

Stereoisomeric Flavor Compounds. 79. Simultaneous Enantioselective Analysis of 3-Butylphthalide and 3-Butylhexahydrophthalide Stereoisomers in Celery, Celeriac, and Fennel

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Using enantioselective multidimensional gas chromatography with heptakis[2,3-di-*O*-acetyl-6-*O*-(*tert*-butyldimethylsilyl)]- β -cyclodextrin as the chiral stationary phase, the simultaneous analysis of the 3-butylphthalide enantiomers and all eight 3-butylhexahydrophthalide stereoisomers was achieved. Enantiomeric distributions of compounds in celery, celeriac, celery seed, and fennel extracts were elucidated.

Keywords: *Enantioselective multidimensional gas chromatography (enantio-MDGC); 3-butyl-(hexahydro)phthalide stereoisomers; celery; celeriac; fennel*

INTRODUCTION

3-Butylphthalide and some hydrogenated derivatives are well-known as character impact flavor compounds of celery and celeriac, both varieties of *Apium graveolens* L. (Ciamician and Silber, 1897a,b; Gold and Wilson, 1963; Bjeldanes and Kim, 1977; Berger et al., 1983). These aroma compounds are frequently described with various concentrations in different food-processing steps (raw or cooked celery and celeriac or celery seed) and are widely used in food production (Barton and De Vries, 1963; Fehr, 1979, 1981; MacLeod et al., 1988; MacLeod and Ames, 1989; Halim et al., 1990; Van Wassenhove and Dirinck, 1990; Van Wassenhove et al., 1990; Manzardo et al., 1996).

As an important flavor compound of some *A. graveolens* species, 3-butylphthalide is investigated in several scientific studies (Mitsuhashi and Muramatsu, 1964; Nagai et al., 1965). The 3*S* enantiomer is generated with a high enantiomeric distribution (Bartschat et al., 1996). Furthermore, 3-butylhexahydrophthalides were investigated in studies on celery and celeriac. Although several investigations are reported in the literature, only two isomers of 3-butylhexahydrophthalides were described and stereochemical features remained unrevealed (MacLeod and Ames, 1989).

Relative configurations of the *cis*-ring-configured 3-butylhexahydrophthalides were described by Nagai and Mitsuhashi (1965), Cocker et al. (1966), and Manzardo et al. (1996). Very recently, the stereoselective analysis of all eight 3-butylhexahydrophthalides has been realized (Bartschat et al., 1997).

Up to now, no experiences with the simultaneous stereoselective analysis of 3-butylphthalide enantiomers and their hexahydroderivatives have been reported.

Enantioselective multidimensional gas chromatography (enantio-MDGC) using the combination of a non-chiral precolumn and a chiral main column has proved to be a powerful method for the direct stereoanalysis of chiral volatiles without any further cleanup or derivatization procedures. This method is frequently used in quality assurance and origin control of flavor and fragrances (Mosandl, 1995, and literature cited therein).

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Table 1. Sample Preparation Parameters of Products Investigated

natural product	used amt (g)	period of extraction (h)	extract vol (mL)	attenuation for enantio-MDGC analysis
celery seed	300	2	4	1:100
German celeriac	500	2	3	neat
Spanish celery	500	2	3	neat
Italian fennel	500	2	0.5	neat

This paper reports the genuine occurrence of 3-butyl-(hexahydro)phthalide stereoisomers in celery, celeriac, celery seed, and fennel using enantio-MDGC with heptakis[2,3-di-*O*-acetyl-6-*O*-(*tert*-butyldimethylsilyl)]- β -cyclodextrin (DIAC-TBDMS- β -CD) as the chiral stationary phase.

MATERIALS AND METHODS

Materials. German celeriac, Spanish celery, and Italian fennel were purchased at the local market. Celery seed was supplied by a commercial flavor company.

Standard compounds of well-known absolute configuration were synthesized as described (Bartschat et al., 1996, 1997).

Sample Preparation. After crushing and homogenization, the natural products were extracted by simultaneous distillation extraction (SDE) (Schultz et al., 1977). An amount of 500 g of plant material (celery seed, 300 g) was suspended in 1.2 L of water. After SDE, the organic layer diethyl ether was separated, dried (MgSO₄), and carefully concentrated, before MDGC investigations (for further preparation parameters see Table 1).

Multidimensional Capillary Gas Chromatography. The MDGC analyses were performed with a SiChromat 2 double-oven system (Siemens, Mannheim, Germany) equipped with a split/splitless injector and two flame ionization detectors (connected to two Shimadzu Chromatopac C-R3A integrators). A PS 268 duran glass column (30 m \times 0.23 mm i.d., film thickness 0.23 μ m) was installed as the precolumn.

Conditions: carrier gas, H₂, 135 kPa; injection, 1 μ L, 120 s; splitless, then split ratio of 1:30; injector temperature, 220 °C; detector temperature, 250 °C; temperature program, 180 °C held for 190 min, increased at 1.2 °C/min to 219 °C, held for 30 min; retention time for **3**, **3'**, **4**, and **4'**, 19.1 min; **1**, **1'**, **2**, and **2'**, 20.7 min; **5** and **5'**, 21.7 min. Transfers onto the main column using cut time intervals were as follows: cut A (transfer of **3**, **3'**, **4**, and **4'**, 18.6–19.7 min; cut B (transfer of **1**, **1'**, **2**, **2'**, **5**, and **5'**, 20.3–22.3 min).

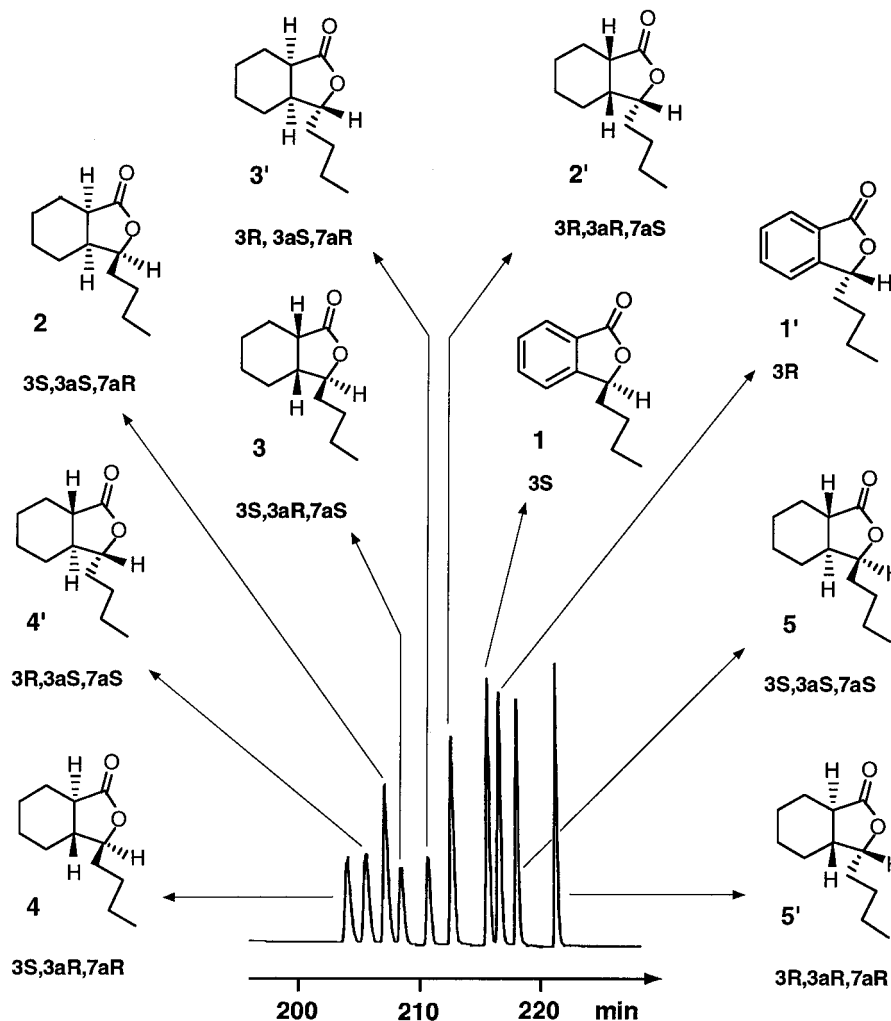


Figure 1. Enantio-MDGC analysis of 3-butyl(hexahydro)phthalides: main column chromatogram (for conditions, see Experimental Procedures).

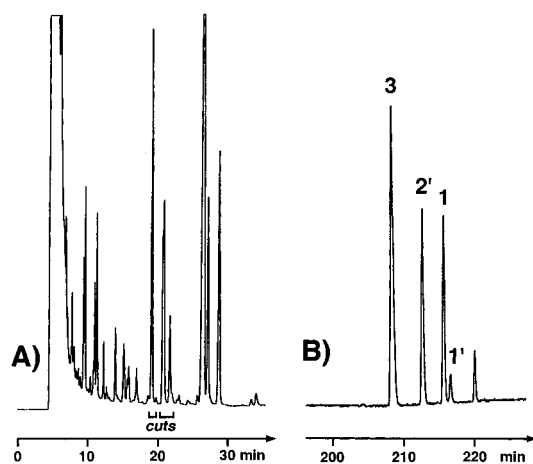


Figure 2. Enantio-MDGC analysis of German celeriac (*A. graveolens* var. *rapaceum*) extract: (A) precolumn chromatogram; (B) main column chromatogram.

A duran glass column (30 m × 0.23 mm i.d., film thickness 0.23 μm) coated with 50% heptakis[2,3-di-*O*-acetyl-6-*O*-(*tert*-butyldimethylsilyl)]-β-cyclodextrin(DIAC-TBDMS-β-CD) (Dietrich et al., 1992; Maas et al., 1994) dissolved in PS 268 was used as the main column.

Conditions: carrier gas, H₂, 110 kPa; detector temperature, 250 °C; temperature program, 95 °C held for 25 min, increased at 2.5 °C/min to 109 °C, held for 160 min, increased at 1.2 °C to 150 °C, held for 30 min. Retention times were as follows: **4**, 204.2 min; **4'**, 205.8 min; **2**, 207.3 min; **3**, 208.7 min; **3'**, 210.9

Table 2. Enantiomeric Distributions (Percent) in Natural Extracts^a

compd	abs config	celery seed	celery	celeriac	fennel
1	3 <i>S</i>	97	88	87	36
1'	3 <i>R</i>	3	12	13	64
2	3 <i>S</i> ,3 <i>aS</i> ,7 <i>aR</i>	nd	nd	nd	nd
2'	3 <i>R</i> ,3 <i>aR</i> ,7 <i>aS</i>	nd	>98	>99	>99
3	3 <i>S</i> ,3 <i>aR</i> ,7 <i>aS</i>	nd	>98	>99	nd
3'	3 <i>R</i> ,3 <i>aS</i> ,7 <i>aR</i>	nd	nd	nd	nd

^a If only one enantiomer was detectable, the enantiomeric purity was described by the detection limit of the method; nd, not detectable.

min; **2'**, 212.8 min; **1**, 215.8 min; **1'**, 216.7 min; **5**, 218.2 min; **5'**, 221.4 min.

Gas Chromatography/Mass Spectrometry. The GC/MS analyses were performed with a Fisons GC 8000 Series equipped with a mass selective detector (Fisons MD 800). A fused silica column (30 m × 0.32 mm i.d., 0.5 μm film thickness, Durabond, J&W Scientific, Folsom, CA) coated with DB-5 was used as stationary phase.

Conditions: carrier gas, He, 20 kPa; injector, split, 42 mL/min; injector temperature, 220 °C; interface temperature, 280 °C; source temperature, 220 °C; temperature program, 100 °C held for 5 min, increased at 2.5 °C to 280 °C, held for 20 min. Retention times were as follows: **3**, **3'**, 32.3 min; **4**, **4'**, 32.5 min; **1**, **1'**, 33.0 min; **2**, **2'**, 33.5 min; **5**, **5'**, 34.1 min.

The following mass spectral data were recorded. *m/z* (relative intensity) **1**, **1'**: 148 (2), 144 (3), 134 (11), 133 (100), 115 (2), 106 (4), 105 (47), 104 (2), 91 (2), 78 (2), 77 (19), 76 (5), 75 (2), 65 (2), 63 (3), 57 (2), 52 (2), 51 (22); **50** (9), 41 (5).

2, 2': 139 (13), 111 (6), 110 (4), 109 (14), 97 (12), 96 (62), 95 (20), 93 (18), 91 (5), 87 (6), 83 (9), 82 (95), 81 (93), 80 (8), 79 (19), 77 (8), 69 (12), 68 (17), 67 (100), 66 (5), 65 (5), 57 (8), 55 (24), 54 (23), 53 (11), 43 (9), 42 (4), 41 (43).

3, 3': 140 (8), 139 (100), 111 (29), 110 (4), 109 (12), 97 (8), 96 (43), 95 (37), 94 (8), 93 (72), 91 (13), 83 (10), 82 (59), 81 (68), 80 (7), 79 (22), 77 (13), 69 (11), 68 (14), 67 (97), 66 (5), 65 (5), 57 (12), 55 (32), 54 (22), 53 (15), 43 (16), 42 (6), 41 (53).

4, 4': 139 (14), 111 (12), 110 (7), 109 (13), 97 (4), 96 (23), 95 (48), 94 (6), 93 (25), 91 (6), 87 (4), 85 (4), 83 (14), 82 (82), 81 (39), 80 (6), 79 (17), 78 (4), 77 (9), 69 (13), 68 (19), 67 (100), 66 (7), 65 (5), 57 (9), 56 (4), 55 (24), 54 (22), 53 (13), 44 (4) 43 (10), 42 (4), 41 (38).

5, 5': 140 (6), 139 (74), 111 (33), 110 (6), 109 (15), 97 (6), 96 (26), 95 (40), 94 (9), 93 (70), 91 (12), 85 (5), 83 (13), 82 (56), 81 (49), 80 (6), 79 (23), 78 (5), 77 (13), 69 (13), 68 (17), 67 (100), 66 (6), 65 (6), 57 (11), 56 (5), 55 (33), 54 (21), 53 (14), 51 (4), 44 (4), 43 (14), 42 (6), 41 (50), 40 (4).

RESULTS AND DISCUSSION

This paper reports on the simultaneous enantio-MDGC analysis of the 3-butylphthalide enantiomers and all eight stereoisomers of 3-butylhexahydrophthalide.

As may be seen from Figure 1, all five enantiomeric pairs are baseline resolved, using DIAC-TBDMS- β -CD as the chiral stationary phase in enantio-MDGC (main column).

To guarantee reliable peak separation in enantio-MDGC, a temperature program resulting in long retention times is in this case absolutely necessary. Chromatographic conditions with shorter retention times yield coelution either of compounds **2** and **3** or of compounds **2** and **3'** or insufficient peak resolution of enantiomers **1** and **1'** or **4** and **4'**.

The structure elucidation of compounds **1,1'** through **5,5'** has been described recently (Bartschat et al., 1996, 1997).

The application of enantio-MDGC to freshly prepared extracts of celery, celeriac, and fennel is reported. As an example, the chromatogram of a celeriac extract is shown in Figure 2.

The occurrence of the identified compounds is always qualitatively assigned by co-injection with racemic standard substances. On this occasion the complete transfer from precolumn to main column of the compounds investigated was ensured. As a further control the genuine compounds were identified by GC/MS analysis.

Table 2 shows the enantiomeric ratios of the compounds investigated. The enantiomeric purities are given in relation to their accuracy limits.

From these results some important conclusions can be drawn:

3-Butylhexahydrophthalides (**2'** and **3**) are detected in celery and celeriac plants, but not in celery seed.

Furthermore, it is astonishing that the hexahydrophthalides **2'** (3*R*,3*aR*,7*aS*) and **3** (3*S*,3*aR*,7*aS*) are generated in high enantiomeric purity, but with different absolute configurations at the C-3 position, while in the case of the phthalide enantiomers **1** and **1'** the 3*S* configuration is preferred (>87%). Therefore, a direct biogenetic pathway for the 3-butylhexahydrophthalides, starting from compounds **1** and **1'**, seems to be improbable. The high enantiomeric purities of compounds **2'** and **3** lead to the supposition that they may be generated with high stereoselectivity, starting from the partially hydrogenated compounds sedanolide, sedanenolide, etc., which are frequently described as

compounds of celery and celeriac. This possibility should be investigated in further biogenetic studies.

An explanation for the lower enantiomeric purity of 3-butylphthalide (**1, 1'**) might be given by partial racemization for this compound, due to resonance-stabilized (benzyl-) anion in the course of the (base-catalyzed) abstraction of the C-3 methine proton (Bartschat et al., 1996). As partial racemization during sample preparation definitely can be excluded, 3-butylphthalide (**1, 1'**) has been stereoanalyzed in celery and celeriac as a genuine enantiomeric ratio favoring the 3*S* configured enantiomer **1** (>87%).

Further, this investigation reports on the structure-function relationship of compounds **2,2'** through **5,5'**. McLeod and Ames (1989) described two isomeric 3-butylhexahydrophthalides with different odor qualities, but stereochemical aspects were not taken into account. As shown by more detailed investigations, using enantio-GC/olfactometry (Bartschat et al., 1997), the odor impression "sweet and sickly" is assigned exclusively to compound **2**, while the odor characteristics "celery, fruity" are attributed with different threshold values to compounds **4, 4'**, and **5**. The genuine compounds **2'** and **3** exhibit no odor impressions.

In summary, the simultaneous enantioselective analysis of all 3-butyl(hexahydro)phthalides (**1,1'** through **5,5'**) and its application to natural products is reported for the first time. For daily routine analysis the analytical procedure may be optimized in view of shorter retention times, if the natural occurrence of the stereoisomers **2, 3', 4, 4', 5**, and **5'** can be excluded.

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