PHOTOLYTIC DEHALOGENATION OF THE NATURAL PRODUCT Q1 AND SCREENING SAMPLES FOR THE OBTAINED HEXACHLORO ISOMERS

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Introduction

Due to their bioaccumulative character and persistency, halogenated natural products (HNPs) are recognized as potential contaminants of marine environmental samples and food.[1] While most environmentally relevant HNPs are brominated or mixed-halogenated compounds, Q1 (Figure 1a) is an exclusively chlorinated HNP.
Q1 was detected in diverse marine samples such as fish, as well as marine mammals and birds at concentrations sometimes exceeding those of anthropogenic halogenated pollutants.[2,3] In theory, 79 mono- to heptachlorinated 1'-methyl-1,2'-bipyrroles are possible. However, next to Q1 only one out of five possible hexachloro congeners (Q1-hex) was identified as a by-product of the lab-synthesis of Q1 (Figure 1b-f).[3,6] In addition, traces of hexachloro isomers but no lower chlorinated homologues were tentatively detected based on the GC/MS screening of predicted mass-to-charge ratios.[4]
The goal of this study was the photolytic production of hexachlorinated homologues from Q1 followed by screening of relevant marine biotic samples in order to determine the environmental relevance for these congeners.

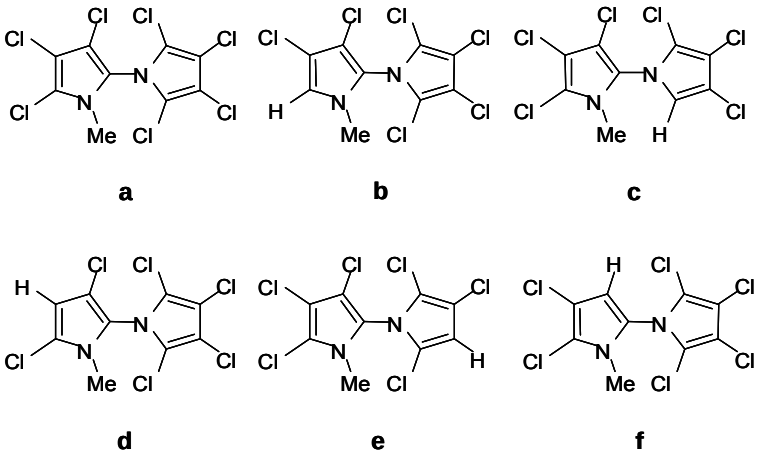

Figure 1: Structures of (a) 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1 or HMBP-isomer #79), (b) 2,3,3',4,4',5-hexachloro-1'-methyl-1,2'-bipyrrole (#74), (c) 2,3,3',4,4',5'-hexachloro-1'-methyl-1,2'-bipyrrole (#75), (d) 2,3,3',4,5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (#76), (e) 2,3,3',4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (#77; Q1-hex, H2), and (f) 2,3,4,4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (#78).

Materials and Methods

Samples and Chemicals: Adipose tissue of an Antarctic brown skua (*C. skua lonnbergi)* and blubber of an Australian melonhead whale blubber sample (*P. electra*) were purified as described elsewhere.[7,8] Q1, Q1-hex, and the internal standard 4,6-dibromo-2-(2´,4´-dibromo)phenoxianisole (BC-2) were recently synthesized and isolated in our research group.[5,9] Solvents, reagents and gases were of best commercially available quality.

Experimental performance, sample preparation and analysis: Dechlorination of Q1 was conducted using UV-light emitted by a 150 W medium pressure mercury vapor lamp (TQ150, Heraeus Noblelight, Hanau, Germany), which was placed in a cooling jacket consisting of quartz glass. No filters were put between the light source and the reaction vessel. The reaction was carried out in quartz beakers (60 mm in height, 30 mm in diameter) which were covered with a penetrated Teflon disk. The beakers were held at room temperature by a constant flow of water. Between 5.1 µg and 102 µg Q1 in 1 mL *n*-hexane, respectively, were irradiated under constant stirring for 5, 10, 15, 20, 25, 30, and 60 min. Control samples without Q1 were irradiated as well. The reaction solutions were transfered in amber 1.5 mL vials, BC-2 was added as internal standard, and the samples were stored in the dark at 4 °C prior to analysis. One microliter of each extract was analyzed by GC/MS. All experiments were performed in duplicates.

GC/ECNI-MS: GC/MS measurements were performed with a CP-3800/1200 GC/MS system (Varian, Darmstadt, Germany). Helium 5.0 was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and transfer line temperatures were set at 250 °C and 280 °C, respectively. The scan rate was two cycles per second and the filament emission was set at 150 µA. GC analyses were performed with a Factor Four™ VF-5ms column (30 m x 0.25 mm i.d. x 0.25 µm d_f, Varian). The GC oven temperature programm started at 70 °C (hold time 1.5 min), which then was raised at 30 °C/min to 140 °C, at 3 °C/min to 210 °C and finally, at 20 °C/min to 270 °C (hold time 4.83 min). The total run time was 35 min. Injections were performed in splitless mode (split opened after 2 min). A solvent delay of 5 min was applied. The electron energy was set at 70 eV and the ion source temperature was set at 200 °C. Methane 5.0 was used as the reagent gas at approximately 8.5 Torr, the SIM peak width was set at 0.5 u. The detector voltage was set at 1200 V. Full scan experiments were carried out in the range *m/z* 30 to *m/*z 650. In the GC/ECNI-MS-SIM mode, we monitored *m/z* 79.0/81.0/159.0/161.0/163.0 for the internal standard BC-2, *m/z* 281.9/282.9/283.9/284.9 for tetrachloro congeners of Q1 (Q1-Cl_4), *m/z* 315.9/316.9/317.9/318.9 for pentachloro congeners of Q1 (Q1-Cl_5), *m/z* 351.8/352.8/353.8/354.8 for hexachloro congeners of Q1 (Q1-Cl_6), and *m/z* 385.8/386.8/387.8/388.8 for Q1.

Results and Discussion

The GC/MS chromatogram of the start sample (0 min; no irradiation) showed the expected peaks for Q1 and the internal standard BC-2 (Figure 2a). No other peaks were detected in the chromatogram of the start sample.

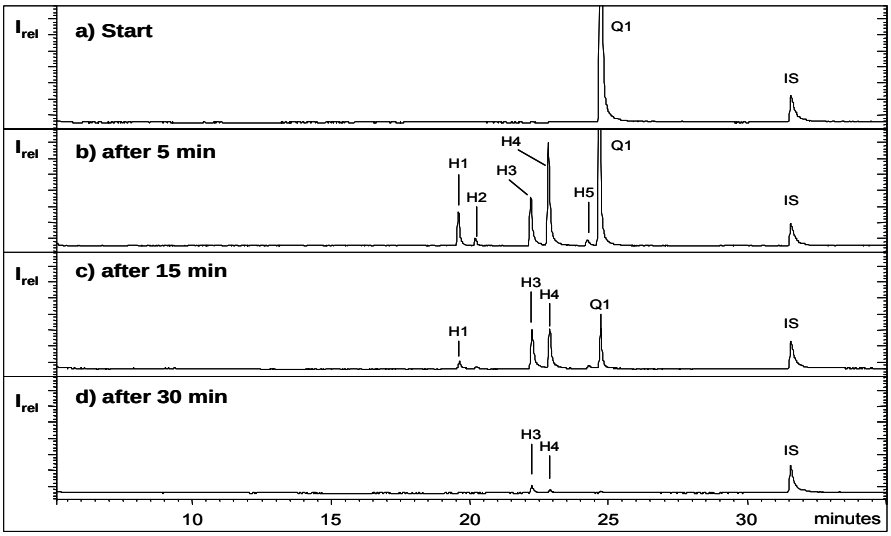

Figure 2: Photolytic Transformation of Q1 at (a) the start (no irradiation) (b) after 5 min, (c) 15 min and (d) 30 min of UV-irradiation. GC/ECNI-MS chromatograms (*m/z* 161+352+354 extracted from the TIC). Labels H1 to H5: hexachloro isomers of Q1. IS: internal standard BC-2. Note that *m/z* 352/354 are only minor fragment ions in the GC/ECNI-MS of Q1. The abundance of Q1 is ~100 fold higher than may be suspected from the mass chromatograms shown.

After 5 min irradiation of the Q1 solution, approximately half of the initial pool of Q1 was detected (Table 1). As can be seen from Figure 2b, all five possible hexachloro congeners (Figure 1b-f) were produced by reductive dechlorination of Q1 (Figure 2b). Using an authentic standard, H2 could be identified as Q1-hex (Figure 1e) but the structures of its isomers remain unknown. H1, H3 and H4 were major hexachloro isomers after 5 min of the photochemical transformation of Q1 whereas H2 and H5 were only detected in minute amounts (< limit of quantitation: < 0.3 %). Additionally, one pentachloro isomer (P1) was at detected (Table 1).

Table 1: Relative retention indices (RRI) of Q1 (defined as 1.000) and its hexachloro (H1 – H5) and one pentachloro (P1) congeners as well as their peak area (%) relative to Q1* as obtained during the photolytical dehalogenation of Q1.

	Q1	**H1**	**H2**	**H3**	**H4**	**H5**	**P1**
RRI	1.000	0.794	0.819	0.899	0,925	0,982	0.694
Start [%]	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
after 5 min [%]	55.2	1.3	0.2	2.1	4.3	0.2	0.1
after 10 min [%]	19.8	0.6	0.1	2.2	3.0	0.2	0.1
after 15 min [%]	7.3	0.2	n.d.	1.5	1.4	0.1	n.d.
after 20 min [%]	2.6	n.d.	n.d.	0.9	0.5	n.d.	n.d.
after 25 min [%]	0.9	n.d.	n.d.	0.4	0.2	n.d.	n.d.
after 30 min [%]	0.3	n.d.	n.d.	0.2	0.1	n.d.	n.d.

* ions of quantitation: Q1 *m/z* 385.8 + 387.8; hexachloro isomers: *m/z* 351.8 + 353.8; pentachloro isomers *m/z* 315.9 + 317.9; n.d.: not detected.

After 15 min irradiation, less than 10 % of the initial pool of Q1 was left (Figure 2c and Table 1). The steadily decreasing amount of Q1 corresponds with half lives of 5-6 min. After the initial formation at 5 min, all hexachloro congeners except H3 showed a continuous decrease over the entire experimental period. H3 concentrations were still on a high level after 15 min but then decreased as well (Table 1). After 30 minutes only traces of Q1, H3 and H4 were detected (Figure 2d). P1 was only found between 5 and 10 min. No further transformation products were detected throughout the experimental phase.

Once the relative retention times were established we screened relevant marine biotic samples from the Antarctic and Australia for the presence of the hexachloro congeners H1 - H5. In addition to the relative retention time, we used the ratio of *m/z* 352 to *m/z* 354 (100.00:80.26) as a second quality assessment parameter. It had to exceed 90 % of the theoretical ratio.

GC/ECNI-MS in the SIM mode proved that H3 and traces of H5 were present in the skua sample from the Antarctic (Figure 3b). Note that H3 had a similar retention time as oxychlordane on DB-5 like columns. Beside oxychlordane there was a second peak eluting prior H3 detected, but neither relative retention time, nor the ratio of *m/z* 352 to *m/z* 354 did match with one of the hexachloro congeners.

In the melonhead whale sample, all hexachloro isomers except H4 were detected (Figure 3c). H3 was the most abundant hexachloro homologue of Q1 in both marine biota samples. This is in contrast with the photochemical degradation experiments which yielded highest amounts for H4 (Figure 3a), the only hexachloro congener of Q1 not detected in the biological samples. Nevertheless, the amounts of H3 were < 0.1% of Q1.

Conclusions

Our irradiation experiments were suitable for the production of low amounts of all possible hexachloro isomers of Q1. Photolysis of higher amounts of Q1 in future work may be suited for the production of sufficient amounts of H1-H5 that will allow NMR structure elucidation. Particularly the identification of H3 will most likely benefit the understanding of the natural occurrence or metabolism of Q1. Irrespectively, it is not known whether H1, H2, H3, and H5 are also naturally produced or metabolites of the HNP Q1.

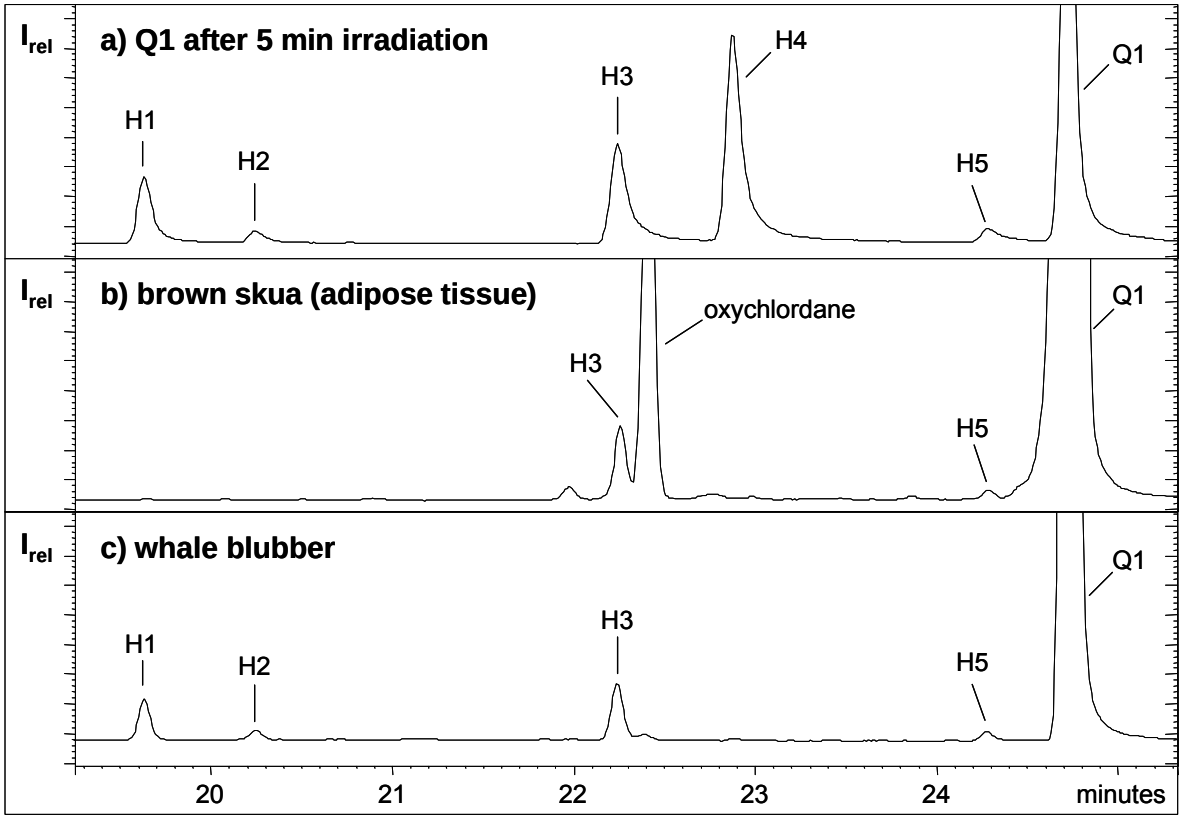

Figure 3: GC/ECNI-MS chromatograms (*m/z* 352+354 extracted from the TIC) of the photochemical transformation of Q1 after 5 min (a) as well as (b) purified extracts of adipose tissue of brown skua (*C. skua lonnbergi)* from the Antartic and (c) blubber of a melonhead whale (*P. electra*) from Australia.

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