

ENANTIOSELECTIVE TRANSFORMATION OF POLYCYCLIC MUSKS
IN AQUATIC BIOTA

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Introduction

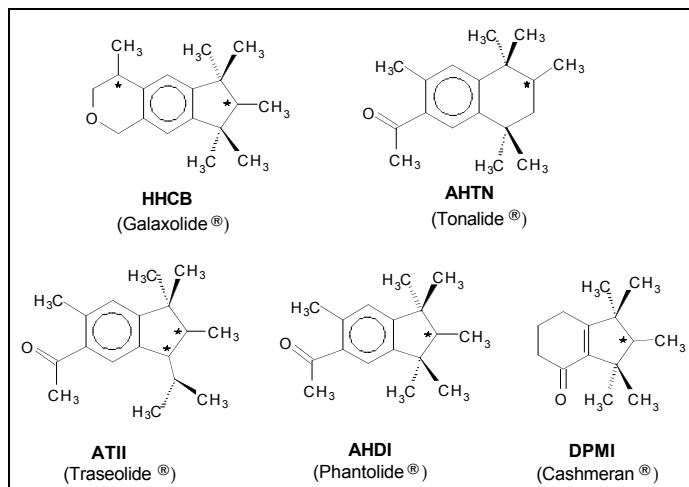
The introduction of modified cyclodextrins in capillary gas chromatography (cGC) and the application of this technique to residual analysis can be considered to represent the historic breakthrough of enantioselective analysis of chiral organochlorine compounds. For a comprehensive survey on the subsequent further development of the chiral stationary modified cyclodextrin phases the reader should refer to the recent review articles by *Vetter and Schurig* [1], *Hühnerfuss* [2], and to the monography by *Kallenborn and Hühnerfuss* [3].

In the present paper, emphasis will be placed upon a new class of chiral xenobiotics, the polycyclic musks, and their enantioselective transformation in different fish species. Additional information, in particular, with regard to the enantiomer separation of these compounds in biota tissue extracts including the GC/MS chromatograms and a preparative enantiomer separation can be inferred from *Gatermann et al.* [4] and *Biselli et al.* [5], respectively.

The chemical structures of the five polycyclic musk compounds investigated herein are shown in Figure 1. The synthetic polycyclic musks HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyrane; galaxolide[®]) and AHTN (1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone; tonalide[®]) are important artificial fragrances used in a large number of perfumes, laundry detergents, fabric softeners, toiletry products, and other household products. Furthermore, the polycyclic musks 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[®]) were included in the present study. All derivatives are chiral compounds, where HHCB and ATII exhibit two asymmetric centres (see asterisks in Figure 1) and thus two diastereomeric pairs of enantiomers.

Meanwhile, these substitutes of the previously applied nitro musks were found in various environmental compartments [6]. According to their application in households the larger part of these compounds reaches the environment via the waste water treatment plants into the rivers,

Figure 1: Short names, trade names, and chemical structures of five polycyclic musk compounds.



lakes and, finally, the marine ecosystem thus particularly interacting with fish and other potential aquatic food.

Materials and Methods

The enantioselective separation of these musk compounds including the diastereomeric pairs of enantiomers was achieved using GC/MS, CE instruments 8560 Mega II gas chromatograph (Milan, Italy) equipped with a capillary column (0.25 mm i.d.) coated with a 1:1 mixture of OV 1701 and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethyl)- β -cyclodextrin including a 2 m precolumn (J&W, Folsom CA). For more experimental details including the chromatograms showing the successful enantioselective separations the reader should refer to the posters by *Gatermann et al.* [4] and *Biselli et al.* [5], this issue, that complement the present paper.

The experimental site of the present study was a sewage treatment plant of the State of Schleswig-Holstein (Germany). At first, the sewage enters the treatment plant, and subsequently the treated waste water flows towards a pond, where it is allowed to remain for some weeks, assuming an average water exchange rate of about 17,000 to 22,000 m³/d and taking into account an area of 128,000 m² and a depth of 4 m. The effluent of the pond is closed with bars thus enabling small fishes only to enter or to escape from the pond. Therefore, equilibrium conditions can be assumed for synthetic musks in fish tissues of larger animals that have to remain within the pond. The 3 rudd, 5 tench, 8 crucian carp, two eel and one pooled mussel samples investigated herein stem from this pond.

Results and Discussion

Concentrations and enantiomeric ratios of HHCB, *cis*- and *trans*-HHCB, AHTN, *trans*-ATII, and AHDI for 18 fish samples and one pooled mussel sample from the pond of the sewage plant are summarised in Table 1. Both the concentrations and the enantiomeric ratios appear to exhibit

Table 1: Concentrations [conc; µg/g lipid] and enantiomeric ratios (ER) of HHCB, *trans*-HHCB, AHTN, *trans*-AHTII, and AHDI in biota caught in the pond of a municipal waste water treatment plant in summer 1997.

| Species | HHCB | <i>trans</i> -HHCB | <i>cis</i> -HHCB | AHTN | | <i>trans</i> -AHTII | | AHDI | |
|-------------------|------|--------------------|------------------|------|------|---------------------|------|------|------|
| | conc | ER | ER | conc | ER | conc | ER | conc | ER |
| Rudd | 6.2 | 0.66 | 1.24 | 5.0 | 0.94 | 0.5 | 0.99 | 0.3 | 0.90 |
| Rudd | 7.1 | 0.55 | 1.10 | 5.7 | 0.91 | 0.5 | 0.98 | 0.3 | 1.12 |
| Rudd | 7.5 | 0.57 | 1.07 | 6.1 | 0.80 | 0.5 | 0.97 | 0.3 | 0.95 |
| Tench | 150 | 1.06 | 0.98 | 30 | 1.65 | 2.3 | 0.60 | 2.2 | 1.31 |
| Tench | 160 | 1.10 | 0.95 | 32 | 1.97 | 2.0 | 0.38 | 1.9 | 1.47 |
| Tench, liver | 72 | 1.01 | 0.85 | 16 | 1.98 | 1.1 | 0.33 | 0.9 | 1.78 |
| Tench | 160 | 0.88 | 1.00 | 35 | 1.74 | 2.2 | 0.51 | 2.1 | 1.30 |
| Tench | 150 | 1.03 | 0.98 | 42 | 1.65 | 2.6 | 0.46 | 2.5 | 1.28 |
| Crucian carp | 39 | 0.12 | 0.44 | 26 | 1.05 | 1.2 | 0.05 | 1.8 | 0.65 |
| Crucian carp | 59 | 0.12 | 0.42 | 31 | 1.24 | 1.5 | 0.08 | 2.7 | 0.81 |
| Crucian carp | 91 | 0.19 | 0.54 | 31 | 1.19 | 1.9 | 0.20 | 3.1 | 0.94 |
| Crucian carp | 71 | 0.14 | 0.48 | 30 | 1.15 | 1.4 | 0.10 | 2.5 | 0.78 |
| Crucian carp | 50 | 0.10 | 0.41 | 34 | 1.19 | 1.6 | 0.06 | 2.4 | 0.71 |
| Crucian carp | 66 | 0.10 | 0.46 | 40 | 1.14 | 1.5 | 0.05 | 2.3 | 0.66 |
| Crucian carp | 84 | 0.13 | 0.51 | 34 | 1.15 | 1.9 | 0.07 | 3.0 | 0.64 |
| Crucian carp, liv | 69 | 0.10 | 0.48 | 31 | 1.29 | 1.6 | 0.06 | 2.5 | 0.63 |
| Eel | 4.8 | 0.98 | 1.27 | 2.6 | 0.87 | 0.3 | 1.95 | 0.2 | 0.73 |
| Eel | 4.6 | 0.79 | 1.15 | 2.7 | | 0.3 | 1.50 | 0.2 | 0.48 |
| Mussel | 120 | 0.91 | 1.26 | 45 | 0.90 | 3.4 | 0.67 | 2.3 | 1.02 |

a species dependency. Clearly distinguishable clusters can be inferred from the data set. For example, for HHCB the following species dependent clusters can be stated: rudd (conc = 6.2 - 7.5 µg/g lipid; ER_{trans} = 0.55 - 0.66; ER_{cis} = 1.07 - 1.24), tench (conc = 150 - 160 µg/g lipid; ER_{trans} = 0.88 - 1.10; ER_{cis} = 0.95 - 1.00), crucian carp (conc = 39 - 91 µg/g lipid; ER_{trans} = 0.10 - 0.19; ER_{cis} = 0.41 - 0.54), eel (conc = 4.6-4.8 µg/g lipid; ER_{trans} = 0.79-0.98; ER_{cis} = 1.15 - 1.27), mussel (conc = 120 µg/g lipid; ER_{trans} 0.91; ER_{cis} = 1.26). Similar clusters can be inferred from Table 1 for concentrations and enantiomeric ratios for AHTN, *trans*-AHTII, and AHDI, respectively.

It is tentatively assumed that high concentrations and enantiomeric ratios close to racemic, i.e., ER ≈ 1, indicate a low metabolization potential of a species for the respective polycyclic musk derivative. On the other hand, caution has to be applied when inferring metabolization potentials exclusively from lipid based concentrations: low concentrations may reflect a stronger metabolization, but it cannot be excluded that specific matrix effects, e.g., high lipid contents like in eel, may pretend a stronger metabolization than actually encountered. An unequivocal parameter for an enantioselective transformation process, however, are ER values clearly different from one. On the basis of these assumptions, rudd appears to exhibit a strong enantioselective metabolization potential for *trans*-HHCB.

Tench shows a low enantioselective metabolization potential for *trans*- and *cis*-HHCB, and a moderate one for AHTN and AHDI. Very strong enantioselective metabolization can be concluded for *trans*- and *cis*-HHCB as well as for *trans*-ATII in crucian carp, while for AHDI a moderate metabolization was observed. With ER values ≤ 0.1 for *trans*-HHCB and *trans*-ATII the highest enantioselectivity in the present study was observed. In eel the high lipid content gives rise to low lipid-normalised concentrations, but the ER values indicate low to moderate enantioselective metabolization for *trans*- and *cis*-HHCB, and AHTN, while for *trans*-ATII and AHDI a stronger enantioselective metabolization capability was found. The values for the pooled mussel sample, known for a low metabolization capability, reflect the water values which were also determined in the present study. For a more detailed discussion of the results obtained herein for crucian carp and tench, the reader should refer to the complementary poster by Gatermann *et al.* [4].

As stated above, the lipid based concentrations for the assignment of metabolization potentials may be misleading. However, in those cases, where both low concentrations and significant deviations of the enantiomeric ratios from one are being encountered, it is justified to assume strong metabolization capacities. This assumption is in line with previous conclusions drawn by Karlsson *et al.* from enantioselective analyses of chlordanes in different fish species [7].

Furthermore, the in part high enantioselectivity in the metabolic capacity for the polycyclic musks and the well-defined binding activities of the polycyclic musks to olfactory receptors, the known effect of natural musk as pheromones and the structure relationship, especially of HHCB, to steroid hormones raise the question whether or not synthetic musks have an effect on the chemical communication and/or the hormone system of species in the aquatic and/or the terrestrial environment. However, this question can only be answered by systematic experiments in which tests with pure enantiomers have to be included.

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