

Structure elucidation of U82, a major octachloro chlordane congener present at higher trophic levels in marine food webs and in human tissue

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Introduction

The presence of the pesticide chlordane in food webs has been confirmed repeatedly [1,2]. Compared to the technical mixture, some important octachloro congeners such as MC5, MC7 and U82 are enriched in human tissue and in marine biota at higher trophic levels [2,3]. This selective accumulation was claimed to be structure related. Dearth and Hites [3] reported that such compounds with a "5+3" chlorine substitution between both rings are more accumulated than "6+2" structures. Furthermore, the low rate of metabolisation of certain chlordane congeners such as MC5 was related to the presence of an endo-chlorine atom at the carbon atom C2 [4].

The isolation of U82 as well as of MC5, MC7 and MC8 by reversed and normal phase high performance liquid chromatography (HPLC) has been described before [5]. The electron capture (ECD) and negative ion chemical ionization (NICI) response factors were also determined for these congeners and the consequences for quantification evaluated [5]. The "5+3" chlorine substitution of U82 was determined earlier by NICI mass spectrometry [6]. However, the exact structure was not elucidated.

Experimental

Standard and solvents: Cyclohexane of pesticide grade was supplied by Malincrodt (Switzerland). For all NMR experiments U82 was re-dissolved in deuterated chloroform with an isotope purity > 99.95 % D (Merck, Germany). The total amount available of U82 was about 300 µg.

Mass spectrometry: Mass spectra were recorded with a Hewlett Packard (HP) 5989B mass spectrometer connected to a 5890 series II gas chromatograph (GC). The ion source temperature was 200°C and of the quadrupole 100°C. The transfer liner temperature was held at 250°C. The electron energy was 70 and 100 eV for electron impact (EI) and NICI, respectively. The scan time for a mass range of m/z 30-500 was 1.25 s. Methane was applied as reagent gas for NICI with a source pressure of 0.6 mbar. The GC was equipped with an Ultra 1 (polymethylsiloxane, HP) capillary column of 22 m length, 0.25 mm ID and a film thickness of 0.14 µm. Splitless injec-

tions were performed with a split time of 2 min and an injector temperature of 250 °C. The temperature program was as follows: 50 °C for 2 min, then 3°C/min to 240°C, isothermal 2 min.

¹H-NMR: All ¹H-NMR experiments were performed on a Bruker ARX 500 spectrometer at 500.13 MHz. The chemical shifts, δ , were determined relative to the solvent peak (δ CDCl₃ = 7.26 ppm relative to TMS). The one dimensional ¹H-NMR spectras were recorded at 4, 22 and 45°C to optimize the peak separation. Homo decoupling experiments were performed to assign the coupling partners in the spin system. Neighborhood correlations were partly established with 2 dimensional nuclear overhauser enhancement spectroscopy (NOESY). The 2d spectrum was recorded with 640 increments and 4096 data points on each increment. The total accumulation time was 38h and the mixing time 200ms. The spin system was computer simulated using the Pearch program package (Department of Chemistry, University of Kupio, Finland).

Results and discussion

Mass spectrometry: The molecular ion of U82 (m/z 406) was the base peak in the NCI mass spectrum. Other abundant fragment ions were m/z 337 ($M-2Cl+H$)⁺, m/z 298 ($M-3HCl$)⁺ and m/z 264 ($M-2Cl-2HCl$)⁺. The molecular ion m/z 406 was also present in the EI mass spectrum, but the intensity was low. Losses of hydrogen and chlorine from the molecular ion were also observed such as m/z 371 ($M-Cl$)⁺, m/z 335 ($M-Cl-HCl$)⁺ and m/z 299 ($M-Cl-2HCl$)⁺. The most intensive fragment ion in the EI mass spectrum was m/z 236 resulting from a retro Diels-Alder cleavage. It the "5+3" chlorine substitution of the rings.

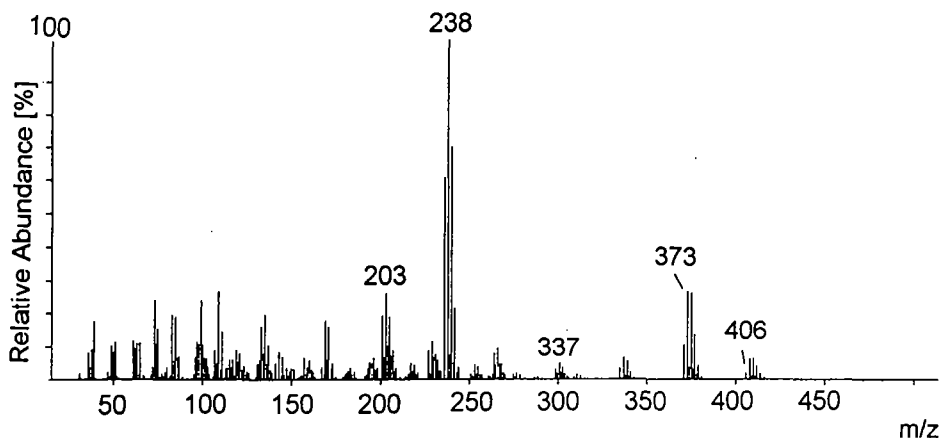


Figure 1: EI mass spectrum of U82

NMR: The interpretation of the ¹H-NMR experiments was based on previously published data for chlordane congeners [7,8]. To achieve the complete assignment of the spin system including values for the coupling constants and chemical shifts a computer simulation of the ¹H-NMR spectrum was made.

Decoupling H2 (see Table 1, Figure 2 and Figure 3) at the chemical shift $\delta = 4.064$ ppm revealed H1 and H3 as spin coupling partners. The spin coupling partnership was further confirmed between H2 and H3 by decoupling H3 ($\delta = 3.633$ ppm). Furthermore, partnership could be established between H7 and H7a by decoupling H7 ($\delta = 3.633$ ppm). Decoupling H3a ($\delta = 3.466$ ppm) affected the spin system of H3.

The information extractable from the 2d NOESY spectrum was limited. Signal overlap made the interpretation of the spectrum rather complex. Nevertheless, neighborhood correlations could be established between H2 and H1 as well as between H2 and H3. Furthermore, H3 and H3a were determined to be neighbors as well as H3a and H7a.

No signal in the $^1\text{H-NMR}$ spectrum had a higher chemical shift than $\delta = 4.2$ ppm implying that U82 has no olefinic protons. These have chemical shifts of $\delta \sim 6$ ppm in chlordane structures [7,8]. Furthermore, U82 has only vicinale protons (each carbon atom are connected to zero or one proton), since no signals appeared at chemical shifts of $\delta < 3.4$ ppm, and all coupling constants in the $^1\text{H-NMR}$ spectrum were below $J = 11$ Hz. Chlordane structures with geminale protons typically have chemical shifts of $\delta 1.5\text{-}2.6$ ppm and coupling constants of $J > 14$ Hz [7].

The isolated double doublet (dd) observed at $\delta = 4.06$ ppm is characteristic for a proton in exo position on carbon C2 (see the comparison with MC5 in Table 1). A corresponding endo proton would give a signal shifted downfield to $\delta \sim 4.5$ ppm [7]. Coupling constants of $J = 8.1$ to 10.1 Hz indicate that the protons on C1, C2 and C3 are positioned endo-exo or exo-endo to each other. Vicinale coupling constants for protons in endo-endo conformation have been determined to $J = 4.1$ Hz for cis-chlordane [7].

Vicinale coupling constants of $J = 10.1$ and $J = 10.3$ Hz, were measured between H1 and H2-exo and between H3 and H2-exo, respectively. This implies that H1 and H3 are positioned endo to H2. The chemical shifts of H1-exo, H2-endo and H3-exo supports this further. H1-endo and H3-endo couples with H7a and H3a, respectively. The coupling constant between these protons was determined to $J \sim 8$ Hz revealing an exo configuration of H3a and H7a. The simulation of the $^1\text{H-NMR}$ spectrum provided the vicinale coupling constant $J = 9.8$ Hz between H3a-exo and H7a-exo, confirming that these protons are located in exo-exo configuration. H7a-exo showed an additional coupling constant of $J = 4$ Hz which relates it to the proton in position C7 with a signal at the chemical shift of $\delta = 3.633$ ppm. The H3a-exo has no additional coupling constant which excludes any protons on carbon C4 and C8.

As shown earlier, U82 is chiral [5]. The H1-endo, H2-exo and H3-endo configuration in combination with chlorines on C5 and C6 exclude any hydrogens connected to C8. The molecule would in this case have a symmetry plane through C2 and C8 and would, therefore, not be chiral.

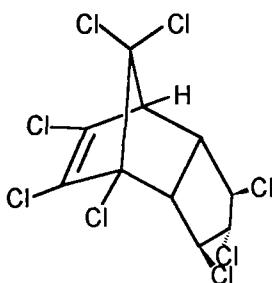


Figure 3: Structure of U82

Table 1. $^1\text{H-NMR}(\text{CDCl}_3)$ data of U82 and other selected chlordanes.

Compound	Chemical shifts(δ , ppm) Coupling constants (J, Hz)							
	H-1 (endo)	H-1 (exo)	H-2 (exo)	H-3	H-3a	H-7a	H-5	H-7
U82 measured	3.56-3.51 (m)		4.064 (t) (10.3)	3.596 (dd) (10.2, 8.1)	3.444 (dd) (9.76, 8.25)	3.495 (dd) (8.4, 3.8)		3.634 (d) (3.7)
U82 simulated	3.534 (dd) (10.29, 8.76)		4,064 (dd) (10.29, 10.23)	3.633 (dd) (10.23, 8.36)	3.466 (dd) (9.82, 8.36)	3.505(ddd) (9.82, 8.76, 3.97)		3.633 (d) (3.97)
MC5*	3.61-3.70 (m)		4.09 (t) (10.1)	3.61-3.70 (m)	3.50-3.53 (m)	3.50-3.53 (m)	6.36 (s)	
cis- chlordane*	1.82 (ddd) (14.7, 8.9, 4.5)	2.43 (ddd) (14.7, 8.2, 2.2)	4.44 (ddd) (4.5, 4.1, 2.2)	3.97 (dd) (8.1, 4.1)	3.55 (dd) (9.7, 8.1)	3.73 (ddd) (9.7, 8.9, 8.2)		

* Values from ref. [7].

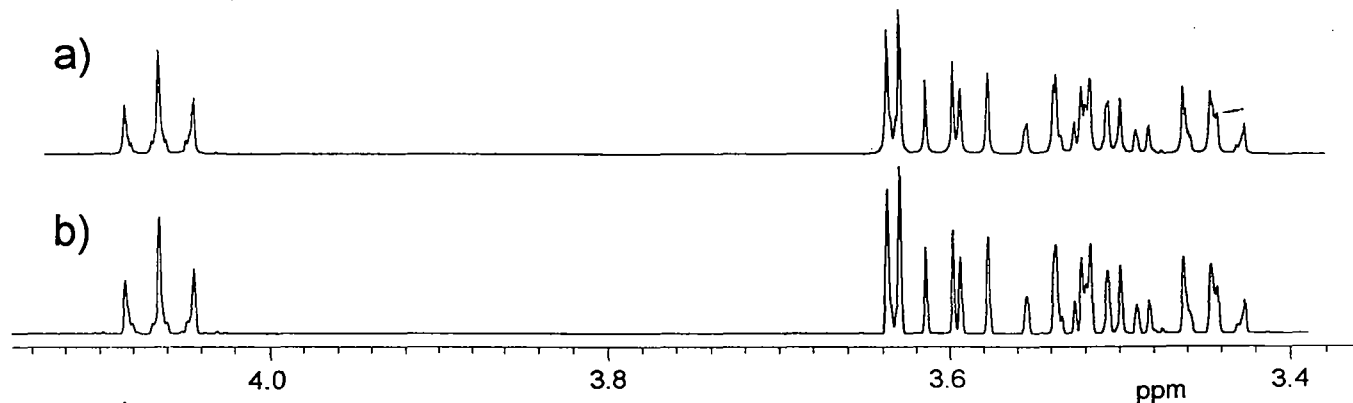


Figure 2. $^1\text{H-NMR}$ spectrum of U82, a) observed, b) simulated with Pearch

Conclusions

According to IUPAC nomenclature, the name of U82 is 1-exo,2-endo,3-exo,4,5,6,8,8-octachloro-3a,7,7a-trihydro-4,7-methanoindane. U82 was confirmed to be "5+3" chlorine substituted in the rings. The C2 position in U82 is endo-chlorine substituted while C1 and C3 both have exo-chlorines. U82 has this in common with congeners such as trans-nonachlor and MC5, which are known to accumulate more in human tissue than for example cis-nonachlor and MC7 [1,3].

The dechlorination of trans-nonachlor into trans-chlordane was determined to be the rate limiting step in both humans and rats [9]. This would also explain the high accumulation factor of U82 in human tissue relative to technical chlordane (about 42, see [3]). Cis- and trans-chlordane together with MC7 have no exo-chlorine at C2. Their accumulation factors are <2 relative to technical chlordane.

Finally, to the best knowledge of the authors, U82 has never been obtained from photochemical experiments in comparison to some other octa-chlordanes such as MC1, MC2, MC3 and MC5 which all were photochemically produced [10].

Acknowledgments

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