



Synthesis, liquid chromatographic fractionation and partial characterization of polybrominated dibenzofuran congeners



Christoph Gallistl, Walter Vetter*

University of Hohenheim, Institute of Food Chemistry (170b), Garbenstr. 28, D-70599 Stuttgart, Germany

ARTICLE INFO

Article history:

Received 18 December 2015
Received in revised form 3 March 2016
Accepted 7 March 2016
Available online 9 March 2016

Keywords:

Polyhalogenated compounds
Polybrominated dibenzofurans (PBDFs)
Synthesis
Countercurrent chromatography
RP-HPLC
Structure elucidation

ABSTRACT

Polybrominated dibenzofurans (PBDFs) are a class of highly toxic environmental contaminants which comprises 135 structurally different congeners. While the gas chromatographic separation and analysis of the most polychlorinated dibenzofurans (PCDFs) are well-documented, comparably little data is currently available in the case of PBDFs. In this study dibenzofuran was brominated to give a mixture of ~40 PBDFs with one to seven bromine atoms. This synthesis mixture was fractionated by both countercurrent chromatography (CCC) with the solvent system *n*-hexane/toluene/acetonitrile and non-aqueous reversed-phase high performance liquid chromatography (RP-HPLC) with acetonitrile as the mobile phase. All together 80 consecutive CCC fractions and 40 HPLC fractions were taken and analyzed for PBDFs by gas chromatography coupled to mass spectrometry (GC/MS). CCC and RP-HPLC offered orthogonal separation of the PBDF mixture. As a consequence, selected CCC fractions were further fractionated by RP-HPLC. In this way, eight PBDFs could be isolated and the structures of twelve PBDFs were elucidated by proton magnetic resonance spectroscopy (¹H NMR).

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Polybrominated dibenzofurans (PBDFs) are a class of polyhalogenated compounds unintentionally formed during different industrial processes [1]. Ten of the 135 possible PBDF congeners feature bromine substituents in 2,3,7,8-position which has been directly linked to elevated toxicity within this class of contaminants [2–4]. In recent years, PBDFs have been detected in various environmental abiotic and biotic samples [1,5–8]. For example, Malmvärn et al. detected and tentatively identified one tribromodibenzofuran (triBDF) besides several low brominated polybrominated dibenzodioxins (PBDDs) in blue mussels (*Mytilus edulis*) from the Baltic Sea [5].

Like their chlorinated relatives, that is the polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), PBDFs are distributed as impurities of industrial chemicals and they are present as products of thermal or combustion processes [9]. PBDF residues have been detected in several brominated flame retardants (BFRs), including polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and tetrabromobisphenol A (TBBP-A) [10–12]. For example, one di- and one triBDF were tentatively detected in a

technical pentabromodiphenyl ether mixture (DE-71) [13]. Moreover, PBDFs can be generated from BFRs and BFR-treated products under thermal or photolytic stress [14–20], for example during the mixing process of BFRs with polymers or the extrusion of flame-retardant polymers [21]. Even moderate temperature conditions like during the heating process of foodstuff can lead to formation of PBDFs from containing BFR residues [22–24]. With BFRs being used in various consumer goods, PBDFs can be formed after disposal of these products, for instance during recycling, waste incineration or storage at landfills, when they are exposed to light and/or higher temperatures [2].

While many PCDF congeners are available as reference standards, there is still a lack of information and availability on their brominated analogues [1]. In model reactions, several mono- to hexasubstituted PBDFs (mono- to hexaBDFs) were generated during the UV irradiation of technical decabromodiphenyl ether (decaBDE) or neat BDE 209 [25,26]. Additionally, mono-, di- and triBDFs were generated by the pyrolysis of monobromophenols, phenol and 2,4-dibromophenol in the presence of CuBr₂ [27] and during exposure of 2,3,7,8-tetraBDF to sunlight under laboratory conditions [28]. In these studies most of the detected PBDF congeners could not be identified due to the lack of fitting reference standards. As a consequence, structural information and GC/MS characteristics were only available for a few PBDF congeners.

* Corresponding author.

E-mail address: walter.vetter@uni-hohenheim.de (W. Vetter).

The aim of this study was to synthesize a PBDF mixture followed by the isolation and structure elucidation of individual PBDF congeners. For this purpose, dibenzofuran was brominated to an average bromine content of ~61% (predominance of di- to tetrabrominated congeners). This mixture was fractionated by countercurrent chromatography (CCC) and non-aqueous reversed-phase high-performance liquid chromatography (RP-HPLC), i.e. techniques which have already successfully been used for the separation of other complex mixtures of polyhalogenated compounds like toxaphene and technical octabromodiphenyl ether [29–32]. CCC fractions rich in selected congeners were purified by RP-HPLC and the isolated PBDF congeners were identified by GC/MS and NMR measurements.

2. Material and methods

2.1. Chemicals and standards

Dibenzofuran (p.a., ≥98%), elemental bromine (A.C.S. grade, ≥99.5%), 2,2,4-trimethylpentane (*iso*-octane, for pesticide residue analysis), iron powder (≥99.9%) and aluminum bromide (≥98%) were supplied from Sigma-Aldrich (Steinheim, Germany). Dichloromethane (DCM, gradient grade for HPLC, ≥99.9%), acetonitrile (ACN, >99.9%, HPLC gradient grade) and *n*-hexane (for pesticide residue analysis, ≥95.0%) were ordered from Th. Geyer (Renningen, Germany) whereas toluene (residue analysis grade), silica gel 60 (for column chromatography) and sodium thiosulfate (A.C.S. grade, ≥99.5%) were from Fluka (Steinheim, Germany). Demineralized water was produced in-house by means of an ELGA purelab classic ultrapure water system (Celle, Germany). Dideuterated dichloromethane (CD₂Cl₂, 99.6%) was from Deutero (Kastellaun, Germany). Perdeuterated α-HCH (α-PDHCH) was synthesized in our laboratory [33]. A solution of 2,3,7,8-tetraBDF, 2,3,4,7,8- and 1,2,3,7,8-pentaBDF, 1,2,3,4,7,8-hexaBDF, 1,2,3,4,6,7,8-heptaBDF and 1,2,3,4,6,7,8,9-octaBDF in nonane (Cambridge Isotope Laboratories, Tewksbury, MA, USA) was kindly provided by CVUA Freiburg (Germany).

2.2. Laboratory safety instructions

Due to the high toxic potential, synthesis of PBDFs in high amounts had to be handled with extreme care. Special attention was given to a separate waste disposal as well as a special treatment for cleaning of contaminated glassware (e.g. heating in a muffle furnace above 600 °C for 24 h). All work was performed in a hood whose exhaust was filtered with concentrations as small as required. Leftovers of synthesis are kept in a safe place.

2.3. Synthesis of a polybrominated dibenzofuran mixture

The PBDF mixture was synthesized using the modified method of Neupert et al. [34]. Dibenzofuran (5 g, 29.8 mmol), aluminum bromide (0.5 g, 1.87 mmol), iron powder (0.5 g, 8.98 mmol) and 2 mL elemental bromine (39.0 mmol) were placed in a 50 mL flask and heated for 60 min to 130 °C. After that, another 2 mL of bromine was added and the solution was again heated for 120 min to 130 °C. The bulk of excess bromine was removed by distillation with a water cooled Liebig condenser at 80 °C. The remaining reaction mixture was dissolved in 200 mL DCM and treated with 100 mL saturated sodium thiosulfate solution to remove remaining bromine. The organic phase was concentrated to 5 mL and further purified by column chromatography [35]. The glass column (inner diameter 2.3 cm) was filled with deactivated silica gel (21 g silica gel, deactivated with 9 g demin. water) and covered with a 1 cm layer of anhydrous Na₂SO₄. PBDFs were eluted with 420 mL *n*-hexane followed by 100 mL *n*-hexane/toluene (95:5, v/v) and 100 mL toluene

into one flask. After removal of the solvent, the final weight of the PBDF mixture was 5.6 g. Its average bromine content was determined by elemental analysis (EA) [36]. Aliquots of the PBDF mixture (~1 mg dissolved in 20 μL *n*-hexane) were filled into 2.0 × 5.0 mm tin capsules for liquid samples (99.9% Sn, IVA Analysentechnik, Meerbusch, Germany) and the solvent was vaporized over night at room temperature before the capsules were sealed and analyzed in the elemental analyzer.

2.4. Gas chromatography coupled to mass spectrometry (GC/MS)

GC/MS measurements were performed with an Agilent 7890/5975c system (Waldbronn, Germany) equipped with a Gerstel CIS-4 PTV injector (Mülheim, Germany) and an Agilent HP-5 MS UI column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The thermally labile octabromodibenzofuran (octaBDF) was analyzed on a shorter HP-5 MS column (15 m × 0.25 mm internal diameter, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The temperatures of transfer line, quadrupole and ion source were set to 300 °C, 150 °C and 150 °C, respectively. The PTV temperature program started at 80 °C (0.01 min), followed by ramps of 700 °C/min to 300 °C (2 min) and 10 °C/min to 260 °C until the end of the run [24]. For both columns the GC oven program started with 50 °C for 1 min. Then, the temperature was raised with 10 °C/min to 300 °C (14 min) (modified from Bendig et al. [24]). Helium (purity 5.0, Westfalen, Münster, Germany) was used as the carrier gas with a constant flow rate of 1.2 mL/min. One microliter was injected and measurements were performed by electron ionization (EI) and electron capture negative ion (ECNI) MS operated in the full scan mode (*m/z* 50–800). In GC/ECNI-MS mode, methane (purity 5.5, Air Liquide, Bopfinger, Germany) was used as reagent gas (2 mL/min, source pressure of 1.6 × 10⁻⁴ Torr).

Confirmatory measurements were carried out on a polar GC column installed in an HP 5890/5971A GC/MS system equipped with an HP 7673A autosampler (Hewlett-Packard, Waldbronn, Germany). The temperatures of the injector and transfer line were set to 250 °C, and 280 °C, respectively. In splitless mode, 1 μL sample solution was injected onto an Rtx-2330 column (60 m length × 0.25 mm internal diameter, 0.1 μm film thickness, Restek, Bellefonte, PA, USA). Helium was used as the carrier gas with a constant flow rate of 1 mL/min. The GC oven program started with a ramp of 6 °C/min from 60 to 150 °C, followed by 4 °C/min to 190 °C and finally 7 °C/min to 250 °C which was held for 7 min [37]. Measurements were performed in full scan mode (*m/z* 50–550).

2.5. Countercurrent chromatography CCC

Analyses were performed with an AECS Quickprep MK8 instrument, consisting of the CCC centrifuge (holding four coils in two bobbins with a total coil volume of 471 mL) (AECS, London, United Kingdom), a ternary Beta 50 pump, a Flash 10 diode array detector set at 231 nm (both from Ecom, Praha, Czech Republic) and a Gilson 203B fraction collector (Middleton, WI, USA) [29]. Rotation speed was set at 865 rpm. The solvent system (*n*-hexane/toluene/ACN, 45:10:45, v/v/v) was used in tail-to-head mode. The mobile phase (upper phase) was pumped at 4 mL/min. Displacement of stationary phase (lower phase) was ~15% of the total coil volume until equilibrium was reached during rotating the CCC centrifuge. An aliquot of the synthesis mixture (350 mg), dissolved in 10 mL of the solvent system, was injected into the CCC system by means of a 10 mL sample loop. After a delay of 51 min, 80 fractions of 7.5 mL (1.875 min per fraction) were collected. The CCC fractions were evaporated to dryness by means of a gentle stream of nitrogen. After weighing (sample weights in Table S1, supporting information), the samples were re-dissolved in 1 mL toluene. Aliquots of the samples

Download English Version:

<https://daneshyari.com/en/article/1198701>

Download Persian Version:

<https://daneshyari.com/article/1198701>

[Daneshyari.com](https://daneshyari.com)