

Technical Notes

Elucidation of a Polychlorinated Bipyrrole Structure Using Enantioselective GC

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A hexachloro congener (Q1-hex) of the natural heptachloro-1'-methyl-1,2'-bipyrrole Q1 was recently observed as a byproduct of the Q1 synthesis. NMR investigation confirmed that Q1-hex has a proton on a carbon in β -position to a nitrogen. Three isomers are possible that fulfill this prerequisite; unfortunately, however, the NMR data were not sufficient to distinguish among the three structural variants. Because only one isomer of Q1-hex is expected to be chiral, we utilized enantioselective gas chromatography and two chiral stationary phases to separate the atropisomers. Baseline separation of the Q1-hex atropisomers was obtained on 10% chemically bonded permethyl- β -cyclodextrin. In the full-scan mode, it was found that Q1-hex was racemic and that both atropisomers had identical mass fragmentation patterns. Partial resolution of the Q1-hex atropisomers was obtained on 25% *tert*-butyldimethylsilylated β -cyclodextrin diluted in PS086. In concert with previous NMR data, these enantioseparations prove that the structure of Q1-hex is 2,3,3',4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (5). To our knowledge, this is the first gas chromatographic separation of atropisomers of an axially chiral 1,2'-bipyrrole derivative.

Chlorinated hydrocarbons continue to interest environmental chemists, toxicologists, and scientists from other disciplines due to significant adverse effects to humans, wildlife, and ecosystems in general.¹ In addition to well-known contaminants such as PCBs, DDT, and other chloropesticides, a new generation of contaminants including polyhalogenated terphenyls, paraffins, naphthalenes, and diphenyl ethers are garnering the attention of environmental chemists.^{2,3} Consequently, any unknown contaminant has to be studied in order to obtain comprehensive knowledge of the threat caused by these xenobiotics. In recent studies, we have

detected the naturally produced heptachloro compound Q1 in various samples, mainly from the Southern Hemisphere.^{4–6} Subsequent studies including one where Q1 was synthesized concluded that this novel compound had a molecular formula $C_9H_3Cl_7N_2$ and a 1'-methyl-1,2'-bipyrrole backbone.^{6,7} Screening of the synthesized Q1 for related compounds resulted in the detection of a hexachloro congener (Q1-hex). Thus, enrichment and elucidation of the structure of Q1-hex was the goal of this study.

EXPERIMENTAL SECTION

Synthesis and Enrichment of Q1-hex. In the final step of the Q1 synthesis, *N*-(1-methyl-1H-pyrrol-2-yl)succinimide was treated with $PCl_5/POCl_3$ at 100 °C. Chromatographic separation and recrystallization from *n*-hexane yielded pure Q1.⁶ Q1-hex was found to elute from silica slightly before Q1. For the present study, we prepared a sample enriched with Q1-hex for 1H NMR investigation. The 1H NMR chemical shifts of Q1-hex were 3.33 (methyl group, s, intensity of 3) and 6.27 ppm (s, intensity of 1).

GC/EI-MS Analysis. Gas chromatography with electron ionization mass spectrometry (EI-MS) was carried out with a Hewlett-Packard 5890 gas chromatograph interfaced to a Hewlett-Packard 5971 mass-selective detector (Waldbronn, Germany). Helium was used as the carrier gas at a column head pressure of 1.1 bar. Sample injections were performed in the splitless mode with the split valve opened 1 min after the start of the analysis. The injector port and the transfer line were heated at 250 and 280 °C, respectively. The GC chiral stationary phase consisted of 10% permethyl- β -cyclodextrin (Chirasil-Dex, Chrompack) chemically bonded to a dimethyl polysiloxane backbone (CB- β -PMCD). The column had a length of 25 m, an internal diameter (i.d.) of 0.25 mm, and a film thickness (d_f) of 0.25 μm . In the full-scan mode (m/z 50–550; 1.5 scans/s), the GC oven program started at 120 °C (2 min), followed by heating rates at 20 °C/min to 150 °C (2 min), at 1.5 °C/min to 200 °C (2 min), and at 10 °C/min to 220 °C (5.17 min). The total run time was 50 min. In the selected ion monitoring mode we recorded m/z 352, 354, 315, 317, 280,

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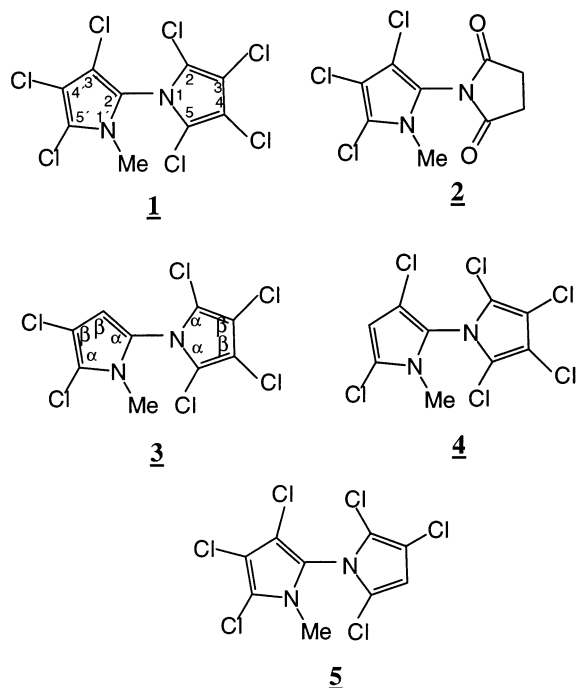


Figure 1. Structures of Q1 (**1**), the intermediate product *N*-(1-methyl-3,4,5-trichloro-1*H*-pyrrol-2-yl)succinimide (**2**), and the three isomers 2,3,4,4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (**3**), 2,3,3',4,5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (**4**), and 2,3,3',4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (**5**). α - and β -positions are denoted in **3**.

and 282 for Q1-hex, as well as m/z 386 and 388 for Q1. The GC oven was programmed as follows (hold times in parentheses): After injection at 80 °C (2 min) the oven was heated at 20 °C/min to 130 °C (2 min), then at 1 °C/min to 165 °C (2 min), and at 10 °C/min to 220 °C (5 min). The total run time was 54 min. A 30 m \times 0.25 mm i.d. fused-silica capillary column coated with 0.2- μ m 25% *tert*-butyldimethylsilylated β -cyclodextrin diluted in 85% methyl/15% phenyl polysiloxane (PS086) obtained from BGB Analytik (β -BSCD; Adliswil, Switzerland) was also used for comparison.⁸

RESULTS AND DISCUSSION

Structure assessment of the hexachloro congener of Q1 was made difficult by limited proton NMR data (only two independent signals) and the lack of previously published data for chlorinated 1,2'-bipyrroles. However, reference ¹H NMR spectra of 2,3,5-trichloro-*N*-methylpyrrole (β -H, 6.25 ppm⁹), 2,5-dichloro-*N*-methylpyrrole (β -H, 5.96 ppm¹⁰), 1,1'-dimethyl-2,2'-bipyrrole (β -H, 6.27 ppm¹¹), and 5,5'-dichloro-1,1'-dimethyl-2,2'-bipyrrole (β -H, 6.18 ppm¹¹) confirmed that the ring proton at 6.27 ppm is located at a β -position relative to pyrrole nitrogen. The chemical shift for protons at α -positions is \sim 0.5 ppm higher than for β -protons (i.e., $>$ 6.5 ppm). Three isomers with this structural feature (Figure 1, **3**–**5**) are possible; however, the true identity could not be distinguished from ¹H NMR spectroscopic data.

Identification of the intermediate product **2** during the Q1 synthesis suggested that the *N*-methyl (left) ring is fully chlorinated, leading us to assume that the ring proton is located on the right pyrrole unit (**5**). However, this could not be unequivocally proven by ¹H NMR. A comparison of the structures shows that Q1 and the hexachloro isomers **3** and **4** possess a symmetrically substituted right ring. In contrast, both ring structures of **5** are asymmetrically substituted. The absence of symmetry elements in orthogonal orientation and the nonplanarity of the two molecule units due to steric hindrance (atropisomerism) are requirements for axial chirality. Assuming that the energy barrier is high enough to hinder the rotation about the pyrrole–pyrrole C–N bond at the elevated temperatures needed to separate this compound on chiral stationary phases, GC enantioseparation is a suitable tool for the structural proof of **5**.

In view of the structural similarity of the hexachloro-1'-methyl-1,2'-bipyrrole (**5**) and atropisomeric PCBs, we checked the literature for CSPs capable of separating atropisomeric xenobiotics.^{12–18} Seventy-eight of the 209 PCB congeners are chiral, and formation of stable atropisomers has been predicted for 19 nonsymmetrically substituted tri- and tetraortho PCBs.¹⁹ A couple of chiral stationary phases (CSPs) have been suggested for the gas chromatographic PCB atropisomer separation after the pioneering work of König et al.¹² and Schurig and Glausch.¹³ Two of the CSPs, which together allow separation of 12 of the 19 stable PCB atropisomers,²⁰ were available in our laboratory (see Experimental Section).

Figure 2a shows the GC/EI-MS chromatogram of the enantio-separation of Q1-hex on CB- β -PMCD, a CSP that was initially synthesized by Schurig and co-workers.²¹ It should be noted also that CB- β -PMCD exhibits vastly different performance characteristics than polysiloxane-diluted permethyl- β -cyclodextrin.²⁰ In the full-scan mode, a near-baseline separation was achieved using CB- β -PMCD. Furthermore, the areas of the peaks were nearly identical, indicating a racemic composition. This was not surprising as the synthesis of Q1 was from an achiral starting material. To our knowledge, this is the first gas chromatographic separation of an axially chiral 1,2'-bipyrrole derivative. The GC/EI-MS spectrum of Q1-hex is shown in Figure 2b. As expected, the mass spectra of both enantiomers were identical, strongly supporting our observation that there is only one hexachloro isomer in the sample. The most abundant ions were (in order of increasing abundance) $[M]^+$ at m/z 350, for $[M - Cl]^+$ at m/z 315, and for $[M - 2Cl]^+$ at m/z 280. Additional fragment ions at m/z 168 (tentatively identified as a tetrachloropyrrole ion) and 132 were

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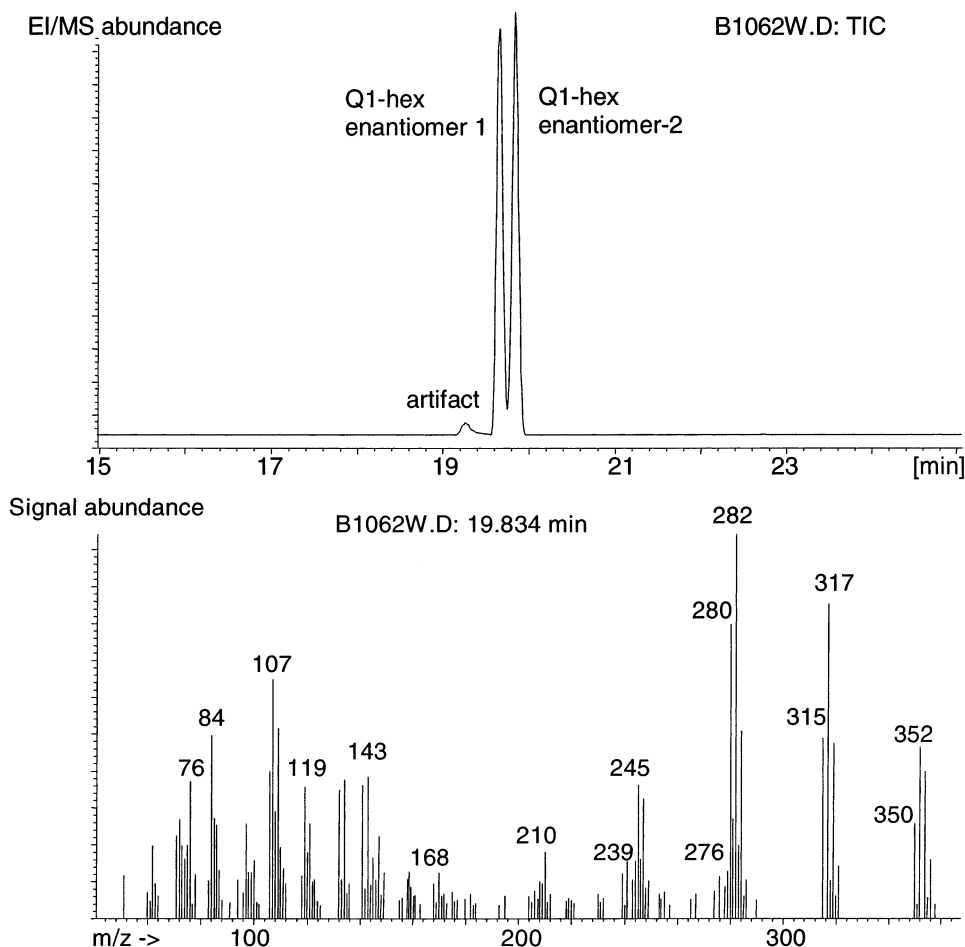


Figure 2. GC/EI-MS full-scan total ion chromatogram of the enantioseparation of Q1-hex on CB- β -PMCD (a) and EI-MS full-scan mass spectrum (m/z 40–550) of enantiomer 2 (b). The elution temperature of the enantiomers was 173 °C.

found in the lower mass range. The fragmentation pattern was comparable with Q1 except for a shift of 34 u to lower m/z values in the upper mass range due to the reduction of one Cl atom on Q1-hex. Modification of the GC oven program resulted in baseline separation of Q1-hex (elution temperature, 158 °C). Additional analysis of Q1-hex on the β -BSCD column resulted in a partial resolution of atropisomers (data not shown). The enantioresolution was clearly weaker relative to CB- β -PMCD. However, both CSPs demonstrated that Q1-hex is chiral. This in turn supports our original contention that the structure of Q1-hex is 2,3,3',4',5,5'-hexachloro-1'-methyl-1,2'-bipyrrole (5, Figure 1).

Chirality was used as early as 1949 as a criterion for the structural elucidation of halogenated compounds when Cristol established the configuration of aaeeee-hexachlorocyclohexane (α -HCH).²² However, in the first half of the 20th century, enantioselective chromatography was not yet available, and Cristol had

to prove the chirality of α -HCH by enantioselective breakdown of the less stable enantiomer with the chiral base brucine followed by establishment of a specific rotation of the enantioenriched solution.²² We therefore submit that the use of enantioselective GC or HPLC is a key tool for investigating the nature and fate of chiral organic compounds.

We employed enantioselective chromatography to elucidate the structure of Q1-hex. In the future, we hope to study the occurrence of Q1-hex enantiomers in the environment. At present, Q1-hex has not been detected in environmental samples, nor is it clear whether any congeners of Q1 (naturally formed or metabolites) exist. Our identification of Q1-hex thus opens the door for additional studies on the enantioselective breakdown of chiral compounds in nature.^{20,23}

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