JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enantioselective Analysis of Methyl-Branched Alcohols and Acids in Rhubarb (*Rheum rhabarbarum* L.) Stalks

MÁRTA DREGUS, HANS-GEORG SCHMARR,† EISUKE TAKAHISA, AND KARL-HEINZ ENGEL*

Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Am Forum 2, D-85350 Freising-Weihenstephan, Germany

The enantiomeric compositions of 2-methylbutanol (**1**), 4-methylhexanol (**2**), 2-methylbutanoic acid (**3**), and 4-methylhexanoic acid (**4**) present in rhubarb (*Rheum rhabarbarum* L.) stalks were determined. Enantiodifferentiation was achieved via multidimensional gas chromatography using heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin as a chiral stationary phase. For all compounds the enantiomeric ratios were in favor of the (*R*)-enantiomers. The alcohols (**1** and **2**) exhibited generally high excesses of the (*R*)-enantiomers, the ratios varying slightly from batch to batch. For the acid (**3**) a rather narrow range averaging 65% (*R*):35% (*S*) was observed. The procedure applied to isolate the volatiles (vacuum headspace technique, simultaneous distillation−extraction, liquid−liquid extraction) had no significant impact on the enantiomeric ratios. The study describes for the first time a plant used as food material in which 2-methyl-branched volatiles are not nearly exclusively present as (*S*)-enantiomers. This information enlarges the current regulatory knowledge regarding the classification of these important flavor compounds as "natural" on the basis of their enantiomeric ratios.

KEYWORDS: Rhubarb; methyl-branched compounds; chirospecific analysis; MDGC

INTRODUCTION

Rhubarb, a member of the family Polygonaceae, is a plant whose parts are used for various purposes. The dried rhizomes of some species, for example, *Rheum officinale* B., *Rheum palmatum* L., *Rheum rhaponticum*, and *Rheum sinense*, are raw materials for the pharmaceutical industry (*1*−*3*). Owing to their taste, extracts obtained from rhizomes of *R. officinale* B., *R. palmatum* L., and *R. rhaponticum* L. are used as flavoring agents for certain bitters (*3*). Another popular use is the consumption of the stalks of *Rheum rhabarbarum* (syn. *undulatum*) L. as a vegetable and their processing into products such as jam, compote, or juice (*1*, *4*).

In a recent study the volatile constituents of rhubarb stalks have been investigated (*5*). In addition to the preponderant compounds with C_6 skeletons, the presence of methyl-branched components turned out to be a typical feature of the spectrum of volatiles isolated by means of vacuum headspace technique (VHS). Representatives in this group comprise 2-methyl-branched compounds (2-methylbutanol and 2-methylbutanoic acid) known to be derived from isoleucine and present in many fruits (*6*−*9*). In addition, the less common 4-methyl-branched homologues (4-methylhexanol and 4-methylhexanoic acid), reported in tobacco (*10*−*13*) and certain animal-derived products (*14*−*16*), are present.

2-Methyl-branched compounds have been considered to be classical examples of chiral volatiles with fixed configurations and high enantiomeric purities. 2-Methylbutanoic acid, the corresponding esters, and the alcohol 2-methylbutanol have been detected as flavor compounds in fresh and processed apples, favoring the (*S*)-configuration and enantiomeric ratios mostly >99% (*7*, *17*−*19*). High enantiomeric excesses of the (*S*)-enantiomer of 2-methylbutanoic acid were also found in other fruits (e.g., strawberry, papaya, black currant, and pineapple), in cheese, and in alcoholic beverages (*18*). 2-Methylbutanol derived from fermented products (e.g., apple, grape, and starch) was also detected as the (*S*)-enantiomer (*20*). Considering the biogenesis of these compounds from L-isoleucine (2*S*,3*S*), their high enantiomeric purities are to be expected. There are only a few exceptions to this general rule. 2-Methylbutanoic acid in Parmesan cheese, for example, exhibits an enantiomeric ratio of 75% (*S*):25% (*R*) (*21*). The only sources in which the (*R*)-acid has been shown to be predominant are medicinal plants, such as *Veratrum* species (e.g., *Veratrum album* and *Veratrum viride*) (*18*, *22*, *23*).

To categorize aroma compounds as "natural" on the basis of their enantiomeric compositions, comprehensive knowledge on genuine enantiomeric ratios is an important prerequisite. Therefore, the aim of this study was to determine the naturally occurring enantiomeric distributions of the methyl-branched acids and alcohols in rhubarb stalks.

* Author to whom correspondence should be addressed (telephone +49-8161-714250; fax +49-8161-714259; e-mail K.H.Engel@lrz.tu-muenchen.de).
† Present address: Staatliche Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Fachbereich Kellerwirtschaft, Breitenweg 71, D-67435 Neustadt/Weinstrasse, Germany.

10.1021/jf030466b CCC: $25.00
Published on Web 10/16/2003

MATERIALS AND METHODS

Materials. Rhubarb stalks (*R. rhabarbarum* L.) were purchased from supermarkets (June 2001) and a local farmers' market (June 2002). The material was stored (for a maximum of 5 days) at 5 °C before analysis.

Chemicals. (*S*)-2-Methylbutanol and (*S*)-2-methylbutanoic acid were purchased from Aldrich, Steinheim, Germany. (*S*)-4-Methyl-1-hexanol was obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Racemic 4-methylhexanol was prepared via hydroboration of 4-methyl-1-hexene (Aldrich) (*24*). Subsequent oxidation with permanganate was applied to yield racemic 4-methylhexanoic acid (*25*). (*S*)-4-Methylhexanoic acid was synthesized according to the same procedure via oxidation of (*S*)-4-methyl-1-hexanol.

Isolation of Volatiles. Volatile constituents were isolated from rhubarb stalks by means of different techniques: vacuum headspace (VHS), liquid−liquid extraction (LLE), and simultaneous distillation−extraction (SDE).

VHS. The material from 2001 was subjected to the isolation procedure as described previously (*5*). To have conditions comparable to those of LLE and SDE, isolations performed in 2002 started from 300 g of rhubarb stalks and included the addition of 300 mL of water to the crushed material.

LLE. Three hundred grams of the cut stalks was homogenized with 300 mL of distilled water in a laboratory blender (2 min). The supernatant obtained after centrifugation (8000*g*, 15 min) was transferred into a liquid−liquid extractor according to the method of Kutscher-Steudel (*26*). Volatiles were extracted for 24 h using 150 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v) as solvent.

SDE. The supernatant obtained according to the procedure described for LLE was transferred into a 2 L round-bottom flask connected to an SDE apparatus (*27*). Distillation−extraction was performed for 2 h, using 150 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v) as solvent.

Each of the extracts obtained was dried over anhydrous sodium sulfate, concentrated at 40−45 °C to a final volume of 0.3 mL using a Vigreux column. One microliter was subjected to analysis by multidimensional gas chromatography (MDGC).

Multidimensional Gas Chromatography. The instrumentation used for enantioselective gas chromatographic analysis of the chiral compounds consisted of two coupled GC 8000 series gas chromatographs (ThermoFinnigan, Carlo Erba Instruments, Egelsbach, Germany) with two independent temperature controls and two flame ionization detectors (FID). The columns were coupled by the moving column stream switching device (MCSS) (*28*, *29*). In the first GC, an achiral precolumn was installed and coupled via the MCSS device and a deactivated fused silica transfer capillary (1 m × 0.25 mm i. d.) to the chiral separation column. A phenylmethyl deactivated fused silica capillary (30 m × 0.25 mm i. d.) was coated in-house with 33% heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin in OV-1701-vi to form a film thickness of 0.25 μm. Synthesis of the cyclodextrin derivative and column preparation were performed as described earlier (*30*, *31*). Injection into the precolumn was done in the split mode at 215 °C (split ratio = 1:15). Carrier gas used was hydrogen at a constant inlet pressure of 165 kPa. The outlet pressure of the precolumn was 98 kPa (measured within the "dome" of the MCSS device via an additional manometer), which also corresponded to the actual inlet pressure for the chiral column in the second GC oven. The monitor detector (precolumn) was set at 260 °C, and the main detector (chiral column) was set at 160 °C. The temperature program for the precolumns [1, DB-5 (28 m × 0.25 mm i.d., 0.25 μm film thickness); 2, DB-Wax (30 m × 0.32 mm i.d. 0.25 μm film thickness, J&W Scientific)] started at 40 °C (5 min hold) and was programmed at 4 °C/min to 240 °C. The temperature program for the main column started at 37 °C (10 min hold) and was programmed at 2 °C/min to 160 °C (precolumn 1), 47 °C (10 min hold), and 2 °C/min to 160 °C (precolumn 2). Control of the MCSS device and data acquisition was done via Chromcard software (ThermoFinnigan).

The limits of detection and determination of the MDGC transfer step were estimated according to previously described procedures (*32*, *33*), using a series of eight dilutions of reference compounds **1**−**4** covering a concentration range from 0.5 to 100 μg/kg.

HRGC—Olfactometry (GC-O). GC-O analysis of 4-methylhexanol and 4-methylhexanoic acid was performed using the above-described column with heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin in OV-1701-vi as chiral stationary phase installed in a Carlo Erba (Fractovap 4200) gas chromatograph equipped with an FID, split injector, and sniffing port. Injection was done in the split mode at 215 °C (split ratio 1:15). Carrier gas used was hydrogen at a constant inlet pressure of 100 kPa. The effluent of the column was split 1:1 via a press-fit splitter (BGB Analytik, Anwil, Switzerland) and short pieces of deactivated fused silica capillaries to the detector and the heated sniffing port (200 °C). The amounts assessed at the sniffing port were ∼200 ng for each enantiomer.

RESULTS AND DISCUSSION

The enantiomeric compositions of 2-methylbutanol, 2-methylbutanoic acid, 4-methylhexanol, and 4-methylhexanoic acid isolated from rhubarb stalks were determined simultaneously via MDGC using heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin (perethyl-β-CD) as chiral main column (**Figure 1a**). The order of elution of the enantiomers was determined by co-injection of optically pure reference compounds. The usefulness of perethyl-β-CD for enantioseparation of 2-alkyl-branched volatiles had been demonstrated before (*34*). The order of elution for the enantiomers of 2-methylbutanol and 2-methylbutanoic acid was in agreement with previously published data (*34*, *35*). The reversal of the order of elution for the alcohol and corresponding acid was also observed for the 4-methyl-branched homologues. Capillary gas chromatographic enantioseparation of 4-methylhexanol is described for the first time. The enantiomers of 4-methylhexanoic acid had been separated on heptakis-(2,3-dimethyl-6-*tert*-butyldimethylsilyl)-β- and -γ-cyclodextrin, however, without assignment of the order of elution (*36*). The methyl ester of this acid had been enantiodifferentiated in the course of the structural elucidation of multibranched long-chain fatty acids from freshwater sponges using heptakis(2,6-dimethyl-3-pentyl)-β-cyclodextrin (*37*).

A typical chromatogram showing the enantiodifferentiation of the four methyl-branched volatiles in a VHS extract from rhubarb stalks using a DB-5 as achiral precolumn in the MDGC system is shown in **Figure 1b**. To confirm these results and to rule out that the cuts on the precolumn would result in the transfer of compounds eventually coeluting with the enantiomers separated on the main column, MDGC analysis was repeated for the same extract using a precolumn of opposite polarity (DB-Wax). A comparison of **Figures 1b** and **2a** shows that the chromatograms obtained on the chiral column differ in the additional presence of two major peaks 5 and 6. Assessment of retention indices and transfer of authentic reference compounds allowed these peaks to be assigned to two main C_6 compounds identified in rhubarb stalks (*5*): using DB-5 as achiral precolumn in the MDGC system, the cut of 2-methylbutanoic acid (KI 880) results in simultaneous transfer of (*E*)-2-hexenol (KI 875). On the other hand, the cut of 2-methylbutanol (KI 1206) from DB-Wax is accompanied by a transfer of (*E*)-2-hexenal (KI 1211). Apart from this difference in cotransferred compounds, the two approaches resulted in the same enantiomeric distributions of the methyl-branched volatiles. Co-injection of the (*S*)-enantiomers confirmed that 2-methylbutanol, 2-methylbutanoic acid, and 4-methylhexanol were detected with optical purities in favor of the (*R*)-enantiomers (**Figure 2b**).

Enantiomeric compositions of methyl-branched compounds determined in extracts obtained by VHS from three batches of rhubarb stalks are listed in **Table 1**. Data originate from duplicate isolation of volatiles for each batch (material from 2001) and triplicate analyses of enantiomers for each extract.

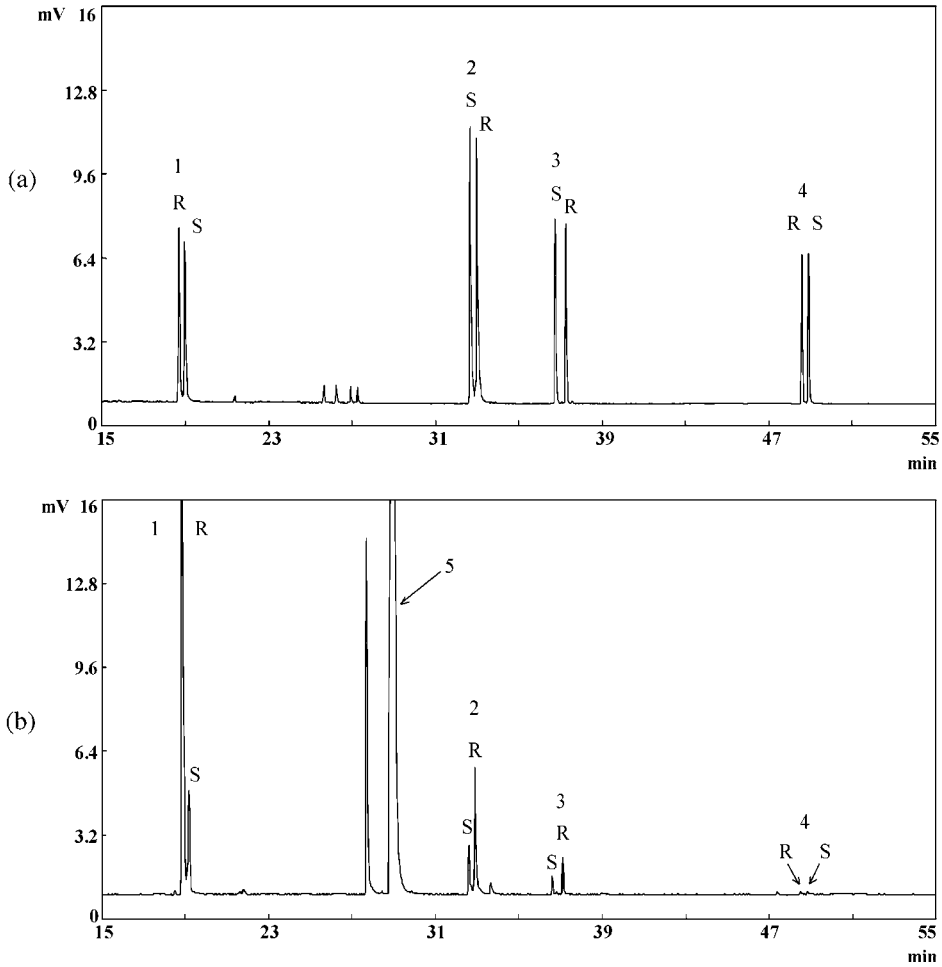

Figure 1. Stereodifferentation of 2-methylbutanol (**1**), 4-methylhexanol (**2**), 2-methylbutanoic acid (**3**), and 4-methylhexanoic acid (**4**) on heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin as chiral stationary phase after transfer from DB-5 (for chromatographic conditions, see Materials and Methods): (a) racemic reference compounds; (b) compounds from a VHS extract of rhubarb stalks. Peak 5 corresponds to (*E*)-2-hexenol, a compound transferred together with 2-methylbutanoic acid.

Statistical evaluation of the MDGC transfer technique (*32*, *33*) revealed limits of determination of 1.5 μg/kg for 2-methylbutanol and 4-methylhexanol, respectively, and 12 μg/kg for 2-methylbutanoic acid. Therefore, the concentrations determined via VHS for 2-methylbutanol (328−367 μg/kg), 2-methylbutanoic acid (43−250 μg/kg), and 4-methylhexanol (18−40 μg/kg) (*5*) were sufficient to fulfill the quantitative requirements for MDGC transfer and subsequent enantiodifferentiation on the chiral stationary phase. 4-Methylhexanoic acid, on the other hand, was present in much lower concentrations (1−2 μg/kg) (*5*). These amounts are below the level of detection (3 μg/kg) and the level of determination (9 μg/kg) observed for this compound (*32*, *33*). Accordingly, accurate quantification of enantiomers and calculation of enantiomeric ratios were not possible (*38*).

For all compounds the enantiomeric ratios determined were in favor of the (*R*)-enantiomer. To confirm this surprising result and to check the potential influence of the isolation procedure on the enantiomeric distributions, extracts obtained from material in 2002 by VHS technique, by SDE, and by LLE were analyzed. Data obtained from three batches are shown in **Table 2**. The isolation procedure applied had no significant impact on the enantiomeric ratios. In particular, the application of SDE, a technique involving thermal treatment of the material under acidic conditions (pH ∼3.5) for 2 h, did not result in enantiomeric ratios being significantly different from those obtained by the other approaches. This is in accordance with the fact that 2-methylbutanoic acid has been isolated without racemization by steam distillation from apple (*17*). Only slight racemization was observed in apple marmalade (*18*) or under harsh conditions of fractional distillation (*39*).

The data obtained in 2002 confirmed the dominance of the (*R*)-enantiomers observed in 2001. The alcohols 2-methylbutanol and 4-methylhexanol exhibited generally high excesses of the (*R*)-enantiomers. The ratios varied only slightly from batch to batch. For 2-methylbutanoic acid a rather narrow range averaging an enantiomeric ratio of 65% (*R*):35% (*S*) was observed. Again, in most cases the concentrations of 4-methylhexanoic acid were too low to allow accurate calculations of enantiomeric ratios.

Sensory Aspects. Alkyl-branched volatiles are examples of chiral compound classes for which differences in the sensory properties of their enantiomers have been reported (*40*). The odor of (*S*)-2-methylbutanoic acid has been described as pleasant, sweet, and fruity, whereas the (*R*)-enantiomer has a penetrating note, reminiscent of cheese and sweat. (*S*)-2-Methylbutanol has been characterized as ethereal and fresh, whereas the optical antipode possesses a fermented, fatty, and musty odor (*40*−*42*). For 4-methylhexanoic acid less pronounced differences in sensory properties between the enantiomers have been reported (*43*). GC-O confirmed both enantiomers to possess acidic, fatty notes, with the (*R*)-enantiomer being more potent. Sensory properties of the enantiomers of 4-methylhexanol had not yet been described. Evaluation via

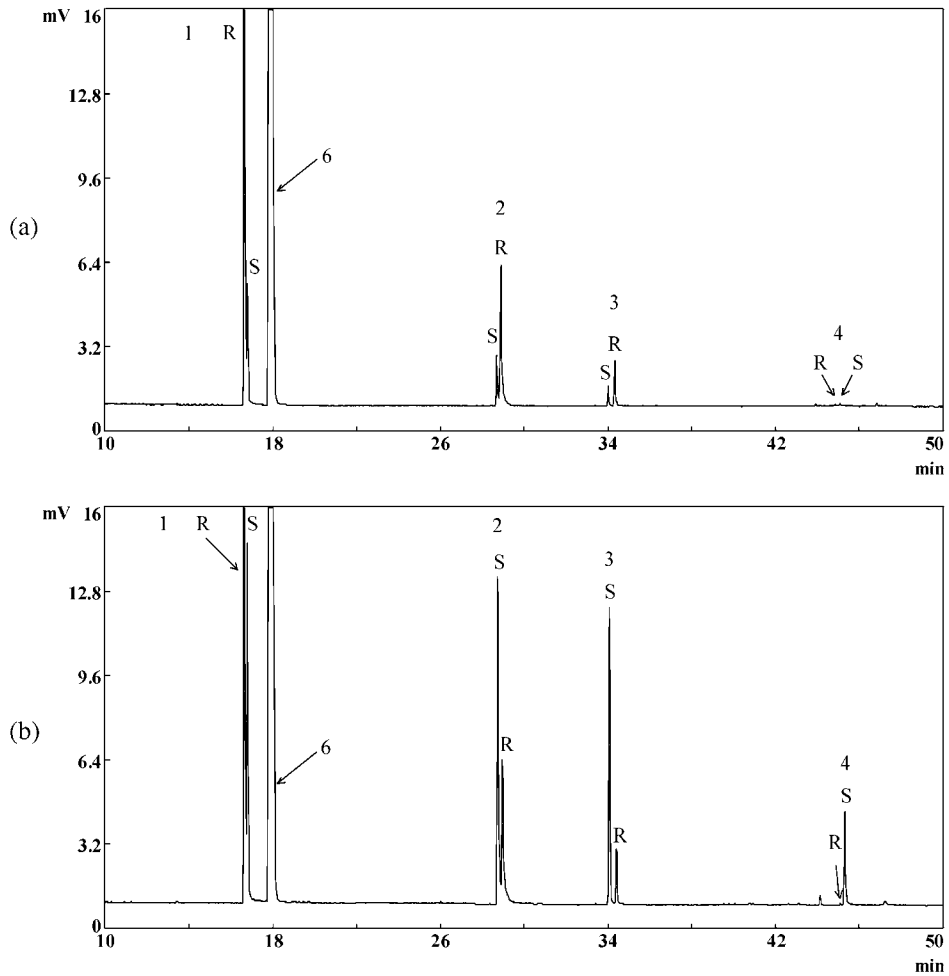

Figure 2. Separation of the enantiomers of 2-methylbutanol (**1**), 4-methylhexanol (**2**), 2-methylbutanoic acid (**3**), and 4-methylhexanoic acid (**4**) on heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin as chiral stationary phase after transfer from DB-Wax (for chromatographic conditions, see Materials and Methods): (a) compounds from a VHS extract of rhubarb stalks; (b) after spiking the VHS extract with (*S*)-configured reference compounds. Peak 6 corresponds to (*E*)-2-hexenal, a compound transferred together with 2-methylbutanol.

Table 1. Enantiomeric Distributions of 2-Methylbutanol (**1**), 4-Methylhexanol (**2**), 2-Methylbutanoic Acid (**3**), and 4-Methylhexanoic Acid (**4**) Isolated by VHS from Extracts of Rhubarb Stalks[a]

	enantiomeric distribution[b] (%)							
	1		**2**		**3**		**4**	
sample	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
batch 1								
VHS1	79	21	92	8	64	36	nd	
VHS2	79	21	93	7	61	39	nd	
batch 2								
VHS1	81	19	92	8	61	39	nd	
VHS2	76	24	93	7	58	42	nd	
batch 3								
VHS1	79	21	88	12	69	31	nd	
VHS2	81	19	84	16	70	30	nd	
mean	*79*	*21*	*90*	*10*	*64*	*36*		

[a] Material from 2001. [b] Means from triplicate experiments (standard deviations $\leq \pm 1$ for all compounds); nd, not detected.

GC-O on a chiral stationary phase revealed a spicy, nutty, slightly green, and sour note for the (*S*)-configured compound and a green, fatty, and nut- and carrot-like odor for the (*R*)-enantiomer. The odor qualities perceived for 2-methylbutanoic acid, 2-methylbutanol, and 4-methylhexanol in the course of the GC-O analysis of VHS extracts obtained from rhubarb stalks (*5*) are in agreement with the analytically determined dominance of the (*R*)-enantiomers.

Biogenetic Aspects. Labeling experiments in various fruits have demonstrated the role of amino acids as precursors of volatile acids, alcohols, and esters (*44*, *45*). Starting from L-isoleucine (2*S*,3*S*), the almost exclusive presence of (*S*)-configured 2-methylbutanoic acid, 2-methylbutanol, and 2-methylbutanoates is plausible. The enantiomeric ratios revealed in this study are in contradiction to this pathway. Either another precursor or a different biogenetic route could be the explanation.

In mammals the role of alloisoleucine as precursor of (*R*)-configured metabolites has been demonstrated. Mechanisms underlying the interconversion of isoleucine and alloisoleucine and the so-called (*R*)-pathway have recently been reviewed (*46*). With regard to plant physiology, administration of ethyl tiglate to apple fruits revealed the enzyme-catalyzed hydrogenation of (*E*)-2-methyl-2-butenyl esters as a source for (*R*)-2-methylbutyl derivatives (*47*).

4-Methylhexanol and 4-methylhexanoic acid are also derived from isoleucine catabolism. Biosynthetic studies in petal tissue of *Nicotiana sylvestris* indicated that 2-methylbutyryl CoA is elongated by the addition of one acetate molecule via fatty acid synthase, followed by reduction to yield 4-methylhexanol (*48*). This biogenetic relationship between 2-methylbutanol and 4-methylhexanol is supported by the rather similar enantiomeric

Table 2. Enantiomeric Distributions of 2-Methylbutanol (**1**), 4-Methylhexanol (**2**), 2-Methylbutanoic Acid (**3**), and 4-Methylhexanoic Acid (**4**) from Extracts of Rhubarb Stalks[a]

	enantiomeric distribution[b] (%)							
isolation	**1**		**2**		**3**		**4**	
procedure	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
batch 4								
VHS1	92	8	85	15	62	38	*	
VHS2	92	8	94	6	66	34	R > S	
SDE1	90	10	82	18	74	26	R > S	
SDE2	90	10	>95		64	36	R > S	
LLE1	92	8	>95		66	34	nd	
LLE2	93	7	>95		63	37	nd	
mean	*92*	*8*			*66*	*34*		
batch 5								
VHS1	79	21	71	29	66	34	*	
VHS2	81	19	75	25	56	44	*	
SDE1	76	24	73	27	68	32	R > S	
SDE2	81	19	78	22	60	40	nd	
LLE1	83	17	81	19	66	34	R > S	
LLE2	82	18	76	24	69	31	nd	
mean	*80*	*20*	*76*	*24*	*64*	*36*		
batch 6								
VHS1	85	15	85	15	67	33	R > S	
VHS2	83	17	82	18	60	40	nd	
SDE1	87	13	87	13	76	24	R > S	
SDE2	88	12	87	13	65	35	nd	
LLE1	87	13	88	12	69	31	nd	
LLE2	82	18	85	15	59	41	*	
mean	*85*	*15*	*86*	*14*	*66*	*34*		

[a] Material from 2002. [b] Means from triplicate experiments (standard deviations $\leq \pm 1$ for all compounds); nd, not detected; *, detected but no ratio calculated because the concentration of at least one of the enantiomers was below the limit of determination; R > S, determined visually but no ratio calculated because the concentration of at least one of the enantiomers was below the limit of determination.

ratios observed, particularly in the rhubarb material analyzed in 2002 (**Table 2**).

The significant proportions of the (*R*)-enantiomers detected in rhubarb stalks indicate that this phenomenon may not be solely due to a minor racemization step in the general (*S*)-pathway starting from L-isoleucine but to a route substantially differing from our present knowledge. In terms of regulatory aspects and the categorization of flavor compounds as "natural" on the basis of enantiodifferentiation, the data presented are to be considered as important pieces of information enlarging our knowledge of the naturally occurring enantiomeric ratios of these important volatiles in food materials.

LITERATURE CITED

(1) Treptow, H. Rhabarber (*Rheum*-Arten) und seine Verwendung. *Ernaehrung (Vienna)* **1985**, *9*, 179−183.

(2) Rimpler, H. *Biogene Arzneistoffe*; Dt-Apotheken Verlag: Stuttgart, Germany, 1995; pp 363−365.

(3) Burdock, G. A. *Fenaroli's Handbook of Flavour Ingredients*, 3rd ed.; CRC Press: Boca Raton, FL, 1995; pp 241−242, 833−843, 860−861.

(4) Burrows, G. Rhubarb. In *Fruit Processing*; Arthey, D., Ashurst, P. R., Eds.; Aspen Publisher: Gaithersburg, MD, 2001; p 169.

(5) Dregus, M.; Engel, K.-H. Volatile constituents of uncooked rhubarb (*Rheum rhabarbarum* L.) stalks. *J. Agric. Food Chem.* **2003**, *51*, 6530−6536.

(6) Idstein, H.; Bauer, C.; Schreier, P. Flüchtige Säuren in Tropenfrüchten: Cherimoya (*Annona cherimolia*, Mill.), Guava (*Psidium guajava*, L.), Mango (*mangifera indica*, L., var. Alphonso), Papaya (*Carica papaya*, L.). *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 394−397.

(7) Schumacher, K.; Asche, S.; Heil, M.; Mittelstädt, F.; Dietrich, H.; Mosandl, A. Methyl-branched flavor compounds in fresh and processed apple. *J. Agric. Food Chem.* **1998**, *46*, 4496−4500.

(8) Rowan, D. D.; Lane, H. P.; Allen, J. M.; Fielder, S.; Hunt, M. B. Biosynthesis of 2-methylbutyl, 2-methyl-2-butenyl, and 2-methylbutanoate esters in Red Delicious and Granny Smith apples using deuterium-labeled substrates. *J. Agric. Food Chem.* **1996**, *44*, 3276−3285.

(9) Pérez, A. G.; Olías, R.; Luaces, P.; Sanz, C. Biosynthesis of strawberry aroma compounds through amino acid metabolism. *J. Agric. Food Chem.* **2002**, *50*, 4037−4042.

(10) Loughrin, J. H.; Hamilton-Kemp, T. R.; Hildebrand, D. F. Headspace compounds from flowers of *Nicotiana tabacum* and related species. *J. Agric. Food Chem.* **1990**, *38*, 455−460.

(11) Kimland, B.; Aasen, A. J.; Almqvist, S.-O.; Arpino, P.; Enzell, C. R. Volatile acids of sun-cured greek *Nicotiana tabacum*. *Phytochemistry* **1973**, *12*, 835−847.

(12) Chuman, T.; Noguchi, M. Acidic aroma constituents of turkish tobacco. *Agric. Biol. Chem.* **1977**, *41*, 1021−1030.

(13) Ha, J. K.; Lindsay, R. C. Quantification of volatile branched-chain and n-chain fatty acids in flue-cured Virginia and blended Turkish tobacco. *Flavour Fragrance J.* **1991**, *6*, 81−85.

(14) Le Quéré, J.-L.; Pierre, A.; Riaublance, A.; Demaizières, D. Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. *Lait* **1998**, *78*, 279−290.

(15) Wong, E.; Nixon, L. N.; Johnson, C. B. Volatile medium chain fatty acids and mutton flavor. *J. Agric. Food Chem.* **1975**, *23*, 495−498.

(16) Ha, J. K.; Lindsay, R. C. Distribution of volatile branched chain fatty acids perinephric fats of various red meat species. *Lebensm.-Wiss. Technol.* **1990**, *23*, 433−440.

(17) Rettinger, K.; Weber, B.; Mosandl, A. XLII. 2-Methylbuttersäure-Enantiomerenverteilung in Äpfeln und apfelhaltigen Lebensmitteln. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 265−268.

(18) Mosandl, A.; Rettinger, K.; Weber, B.; Henn, D. Untersuchungen zur Enantiomerenverteilung von 2-Methylbuttersäure in Früchten und anderen Lebensmitteln mittels multidimensionaler Gaschromatographie (MDGC). *Dtsch. Lebensm. Rundsch.* **1990**, *86*, 375−379.

(19) Karl, V.; Rettinger, K.; Dietrich, H.; Mosandl, A. 2-Alkylverzweigte Aromastoffe − Struktur, Geruch und chirospezifische Analyse. *Dtsch. Lebensm. Rundsch.* **1992**, *88*, 147−149.

(20) Schumacher, K.; Hener, U.; Patz, C.; Dietrich, H.; Mosandl, A. Authenticity assessment of 2- and 3-methylbutanol using enantioselective and/or $^{13}C/^{12}C$ isotope ratio analysis. *Eur. Food Res. Technol.* **1999**, *209*, 12−15.

(21) Werkhoff, P.; Brennecke, S.; Bretschneider, W.; Güntert, M.; Hopp, R.; Surburg, H. Chirospecific analysis in essential oil, fragrance and flavor research. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 307−328.

(22) Weisenborn, F. L.; Bolger, J. W.; Rosen, D. B.; Mann, L. T.; Johnson, L.; Holmes, H. L. Synthetic hypotensive esters from germine. *J. Am. Chem. Soc.* **1954**, *76*, 1792−1793.

(23) Fieser, L. F.; Fieser, M. In *Organische Chemie*; Verlag Chemie: Weinheim, Germany, 1976; p 1660.

(24) Lane, C. F. Organic synthesis using borane-methyl sulfide. The hydroboration−oxidation of alkenes. *J. Org. Chem.* **1974**, *39*, 1437−1438.

(25) *Organikum*, 20th ed.; Becker, H. G. O., Berger, W., Domschke, G., Eds.; Johann Ambrosius Barth Verlag: Heidelberg, Germany, 1996.

(26) *Gattermann-Wieland Die Praxis des organischen Chemikers. Teil 1. Allgemeine Arbeitsanweisungen*, 42nd ed.; Wieland, T., Mayer, C., Eds.; de Gruyter Verlag: Berlin, Germany, 1972; pp 71−73.

(27) Schultz, T. H.; Flath, R. A.; Mon, T. R.; Eggling, S. B.; Teranishi, R. Isolation of volatile components from a model system. *J. Agric. Food Chem.* **1977**, *25*, 446−449.
(28) Sulzbach, H. Controlling chromatograph gas streams − by adjusting relative positioning off supply columns in connector leading to detector; Carlo Erba Strumentazione GmbH, Germany, 1991; DE 4017909.
(29) Sulzbach, H. Multidimensionale Kapillarsäulen-Gaschromatographie. *GIT Fachz. Lab.* **1996**, *40*, 131.
(30) Schmarr, H.-G. Beiträge zur On-line LC-GC Kopplung und modifizierte Cyclodextrine als chirale stationäre Phasen in der Kapillar-GC. Dissertation, Goethe Universität, Frankfurt/Main, Germany, 1992.
(31) Askari, C.; Hener, U.; Schmarr, H.-G.; Rapp, A.; Mosandl, A. Stereodifferentiation of some chiral monoterpenes using multidimensional gas chromatography. *Fresenius' J. Anal. Chem.* **1991**, *340*, 768−772.
(32) Hädrich J.; Vogelgesang, J. Konzept 2000 − Ein statistischer Ansatz für die analytische Praxis. Teil 1: Nachweisgrenze, Erfassungsgrenze und Bestimmungsgrenze (1. Mitteilung). *Dtsch. Lebensm. Rundsch.* **1999**, *95*, 428−436.
(33) Hädrich J.; Vogelgesang, J. Konzept 2000 − Ein statistischer Ansatz für die analytische Praxis. 2. Mitteilung: Berechnungsbeispiele. *Dtsch. Lebensm. Rundsch.* **1999**, *95*, 495−499.
(34) Rettinger, K. R.; Karl, V.; Schmarr, H.-G.; Dettmar, F.; Hener, U.; Mosandl, A. Chirospecific analysis of 2-alkyl-branched alcohols, acids, and esters: chirality evaluation of 2-methylbutanoates from apples and pineapples. *Phytochem. Anal.* **1991**, *2*, 184−188.
(35) Karl, V.; Schmarr, H.-G.; Mosandl, A. Simultaneous stereoanalysis of 2-alkyl-branched acids, esters and alcohols using a selectivity-adjusted column system in multi-dimensional gas chromatography. *J. Chromatogr.* **1991**, *58*, 347−350.
(36) Maas, B.; Dietrich, A.; Karl, V.; Kaunzinger, A.; Lehmann, D.; Köpke, T.; Mosandl, A. *tert*-Butyldimethylsilyl-substituted cyclodextrin derivatives as versatile chiral stationary phases in capillary GC. *J. Microcolumn Sep.* **1993**, *5*, 421−427.
(37) Řezanka, T.; Dembitsky, V. M. Multibranched polyunsaturated and very-long-chain fatty acids of freshwater Israeli sponges. *J. Nat. Prod.* **2002**, *65*, 709−713.
(38) Lebensmittelchemische Gesellschaft (GDCh). Enantioselektive Analyse zur Natürlichkeitsbewertung von Aromastoffen. *Lebensmittelchemie* **2001**, *55*, 24−26.
(39) Gatfield, I. L.; Hilmer, J.-M.; Werkhoff, P.; Bertram, H.-J. Mechanistic aspect of the formation of natural 2-methylbutanoic acid via fermentative oxidation of the corresponding alcohol. *Adv. Food Sci.* **2000**, *22*, 100−106.
(40) Boelens, M. H.; Boelens, H.; von Gemert, L. J. Sensory properties of optical isomers. *Perfum. Flavor.* **1993**, *18*, 2−16.
(41) Mosandl, A. Review. Capillary gas chromatography in quality assessment of flavours and fragrances. *J. Chromatogr.* **1992**, *624*, 267−292.
(42) Koppenhoefer, B.; Behnisch, R.; Epperlein, U.; Holzschuh, H.; Bernreuther, A.; Piras, P.; Roussel, C. Enantiomeric odor differences and gas chromatographic properties of flavours and fragrances. *Perfum. Flavor.* **1994**, *19*, 1−14.
(43) Ohloff, G. Odorous properties of enantiomeric compounds. In *Olfaction and Taste IV, Proceedings of the Fourth International Symposium*; Schneider, D., Ed.; Wiss. Verlagsgesellschaft: Stuttgart, Germany, 1972; pp 156−160.
(44) Schreier, P. Amino acids as precursors of volatiles. In *Chromatographic Studies of Biogenesis of Plant Volatiles*; Schreier, P., Ed.; Dr. Alfred Hüthig Verlag: Heidelberg, Germany, 1984; pp 76−84.
(45) Sanz, C.; Olias, J. M.; Perez, A. G. Aroma biochemistry of fruits and vegetables. In *Phytochemistry of Fruit and Vegetables*; Tomás-Barberán, F. A., Robins, R. J., Eds.; Clarendon Press: Oxford, U.K., 1997; pp 125−155.
(46) Mamer, O. A. Review. Initial catabolic steps of isoleucine, the *R*-pathway and the origin of alloisoleucine. *J. Chromatogr. B* **2001**, *758*, 49−55.
(47) Hauck, T.; Weckerle, B.; Schwab, W. Metabolism of ethyl tiglate in apple fruits leads to the formation of small amounts of (*R*)-ethyl 2-methylbutanoate. *Enantiomer* **2000**, *5*, 505−512.
(48) Kandra, L.; Wagner, G. J. Pathway for the biosynthesis of 4-methyl-1-hexanol volatilized from petal tissue of *Nicotiana sylvestris*. *Phytochemistry* **1998**, *49*, 1599−1604.

Received for review June 27, 2003. Revised manuscript received August 15, 2003. Accepted August 17, 2003.

JF030466B