JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of the Key Aroma Compounds in an American Bourbon Whisky by Quantitative Measurements, Aroma Recombination, and Omission Studies

LUIGI POISSON AND PETER SCHIEBERLE*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Thirty-one of the 45 odor-active compounds previously identified by us in an American Bourbon whisky were quantified by stable isotope dilution assays. Also for this purpose, new synthetic pathways were developed for the synthesis of the deuterium-labeled whisky lactone as well as for γ-nona- and γ-decalactone. To obtain the odor activity values (OAVs), the concentrations measured were divided by the odor thresholds of the odorants determined in water/ethanol (6:4 by vol.). Twenty-six aroma compounds showed OAVs >1, among which ethanol, ethyl (*S*)-2-methylbutanoate, 3-methylbutanal, 4-hydroxy-3-methoxybenzaldehyde, (*E*)-β-damascenone, ethyl hexanoate, ethyl butanoate, ethyl octanoate, 2-methylpropanal, (3*S*,4*S*)-*cis*-whiskylactone, (*E*,*E*)-2,4-decadienal, 4-allyl-2-methoxyphenol, ethyl-3-methylbutanoate, and ethyl 2-methylpropanoate showed the highest values. The overall aroma of the Bourbon whisky could be mimicked by an aroma recombinate consisting of the 26 key odorants in their actual concentrations in whisky using water/ethanol (6:4 by vol.) as the matrix. Omission experiments corroborated the importance of, in particular, 4-hydroxy-3-methoxybenzaldehyde, (3*S*,4*S*)-*cis*-whiskylactone, ethanol, and the entire group of esters for the overall aroma of the Bourbon whisky.

KEYWORDS: Bourbon whisky; stable isotope dilution analysis; odor activity value; aroma recombination; ethyl (*S*)-2-methylbutanoate; (3*S*,4*S*)-*cis*-whiskylactone

INTRODUCTION

Whisky is famous for its unique aroma combining smoky, malty odors with a characteristic sweet, vanilla-like flavor note, in particular, in American Bourbon-type spirits. Numerous publications have dealt with the identification of volatile components in several types of whisky so far (*1*), but only a few studies have made efforts to obtain quantitative data for whisky aroma compounds, and to evaluate their aroma contribution, for example, on the basis of odor activity values (ratio of concentration to odor threshold). Salo et al. (*2*) were the first to determine odor thresholds for Scotch whisky components. The authors calculated odor units (equivalent to odor activity values), but unfortunately, only quantitative data determined by other authors were used in the calculations. A whisky model was prepared, and the aroma contribution of compound groups was judged in sensory tests by means of omission experiments. These data suggested carbonyl compounds such as butanal, 2-methylpropanal, pentanal, 2-methylpentanal, and 2,3-butandione as well as straight-chain ethyl esters as important whisky aroma compounds (*2*–*4*). In a following study, the same group (*5*) quantified various phenols in Bourbon whisky and found that the concentrations of 2-methoxyphenol, 4-ethylphenol, 4-ethyl-2-methoxyphenol, and 4-allyl-2-methoxyphenol exceeded their thresholds determined in a water/ethanol mixture. However, because, for example, no GC-Olfactometry was used in the identification experiments, the selection of compounds chosen for quantification was somewhat arbitrary with respect to their aroma contribution.

In our recent study (*6*), the key aroma compounds in an American Bourbon whisky were identified on the basis of an odor intensity ranking by application of aroma extract dilution analysis and aroma dilution analysis. A total of 45 odor-active areas were located, of which 42 odorants could be identified.

The aim of the present study was, consequently, (i) to quantify the aroma compounds previously characterized with the highest FD factors using stable isotope dilution assays, (ii) to calculate their odor activity values on the basis of their odor thresholds in water/ethanol, and (iii) to verify the results by means of aroma recombination and omission experiments.

MATERIALS AND METHODS

American Bourbon Whisky. The whisky under investigation was an American Kentucky Straight Bourbon whisky, which according to

* Corresponding author. Tel: +49 89 289 13265. Fax: +49 89 289 14183. E-mail: Peter.Schieberle@ch.tum.de.

10.1021/jf800383v CCC: $40.75
Published on Web 06/27/2008

the label had been produced according to the sour mesh method and had been stored in new, heat-charred oak casks for at least three years. The whisky samples were purchased at a local supermarket. Mentioning of a brand name does not imply any research contact with the whisky manufacturer nor is it for advertising purposes.

Chemicals. The reference compounds of the odorants were obtained from the commercial sources as recently reported (*6*). Anhydrous sodium sulfate, ethyl acrylate, ethyl crotonate, *n*-heptane, hydrochloric acid (37%), and *tert*-butylalcohol were from Merck, Darmstadt, Germany. (*E*)-2-heptenal, (*E*)-2-hexenal, (*E*)-2-pentenal, sodium thiosulfate, and samarium-(II)-diiodide (0.1 M in tetrahydrofurane) were from Aldrich, Sigma-Aldrich Chemie (Taufkirchen, Germany). Lindlar-catalyst and tetrahydrofurane were from Fluka, Sigma-Aldrich Chemie (Taufkirchen, Germany). Deuterium gas (99.7%) and Argon (99.996%) were purchased from Air Liquide (Düsseldorf, Germany). Diethyl ether and *n*-pentane (Merck, Darmstadt, Germany) were freshly distilled before use. Silica 60 (Merck, Darmstadt, Germany) was purified according to a procedure described by Esterbauer (*7*).

Syntheses of Labeled Internal Standard. *[$^2H_2$]-cis/trans-Whiskylactone. [2,3-$^2H_2$]-Pentanal.* A mixture of (*E*)-2-pentenal (1 g), dissolved in *n*-heptane (30 mL), and Lindlar-catalyst (500 mg) was deuterated in a laboratory autoclave (Roth, Karlsruhe, Germany) at 2 × 10^5 Pa for 1 h at room temperature. After filtration and addition of water (100 mL), the target compound was isolated by extraction with diethyl ether (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was distilled off using a Vigreux column.

[2,3-$^2H_2$]-Pentanal (5 mmol), ethyl crotonate (5 mmol), and *tert*-butylalcohol (5 mmol) dissolved in tetrahydrofurane (15 mL) were added to a solution of samarium-(II)-diiodide (10 mmol) in tetrahydrofurane (100 mL) at 0 °C under an argon atmosphere and stirred for 5 h. After the addition of aqueous hydrochloric acid (2 mol/L, 50 mL), the target compound was extracted with diethylether (3 × 50 mL). The organic layer was washed with aqueous sodium thiosulfate (20% in water, 50 mL), followed by water (3 × 50 mL), and finally dried over anhydrous sodium sulfate. The solvent was distilled off using a Vigreux column at 40 °C. The remaining yellow oil was diluted with *n*-pentane (1 mL) and applied onto a glass column with cooling jacket (30 cm × 1 cm) filled with silica 60 (30 g) in *n*-pentane. To yield fractions 1−10, the solution was fractionated at 12 °C using 10 *n*-pentane/diethyl ether mixtures of increasing polarity (50 mL each 95:5, 90:10, 80:20, 80:20, 60:40, 40:60, 20:80, 20:80 v/v). Each fraction was dried over anhydrous sodium sulfate and concentrated to 1 mL using a Vigreux column (50 cm × 1 cm i.d.). The target compound was detected by HRGC-O in fractions 5 and 6.

[$^2H_2$]-*cis*-Whiskylactone was characterized by the following mass spectral data: MS-EI, *m/z* (%): 99 (100), 42 (39), 71 (33), 43 (23), 70 (19), 41 (16), 89 (13), 56 (10); MS-CI, *m/z* (%): 159 (100).

[$^2H_2$]-*trans*-whiskylactone was characterized by the following mass spectral data: MS-EI, *m/z* (%): 99 (100), 42 (27), 71 (33), 43 (22), 70 (12), 41 (13), 100 (10), 89 (9), 56 (5); MS-CI, *m/z* (%): 159 (100).

[$^2H_2$]-γ-Nonalactone. [$^2H_2$]-γ-Nonalactone was synthesized following the procedure described above for [$^2H_2$]-*cis/trans*-whiskylactone, but using (*E*)-2-hexenal instead of (*E*)-2-pentenal and ethyl acrylate instead of ethyl crotonate. After purification by column chromatography as described above, the target compound was detected by HRGC-O in fractions 5 and 6 and analyzed by mass spectrometry, yielding the following mass spectral data: MS-EI, *m/z* (%): 85 (100), 86 (11), 56 (7), 57 (7), 42 (5); MS-CI, *m/z* (%): 159 (100).

[$^2H_2$]-γ-Decalactone. [$^2H_2$]-γ-Decalactone was also synthesized according to the procedure described for [$^2H_2$]-*cis/trans*-whiskylactone, but using (*E*)-2-heptenal instead of (*E*)-2-pentenal and ethyl acrylate instead of ethyl crotonate. After purification by column chromatography as described above, the target compound was detected by HRGC-O in fractions 6 and 7. The analyte was characterized by the following mass spectral data: MS-EI, *m/z* (%): 85 (100), 86 (16), 56 (8), 57 (6), 41 (5); MS-CI, *m/z* (%): 173 (100).

Determination of the Concentrations of the Synthesized Labeled Compounds. Because the syntheses were performed on a microscale basis, it was impossible to determine the concentrations of the target compounds by weight. Thus, the following procedure was used: Defined amounts of the respective unlabeled compound and methyl octanoate were analyzed by GC-FID yielding an FID response factor. Then, a defined amount of methyl octanoate was added to a defined volume of the solution containing synthesized labeled compound and again analyzed by GC-FID. The concentration of the labeled compound was then calculated from the GC peak areas using the FID response factor determined for the unlabeled compound.

The following compounds were synthesized according to the literature given in parentheses: [$^{13}C_4$]-2,3-butandione and [$^2H_3$]-ethyl butanoate (*8*), [$^2H_{5-7}$]-(*E*)-β-damascenone (*9*), [$^2H_2$]-(*E,E*)-2,4-nonadienal, [$^2H_2$]-(*E,Z*)-2,6-nonadienal, [$^2H_2$]-(*E*)-2-nonenal, [$^2H_4$]-(*E,E*)-2,4-decadienal and [$^2H_2$]-(*E*)-2-decenal (*10*), [$^{13}C_2$]-1,1-diethoxyethane, [$^2H_5$]-*trans*-ethyl cinnamate and [$^2H_3$]-ethyl hexanoate (*11*), [$^2H_3$]-4-ethyl-2-methoxyphenol, [$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde, and [$^2H_3$]-4-vinyl-2-methoxyphenol (*12*), [$^2H_3$]-ethyl 2-methylbutanoate, [$^2H_3$]-ethyl 3-methylbutanoate and [$^2H_3$]-ethyl 2-methylpropanoate (*13*), [$^2H_3$]-ethyl octanoate and [$^2H_2$]-2-phenylethanol (*14*), [$^2H_3$]-ethyl propanoate, [$^{13}C_2$]-3-methylbutyl acetate, and [$^{13}C_2$]-2-phenylethyl acetate (*15*), [$^2H_3$]-2-methoxyphenol (*16*), [$^2H_2$]-3-methylbutanal (*17*), [$^2H_2$]-3-methylbutanol (*18*), and [$^2H_7$]-2-methylpropanal (*19*).

Quantitation by Stable Isotope Dilution Assays in Combination with Two-Dimensional High Resolution Gas Chromatography (TD-HRGC-SIDA). The labeled internal standards (2 to 10 μg, respectively) dissolved in diethyl ether (0.5 mL) were added to aliquots of the whisky containing the respective analytes in similar concentrations as determined in preliminary experiments. After the addition of tap water and stirring for 1 h, the volatiles and the internal standards were isolated by extraction with diethyl ether. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 100 mL at 37 °C using a Vigreux column (50 cm × 1 cm ID). The nonvolatile material was removed by SAFE distillation at 40 °C (*20*), and the distillate was concentrated to 100 μL using a Vigreux column and a micro distillation apparatus (*21*).

TD-HRGC-MS was performed using a GC Mega 2 Series (Fisons, Mainz, Germany) connected to a GC 5160 (Carlo Erba, Hofheim, Germany). In the first dimension, the separation of the distillate was achieved on a DB-5 column (neutral/basic fraction = NBF) or on an FFAP column (acidic fraction = AF), respectively. The elution range containing the selected odorant and the internal standard was transferred into a cold trap (−100 °C) by means of a MCSS system (moving capillary stream switching) (Thermo, Dreieich, Germany). After complete trapping, the analyte and the internal standard were transferred onto the second column (DB-FFAP for NBF and OV-1701 for AF; 30 m × 0.32 mm fused silica capillary DB 1701, 0.25 μm (Chrompack, Mühlheim, Germany), by heating the trap to 200 °C. For mass chromatography, the second column was coupled in the open-split mode to an ITD 800 ion trap detector (Finnigan MAT, Bremen, Germany) running in the chemical ionization mode (CI) with methanol as reactant gas (ionization energy 115 eV). The selected ions of the labeled standard and the aroma compound were monitored (**Table 1**), and their intensities were calculated by means of a computer program. Concentrations were calculated and corrected using MS response factors obtained by measuring defined mixtures of the respective labeled and unlabeled compound.

Determination of Ethanol. Ethanol was determined on the basis of density by weighing exactly 20 mL of a steam-distillate of whisky (20 mL).

Determination of Odor Thresholds. To guarantee the absence of contaminating odorants, all reference compounds were first analyzed by HRGC-O (*6*) and purified by distillation, if necessary. A defined amount of the odorant in ethanol (10 μL) was then pipetted into a Teflon vessel containing 25 mL of ethanol/water (6:4 by vol.) and stirred for 2 min. Then, the sample was judged by 10 trained assessors as described below. Triangular tests using 25 mL of water/ethanol (6:4 by vol.) as the control were performed, and the samples were presented with increasing concentrations of the odorant. Odor thresholds were calculated according to the method of 35 LMBG, methods 00.90−7 and 00.90−9 (*22*).

Descriptive Profile Tests. Assessors for the descriptive profile tests were recruited from the German Research Center for Food Chemistry at Garching and were trained to describe and recognize the odor qualities of about 40 odorants. The assessors were subjected to a ranking test

Table 1. Selected Ions (*m/z*) of Analytes and Isotopically Labeled Standards (IST) Used in the Stable Isotope Dilution Assays

compound	analyte (*m/z*)	isotope label	IST (*m/z*)	MS response factor
2-methylpropanal	73	$^2H_7$	80	0.98
3-methylbutanal	87	$^2H_2$	89	1.04
2,3-butandione	87	$^{13}C_4$	91	0.87
ethyl propanoate	103	$^2H_3$	106	1.00
ethyl butanoate	117	$^2H_3$	120	1.06
ethyl hexanoate	145	$^2H_3$	148	1.00
ethyl octanoate	173	$^2H_3$	176	1.07
ethyl 2-methylpropanoate	117	$^2H_3$	120	1.00
(*S*)-ethyl 2-methylbutanoate	131	$^2H_3$	134	0.97
ethyl 3-methylbutanoate	131	$^2H_3$	134	1.02
trans-ethyl cinnamate	177	$^2H_5$	182	0.60
(*E*)-damascenone	191	$^2H_{5-7}$	196−198	0.75
3-methylbutanol	71	$^2H_2$	73	0.98
2-phenylethanol	105	$^2H_2$	107	0.98
2-phenylethyl acetate[a]	182	$^{13}C_2$	184	0.89
1,1-diethoxyethane	73	$^{13}C_2$	75	0.94
3-methylbutyl acetate[a]	148	$^{13}C_2$	150	0.72
(*E*)-2-nonenal	141	$^2H_2$	143	0.89
(*E*)-2-decenal	155	$^2H_2$	157	0.97
(*E,E*)-2,4-nonadienal	139	$^2H_2$	141	1.04
(*E,Z*)-2,6-nonadienal	121	$^2H_2$	123	1.00
(*E,E*)-2,4-decadienal	153	$^2H_4$	157	0.83
trans-whiskylactone	157	$^2H_2$	159	0.90
cis-whiskylactone	157	$^2H_2$	159	0.69
γ-nonalactone	157	$^2H_2$	159	0.75
δ-decalactone	171	$^2H_2$	173	0.95
4-hydroxy-3-methoxybenzaldehyde	153	$^2H_3$	156	0.97
4-allyl-2-methoxyphenol[b]	165	$^2H_2$	154	0.84
2-methoxyphenol	125	$^2H_3$	128	1.02
4-ethyl-2-methoxyphenol	153	$^2H_3$	156	1.02

[a] Ammonia was used as the reactant gas $[M + 18]^+$. [b] $[^2H_3]$-4-Vinyl-2-methoxyphenol was used as the isotope labeled standard.

with a series of eight supra-threshold aqueous solutions (25 mL in Teflon vessels) of 3-methylbutanal (malty), (*E,E*)-2,4-decadienal (fatty), γ-nonalactone (coconut-like), ethyl butanoate (fruity), 2-phenylethanol (flowery), 4-hydroxy-3-methoxybenzaldehyde (vanilla-like), 2-methoxyphenol (smoky), and 4-ethylphenol (phenolic), and were asked to rate the odor intensities as 0 (not perceivable), 1 (weak), 2 (significant), and 3 (strong) using a seven point scale of 0, 0.5, 1.0, 1.5 . . . 3.0. Ten panelists were then selected for the evaluation of Bourbon whisky and the aroma model mixtures. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C in three different sessions.

The evaluation of the odor (orthonasal) of the whisky was performed in the following way: The panelists were asked to evaluate the intensities of the eight odor qualities represented by the chemicals given above using the seven point linear scale. The results obtained at three different sessions were averaged for each odor note and plotted in a spider web diagram. The values judged by the single assessors differed by not more than 20%.

An aroma recombinate consisting of 26 whisky odorants with OAVs > 1 was prepared in water/ethanol (6:4 by vol.), and the pH was adjusted to 4.1. The overall aroma profile of the model mixture was determined in the same way as described above for whisky. In a separate session, the overall similarity of the whisky aroma and the aroma recombinate were compared. The similarity was estimated using a seven point scale from 0 to 3.

To obtain the odorless whisky matrix, a whisky sample was freeze-dried, and after the addition of diethyl ether, the volatile components were distilled off by SAFE distillation until the residue was odorless. Then, an aliquot of the whisky residue, corresponding to the respective amount of volatiles was added to the whisky aroma recombinate, and the descriptive profile test was performed as described above. Again, in a separate session, the overall similarity of the whisky aroma and the aroma recombinate in whisky matrix were compared.

Omission Experiments. Mixtures were prepared by omitting selected components from the complete recombinate and were presented to the sensory panel in comparison to the complete model in a triangel test as described in ref *22*. The significance α of the difference detected was calculated according to ref *23*. The sensory panel for the omission experiments was the same as that for the descriptive profile tests.

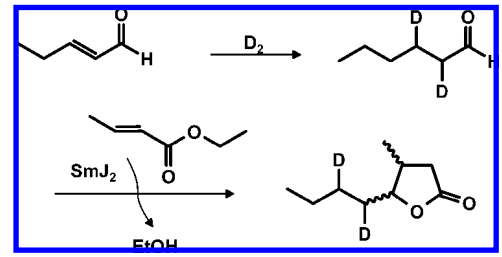

Figure 1. Synthetic route used in the preparation of $[^2H_2]$-whisky lactone.

RESULTS AND DISCUSSION

By application of an aroma extract dilution analysis (AEVA) on the same Bourbon whisky (*6*), 42 odor-active compounds have been identified in our previous study among which ethanol, 3-methylbutanal, ethyl 2-methylpropanoate, (*E*)-β-damascenone, α-damascone, γ-nonalactone, γ-decalactone, (*Z*)-6-dodeceno-γ-lactone, (3*S*,4*R*)-*trans*-whiskylactone, (3*S*,4*S*)-*cis*-whiskylactone, 4-allyl-2-methoxyphenol, 4-hydroxy-3-methoxy-benzaldehyde, 3-methyl-1-butanol, 2-phenylethanol, and (*S*)-ethyl 2-methylbutanoate were identified with the highest flavor dilution (FD) factors. Although dilution to odor threshold techniqes, such as the aroma extract dilution analysis and the aroma dilution analysis, are useful methods for the screening of important odorants in foods, these methods neither permit a study on the influence of the food matrix on odorant binding nor on the interactions of odorants when matching the overall odor impression of the food. For this reason, first, the odor activity value concept (*21*) was applied in this study to the odorants of the Bourbon whisky analyzed in the previous study.

Thirty-one odorants that had shown high FD factors during AEVA or AVA, respectively (*6*), were quantified by means of stable isotope dilution assays. 2,3-Butandione was included in the quantification experiments because it was frequently described as an important whisky aroma compound in the literature (*3, 5*).

In particular, for the synthesis of the deuterium labeled lactones, a synthetic route starting from a labeled aldehyde and an unsaturated ester in the presence of Samarian diiodide was applied. The reaction route is exemplified for the synthesis of the whiskylactone in **Figure 1**; the principle of the reaction is a reductive coupling of an α,β-unsaturated ester with the Deuterium-labeled aldehyde. The mass spectra (MS-EI, MS-CI) obtained for the $[^2H_2]$-whiskylactone are displayed in **Figure 2A** and **B**. The shift of two mass units in the molecular ion (*m/z* 159) as compared to the analyte (*m/z* 157; data not shown) confirmed the presence of two deuterium atoms in the labeled internal standard.

The results of the quantitative measurements (**Table 2**) revealed ethanol (316 g/L) and 3-methylbutanol (1060 mg/L) as the compounds with the highest concentrations, followed by 1,1-diethoxyethane (15.3 mg/L), 2-phenylethanol (13.9 mg/L), ethyl octanoate (8.34 mg/L), 3-methylbutyl acetate (2.59 mg/L), (3*S*,4*S*)-*cis*-whiskylactone (2.49 mg/L), 4-hydroxy-3-methoxybenzaldehyde (2.13 mg/L), ethyl hexanoate (1.99 mg/L), and 2-phenylethyl acetate (1.94 mg/L). However, some components were found in extremely low concentrations, such as (*E,Z*)-2,6-nonadienal (0.9 μg/L) or γ-decalactone (1.6 μg/L). The standard

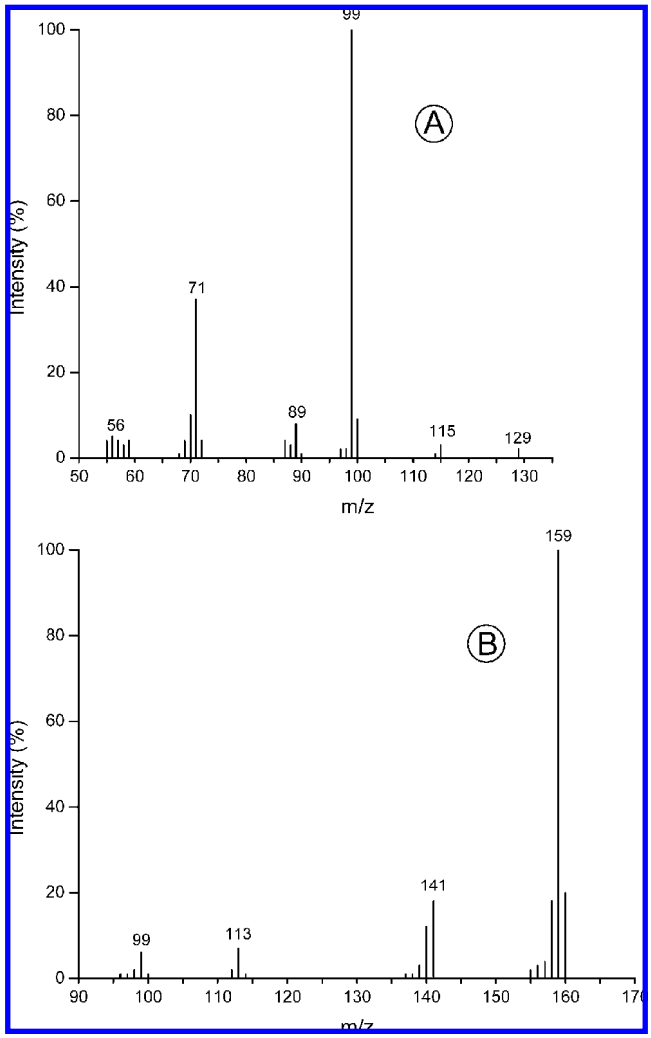

Figure 2. Mass spectra of [$^2H_2$]-whiskylactone. **(A)** MS-EI. **(B)** MS-CI.

deviation calculated from the results of three separate samples taken from the same bottle was ≥10%.

Because the composition of whisky may be influenced by many factors during the manufacturing process, it is customary to mix the distillates of several casks and different vintages to yield a standardized product. To evaluate the reproducibility of the production process, 15 selected whisky odorants were quantified in 2 whisky samples originating from the same brand, but from 2 different batches, which, according to the label, were produced in 1996 and 1998. The data indicated (**Table 3**) that the differences in the concentrations of the majority of the odorants in the two whiskies were comparably small, except for 2-methylpropanal, (3*S*,4*S*)-*cis*-whiskylactone, and 4-hydroxy-3-methoxybenzaldehyde, for which the concentrations were by factors of 1.8 and 1.4, respectively, higher in the whisky from 1998. However, the concentration of 3-methylbutanal was lower by a factor of 1.4 in the 1998 whisky than in the one from 1996. Yet, although only two samples originating from two different whisky batches were compared in this experiment, these data suggest that whisky production by the same manufacturer is quite reproducible when different batches are compared.

Odor activity values (ratio of concentration to odor threshold) are a good means to correlate quantitative data with the volatility of a compound from the respective matrix (*21*). However, it is necessary that the thresholds of single components are determined in a matrix as close as possible to the food itself. For this reason, the odor thresholds for all aroma components under investigation were determined in water/ethanol (6:4 by vol.), representing the whisky matrix.

By far, the highest number was calculated for ethanol (12690) (**Table 4**). This was determined using the odor threshold of ethanol in water and mirrors the dominating aroma impression of ethanol in alcoholic beverages. However, the results suggested that 25 odorants should additionally contribute to the characteristic aroma of American Bourbon whisky because their concentrations clearly exceeded their odor thresholds in water/ethanol (**Table 4**).

Table 2. Concentrations of 32 Potent Odorants in an American Bourbon Whisky

compound	concentration [μg/L][a]	standard deviation [%][b]
ethanol	316000000	
3-methylbutanol	1060000	2
1,1-diethoxyethane	15300	1
2-phenylethanol	13900	2
ethyl octanoate	8340	9
3-methylbutyl acetate	2590	7
(3*S*,4*S*)-*cis*-whiskylactone	2490	6
4-hydroxy-3-methoxybenzaldehyde	2130	2
ethyl hexanoate	1990	1
2-phenylethyl acetate	1940	2
ethyl propanoate	793	4
ethyl butanoate	551	5
3-methylbutanal	342	10
(3*S*,4*R*)-*trans*-whiskylactone	337	8
4-allyl-2-methoxyphenol	240	1
2-methylpropanal	233	9
ethyl 2-methylpropanoate	134	10
γ-nonalactone	120	10
4-ethyl-2-methoxyphenol	59	3
2-methoxyphenol	56	7
ethyl 3-methylbutanoate	52	6
(*E*,*E*)-2,4-decadienal	39	6
2,3-butandione	33	10
(*S*)-ethyl 2-methylbutanoate	30	10
(*E*)-damascenone	11	5
(*E*)-2-nonenal	9	5
(*E*,*E*)-2,4-nonadienal	2.4	10
(*E*)-2-decenal	1.8	3
trans-ethyl cinnamate	1.7	10
γ-decalactone	1.6	10
(*E*,*Z*)-2,6-nonadienal	0.9	10

[a] The mean value obtained by analyzing three different samples taken from the same bottle. [b] The standard deviation of the mean value [%].

Table 3. Comparison of the Concentrations of 15 Selected Odorants in 2 Batches of Bourbon Whisky Produced in 1996 and 1998

	conc. [μg/L][a]	
compound	1996	1998
ethyl octanoate	8340	10100
(3*S*,4*S*)-*cis*-whiskylactone	2490	3880
4-hydroxy-3-methoxybenzaldehyde	2130	3060
ethyl hexanoate	1990	2390
ethyl butanoate	551	668
3-methylbutanal	342	242
(3*S*,4*R*)-*trans*-whiskylactone	337	364
4-allyl-2-methoxyphenol	240	194
2-methylpropanal	233	417
ethyl 2-methylpropanoate	134	143
ethyl 3-methylbutanoate	52	51
2,3-butandione	33	32
(*S*)-ethyl-2-methylbutanoate	30	35
(*E*)-β-damascenone	11	9
(*E*)-2-nonenal	9	12

[a] Data are the mean values of triplicates differing not more than ± 10%.

Table 4. Orthonasal Odor Thresholds and Odor-Activity Values (OAV) of 31 Odorants in Bourbon Whisky

compound	odor threshold (μg/L) in water/ethanol (6:4 by vol.)	OAV[a]
ethanol	[b]	12690
(*S*)-ethyl 2-methylbutanoate	0.2	138
3-methylbutanal	2.8	122
4-hydroxy-3-methoxybenzaldehyde	22	97
(*E*)-damascenone	0.1	79
ethyl hexanoate	30	67
ethyl butanoate	9.5	58
ethyl octanoate	147	57
2-methylpropanal	5.9	39
(3*S*,4*S*)-*cis*-whiskylactone	67[c]	37
(*E*,*E*)-2,4-decadienal	1.1	35
4-allyl-2-methoxyphenol	7.1	34
ethyl 3-methylbutanoate	1.6	33
ethyl 2-methylpropanoate	4.5	30
1,1-diethoxyethane	719	21
3-methyl-butanol	56100	19
2-phenylethyl acetate	108	18
(*E*)-2-nonenal	0.6	16
2,3-butandione	2.8	12
3-methylbutyl acetate	245	11
4-ethyl-2-methoxyphenol	6.9	9
γ-nonalactone	21	6
2-methoxyphenol	9.2	6
2-phenylethanol	2600	5
(*E*,*Z*)-2,6-nonadienal	0.3	3
trans-ethyl cinnamate	0.7	2
ethyl propanoate	3452	<1
(3*S*,4*R*)-*trans*-whiskylactone	790[c]	<1
(*E*,*E*)-2,4-nonadienal	2.6	<1
(*E*)-2-decenal	5.2	<1
γ-decalactone	21	<1

[a] Odor-activity values were calculated by dividing the concentrations (see **Table 2**) by the respective odor threshold in water/ethanol (6:4 by vol.). [b] The odor activity value for ethanol was calculated by dividing its concentration by its odor threshold in water. [c] Odor thresholds were taken from ref *25*.

Relatively high odor activity values were also calculated for ethyl (*S*)-2-methylbutanoate (138) and 3-methylbutanal (122), followed by 4-hydroxy-3-methoxybenzaldehyde, (*E*)-β-damascenone, ethyl hexanoate, ethyl butanoate, and ethyl octanoate with OAVs between 50 and 100. With odor activity values between 10 and 50, 2-methylpropanal, (3*S*,4*S*)-*cis*-whiskylactone, (*E*,*E*)-2,4-decadienal, 4-allyl-2-methoxyphenol, ethyl 3-methylbutanoate, ethyl 2-methylpropanoate, 1,1-diethoxyethane, 3-methyl-1-butanol, 2-phenylethyl acetate, (*E*)-2-nonenal, 2,3-butandione, and 3-methylbutyl acetate should also contribute to whisky aroma. However, for ethyl propanoate, (3*S*,4*R*)-*trans*-whiskylactone, (*E*,*E*)-2,4-nonadienal, (*E*)-2-decenal, and γ-decalactone, OAVs <1 were calculated suggesting that no aroma contribution is to be expected from these components.

To confirm the contribution of the 26 key odorants to the overall whisky aroma, an aroma recombinate was prepared consisting of all whisky odorants with OAVs >1 in water/ethanol (6:4 by vol.) in their natural concentrations (**Table 2**). Descriptive profile tests were performed for the aroma recombinate in comparison with Bourbon whisky by evaluating eight odor attributes as well as the overall similarity.

The comparison of the aroma profiles of the aroma recombinate and the whisky showed a good similarity (**Figure 3**). This result is also expressed by the evaluation of the overall similarity between the model mixture and the original whisky that was judged to be 2.7 out of 3.0 points.

To evaluate the influence of the nonvolatile fraction of whisky on, for example, aroma release phenomena, the deodorated, nonvolatile residue of a whisky sample representing the real whisky matrix was added to the corresponding amount of the aroma model mixture, and again descriptive profile tests against the original whisky were performed. The data (**Figure 3**) showed that the aroma model with matrix addition gave an even better similarity to the whisky sample and was judged with a score of 2.8 points out of 3.0. However, because the similarities of the whisky aroma models with and without matrix to the whisky only showed small differences, the results suggest that the nonvolatile fraction is not a crucial factor, for example, in binding volatile whisky components.

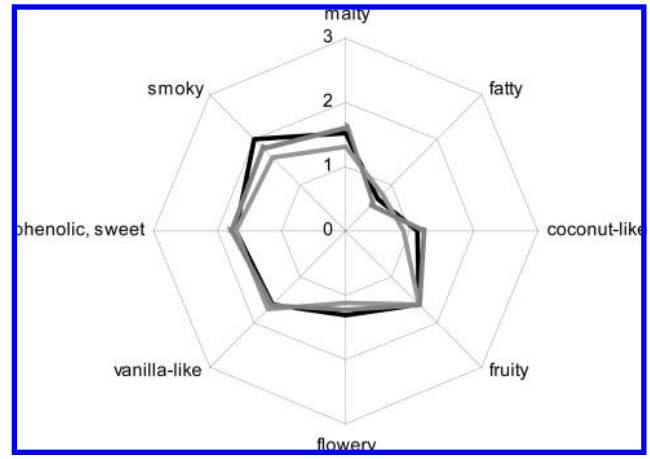

Figure 3. Aroma profile analysis of the American Bourbon whisky (black), the aroma recombinate in water/ethanol (6:4 by vol.) (gray) and the aroma recombinate in water/ethanol (6:4 by vol.) also containing the nonvolatile, odorless matrix from whisky (dark gray).

Table 5. Omission Experiments from the Complete Model Mixture

no.	odorants omitted from the complete model mixture	significance α (%)[a]
1	all ethyl esters and 3-methylbutyl acetate	0.1
2	4-hydroxy-3-methoxybenzaldehyde	1.0
3	2-methylpropanal, 3-methylbutanal	>5.0
3 A	2-methylpropanal, 3-methylbutanal, 3-methylbutanol	>5.0
4	(3*S*,4*S*)-*cis*-whiskylactone, γ-nonalactone	1.0
4 A	(3*S*,4*S*)-*cis*-whiskylactone	0.1
4 B	γ-nonalactone	>5.0
5	(*E*)-β-damascenone	>5.0
6	2,3-butandione	>5.0
7	ethanol	0.1

[a] Significance value α (%) 0.1, very highly significant; 1.0, highly significant; 5.0, significant; and >5.0, not significant.

To gain deeper insight into the interaction of whisky odorants leading to the final aroma, omission experiments were carried out. For this purpose, the aroma of the complete recombinate containing all 26 key aroma compounds (OAV $\geq$1) was compared to the aroma of model mixtures missing either single components or groups of components. The data (**Table 5**) showed that the panel was able to detect the omission of the entire group of ethyl esters with a very high significance. This result indicated the important role of these fruity-smelling compounds for the overall whisky aroma. A model mixture without 4-hydroxy-3-methoxybenzaldehyde (vanilla-like) was also evaluated as significantly different in aroma, but the omission of the Strecker aldehydes 2-methylpropanal and 3-methylbutanal could not be detected significantly by the sensory panel (model no. 3; **Table 5**). Even if these were omitted together with the malty smelling 3-methylbutanol (model no. 3A), no significant difference was detected. Thus, the distinct malty aroma note present in the aroma profile of the entire model

Table 6. Comparison of Odor Thresholds of Selected Odorants in Water and in Water/Ethanol (6:4 by Vol.)

compound	odor threshold [μg/L] in water	odor threshold [μg/L] in ethanol/water
(*E*)-β-damascenone	0.0007[a]	0.1
(3*S*,4*R*)-*trans*-whiskylactone	64[b]	790[b]
(3*S*,4*S*)-*cis*-whiskylactone	28[b]	67[b]
3-methylbutanol	1000[a]	56100
2-methylpropanal	1[a]	6
ethyl propanoate	20[c]	3452
ethyl butanoate	14[a]	9
ethyl hexanoate	2[c]	30
ethyl 2-methylpropanoate	0.02[c]	4
(*S*)-ethyl-2-methylbutanoate	0.006[d]	0.2
ethanol	24900[a]	

[a] Data taken from ref *24*. [b] Data taken from ref *25*. [c] Data taken from ref *26*. [d] Data taken from ref *27*.

mixture (**Figure 3**) could not be linked to certain aroma components.

A model mixture lacking in both, (3*S*,4*S*)-*cis*-whiskylactone and γ-nonalactone (model no. 4) was evaluated as significantly different compared to the entire model mixture. A mixture only lacking γ-nonalactone (model no. 4B), however, was not detected as different from the entire model, while a mixture without (3*S*,4*S*)-*cis*-whiskylactone (model no. 4B) was detected with very high significance. This indicates that (3*S*,4*S*)-*cis*-whiskylactone rather than γ-nonalactone is responsible for the coconut-like note of the whisky. Despite the relatively high odor activity value, which had been calculated for (*E*)-β-damascenone, its omission was not detected by the sensory panel in the respective model mixture (model no. 5 in **Table 4**), which might be due to the very intense fruity aroma note of the esters in the aroma model. The model lacking in 2,3-butandione (model no. 6) was also not judged as significantly different, which agrees with the fact that diacetyl had not been detected by GC-O (*6*). However, in model no. 7 lacking in ethanol, the sensory panel was able to detect a highly significant difference between the samples, thereby confirming the crucial role of ethanol for the whisky aroma. This was in agreement with the extremely high odor activity value determined for the alcohol (**Table 5**).

A comparison of odor thresholds for selected odorants in water and in ethanolic solution (40%) shows (**Table 6**) that the values for some compounds differed by magnitudes (e.g., (*E*)-β-damascenone, ethyl propanoate), whereas for others these were quite similar (ethyl butanoate, (3*S*,4*S*)-*cis*-whiskylactone). Thus, the data clearly show that only the determination of odor thresholds in a matrix similar to the food under investigation will give reliable results on the aroma contribution of single odorants.

2-Methylpropanal, 3-methylbutanal, and straight-chain ethyl esters, such as ethyl hexanoate and ethyl octanoate have been suggested by Salo as important aroma compounds in Scottish whisky (*3, 5*). Our studies showed that these also contribute significantly to the flavor of American Bourbon whisky. However, additionally, the previously unknown whisky constituents ethyl (*S*)-2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanoate, (*E*)-β-damascenone, and (*E*,*E*)-2,4-decadienal were confirmed as major aroma compounds of American Bourbon whisky. Particularly for ethyl (*S*)-2-methylbutanoate, which has been newly detected as a whisky flavor compound in our previous studies (*6*), a high odor activity value was calculated indicating its important role in whisky aroma. Straight-chained ethyl esters such as ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were previously quantified in American Bourbon whisky (*28*), but the concentrations of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and ethyl 2-methylpropanoate had not been determined yet in whisky. Schreier et al. (*29*) quantified ethyl 2-methylbutanoate and ethyl 3-methylbutanoate in brandy in concentration ranges of 61−123 μg/L and 114−219 μg/L, respectively. In Bourbon whisky, these compounds were present in clearly lower concentrations (30 and 52 μg/L, respectively; **Table 2**).

The contribution of various phenols to the flavor of Bourbon whisky, which has previously been studied by Jounela-Eriksson (*5*), was confirmed by our experiments. Particularly, for 4-allyl-2-methoxyphenol, a relatively high odor activity value was determined. Also results of Connor (*30*), who had suggested the importance of (3*S*,4*S*)-*cis*-whiskylactone and 4-hydroxy-3-methoxybenzaldehyde for the aroma of American Bourbon whisky by application of SPME/GC-O techniques, were in agreement with the whisky used in our studies.

The role of ethanol for the aroma of whisky, however, seems to have been underestimated in the literature so far since an aroma model mixture lacking in ethanol was judged as significantly different as compared to a complete aroma model. Furthermore, ethanol seems to have a masking effect, especially regarding the fruity aroma notes because an aroma model lacking in ethanol showed a more pronounced fruitier note as the complete model. This is in good agreement with studies on wine aroma (*10*), where a significantly fruitier aroma was found after reducing the ethanol concentration of model mixtures.

In summary, the aroma simulation experiments demonstrated that it is possible to create the typical aroma of American Bourbon whisky by mixing 26 odorants in their actual concentrations in a water/ethanol matrix. Moreover, this result shows that the key aroma compounds of American Bourbon whisky have been correctly identified during the application of the odor activity value concept using dilution of odor techniqes followed by exact quantitations and relating them to odor thresholds.

ACKNOWLEDGMENT

We thank Dr. Petra Steinhaus for her valuable help in the preparation of the manuscript.

LITERATURE CITED

(1) The Netherlands Organisation for Applied Scientific Research TNO: Volatile Compounds in Food, Version 9.2. http://www.vcf-online.nl (2007).
(2) Salo, P.; Nykaenen, L.; Suomalainen, H. Odor thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whisky. *J. Food Sci.* **1972**, *37*, 394–398.
(3) Jounela-Eriksson, P. the Aroma Composition of Distilled Beverages and the Perceived Aroma of Whisky In *Flavor of Foods and Beverages*; Charalambous, G., Inglett, G. Eds.; Academic Press Inc.: New York, 1978; pp 339−354.
(4) Salo, P. Interactions among volatile aroma compounds in a whiskey imitation. *Lebensm. Wiss. Technol.* **1973**, *6*, 52–58.
(5) Jounela-Eriksson, P.; Lehtonen, M. Phenols in the Aroma of Distilled Beverages, In The Quality of Foods and Beverages. *Proceedings of the 2nd International Flavor Conference*, Greece; Charalambous, G., Ed.; Inglett: New York, 1981, pp. 167–181.
(6) Poisson, L.; Schieberle, P. Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* 5813–5819.
(7) Esterbauer, H. On the autoxidation of methyl linoleate in water. *Fette, Seifen, Anstrichmittel* **1968**, *70*, 1–4.
(8) Hofmann, T.; Schieberle, P. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements

and senory studies on model mixtures. *J. Agric. Food Chem.* **1997**, *45*, 227–232.
(9) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assays. *Flavour Fragr. J.* **1991**, *192*, 541–547.
(10) Guth, H.; Grosch, W. Deterioration of soya-bean oil; quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513–522.
(11) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
(12) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assay. *Flavour and Fragr. J.* **1995**, *10*, 1–7.
(13) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513–518.
(14) Gassenmeier, K.; Schieberle, P. Potent aromatic compounds in the crumb of wheat bread (French-type)- influence of preferments and studies on the formation of key odorants during dough processing. *Z. Lebensm.-Unters.-Forsch.* **1995**, *201*, 241–248.
(15) Fuhrmann, E. Studies on the Aroma of Apples and Structure/Activity Correlations of Esters Contributing to Apple Aroma (in German). Ph.D. Thesis, Technical University Munich, Germany, 1998.
(16) Zimmermann, M. Important Flavor Compounds of Bell Pepper (*Capsicum annuum* var. Annuum). Ph.D. Thesis, Technical University Munich, Germany, 2001.
(17) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odourants during storage of white bread. *Flavour Fragr. J.* **1992**, *7*, 213–218.
(18) Schieberle, P. Primary odorants of pale lager beer. *Z. Lebensm.-Unters.-Forsch.* **1991**, *193*, 558–565.
(19) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366–2371.
(20) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
(21) Schieberle, P. Recent Developments in Methods for Analysis of Flavor Compounds and Their Precursors In *Characterization of Food: Emerging Methods*; Goankar, A. Ed.; Elsevier: Amsterdam, The Netherlands 1995; pp 403−431.
(22) Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach 35 LMBG, volume I/1, methods 00.90-7 and 00.90-9, Beuth Verlag, Berlin, Germany, 1996.
(23) Jellinek, G. *Sensory Evaluation of Food*; VCH Verlagsgesellschaft: Weinheim, Germany, 1985.
(24) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; German Research Center for Food Chemistry, Garching, Germany, 1998.
(25) Otsuka, K.; Zenibayashi, Y.; Itoh, M.; Totsuka, A. Presence and significance of two diastereomers of β-methyl-γ-octalactone in aged distilled liquors. *Agric. Biolog. Chem.* **1974**, *38*, 485–490.
(26) Schnabel, K.-O.; Belitz, H. D.; von Ranson, C. Untersuchungen zur Struktur-Aktivitätsbeziehung bei Geruchsstoffen. 1. Mitteilung: Wahrnehmungs-schwellenwerte und Geruchsqualitäten von gesättigten aliphatischen und alicyclischen Verbindungen mit Sauerstofffunktion. *Z. Lebensm.-Unters.-Forsch.* **1988**, *187*, 215–223.
(27) Takeoka, G. R.; Buttery, R. G.; Flath, R. A. Volatile constituents of asian pear (*Pyrus serotina*). *J. Agric. Food Chem.* **1992**, *40*, 1925–1929.
(28) Martin, G. E.; Dyer, T. H.; Buscemi, P. C. Quantitative gas-liquid chromatographic determination of ethyl esters and isoamyl acetate in whiskies and rums and confirmation by mass spectrometry. *J. Assoc. Offic. Anal. Chem.* **1974**, *57*, 610–613.
(29) Schreier, P.; Drawert, F.; Winkler, F. Composition of neutral volatile constituents in grape brandies. *J. Agric. Food Chem.* **1979**, *27*, 365–372.
(30) Connor, J.; Reid, K.; Richardson, G. SPME Analysis of Flavor Components in the Headspace of Scotch Whiskey and Their Subsequent Correlation with Sensory Perception In *Gas Chromatography-Olfactometry: State of the Art*; Leland, J. V., Schieberle, P. Eds.; ACS Symposium Series 782; American Chemical Society: Washington DC, 2001; pp 113−121.

Received for review February 7, 2008. Revised manuscript received April 23, 2008. Accepted April 27, 2008.

JF800383V