of water (2 mL), the ester was extracted with pentane (3 mL). The organic layer was washed twice with

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Table 1. Thin-Film Capillaries, Selected Ions, and Calibration Factors for Mass Chromatography of the Odorants

odorant[a,b]	capillary	selected ion (*m/z*)	int std[c]	selected ion (*m/z*)	calibrn factor
1,1-diethoxyethane (**1**)	DB-5	73	1,1-diethoxy-[$^{13}C_2$]ethane (**c-1**)	75	1.00
ethyl isobutyrate (**2**)	DB-5	117	[2,2,2-$^2H_3$]ethyl isobutyrate (**d-2**)	120	0.92
butane-2,3-dione (**3**)	DB-5	87	[1,4-$^{13}C_2$]butane-2,3-dione (**c-3**)	89	1.00
ethyl butyrate (**4**)	DB-5	117	[2,2,2-$^2H_3$]ethyl butyrate (**d-4**)	120	1.00
ethyl 2-methylbutyrate (**5**)	DB-5	131	[2,2,2-$^2H_3$]ethyl 2-methylbutyrate (**d-5**)	134	1.12
ethyl 3-methylbutyrate (**6**)	DB-5	131	[2,2,2-$^2H_3$]ethyl 3-methylbutyrate (**d-6**)	134	0.95
2-methylpropanol (**7**)	DB-FFAP	57	2-methyl[2,3-$^2H_2$]propanol (**d-7**)	59	0.75
3-methylbutyl acetate (**8**)	DB-5	71	3-methyl[3,4-$^2H_2$]butylacetate (**d-8**)	73	0.79
3-methylbutanol (**9**)	DB-FFAP	71	3-methyl[3,4-$^2H_{2-5}$]butanol (**d-9**)	72−75	1.08
ethyl hexanoate (**10**)	DB-5	145	[2,2,2-$^2H_3$]ethyl hexanoate (**d-10**)	148	1.00
cis-rose oxide (**11**)	DB-5	99	*cis*-[$^2H_2$]rose oxide (**d-11**)	101	0.90
hexanol (**12**)	DB-FFAP	85	[3,4-$^2H_4$]hexanol (**d-12**)	89	0.90
4-mercapto-4-methylpentan-2-one (**13**)	DB-FFAP	99	4-mercapto-4-[^{13}C]methyl[1,3,5-$^{13}C_3$]pentan-2-one (**c-13**)	103	1.00
(*Z*)-3-hexenol (**14**)	DB-FFAP	83	(*Z*)-3-[3,4−^{2}H]hexenol (**d-14**)	85	0.73
ethyl octanoate (**15**)	DB-5	173	[2,2,2-$^2H_3$]ethyl octanoate (**d-15**)	176	1.00
acetic acid (**16**)	DB-FFAP	61	[$^{13}C_2$]acetic acid (**c-16**)	63	1.00
linalool (**17**)	DB-FFAP	137	tetrahydrolinalool	141	2.13
isobutyric acid (**18**)	DB-FFAP	89	[3,4-$^2H_{3-4}$]butyric acid (**d-19**)	91−93	0.89
butyric acid (**19**)	DB-FFAP	89	[3,4-$^2H_{3-4}$]butyric acid (**d-19**)	91−93	0.89
2-/3-methylbutyric acid (**20**)	DB-FFAP	103	3-methyl[3,4-$^2H_2$]butyric acid (**d-20**)	105	0.59
3-(methylthio)-1-propanol (**21**)	DB-FFAP	89	3-([$^2H_3$]methylthio)-1-propanol (**d-21**)	92	1.05
citronellol (**22**)	DB-FFAP	83	tetrahydrolinalool	141	1.70
(*E*)-*β*-damascenone (**23**)	DB-5	191	(*E*)-*β*-[$^2H_6$]damascenone (**d-23**)	195−198	0.69
2-phenylethyl acetate (**24**)	DB-FFAP	165	2-phenylethyl [1,2-$^{13}C_2$]acetate (**c-24**)	167	0.91
hexanoic acid (**25**)	DB-FFAP	117	[3,4-$^2H_{2-4}$]hexanoic acid (**d-25**)	119−121	0.73
geraniol (**26**)	DB-FFAP	137	tetrahydrolinalool	141	1.48
2-methoxyphenol (**27**)	DB-FFAP	125	2-[$^2H_3$]methoxyphenol (**d-27**)	128	1.00
2-phenylethanol (**28**)	DB-FFAP	105	2-phenyl[1,1-$^2H_2$]ethanol (**d-28**)	107	1.02
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**29**)	DB-FFAP	129	4-hydroxy-2,5-[$^{13}C_2$]dimethyl-3(2*H*)-furanone (**c-29**)	131	1.00
5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**30**)	DB-OV-1701	143	5-[2,2,2-$^2H_3$]ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**d-30**)	146	1.00
trans-ethyl cinnamate (**31**)	DB-FFAP	177	*trans*-[2,2,2-$^2H_3$]ethyl cinnamate (**d-31**)	180	1.00
4-allyl-2-methoxyphenol (**32**)	DB-FFAP	165	2-[$^2H_3$]methoxy-4-vinylphenol (**d-34**)	154	0.40
3-ethylphenol (**33**)	DB-FFAP	123	3-[1,1-$^2H_2$]ethylphenol (**d-33**)	125	1.01
2-methoxy-4-vinylphenol (**34**)	DB-FFAP	151	2-[$^2H_3$]methoxy-4-vinylphenol (**d-34**)	154	1.00
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**35**)	DB-FFAP	129	3-hydroxy-4,5-[$^{13}C_2$]dimethyl-2(5*H*)-furanone (**c-35**)	131	1.00
wine lactone (**36**)	DB-FFAP	167	[$^2H_3$]wine lactone (**d-36**)	170	1.00
decanoic acid (**37**)	DB-FFAP	173	[4,5-$^2H_{2-4}$]decanoic acid (**d-37**)	175−179	0.90
(*Z*)-6-dodeceno-*γ*-lactone (**38**)	DB-FFAP	197	(Z)-6-[6,7-^{2}H]dodeceno-*γ*-lactone (**d-38**)	199	0.89
4-hydroxy-3-methoxybenzaldehyde (**39**)	DB-FFAP	153	4-hydroxy-3-[$^2H_3$]methoxybenzaldehyde (**d-39**)	156	1.01
acetaldehyde (**40**)	DB-FFAP	45	[$^{13}C_2$]acetaldehyde (**c-40**)	47	1.00
dimethyl sulfide (**41**)	DB-FFAP	63	[$^2H_6$]dimethyl sulfide (**d-41**)	69	1.10
ethyl acetate (**42**)	DB-FFAP	89	[2,2,2−$^2H_3$]ethyl acetate (**d-42**)	92	1.00
dimethyl trisulfide (**43**)	DB-FFAP	127	[$^2H_6$]dimethyl trisulfide (**d-43**)	133	1.00
ethanol (**44**)	DB-FFAP	47	[2,2,2-$^2H_3$]ethanol (**d-44**)	50	0.75

[a] The numbering of the odorants refers to Tables 2 and 3. [b] Compounds were determined with their internal standards by the MS system INCOS XL in the chemical ionization mode (CI) with methane (compounds **1**−**22** and **25**−**44**), and with isobutane (compound **24**) as reagent gas, respectively. Compound **23** was determined in the CI mode by the ion trap detector ITD-800 with methanol as reagent gas. [c] Abbreviation of the labeling: c, carbon-13; d, deuterium.

aqueous sodium bicarbonate (0.5 mol/L; 3 mL) and then dried over anhydrous Na_2SO_4.

MS(EI) of **c-24**: *m/z* (%) 104 (100), 45 (80), 91 (20).

MS(CI, isobutane) of **c-24**: *m/z* (%) 167 ($M^+ + 1$,100).

*3-[1,1-$^2H_2$]Ethylphenol (**d-33**).* The synthesis of **d-33** followed the indications of Nystrom and Berger (1958): reduction of 3-hydroxyacetophenon with lithium aluminum deuteride in the presence of anhydrous $AlCl_3$.

Anhydrous $AlCl_3$ (1 mmol, 133 mg) in diethyl ether (3 mL) was dropped into a solution of lithium aluminum deuteride (1 mmol, 42 mg) in diethyl ether (3 mL). After 5 min, a solution of 3-hydroxyacetophenone (0.8 mmol, 109 mg) and $AlCl_3$ (0.8 mmol, 109 mg) in diethyl ether (6 mL) was added dropwise and then stirred for 2 h. After cooling at 0 °C, aqueous H_2SO_4 (1 mol/L) was added until the residue was dissolved. The organic layer was removed, washed with water (2 × 10 mL), and dried over anhydrous Na_2SO_4. The solvent was distilled off on a Vigreux column (50 × 1 cm), and the residue was purified by column chromatography. The sample was applied onto a column (30 × 1.5 cm) packed with silica gel, which was purified according to the procedure of Esterbauer (1968). Stepwise elution was performed with 95:5 (v/v) and 60:40 (v/v) pentane/diethyl ether (200 mL each); **d-34** appeared in the elution range 200−400 mL.

MS(EI) of **d-33**: *m/z* (%) 109 (100), 124 (M^+, 50), 108 (15), 78 (12), 79 (10).

*[$^2H_3$]Wine Lactone [**d-36**; (3SR,3aSR,7aRS)-3a,4,5,7a-tetrahydro-3[$^2H_3$]-6-dimethylbenzofuran-2(3H)-one].* The labeled compound was prepared starting from isoprene and ethyl prop-2-enoate as previously reported for the unlabeled compound (Guth, 1996). The labeling was performed by using [$^2H_3$]-iodomethane instead of iodomethane for the alkylation of (3*aRS*,7*aSR*)-3a,4,5,7a-tetrahydro-6-methylbenzofuran-2(3*H*)-one.

MS(EI) of **d-36**: *m/z* (%) 154 (100), 93 (70), 169 (M^+, 34), 78 (30), 58 (28), 91 (28), 107 (24), 126 (24), 110 (20), 141 (10).

*[4,5-$^2H_{2-4}$]Decanoic Acid (**d-37**).* The acid was obtained by deuteration of (*Z*)-4-decenoic acid in [^{2}H]methanol as described for [3,4-$^2H_{2-4}$]hexanoic acid (Guth and Grosch, 1993b).

MS(EI) of **d-37**: *m/z* (%) 61 (100), 73 (88), 74 (88), 60 (82), 43 (48), 75 (47), 58 (46), 44 (36), 131 (30), 132 (28), 175 (M^+, 15).

*[2,2,2-$^2H_3$]Ethyl Butyrate (**d-4**), [2,2,2-$^2H_3$]Ethyl 3-Methylbutyrate (**d-6**), [2,2,2-$^2H_3$]Ethyl Hexanoate (**d-10**), [2,2,2-$^2H_3$]-Ethyl Octanoate (**d-16**), [2,2,2-$^2H_3$]Ethyl Cinnamate (**d-31**), [2,2,2-$^2H_3$]Ethyl Acetate (**d-42**), and 3-Methyl[3,4-$^2H_2$]butyl Acetate (**d-8**).* The esters were obtained by a proton-catalyzed reaction of the corresponding acid (butyric acid, 3-methylbutyric acid, hexanoic acid, octanoic acid, cinnamic acid, and acetic acid) with *[2,2,2-$^2H_3$]*ethanol, according to the method of Guth and Grosch (1993c): The mixture consisting of acid (50 mmol), *[2,2,2-$^2H_3$]*ethanol (5 mmol) and concentrated H_2SO_4 (50 mg) was refluxed for 4 h. After addition of water (20 mL), the ester was extracted with diethyl ether (30 mL). The organic layer was washed twice with aqueous sodium bicarbonate (0.5 mol/L; 30 mL) and then dried over anhydrous Na_2SO_4.

*3-Methyl[3,4-$^2H_2$]butyl acetate (**d-8**)* was prepared from acetic acid (50 mmol) and 3-methyl[3,4-$^2H_2$]butanol (5 mmol) as described above.

The following odorants were synthesized as reported in the literature: (3*S*,3a*S*,7a*R*)-3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3)-one (**36**, wine lactone) (Guth, 1996); dimethyl trisulfide (**43**) (Milligan et al., 1963); (*Z*)-6-dodeceno-γ-lactone (**38**) (Widder et al., 1991); [3,4-$^2H_{2-4}$]butyric acid (**d-20**), (*Z*)-6-[6,7-^{2}H]dodeceno-γ-lactone (**d-38**), and [1,4-$^{13}C_2$]butane-2,3-dione (**c-3**) (Schieberle et al., 1993); 3-([$^2H_3$]methylthio)-1-propanol (**d-21**) (Sen and Grosch, 1991); 2-methyl[2,3-$^2H_2$]propanol (**d-7**) (Guth and Grosch, 1993d); 3-methyl[3,4-$^2H_{2-5}$]-butanol (**d-9**) and 3-methyl[3,4-$^2H_2$]butyric acid (**d-20**) (Guth and Grosch, 1994); (*Z*)-3-[3,4-^{2}H]hexenol (**d-14**) (Guth and Grosch, 1990); 2-phenyl[1,1-$^2H_2$]ethanol (**d-28**) (Schieberle, 1991); [2,2,2-$^2H_3$]ethyl 2-methylbutyrate (**d-5**) and [2,2,2-$^2H_3$]-ethyl isobutyrate (**d-2**) (Guth and Grosch, 1993c); 2-[$^2H_3$]-methoxyphenol (**d-27**), 4-hydroxy-3-[$^2H_3$]methoxybenzaldehyde (**d-39**), [3,4-$^2H_4$]hexanol (**d-12**), and [3,4-$^2H_{2-4}$]hexanoic acid (**d-25**) (Guth and Grosch, 1993b); 4-hydroxy-2,5-[$^{13}C_2$]dimethyl-3(2*H*)-furanone (**c-29**) (Sen et al., 1991a); 3-hydroxy-4,5-[$^{13}C_2$]-dimethyl-2(5*H*)-furanone (**c-35**) (Blank et al., 1993); 5-[2,2,2-$^2H_3$]ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**d-30**) (Preininger and Grosch, 1994); (*E*)-β-[$^2H_6$]damascenone (**d-23**) (Sen et al., 1991b); [$^2H_6$]dimethyl trisulfide (**d-43**) (Milo and Grosch, 1996); 2-[$^2H_3$]methoxy-4-vinylphenol (**d-34**) (Semmelroch et al., 1995).

Concentrations of Labeled Compounds. The concentrations of compounds **c-1**, **d-4**, **d-6**−**8**, **d-10**, **d-11**, **d-15**, **c-24**, **d-31**, **d-33**, and **d-37** were gas chromatographically (GC) determined with methyl octanoate as internal standard (Guth and Grosch, 1993c). The concentrations of **c-13** and **d-36** were determined by GC with 4-methyl-2-penten-2-one and δ-decalactone, respectively, as internal standards.

Quantitative Analyses. *Odorants **1**−**10**, **12**, **14**−**22**, **24**−**28**, **32**, **34**, **37**, and **39**.* The wine sample (100 mL) was spiked with known amounts of the internal standards listed in Table 1. The procedure of spiking was the same as reported for the analysis of flavor compounds from coffee brews (Semmelroch et al., 1995). The solvent extract was separated in neutral and acidic fractions (Guth, 1997) and each fraction was concentrated to a volume of 100−500 μL by distilling off the solvent on a Vigreux column (50 × 1 cm) and by microdistillation according to the procedure of Bemelmans (1979). Aliquots (0.5 μL) of the volatile fractions were separated by HRGC on capillaries detailed in Table 1 and analyzed by MS (Table 1). Compounds **16**, **18**−**20**, **25**, **37**, and **39** were analyzed in the acidic fraction and the remaining compounds in the neutral fraction.

*Odorants **29**, **30**, and **35**.* The wine sample (200 mL) was spiked with known amounts of the internal standards listed in Table 1. After addition of NaCl (20 g) the solution was extracted with diethyl ether (2 × 100 mL). The organic layer was washed with brine (2 × 50 mL) and separated in neutral and acidic fractions (Guth, 1997). The acidic fraction was concentrated to a volume of 200 μL by distilling off the solvent on a Vigreux column (50 × 1 cm) and by microdistillation according to the procedure of Bemelmans (1979). Compounds **29**, **30**, and **35** were enriched by HPLC using a Lichrospher 100 Diol column (Guth, 1997). Fraction AIII was separated by HRGC on capillary DB-FFAP for the determination of **29** and **35** and on capillary DB-OV-1701 for the determination of **30**.

*Odorants **11**, **13**, **23**, **31**, **33**, **36**, **38**, and **43**.* The wine sample (800 mL) was spiked with known amounts of the internal standards listed in Table 1. The solvent extract was separated in neutral and acidic fractions, and then the neutral fraction was subjected to column chromatography (CC) on silica gel as described in Guth (1997). Aliquots (0.5 μL) of the CC subfraction NI were separated by HRGC (Table 1) for the determination of **31** and **43**, subfraction NII for the determination of **11** and **13**, subfraction NIV for the determination of **33** and **36**, and subfraction NV for the determination of **38**. Quantitation of **23** was performed by multidimensional gas chromatography (MDGC) coupled with the MS system ITD-800, running in the chemical ionization mode (CI) with methanol as reagent gas (Sen et al., 1991b). A DB-FFAP column (30 m × 0.32 mm, 0.25 μm film thickness) was used as the precolumn, installed into a Mega 2 gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany), and a DB-5 column (30 m × 0.32 mm, 0.25 μm film thickness) as the main column, installed into a Fisons GC 5160. The precolumn was coupled with the main column via a moving capillary switching (MCSS) system (Fisons Instruments) and a methyl-deactivated transfer line, which was held at −100 °C. The flow adjustments of the MCSS coupling system are described by Weber et al. (1995). Aliquots (0.5 μL) of CC subfraction NI were applied by on-column injection onto the precolumn at 35 °C and held for 2 min, and then the temperature of the oven was raised at 40 °C/min to 60 °C, held for 1 min, and then raised at 6 °C/min to 250 °C. Using a cut time interval of 19−20 min, **23** was transferred into the liquid nitrogen cooled trap. To start the second GC run on the main column, the trap was heated very rapidly to 200 °C. The temperature program of the main column was as follows: 50 °C, held for 1 min, and then raised at 40 °C/min to 60 °C, held for 1 min, and then raised at 6 °C/min to 250 °C (retention time of **23** = 16 min).

*Odorants **40**−**42** and **44**.* Wine (5 mL) was poured into a vessel (20 mL). After addition of the internal standards (Table 1), the vessel was sealed with a septum. In a headspace volume of 10 mL, which was drawn with a gastight syringe, **40** was determined by SHA coupled with the MS system Incos XL as described by Guth and Grosch (1994). Odorants **41** and **42** were analyzed in a headspace volume of 1 mL. For the determination of **44** the wine sample was diluted with water (1 + 100) and then analyzed by the injection of 100 μL of headspace.

High-Resolution Gas Chromatography (HRGC)/Mass Spectrometry (MS). HRGC was performed with a Type 5300 gas chromatograph (Fisons Instruments) by using the following capillaries: DB-FFAP, DB-1701, and DB-5 (30 m × 0.32 mm, 0.25 μm film thickness; J&W Scientific, Fisons Instruments, Mainz, Germany). The samples were applied by on-column injection technique at 35 °C and held for 1 min, and then the temperature of the oven was raised at 40 °C/min to 60 °C, held for 1 min, then raised at 6 °C/min to 250 °C, and held for 10 min isothermally.

MS analyses were performed with a MS-8230 (Finnigan, Bremen, Germany) and by the MS system Incos XL (Finnigan) in tandem with the capillaries described above (Table 1).

The mass spectra in the chemical ionization mode (MS/CI) were obtained at 115 eV with isobutane and methane, respectively, as reagent gas (Table 1).

Odor Detection Thresholds. A defined amount of each compound (**1**−**43**), dissolved in ethanol (100 μL), was added to a mixture of water/ethanol (90 + 10, v/v; 1 L). After stirring for 30 min, this stock solution was diluted (1 + 1, v/v) stepwise with water/ethanol (90 + 10, v/v) and stirred for 10 min after each dilution step. Immediately after preparation, the diluted samples (2 mL) were presented in covered glass beakers (diameter, 10 mm; capacity 10 mL) at 21 °C to individual panel members (six trained judges). The cap was removed, the sample rinsed into the mouth, and the odor then retronasally perceived. Sensitivity odor threshold values were determined by the triangle test (Guth and Grosch, 1993c) by using water/ethanol (90 + 10, v/v) as a blank. The samples were presented

Table 2. Odor Threshold Values, Concentrations, and Odor Activity Values of Potent Odorants of Scheurebe and Gewürztraminer Wines

odorant	odor threshold value[a]	concentration[b] (mg/L)		odor activity value[c]	
		Scheurebe	Gewürztraminer	Scheurebe	Gewürztraminer
1,1-diethoxyethane (**1**)	50	n.a.	375		8
ethyl isobutyrate (**2**)	15	480	150	32	10
butane-2,3-dione (**3**)	100	180	150	2	2
ethyl butyrate (**4**)	20	184	210	9	11
ethyl 2-methylbutyrate (**5**)	1	4.5	4.4	5	4
ethyl 3-methylbutyrate (**6**)	3	2.7	3.6	1	1
2-methylpropanol (**7**)	40000	108000	52000	3	1
3-methylbutyl acetate (**8**)	30	1450	2900	48	97
3-methylbutanol (**9**)	30000	109000	127800	4	4
ethyl hexanoate (**10**)	5	280	490	56	98
cis-rose oxide (**11**)	0.2	3.0	21	15	105
hexanol (**12**)	8000	1890	1580	<1	<1
4-mercapto-4-methylpentan-2-one (**13**)	0.0006	0.40	<0.01	667	<1
(*Z*)-3-hexenol (**14**)	400	74	74	<1	<1
ethyl octanoate (**15**)	2	270	630	135	315
acetic acid (**16**)	200000	255000	280000	1	1
linalool (**17**)	15	307	175	20	12
isobutyric acid (**18**)	200000	4160	2040	<1	<1
butyric acid (**19**)	10000	1290	1580	<1	<1
2-/3-methylbutyric acid (**20**)	3000	550	750	<1	<1
3-(methylthio)-1-propanol (**21**)	500	1040	1415	2	3
citronellol (**22**)	100	15	42	<1	<1
(*E*)-β-damascenone (**23**)	0.05	0.98	0.84	20	17
2-phenylethyl acetate (**24**)	250	262	112	1	<1
hexanoic acid (**25**)	3000	2470	3230	<1	1
geraniol (**26**)	30	38	221	1	7
2-methoxyphenol (**27**)	10	2.2	3.6	<1	<1
2-phenylethanol (**28**)	10000	21600	18000	2	2
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (**29**)	500	1.8	4.2	<1	<1
5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (EHMF) (**30**)	500	117	53	<1	<1
trans-ethyl cinnamate (**31**)	1	2.3	2.0	2	2
4-allyl-2-methoxyphenol (eugenol) (**32**)	5	0.5	5.4	<1	1
3-ethylphenol (**33**)	0.5	0.1	0.1	<1	<1
2-methoxy-4-vinylphenol (vinylguaiacol) (**34**)	40	4.5	25	<1	<1
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon (**35**)	5	3.3	5.4	<1	1
wine lactone (**36**)	0.01	0.10	0.10	10	10
decanoic acid (**37**)	15000	930	1270	<1	<1
(*Z*)-6-dodeceno-γ-lactone (**38**)	0.1	0.14	0.27	1	3
4-hydroxy-3-methoxybenzaldehyde (vanillin) (**39**)	200	17	45	<1	<1
acetaldehyde (**40**)	500	1970	1860	4	4
dimethyl sulfide (**41**)	10	7.1	14	<1	1
ethyl acetate (**42**)	7500	22500	63500	3	8
dimethyl trisulfide (**43**)	0.2	0.09	0.25	<1	1
ethanol (**44**)		77.2 g/L	97.8 g/L		

[a] The odor threshold values (μg/L) were determined in water/ethanol (90 + 10, w/w). [b] The data are mean values of duplicates (maximum SD: ±10%). [c] The odor activity values were calculated by dividing the concentration by the odor threshold value of the compound in water/ethanol.

in order of decreasing concentrations, and the odor threshold values evaluated in two sessions were averaged (Table 2).

Sensory Experiments. Compounds **1**−**43** in ethanol (2 mL) were added to a water/ethanol mixture (90 + 10, v/v; 998 mL) in concentration levels equal to those determined in Gewürztraminer and Scheurebe (Table 2), respectively. After stirring for 30 min, the model mixtures were compared nasally in covered glass beakers (diameter, 40 mm; capacity, 45 mL) with the original wines.

RESULTS AND DISCUSSION

Quantitative Analyses. To gain an exact insight into the flavor differences of the two wine varieties, 43 compounds revealed by AEDA and SHA/O (Guth, 1997) as potent wine odorants were selected for quantitative measurements. Compounds **1**−**16**, **19**−**21**, **23**−**25**, **27**−**31**, **33**−**44** were determined by a stable isotope dilution assay (IVA). Compounds **17**, **18**, **22**, **26**, and **32** were evaluated by using similar internal standards (Table 1) for the quantitation experiment. The amounts of 44 odorants (including ethanol) found in the varieties Scheurebe and Gewürztraminer are listed in Table 2. Acetic acid (**16**) was the major compound in both wine samples; Scheurebe wine contained 255 mg/L and Gewürztraminer 280 mg/L. Differences between the two varieties were found for ethyl isobutyrate (**2**), which was higher in Scheurebe wine (480 μg/L) than in Gewürztraminer (150 μg/L), whereas *cis*-rose oxide (**11**) predominated in the latter, with 21 μg/L compared to 3.0 μg/L in the former wine. Significant difference was found for 4-mercapto-4-methylpentan-2-one (**13**), which was present only in the variety Scheurebe (0.4 μg/L) but not in Gewürztraminer (<0.01 μg/L).

Odor Activity Values (OAVs). To estimate the sensory contribution of the 43 odorants to the overall flavor of the wine samples, their OAVs were calculated (Table 2). To take into account the influence of ethanol, the odor threshold values of wine odorants were determined in a mixture of water and ethanol (Table 2) and were used to calculate the OAVs for each compound. According to the results in Table 2, the thiol **13**, ethyl octanoate (**15**), ethyl hexanoate (**10**), 3-methylbutyl acetate (**8**), ethyl isobutyrate (**2**), (*E*)-β-damascenone (**23**), *cis*-rose oxide (**11**), and wine lactone (**36**) showed

Table 3. Odor of Gewürztraminer Wine Model Mixtures Affected by the Absence of One Component

expt no.	model[a]	similarity[b]
1	complete model mixture (Gewürztraminer, 42 odorants)	3
2	*cis*-rose oxide (**11**)	1
3	wine lactone (**36**)	2
4	ethyl hexanoate (**10**)	2
5	ethyl octanoate (**15**)	2
6	3-methylbutyl acetate (**8**)	2
7	acetic acid (**16**)	2
8	acetaldehyde (**40**)	2.5
9	(*E*)-β-damascenone (**23**)	2.5
10	geraniol (**26**)	2.5
11	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**35**)	2.5
12	complete model mixture (Scheurebe, 42 odorants)	3
13	4-mercapto-4-methylpentan-2-one (**13**)	0.5

[a] The composition of the model is described under Sensory Experiments. The numbering in parentheses refers to Tables 1 and 2. [b] The similarity in the overall flavor (quality and intensity of the model was scored on the following scale: 0, absent; 1, weak; 2, medium; 3, strong). The results obtained by six panelists were averaged. The maximum deviation from the mean value was ±0.5.

the highest OAVs in the Scheurebe wine. With the exception of 4-mercapto-4-methylpentan-2-one (**13**), the above-mentioned odorants also showed the highest OAVs in Gewürztraminer wine. However, the rankings of the OAVs were different because of differences in concentration levels. The sequence of the most potent odorants of Gewürztraminer was **15** followed by **11**, **10**, **8**, **23**, **4**, **2**, and **36**. No importance in either variety was found for **12**, **14**, **18**−**20**, **22**, **27**, **29**, **30**, **33**, **34**, **37**, and **39** because the concentrations of the odorants did not reach their odor thresholds in the water/ethanol mixture.

Screening of the two varieties by AEDA had revealed wine lactone (**36**), sotolon (**35**), 3-ethylphenol (**33**), 2-phenylethanol (**28**), 3-methylbutanol (**9**), and ethyl 2-methylbutyrate (**5**) as the odorants with the highest flavor dilution (FD) factors (Guth, 1997). With the exception of **36**, these results are in contrast to the relatively low OAVs of the remaining compounds. As discussed by Grosch (1993), the difference between the FD factor of a compound and its OAV is afflicted by simplifications implicit in AEDA. For example, the FD factor is not corrected for losses of the odorants during isolation and concentration steps. Furthermore, the compounds are completely volatilized during GC analysis, whereas the headspace concentrations of the odorants in wine samples depend on their volatility and solubility.

Sensory Experiments. To clarify, whether the odorants showing high OAVs are actually the key aroma compounds of Gewürztraminer and Scheurebe, the odorants **1**−**43** in concentrations equal to those in wine (Table 2) were dissolved in a water/ethanol mixture. The quality and intensity of the aroma of this model were compared with the corresponding original wine. The results in Table 3 indicate that the aroma of Gewürztraminer (experiment 1) and Scheurebe models (experiment 12), respectively, showed good agreement with the original wines. To investigate whether the odorants contribute to the overall flavor of the wines, one after the other was omitted in the Gewürztraminer model (Table 3). In experiment 2, the absence of *cis*-rose oxide diminished strongly the similarity with that of the original Gewürztraminer wine. Also, omission of wine lactone (experiment 3), ethyl octanoate (experiment 5), acetic acid (experiment 7), 3-methylbutyl acetate (experiment 6), and ethyl hexanoate (experiment 4) led to a decreasing similarity with the original Gewürztraminer. The respective absence of the compounds **1**−**7**, **9**−**14**, **17**−**22**, **24**, **25**, **27**−**34**, **37**−**39**, and **41**−**43** (Table 2) was not noticed by the assessors (data not shown).

For the Scheurebe model (Table 3) the lack of 4-mercapto-4-methylpentan-2-one (experiment 13) has a drastic effect, as the odor of the remaining 41 odorants was completely different from that of the original wine.

CONCLUSION

The study has revealed potent odorants that are responsible for the overall flavor of Scheurebe and Gewürztraminer wines. Quantitation, calculation of OAVs, and sensory studies indicated that significant differences in odor profiles of both varieties were mainly caused by *cis*-rose oxide (**11**) in Gewürztraminer and by 4-mercapto-4-methylpentan-2-one (**13**) in Scheurebe. These compounds are suitable indicators for the objectification of flavor differences of the two wine varieties. Methods for an accurate quantification of wine odorants, which partly occurred as trace components, are reported.

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