

Species-dependent enantioselective transformation of HHCB, AHTN and ATII in crucian carp (*Carassius carassius*) and tench (*Tinca tinca*)

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Introduction

The synthetic polycyclic musks HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*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. The chemical structures of the five polycyclic musk compounds investigated herein can be found in Figure 1 of the complementary paper by Hühnerfuss et al. [1]. Meanwhile, these substitutes of the previously applied nitro musks were found in various environmental compartments [2]. All derivatives are chiral compounds, where HHCB and ATII exhibit two asymmetric centres and thus two diastereomeric pairs of enantiomers. For HHCB the enantioselective syntheses and separations of all four stereoisomers was achieved, and thus it could be proved that the 4*S*,7*R*/*S*-isomers are the powerful, musky components [3]. The successful enantioselective GC and HPLC separations of the five polycyclic musks, including the diastereomeric pairs of enantiomers are presented in the complementary paper by Biselli et al. [4]. In the present paper we report the detailed discussion of the enantiomeric ratios (ER) analysed in tissue extracts of two different fish species, which were caught in a pond of a municipal sewage plant in Schleswig-Holstein (Germany).

Materials and Methods

Enantioselective GC/MS was carried out with a 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/heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethyl)- β -cyclodextrin including a 2 m pre-column (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 on column (2 mL 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. The five tench (*Tinca tinca*) and eight crucian carps (*Carassius carassius*) investigated herein were caught in the pond of a municipal sewage plant in Schleswig-Holstein (Germany) which is described in the complementary paper by Hühnerfuss et al. [1].

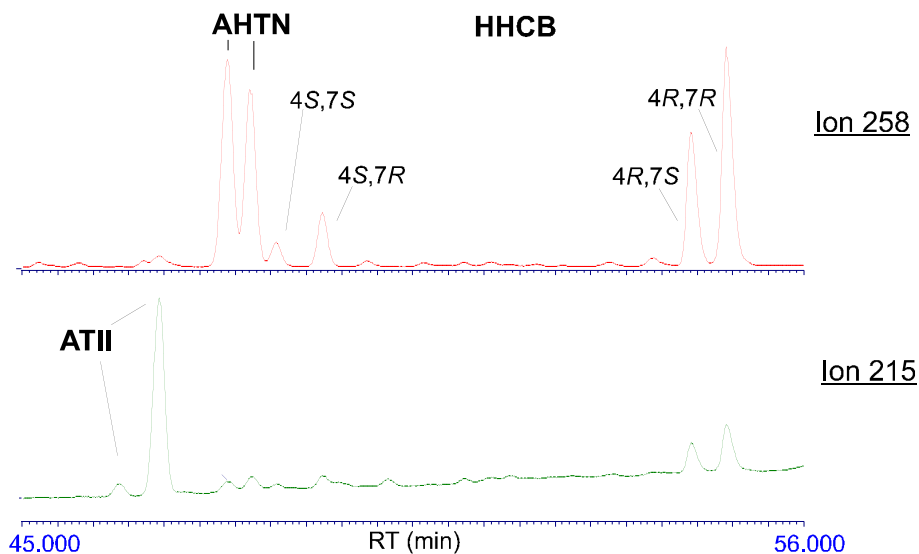
Results and Discussion

An example of two single ion monitoring (SIM) fragmentograms (electron impact mode) of a crucian carp and a tench sample extract, respectively, containing HHCB, AHTN, and ATII is shown in Figure 1. The concentrations and the enantiomeric ratios of HHCB, AHTN, *trans*-ATII, and AHDI in the tissue extracts of all 18 fish species and one pooled mussel caught in the pond of the municipal waste water treatment plant can be found in Table 1 of ref. [1], this issue. Both the concentrations and the enantiomeric ratios appear to exhibit a species dependency. Clearly distinguishable fingerprint-like clusters can be inferred from the data set as illustrated in Figure 2. In the tench samples higher concentration levels and only slight differences in the ER compared to water samples were found. Therefore, for tench a low metabolisation capacity for polycyclic musks is suggested. On the other hand, in the crucian carp the highest enantioselectivity was found. The first eluting enantiomers of *cis*- and *trans*-HHCB, and *trans*-ATII were preferentially metabolised resulting in strong enantiomeric excesses of the second eluting one. Especially for *trans*-HHCB and *trans*-ATII with ratios at or below 0.1 a very strong enantioselectivity was determined.

In order to support the conclusion that the lower concentrations encountered in crucian carps are caused by enantioselective degradation, theoretical concentrations for passive bioconcentration were calculated on the following basis: it was tentatively assumed that only one enantiomer is being degraded and that the prevailing enantiomer represents 50 % of a „theoretical concentration“ without metabolisation. In the case of *trans*-ATII, an ER of 0.8 (found in the water samples) instead of a racemic distribution was used as basic value. The calculated values for each species as well as mean experimental values are summarized in Table 1. In the case of AHTN, the measured values in crucian carp and tench differ not significantly and are comparable with the values in mussels, which implies that the concentration levels are explainable with passive bioconcentration only and no or little metabolisation. On the other hand, in crucian carp significantly lower concentrations of HHCB and ATII compared to tench as well as lower experimental than calculated values indicate strong enantioselective transformation of the first eluting *trans*-ATII enantiomer in crucian carp. In the case of HHCB, the calculation is more sophisticated, because the measured concentration is the sum of two diastereomers, *cis*- and *trans*-HHCB. The comparison of the stereochemical patterns in the crucian carp sample extracts and in standard solutions shows a different behaviour of the *cis* and the *trans* isomer: For the *cis* isomer both enantiomers seem to be degraded compared to the latest eluting enantiomer of *trans*-HHCB. Therefore, it was decided to choose this stereoisomer (4*R*,7*R*) as the basic value for the calculation. The mean calculated value (122 µg/g lipid) is as high as the mussel value and only slightly lower than the mean value of the tench samples (152 µg/g lipid). Thus, it can be concluded that also for HHCB enantioselective transformation is the most important process resulting in lower concentrations for crucian carp. In addition, a higher selectivity of the *trans* compared to the *cis* isomer can be assumed.

Figure 1: Single ion monitoring (SIM) fragmentograms (electron impact mode) of a crucian carp and a tench sample containing HHCB, AHTN, and ATII; stationary phase: 1:1 mixture of OV 1701 and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethyl)- β -cyclodextrin.

Sample: Crucian carp



Sample: Tench

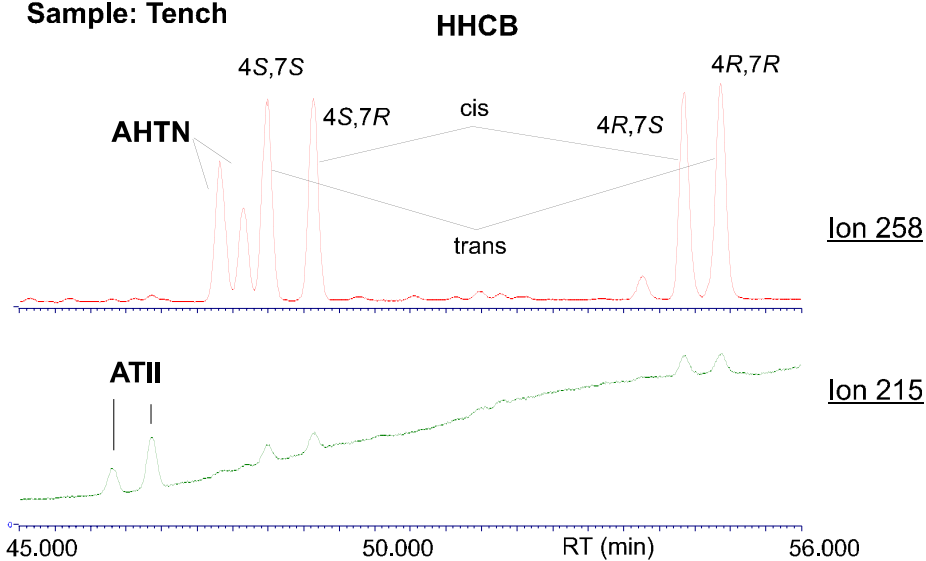


Figure 2: Mean ER values of *cis*- and *trans*-HHCB, *trans*-ATII, and AHTN for different species.

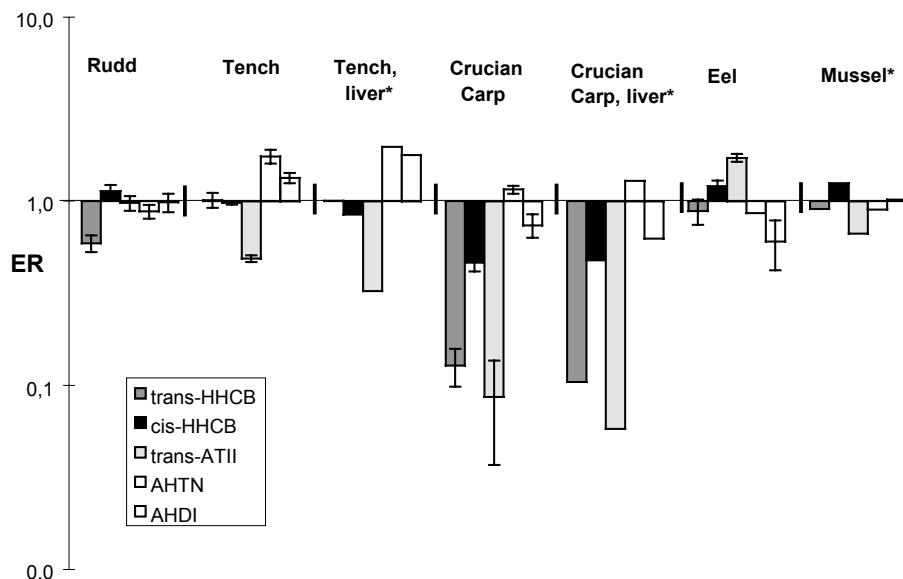


Table 1: Experimental and calculated concentrations of HHCB, AHTN, and ATII ($\mu\text{g/g}$ lipid)

Species	HHCB conc		AHTN conc		ATII conc	
	experim.	calculated	experim.	calculated	experim.	calculated
Tench	152	-	35	44	2.3	2.8
Tench, liver	72	-	16	21	1.1	1.7
Crucian carp	66	122	32	35	1.6	3.0
Crucian carp, liver	69	135	31	35	1.6	3.0
Mussel	120	-	45	-	3.4	-

Acknowledgements

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References

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