Separation of HHCB, AHTN, ATII, AHDI and DPMI by enantioselective capillary gas chromatography and preparative separation of HHCB and ATII by enantioselective HPLC

Scarlett Biselli[1], Helmut Dittmer[1], Robert Gatermann[2], Roland Kallenborn[2], Wilfried A. König[1], and Heinrich Hühnerfuss[1]

[1]Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany
[2]Norwegian Institute for Air Research, The Polar Environmental Centre, N-9296 Tromsø, Norway

Introduction

The widely used synthetic polycyclic musk fragrances HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[*g*]-2-benzopyrane; galaxolide®), AHTN (1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone; tonalide®), ATII 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methyl-ethyl)-1*H*-inden-5-yl]-ethanone; traseolide®), DPMI (1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4*H*-inden-4-one cashmeran®) and AHDI (1-(2,3-dihydro-1,1,2,3,3,6-hexamethyl-1*H*-inden-5-yl)-ethanone; phantolide®) are chiral compounds, where HHCB and ATII exhibit two asymmetric centres (see asterisks in Figure 1) and thus two diastereomeric pairs of enantiomers. The separation of the diastereomers can be hardly attained on capillary columns commonly used in pesticide and PCB residue analysis, only in two studies the diastereomers of HHCB were successfully separated (1,2). In the present investigation a comparison of two capillary columns coated with differently modified cyclodextrins as chiral stationary phases is presented. A method for the enantioselective separation of the chiral polycyclic musks and their technical by-products by GC-MS is shown.

Furthermore, we also present a method for the enantioselective separation of larger quantities of ATII and HHCB by preparative HPLC. This aspect is of particular interest to the investigation of enantioselective transformation processes in biota, where pure standard compounds as well as their separated enantiomers are urgently needed. In pursuing studies, this reference material will also be applied to addressing the toxicological relevance of the respective isomers and enantiomers by systematic studies, in particular, with regard to the problem whether or not comparable effects are encountered in the presence of the 4*S*-isomers of HHCB and of 5α-androst-16-en-3-one, respectively.

Materials and Methods

Enantioselective GC/MS was carried out with a CE instruments 8560 Mega II gas chromatograph (Milan, Italy) was used equipped with a capillary column (0.25 mm i.d.) coated with a 1:1 mixture of OV 1701/heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethyl)-*β*-cyclodextrin including a 2 m precolumn (J&W, Folsom CA). Temperature program: 343 K (70 °C, 2 min. isotherm) then rising by 10 K/min to 418 K (145 °C), 0.5 K/min to 453 K (180 °C) and 10 K/min to 230 K (503 °C, 10 min isotherm); helium 5.0 as carrier gas, flow velocity: 1.0 mL/min. The sample was injected in on column mode (2 µL injection volume). The GC was coupled to a Finnigan MD 800 quadrupole low-resolution mass spectrometer (San Jose, USA). The MS was used in electron

impact mode (EI) with the ion source temperature set on 453 K (180 °C) applying SIM mode with a dwell time of 100 ms for each ion.

For the preparative enantioselective HPLC separation a Merck-Hitachi (Darmstadt) HPLC-L6200 with HPLC-UV detector L4000 (210/ 260 nm) was used equipped with a packed (8 mm i.d; 200 mm length) column with a stationary phase of monofunctionalized permethyl-β-cyclodextrin on 3-aminopropyl silica gel (5µm particle size; 10 nm pore size). The mobile phase comprised a mixture of acetonitrile and water (1:4), residue grade received from Merck (Darmstadt), with a flow of 1 L/min; injection volume: 150 µL.

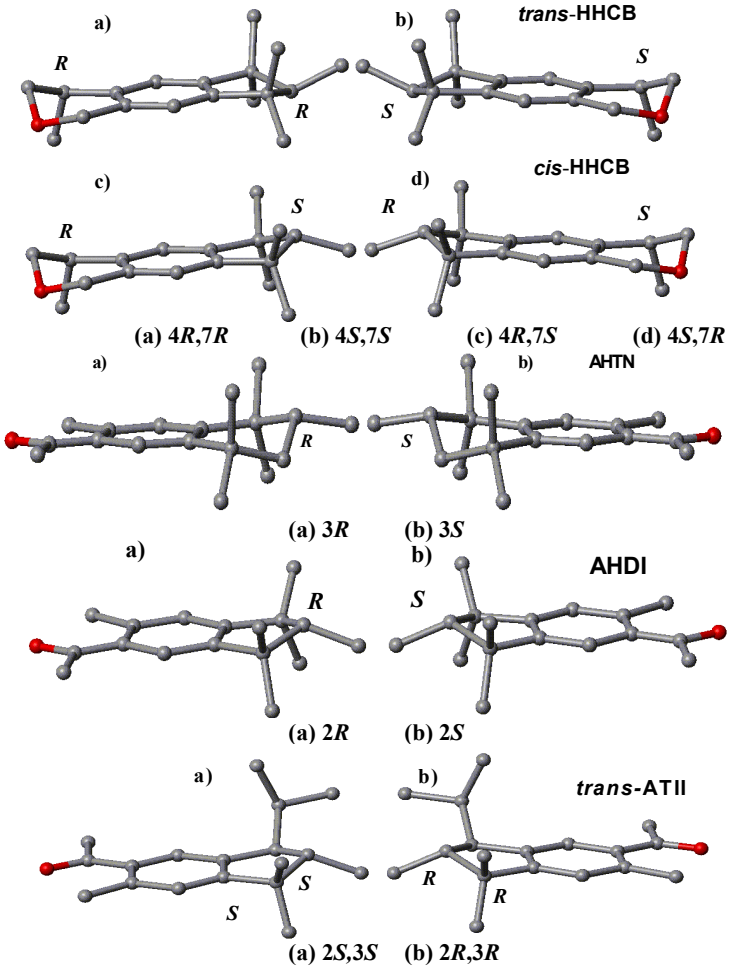

Figure 1: The stereochemical structures of HHCB, AHTN, AHDI, and ATII

Results and Discussion

1. Separation of the standard substances

In the present investigation two different cyclodextrin type chiral stationary phases were tested. A satisfactory enantiomeric separation of all chiral polycyclic musks was achieved with a column coated with a 1:1 mixture of OV 1701/heptakis(2,3-di-*O*-methyl-6-*O*-*tert*hexyldimethyl)-*β*-cyclodextrin. Selected ion monitoring (SIM) fragmentograms (m/z = 213; m/z = 215, m/z = 243 and m/z = 258) of a standard mixture containing HHCB, AHTN and ATII (1 ng/µL each) are shown in figure 2.

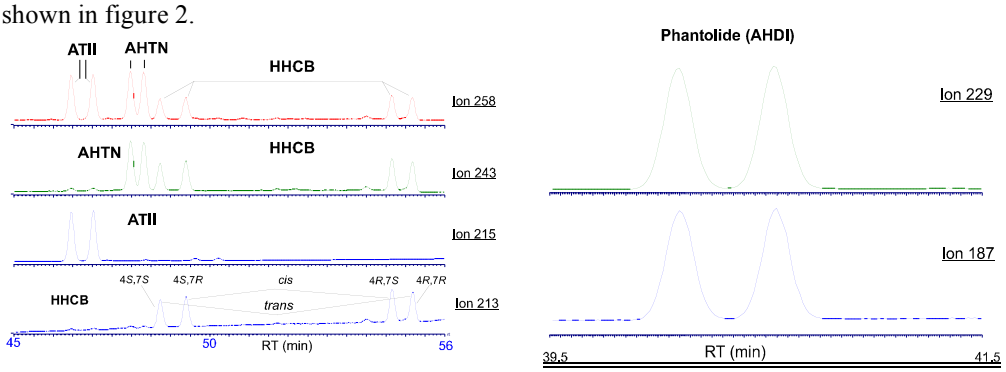

Figure 2: SIM fragmentograms of a standard mixture containing HHCB, AHTN, ATII, and AHDI (1 ng/µL, each), column: 1:1 mixture of OV 1701 and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethyl)-*β*-cyclodextrin

Using a column coated with a 1:4 mixture of OV 1701/octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin, only a baseline separation of the HHCB diastereomers was achieved, where *cis*-HHCB was the first eluting isomer. The enantiomers of ATII were partly separated, whereas no separation of the AHTN enantiomers was obtained (figure 3). The technical formulation of HHCB used in fragrances contains different chiral by-products with the same fragmentation ions m/z = 258 and m/z = 243 (12). Therefore, possible coeluation of these by-products and the polycyclic musks themselves were investigated by using the authentic mixture. As shown in figure 2, no coelution between HHCB and the by products was found.

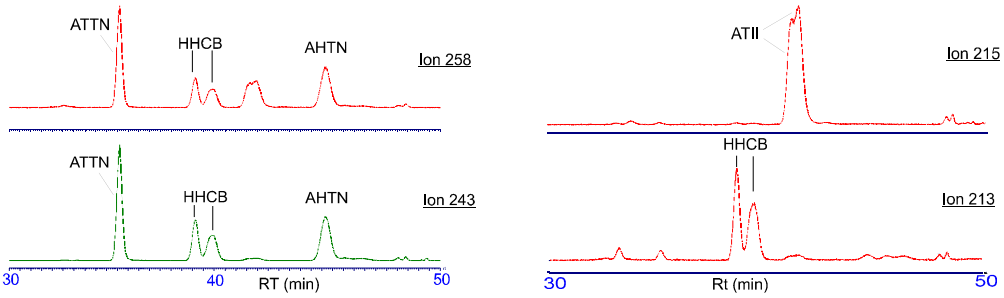

Figure 3: SIM fragmentograms of a standard mixture containing HHCB, AHTN, ATTN and ATII (1 ng/µL, each), stationary phase: 25 % octakis(2,3,6-tri-*O*-ethyl)-γ-cyclodextrin in OV 1701

The assignments of the peaks to the diastereomeric pairs of HHCB (Fig. 2) was achieved by comparing the retention times of the authentic separated stereoisomers with those of the different

compounds in the racemic standard. The two diastereomers responsible for the significant musky odour (4*S* configuration, see Fig. 1) are eluting first on the *β*-CD column.

Technical HHCB consists of a nearly 1:1 mixture of both diastereomers with a slight excess of the *cis* isomer, while technical ATII contains more than 95 % of the *trans* isomer. Thus, the separation of HHCB and ATII on the chiral column is different. The ATII enantiomers of each diasteriomeric pair eluted closely together, whereas the *cis* and *trans* isomers were widely separated. However, for HHCB the 4*S* and the 4*R* diastereomers eluted closely together, whereas the enantiomers were extremely widely separated (see Fig. 2). In addition, the separation of the enantiomers of AHDI is shown in Fig. 2 (m/z = 229 and m/z = 187).

2. Preparative Separation of HHCB and ATII by enatioselective HPLC

The HPLC separation of a higher amount of the polycyclic musks as pure enantiomeric standards was successfully attained by a monofunctionalized permethyl-*β*-cyclodextrin stationary phase for HHCB and ATII as well as for their chiral by-products. Figure 4 shows the chromatograms with good separation of the by-products and baseline separation for the third and fourth eluting isomers of HHCB. The isomers of the *trans*-ATII are nearly baseline separated, and there is also a good partition of the thus far unknown compound of the technical product of ATII. The absolute configuration of the isomers of ATII is not yet known, but we intend to determine it by X-ray diffraction.

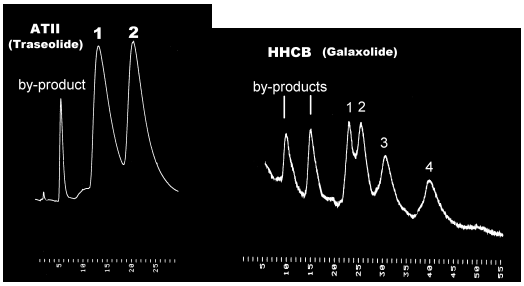

Figure 4 : Preparative enantioselective separation ofATII and HHCB by HPLC, stationary phase permethyl-β-cyclo dextrin on 3-aminopropyl silica gel.

Acknowledgements

Robert Gatermann appreciates the support of a guest researcher fellowship of the Norwegian Research council (NFR) under the project: 125745/720:"Ecotoxicological considerations concerning nitro and polycyclic musk compounds in the Norwegian environment".

References

1. Müller S, Schmid P and Schlatter Ch; *Chemosphere* **1996**, 33, 17-28
2. Draisci R, Marchiafava C, Ferretti E, Palleschi L, Catellani G and Anastasio A; *J. Chromatogr.* **1998**, A814, 187-197
3. Dittmer H and König WA, *Carbohydr. Res.* submitted