ENANTIOSELECTIVE ANALYSIS OF THE HHCB METABOLITE HHCB-LACTONE IN ENVIRONMENTAL SAMPLES

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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 xenobiotics. For a comprehensive survey on the subsequent further development of the chiral stationary phases the reader should refer to the recent review articles by *Vetter and Schurig* [1], *Hühnerfuss* [2], and to the monograph by *Kallenborn and Hühnerfuss* [3]. In the present paper, emphasis will be placed upon a new class of chiral xenobiotics, the synthetic polycyclic musks HHCB (e.g., galaxolide®) and AHTN (e.g., tonalide®), which are important artificial fragrances used in a large number of perfumes, laundry detergents, fabric softeners, toiletry products, and other household products [4], as well as the polycyclic musk ATII (e.g., traseolide®). All derivatives are chiral compounds, where HHCB and ATII exhibit two stereogenic centres and thus two diastereomeric pairs of enantiomers. Herein, the focus will be upon the main HHCB metabolite, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[*g*]-2-benzopyrane-1-one (HHCB-lactone, in some refs 'galaxolidone'; Fig. 1). Franke et al. [5] synthesised the racemic standard compound by oxidation of racemic HHCB using a finely powdered mixture of potassium permanganate and copper sulfate pentahydrate. Thus, they were for the first time able to verify the presence of this compound in environmental samples. A re-evaluation of existing cGC/MS analyses showed that this metabolite is very common in surface waters including water from the Odra and Elbe rivers. Franke et al. conjectured that HHCB-lactone is likely to be formed by autoxidation of the benzylic methylene group of HHCB, and so its occurrence in aquatic environmental samples can be explained by an abiotic process. Enzymatic oxidation at the benzylic position of HHCB may also occur, but the formation of HHCB-lactone is certainly not restricted to biotransformation reactions. For example, in laboratory experiments Itrich et al. [6] observed the oxidation of HHCB to the lactone in activated sewage sludge (i.e., biotransformation) and in abiotic controls.

Materials and Methods

The enantioselective separation of the musk compounds including the diastereomeric pairs of enantiomers was achieved using GC/MS, CE instruments 8560 Mega II GC (Milan, Italy) equipped with a capillary column (0.25 mm i.d.; length 25 m) 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 pre-

column (J&W, Folsom CA) coupled to a low resolution MS detector (MD800, Finnigan, San Jose CA) run in EI mode.

The experimental site of the present study was a sewage treatment plant of the Federal 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 (depth 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, 4 tench, 7 crucian carp, two eel, one pooled mussel and 9 SPMD samples investigated herein stem from this pond.

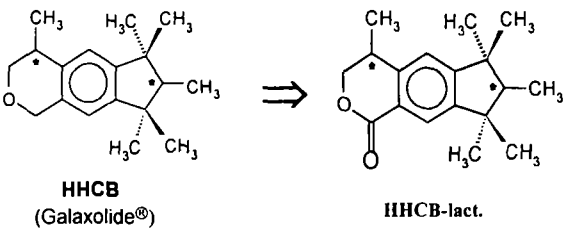

Fig. 1: The transformation of HHCB to HHCB-lactone

Results and Discussion

As HHCB-lactone exhibits two stereogenic centres (Fig. 1), two diastereomeric pairs of enantiomers may be expected, which in turn may give rise to at maximum four peaks in the gas chromatogram. The enantioselective separation of this compound including the diastereomeric pairs of enantiomers was achieved using a stationary phase consisting of a 1:1 mixture of OV 1701/heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (TBDMS; see above). Selected ion monitoring (SIM) fragmentograms (m/z = 257) of the HHCB-lactone standard and chromatograms (TIC) of a standard mixture containing HHCB, AHTN, ATII and the HHCB-lactone are shown in Fig. 2. It is worth noting that the stationary phase used herein allowed both an enantioselective separation of HHCB-lactone, of its parent compound HHCB and of the polycyclic musks AHTN and ATII without any significant coelutions. Detailed photochemical transformation experiments, i.e., UV irradiation of the respective HHCB enantiomers, revealed that the elution sequence of the HHCB-lactone enantiomers corresponds exactly with that of the respective parent enantiomers of HHCB, i.e., the *trans*-HHCB-lactone enantiomers (4*S*,7*S* and 4*R*,7*R*) and of the *cis*-HHCB-lactone enantiomers (4*S*,7*R* and 4*R*,7*S*) exhibit the same elution sequence as those of the respective HHCB stereoisomers (Fig. 2).

As an example for environmental samples, SIM fragmentograms are shown for tench, zebra mussel, rudd, eel, crucian carp and SPMD extracts in Fig. 3. For comparison, the fragmentogram for the parent compound HHCB as determined in the same crucian carp extract is also included in Fig. 3. Average concentrations of HHCB-lactone as well as the enantiomeric ratios of both *trans*- and *cis*-HHCB-lactone are summarised in Table 1 for rudd, tench, crucian carp, eel, one pooled zebra mussel sample and SPMD extracts, all stemming from the pond of the sewage plant. For comparison, average concentrations of the parent compound HHCB are also included in Table 1.

In the SPMD sample extracts, which largely reflect the situation in the water, no pronounced enantiomeric shifts were found both for *trans*- and *cis*-HHCB-lactone. The most intensive deviation of the enantiomeric ratios from racemic was encountered for crucian carp extracts. Basically, the latter result is in line with the corresponding result for the parent compound HHCB, though the exact ER values summarised in Table 1 indicate that the enantiomeric shifts are less pronounced for the metabolite than for the parent compound. However, it is interesting to note that both for HHCB and its transformation product an enantioselective transformation of the 4*S* enantiomers of the diastereomeric pairs was observed, i.e., the HHCB stereoisomers that are preferentially formed are being subject to preferential further transformation. In this case, an enantioselective bioconcentration from the water can be excluded, because the enantiomeric shifts for the diasteromeric pairs

of HHCB enantiomers in water were negligible. Similar coincidences of the preferential transformation of the same stereoisomers of the parent compound and the metabolite can be inferred from the results obtained for rudd and eel sample extracts. However, in these two cases, the diastereomers show a different metabolisation tendency: while for *trans*-HHCB-lactone the 4*S* enantiomer is being preferentially transformed, in the case of the *cis*-HHCB-lactone the 4*R* enantiomer is enantioselectively transformed. Summarising, the detailed analysis of the enantiomeric ratios of the polycyclic musk HHCB and its metabolite HHCB-lactone reveals the different enzymatic transformation pathways occurring in different fish species.

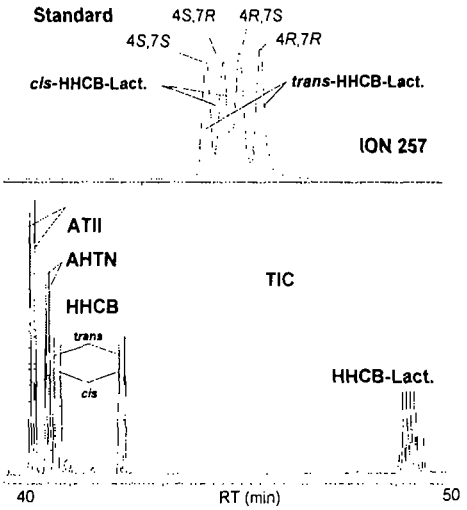

Fig. 2: top: SIM fragmentogram of a HHCB-lactone standard (ion 257); below: standard mixture containing HHCB, AHTN, ATII, and HHCB-lactone (total ion chromatogramme , TIC)

Table 1: Average concentrations for HHCB and HHCB-lactone, enantiomeric ratios (ER) of HHCB-lactone in different samples stemming from the pond of a sewage treatment plant.

Sample	HHCB conc [µg/g lipid]	HHCB-lact. conc [µg/g lipid]	*trans*-HHCB-lact. ER	*cis*-HHCB-lact. ER
Rudd (n = 3)	6.9	8	0.65	1.19
Tench (n = 4)	155	33	0.68	0.99
Crucian carp (n = 7)	66	24	0.39	0.83
Eel (n = 2)	4.7	4	0.59	1.51
Zeb.mussel (pooled)	120	33	0.81	0.91
SPMD (n = 9)	166 (n = 12)	8	0.81	1.01

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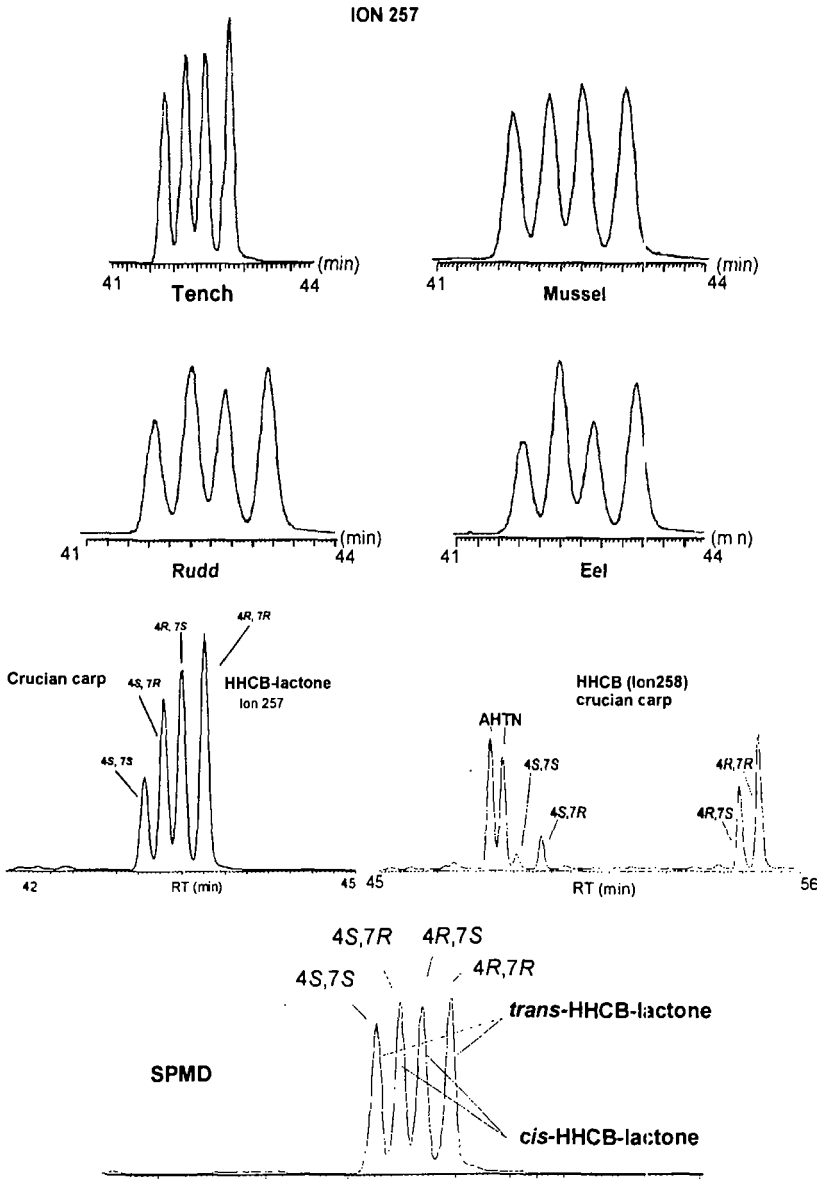

Fig. 3: SIM fragmentograms of HHCB (crucian carp) and HHCB-lactone in various fish, zebra mussel and SPMD extracts.

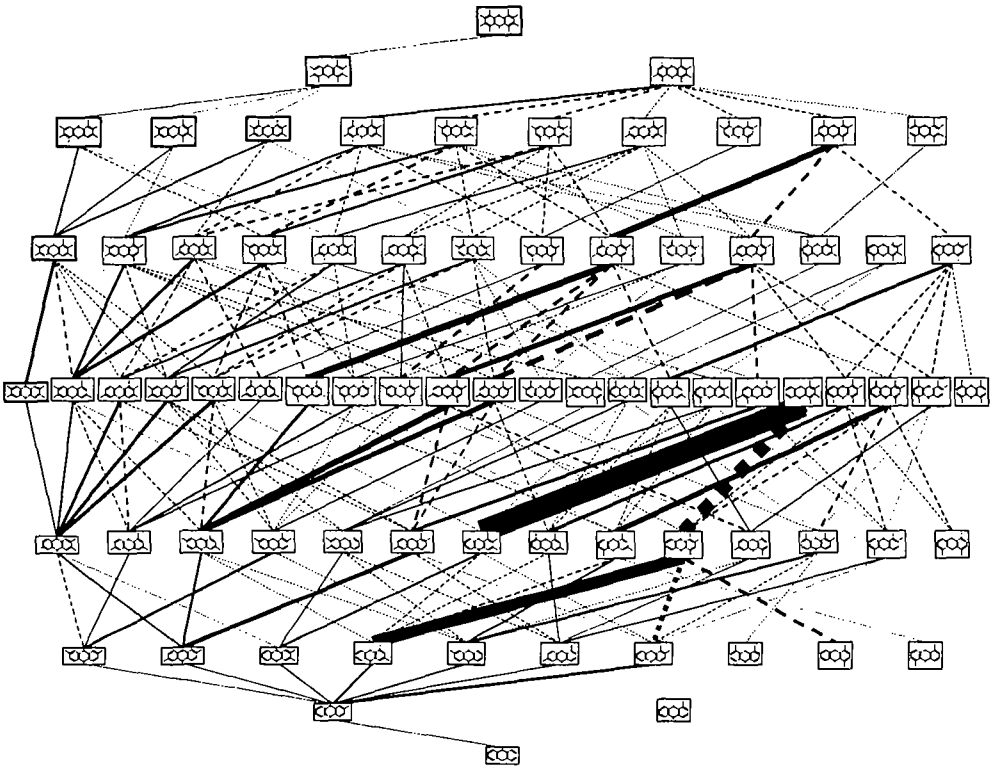

Figure 1 Proposed pathways of the photodegradation of OCDD in hexane solution with irradiation of 254 nm UV-ray
The solid line shows main pathways. Line-width indicates the reactivity.

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