Synthesis and Analysis of Thio-, Thiono-, and Dithio-Derivatives of Whiskey Lactone

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Cis- and *trans*-3-methyl-4-octanolide (**1**, whiskey lactones) were converted into their thio- (**2**), thiono- (**3**), and dithio- (**4**) derivatives by reaction with phosphorus pentasulfide. The reaction products were characterized by GC−mass spectrometry, ^{1}H NMR spectroscopy, and GC−olfactometry. Two-dimensional NOESY spectra showed that sulfur is incorporated into the ring with reversal of the absolute configuration at C-4, whereas substitution of the keto-oxygen atom by sulfur occurs with retention of ring configuration. The *cis*- and *trans*-pairs of **2**, **3**, and **4** were separated into enantiomers by GC on heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin and heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-*β*-cyclodextrin as chiral stationary phases. GC−olfactometry revealed a sweet coconut-like odor for the *cis*-thio- and pleasant mushroom-like flavors for the *cis*-thiono- and *trans*-dithio-derivatives of whiskey lactone.

Keywords: *Whiskey lactone; thio-whiskey lactone; thiono-whiskey lactone; dithio-whiskey lactone; enantioselective GC; GC−MS; ^{1}H NMR*

INTRODUCTION

Lactones are important contributors to the flavor of many fruits, such as apricots, peaches, and strawberries, as well as of dairy products (*1*). "Whiskey lactone" (3-methyl-4-octanolide, 5-butyldihydro-4-methylfuran-2(3*H*)-one), also known as "oak" or "*Quercus*" lactone, can be found in oak wood and spirits or wines stored in casks made from oak wood (*2*−*5*). Isolation, structure elucidation, and sensory characterization of the four stereoisomers of whiskey lactone have been described (*6*−*10*). Sulfur-containing volatiles are among the most important and potent flavor compounds known and play outstanding roles in the aroma patterns of several fruits, such as passion fruit (*11*−*13*), blackcurrant (*14*), and grapefruit (*15*, *16*). Recently, synthesis and analysis by enantioselective gas chromatography of various sulfur-containing aliphatic lactones, such as *γ*- and *δ*-thiolactones (*17*−*19*) and *γ*- and *δ*-thiono- (*20*, *21*) or *γ*-dithiolactones (*22*) have been described. Some of these compounds exhibit strong and interesting flavor properties. Thionolactones can be synthesized by the reaction of lactones with 2,4-bis(4-methoxy-phenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent) (*23*). Reaction of lactones with phosphorus pentasulfide is known to afford all three types of sulfur-containing lactones, i.e. thio-, thiono-, and dithio-lactones (*23*, *24*). This study reports on the synthesis, structure elucidation by GC−MS and ^{1}H NMR, enantioseparation on different chiral stationary phases, and olfactometric characterization of thio-, thiono-, and dithio-derivatives of whiskey lactone.

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MATERIALS AND METHODS

Reagents. Whiskey lactone (**1**) was purchased from Aldrich, Steinheim, Germany. Separation of whiskey lactone into *cis*-**1** and *trans*-**1** was achieved according to published procedures (*7*, *9*) by preparative chromatography on a column (55 × 2.5 cm) packed with silica gel (silica gel 60, 0.063−0.200 mm, Merck, Darmstadt, Germany) using petrol ether/diethyl ether mixtures of increasing polarity (285:15 (v/v), 450:55 (v/v), 180:45 (v/v), 360:90 (v/v)). Fractions of about 10 mL were collected and monitored by gas chromatographic analysis (GC system ii, described below). 2,4-Bis(4-methoxy-phenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent) and phosphorus pentasulfide were purchased from Fluka, Neu-Ulm, Germany.

Reaction of Whiskey Lactone with Phosphorus Pentasulfide. The reaction of *cis*-**1** and *trans*-**1** with phosphorus pentasulfide followed the method described by Fries and Mengel (*24*) and is illustrated in Figure 1. Mixtures of 1 g of *trans*-**1** or 0.85 g of *cis*-**1** and 250 mg of freshly ground phosphorus pentasulfide were heated in small screw-cap vials (11 mL) on thermostated metal plates at 140−150 °C for 10 min. After the mixture cooled and another 250 mg of phosphorus pentasulfide was added, heating was continued at 180−200 °C for another 10−20 min. It is known that the selectivity of the reaction with phosphorus pentasulfide strongly depends on temperature and time (*25*). Therefore, excessive heating was avoided. The dark red (almost black) mixtures were cooled and extracted three times with 30 mL of diethyl ether. The extract was filtered and concentrated under reduced pressure. Analysis by gas chromatography (GC systems i and ii, described below) and by GC−MS revealed three major products in each of the reaction mixtures. The concentrated extract was applied to a column of silica gel (55 × 2.5 cm; silica gel 60) and eluted with mixtures of petrol ether/diethyl ether of increasing polarity (285:20 (v/v), 450:50 (v/v), 250:50 (v/v), 150:100 (v/v)). Fractions of about 10 mL were collected and monitored by gas chromatographic analysis. By increasing the polarity of the eluent, three products were isolated from each reaction mixture (Ia−IIIa, starting from *trans*-**1** and Ib−IIIb, starting from *cis*-**1**). Fractions containing the individual compounds were pooled and concentrated under reduced

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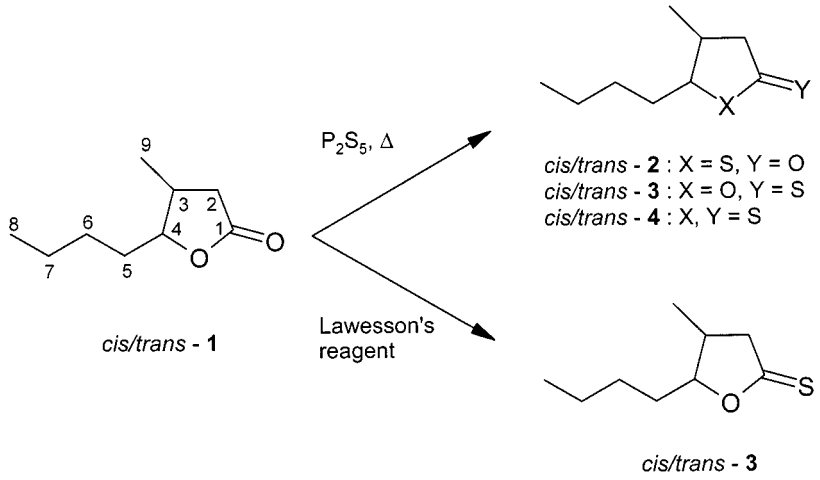

Figure 1. Synthesis of sulfur-containing derivatives of whiskey lactone.

Table 1. ^{1}H NMR Data of cis-Configured Compounds 1−4

	cis-**1**			*cis*-**2**			*cis*-**3**			*cis*-**4**		
position	δ^a	J^b	rel. NOE^c (%)	δ	J	rel. NOE (%)	δ	J	rel. NOE (%)	δ	J	rel. NOE (%)
2	2.65 (dd)	17.0 7.9	15	2.70 (m)	nd^d		3.17 (dd)	18.2 7.5	14	3.15 (dd)	17.6 6.3	11
2′	2.13 (dd)	17.0 4.0	6	2.39 (dd)	nd		2.88 (dd)	18.2 3.2	3	2.95 (dd)	17.5 5.4	2
3	2.54 (m)	nd^c	28	2.69 (m)	nd		2.64 (m)		27	2.87 (m)		21
4	4.38 (ddd)	nd		3.89 (ddd)	10.2 5.2 5.2		4.80 (ddd)	10.3 8.9 5.2		4.14 (ddd)	10.4 5.4 5.4	
5	1.65−1.25 (m)	nd		nd	nd		1.81 (m)	nd	13	1.81 (m)	nd	14
5′	nd	nd		nd	nd		1.67−1.52 (m)	nd	31	1.70 (m)	nd	10
6	1.65−1.25 (m)	nd		nd	nd		1.67−1.52 (m)	nd	31	1.45−1.32 (m)	nd	26
6′	nd	nd		nd	nd		1.47−1.36 (m)	nd	24	nd	nd	
7	1.65−1.25 (m)	nd		nd	nd		1.47−1.36 (m)	nd	24	1.45−1.32 (m)	nd	26
8	0.87 (t)	7.1		nd	nd		0.95 (t)	7.1		0.95 (t)	6.9	2
9	0.96 (d)	7.1	7	1.11 (d)	6.9	weak	1.01 (d)	7.1	7	1.10 (d)	6.9	6

^{a} δ in ppm (calibrated on residual proton in $CDCl_3$). ^{b} J in Hz. ^{c} Relative cross-peak intensities were determined as described in Materials and Methods. ^{d}nd, Not determined.

pressure. Final purities determined by GC were at least 93%. In the case of the reaction of *cis*-**1** with phosphorus pentasulfide, compounds IIb and IIIb could not be separated sufficiently; additional purification was achieved by semipreparative high-performance liquid chromatography (HPLC) using a Varian 9001 pump with a model 9050 Varian UV-detector operated at 220 nm (Varian, Darmstadt, Germany). The mobile phase was petrol ether/diethyl ether (250:5; v/v) at a flow rate of 4 mL/min. The separation column was a 25 cm × 8 mm i.d. column packed with Nucleosil 50-5 (C. S. Chromatographie Service, Langerwehe, Germany). For characterization of their chemical structures, the isolated compounds were subjected to ^{1}H NMR analysis.

Reaction of Whiskey Lactone with Lawesson's Reagent. In a micro-preparation, following the procedure described by Scheibye (*23*), 25 μL of *cis*-**1** and *trans*-**1**, respectively, and 30 mg of 2,4-bis(4-methoxy-phenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson's reagent) were dissolved in 1 mL of dry toluene in a GC sample vial. This mixture was reacted for 2 h under reflux using a condenser (a piece of 15 cm × 0.53 mm i.d. fused silica which was inserted through the vial septum) and a metal heating plate. After the mixture cooled, an aliquot was diluted and analyzed by gas chromatography.

Nuclear Magnetic Resonance Spectroscopy. ^{1}H NMR spectra were recorded at 500.13 MHz with a Bruker DRX 500 spectrometer (Bruker, Karlsruhe, Germany). The solvent was deuterated chloroform. The chemical shifts were referenced to the solvent signal. Acquisition and processing parameters for one-dimensional experiments and two-dimensional NOESY experiments were according to standard Bruker software (XWIN-NMR). The mixing time was 1 s in NOESY experiments. The intensities of cross-signals in the phase- and baseline-corrected two-dimensional spectra were determined and referenced to the cross-peak between H-2 and H-2′ with a relative intensity of 100 arbitrary units. Relative intensities of cross-peaks between the H-4 signal and the respective indexed signals are given in Tables 1 and 2.

Gas Chromatography−Mass Spectrometry. Mass spectral data were acquired on a GC 8000 Top (C. E. Instruments) gas chromatograph coupled to a Voyager mass selective detector (Thermoquest−Finnigan, Egelsbach, Germany). The mass spectrometer interface temperature was set to 240 °C and the electron ionization mode (EI) was used at 70 eV. The separation column was a 30 m × 0.25 mm i.d. DB-WAXetr (J&W, Folsom, CA) fused silica capillary with a film thickness of 0.5 μm. The oven temperature was programmed from 40 °C (5 min hold) to 240 °C (25 min isothermal) at 4 °C/min. Helium was used as carrier gas at a constant inlet pressure of 75 kPa. Data acquisition was with the Mass-Lab system (Thermoquest−Finnigan).

Gas Chromatography. Capillary GC was performed on four different GC systems as follows: (i) A Carlo Erba Mega II 8575 (Thermoquest, C. E. Instruments, Rodano, Italy) was equipped with a flame ionization detector (FID) and a flame photometric detector (FPD 80, C. E. Instruments). Parallel detection was achieved by dividing the effluent of the column (DB-Wax, J&W; 60 m × 0.32 mm i.d., 0.25 μm film thickness) via a press-fit splitter (BGB-Analytik, Anwil, Switzerland) and short pieces of deactivated fused silica capillaries (BGB-

Table 2. ^{1}H NMR Data of trans-Configured Compounds 1−4

	trans-**1**			*trans*-**2**			*trans*-**3**			*trans*-**4**		
position	δ^a	J^b	rel. NOE^c (%)	δ	J	rel. NOE (%)	δ	J	rel. NOE (%)	δ	J	rel. NOE (%)
2	2.68 (m)	nd[d]		2.73 (dd)	16.4	3	3.30 (dd)	18.5	3	3.29 (dd)	17.8	5
					6.3			7.9			6.3	
2′	2.21 (m)	nd		2.36 (dd)	16.4	10	2.695 (dd)	18.5	9	2.78 (dd)	17.8	23
					10.1			9.5			9.9	
3	2.21 (m)	nd		2.28 (m)	nd	6	2.30 (m)	nd	7	2.49 (m)		12
4	4.02 (ddd)	11.8		3.495 (ddd)	10.2		4.40 (ddd)	12.7		3.74 (ddd)	10.1	
		7.7			8.0			7.8			8.3	
		3.9			3.7			4.8			3.9	
5	1.74−1.31 (m)	nd		1.99 (m)	nd	20	1.80−1.34 (m)	nd		1.97 (m)	nd	30
5′	nd	nd		1.62 (m)	nd	13	1.80−1.34 (m)	nd		1.68 (m)	nd	18
6	1.74−1.31 (m)	nd		1.46−1.32 (m)	nd	24	1.80−1.34 (m)	nd		1.45−1.32 (m)	nd	41
6′	nd	nd		nd	nd		1.80−1.34 (m)	nd		nd	nd	
7	1.74−1.31 (m)	nd		1.46−1.32 (m)	nd	24	1.80−1.34 (m)	nd		1.45−1.32 (m)	nd	41
8	0.93 (t)	7.2		0.94 (t)	7.1	2	0.95 (t)	7.2		0.95 (t)	7.1	3
9	1.15 (d)	6.5	strong	1.21 (d)	6.5	22	1.15 (d)	6.7	22	1.21 (d)	6.7	37

[a] δ in ppm (calibrated on residual proton in $CDCl_3$). [b] J in Hz. [c] Relative cross-peak intensities were determined as described in Materials and Methods. [d] nd, Not determined.

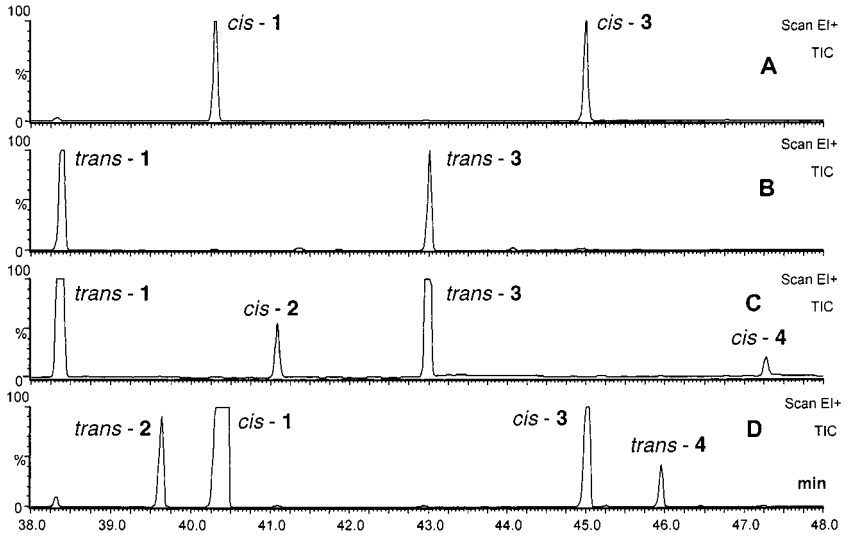

Figure 2. GC−MS chromatograms of the reaction mixtures of **1** with Lawesson's reagent (A, *cis*-**1**; B, *trans*-**1**) and phosphorus pentasulfide (C, *trans*-**1**; D, *cis*-**1**). Conditions as described in Materials and Methods.

Analytik) to the two detectors. Split injection was performed at 220 °C, and column temperature was programmed from 40 °C (5 min hold) to 230 °C (15 min hold) at 4 °C/min. Hydrogen was used as carrier gas at a constant inlet pressure of 105 kPa. (ii) A Carlo Erba Fractovap 4160 with FID and split injector; the column used was a 30 m × 0.25 mm i.d. fused silica capillary column coated in-house (*26*) by the static procedure with 25% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (synthesized as described in the literature (*27*)) and 75% SE 54 (Riedel de Haen, Seelze, Germany) to form a film thickness of 0.25 μm; column: DiMe-β-CD. Split injection was performed at 220 °C, and the column temperature was programmed from 120 °C (2 min hold) to 205 °C (5 min hold) at 2 °C/min. Hydrogen was used as carrier gas at a constant inlet pressure of 100 kPa. (iii) A Carlo Erba Vega 6000 with FID and split injector; the column used was a 30 m × 0.25 mm i.d. fused silica capillary column coated in-house by the static procedure with 50% heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (synthesized as described in the literature (*28*) and 50% OV-1701-vi (Supelco, Germany) to form a film thickness of 0.25 μm; column: DiAc-β-CD. Split injection was performed at 220 °C, and the column temperature was programmed from 120 °C (2 min hold) to 205 °C (5 min hold) at 2 °C/min. Hydrogen was used as the carrier gas at a constant inlet pressure of 100 kPa. Conditions for the determination of alpha and resolution values are given in the corresponding table. For the description of odor qualities, a system suitable for GC−olfactometry (GC/O) was used: (iv) A Carlo Erba Fractovap 4200 equipped with FID and split injector. The effluent of a 55 m × 0.32 mm i.d., 0.25 μm DB-Wax fused silica capillary was split via a press-fit splitter and short pieces of deactivated fused silica capillaries to the FID and to a heated sniffing-port. The column temperature was programmed from 60 °C (5 min hold) to 230 °C (10 min hold) at 4 °C/min. Hydrogen was used as the carrier gas at a constant inlet pressure of 100 kPa. The evaluations of retention indices were performed on a DB-Wax (GC system i) and an apolar column (30 m × 0.32 mm i.d. fused silica capillary column) coated in-house by the static procedure with 0.25 μm of a dimethyl polysiloxane (PS-255, Fluka) which was installed in the GC used in system iii. Data were processed via Chromcard Software (C. E. Instruments).

RESULTS AND DISCUSSION

Commercially available whiskey lactone (**1**) was separated into *cis*-**1** (3*R*,4*R* and 3*S*,4*S*) and *trans*-**1** (3*S*,4*R* and 3*R*,4*S*) by liquid chromatography. Elution order on silica gel and gas chromatographic behavior on a methyl silicon and a carbowax stationary phase (*trans*-**1** before *cis*-**1**), as well as ^{1}H NMR data (Tables 1 and 2), were in accordance with previous reports (*9, 29, 30*).

Characterization of Thio-, Thiono-, and Dithio-Derivatives of Whiskey Lactone. The reaction mix-

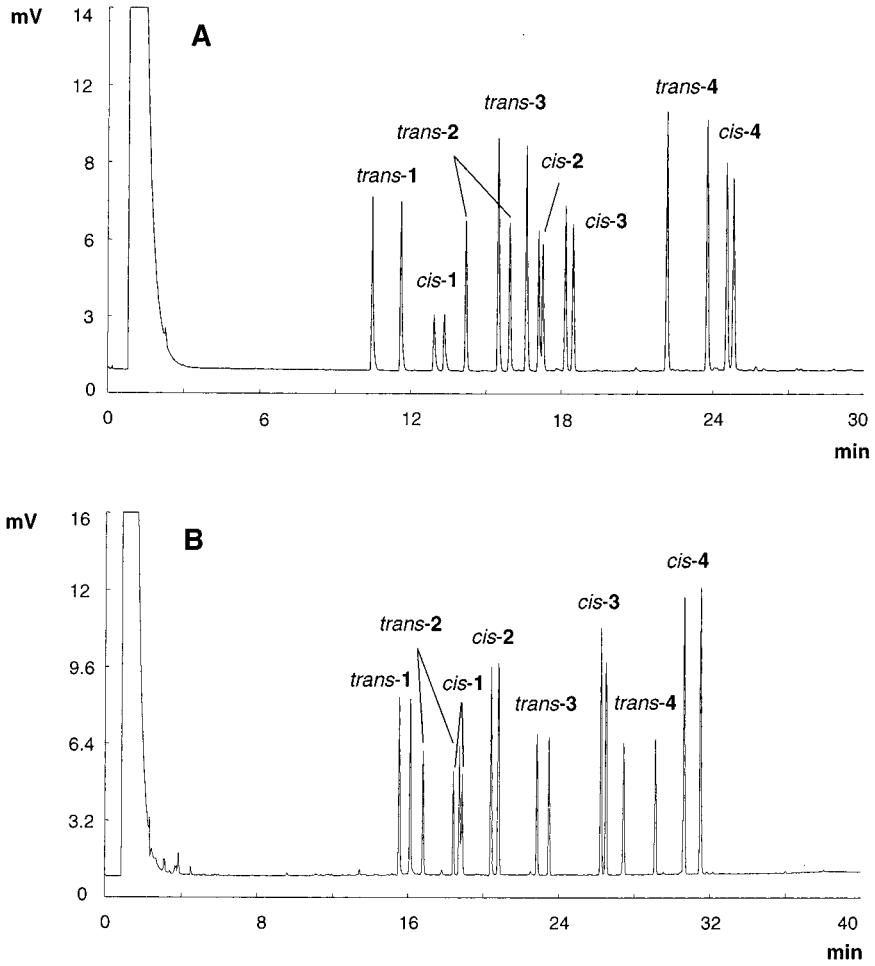

Figure 3. Chiral separation of compounds **1**−**4** (A, DiMe-*β*-CD; B, DiAc-*β*-CD). Hydrogen as carrier gas at 100 kPa; temperature programmed from 100 °C (2 min hold) at 2°/min to 200 °C.

Table 3. Mass Fragmentation of the Sulfur-Containing Derivatives of Whiskey Lactone

compound	*m/z* (% relative intensity)
cis-**2**	172 (6, M^+); 55 (100), 128 (96), 69 (57), 101 (55), 41 (50), 56 (29), 39 (23), 60 (18), 45 (18), 27 (16)
cis-**3**	172 (69, M^+); 56 (100), 55 (96), 69 (69), 41 (60), 81 (35), 97 (34), 96 (28), 57 (24), 39 (21), 29 (21)
cis-**4**	188 (100, M^+); 55 (98), 41 (66), 56 (56), 97 (55), 173 (52), 85 (48), 117 (46), 69 (35), 45 (30), 96 (28)
trans-**2**	172 (3, M^+); 55 (100), 128 (86), 101 (54), 41 (41), 69 (40), 39 (21), 115 (20), 45 (16), 56 (15), 29 (14), 27 (14)
trans-**3**	172 (45, M^+); 55 (100), 58 (89), 69 (63), 56 (61), 41 (59), 81 (35), 97 (31), 96 (27), 29 (24), 39 (23)
trans-**4**	188 (98, M^+); 55 (100), 97 (58), 41 (57), 85 (46), 173 (44), 56 (42), 96 (33), 131 (31), 117 (31), 69 (30)

tures were analyzed by GC and GC−MS (Figure 2). The selective synthesis of thiono-compounds with Lawesson's reagent (*23*) allowed the unambiguous determination of the thiono-derivatives of whiskey lactone (*cis*-**3** and *trans*-**3**) in the chromatograms. Assignment of thio- and dithio-derivatives of whiskey lactone was then based on GC−MS analysis. Mass spectral data of *cis*- and *trans*-**2**−**4** are given in Table 3. The elution orders of whiskey lactone and its sulfur-containing derivatives on a carbowax or a methyl silicon stationary phase were determined as follows (Kováts indices given on DB-Wax/PS-255): *trans*-**1** (1861/1258); *trans*-**2** (1926/1362); *trans*-**3** (2034/1391); and *trans*-**4** (2168/1543); and *cis*-**1** (1951/1286); *cis*-**2** (1961/1387); *cis*-**3** (2127/1423); and *cis*-**4** (2200/1567). The mixtures of thio-, dithio-, and thiono-derivatives of whiskey lactone (*cis*- and *trans*-**2**−**4**), prepared by the reaction with phosphorus pentasulfide, were separated by liquid chromatography. The fractions obtained were analyzed by GC and characterized by ^{1}H NMR. Elution orders after liquid chromatography on silica gel could be assigned as follows: (a) elution of the reaction products of *trans*-**1** with phosphorus pentasulfide with increasing polarity of the eluent yielded *cis*-**4** (Ia) before *trans*-**3** (IIa) and *cis*-**2** (IIIa); (b) elution of the reaction products of *cis*-**1** yielded *trans*-**4** (Ib) before *trans*-**2** (IIb) and *cis*-**3** (IIIb). The assignment of the cis/trans-diastereomers was achieved by ^{1}H NMR analysis of the purified compounds and evaluation of cross-peak intensities in two-dimensional NOESY spectra. Typically, strong NOEs were detected between the signals accounting for H-4 and H-3 in the cis-configured compounds, whereas strong NOEs were observed between H-4 and the methyl signals in the trans-configured compounds (Tables 2 and 3). The relative intensities of additional cross-peaks are summarized in Tables 2 and 3, confirming the assignments. In contrast to a previous preliminary assignment of the cis/trans-configurations (*31*), the absolute configuration at C-4 of the ring system is reversed due to the exchange of oxygen by sulfur in the course of the reaction of whiskey lactone with phosphorus pentasulfide. The thionation reactions of *cis*-**1** and *trans*-**1** with Lawesson's reagent, as well as with phosphorus pentasulfide, yield *cis*-**3** and *trans*-**3**, respectively. On the other hand, reaction of *cis*-**1** and *trans*-**1** with phosphorus pentasulfide affords *trans*-**2**

Table 4. Chromatographic Performance of Whiskey Lactone and its Sulfur-Containing Derivatives on DiMe-β-CD and DiAc-β-CD (temperature 140 °C, isothermal; hydrogen at 56 cm/sec)

	DiMe-β-CD			DiAc-β-CD		
compound	α	K_1	cR_s	α	K_1	cR_s
cis-**1**	1.056	7.7	2.1	1.057	15.9	4.4
cis-**2**	1.021	13.3	1.3	1.047	20.4	3.6
cis-**3**	1.033	15.1	1.6	1.030	40.7	2.1
cis-**4**	1.032	30.8	1.8	1.095	20.5[a]	7.0
trans-**1**	1.145	5.7	4.2	1.070	11.1	4.9
trans-**2**	1.210	9.5	7.8	1.233	13.0	15.2
trans-**3**	1.132	11.1	5.7	1.086	26.3	6.0
trans-**4**	1.203	22.8	9.1	1.166	14.5[a]	11.8

[a] Isothermal at 160 °C.

Table 5. GC/O of Sulfur-Containing Derivatives of Whiskey Lactone

compound	odor description[a]
cis-**2**	sweet, coconut-like (faint), candied almonds
cis-**3**	mushroom (*Boletus edulis*)
cis-**4**	mushroom, moldy, forest soil
trans-**2**	sweet, coconut-like
trans-**3**	moldy, carrot-like, tartish
trans-**4**	mushroom, nutty

[a] About 50−100 ng each.

and *trans*-**4** and *cis*-**2** and *cis*-**4**, respectively, as reaction products (Figure 2).

Enantioselective GC. Separation of the enantiomers of all investigated compounds could be achieved on columns with heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DiMe-β-CD; Figure 3A) or heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DiAc-β-CD; Figure 3B) as chiral stationary phases. Either column is suitable for the enantioseparation of these compounds. However, under the conditions used, a partial coelution occurs for *cis*-**1** and *trans*-**2** on DiAc-β-CD. In general, the trans-configured compounds show higher resolutions than the cis-configured compounds on both stationary phases. The separation factors (α) and values for the chiral resolution (cR_s) (*32*) on these two stationary phases are summarized in Table 4. The high resolution achieved for some compounds, in particular the *trans*-**2** and *trans*-**4** derivatives, is remarkable. Determination of the elution order of the enantiomers is part of ongoing research; the results will be published in a separate paper.

GC−Olfactometry (GC/O). Evaluation of the sensory properties of the sulfur-containing whiskey lactone derivatives was performed by sniffing of the substances after chromatographic separation on DB-Wax. Odor characteristics perceived are described in Table 5. In analogy to aliphatic lactones (*17*, *18*), both thio-derivatives (*cis*- and *trans*-**2**) showed a faint, but still perceptible, sweet, coconut-like odor, reminiscent of the basic character of the oxygen-containing lactone. The thiono- and dithio-derivatives exhibited mushroom-like notes as predominating aroma impressions. The mushroom-like odor of the thiono-derivative *cis*-**3** was intense and pleasant, reminiscent of the well-known edible mushroom *Boletus edulis*. A mushroom-like note, resembling moldy forest soil, was determined for *cis*-**4**. Interestingly, mushroom-like notes for aliphatic sulfur-containing lactones, e.g. the thio-derivative of γ-octalactone (*17*, *18*), the thiono-derivatives of γ- and δ-octalactone (*21*), and the dithio-derivative of γ-octalactone (*22*), have also been described by others. Obviously, ring size and the length of the side chain are important factors for this aroma impression. In the case of sulfur-containing whiskey lactone derivatives, the stereochemistry of the methyl-group at C-3 influences the aroma quality, as only the cis-configured thiono-derivative showed the typical *B. edulis* odor. Determination of odor thresholds and sensory evaluations of the different enantiomers are subjects of current investigations.

The results of this study emphasize the importance of sulfur to the sensory properties of volatiles. This type of data broadens our knowledge of structure−sensory activity relationships and should initiate research activities to look for the occurrence of some of these attractive compounds in nature.

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