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NMR spectroscopy in environmental chemistry: ^{1}H and ^{13}C NMR parameters of tricyclic polychlorinated C_{10} hydrocarbons and their oxy derivatives based on two-dimensional NMR techniques

Erkki Kolehmainen,* Jari Koivisto, Katri Laihia, Reijo Kauppinen and Jaakko Paasivirta

Department of Chemistry, University of Jyväskylä, P.O.B. 35, FIN-40351 Jyväskylä, Finland

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ABSTRACT: Two-dimensional homo- and heteronuclear NMR chemical shift correlation techniques were applied in the characterization of five tricyclic polychlorinated C_{10} hydrocarbons, chlordene (**1**), heptachlor (**2**), *trans*-nonachlor (**3**), α-chlordene (**4**) and γ-chlordene (**5**), which are spread globally in the environment owing to their use as insecticides. Approximate and partly contradictory ^{1}H and ^{13}C NMR chemical shifts reported in the literature were corrected in this work. The chemical shift assignments of **1–5** were based on DQF COSY, HMQC and HMBC experiments. In addition, an INADEQUATE experiment was needed to ascertain the ^{13}C chemical shifts assignment of **2**. The ^nJ(H,H)s of **1–5** were solved by computer-assisted spectral analyses. Further, complete ^{1}H and ^{13}C NMR parameter sets of three oxy derivatives, heptachlor *exo*-epoxide (**6**), 1-*exo*-hydroxychlordene (**7**) and its acetate (**8**), were also determined. Compounds **7** and **8** were synthesized to be used as model compounds, and their NMR parameters are reported for the first time. By using solvent susceptibility matched symmetrical micro-NMR tubes and HMQC and HMBC experiments, **1–5** can be reliably characterized at submilligram levels by their ^{1}H and ^{13}C NMR parameters with a 500 MHz (11.8 T) spectrometer and a 5 mm diameter standard probehead. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; two-dimensional NMR; tricyclic polychlorinated C_{10} hydrocarbons; oxy derivatives; computer-assisted spectral analysis; solvent susceptibility matched micro-NMR tubes

INTRODUCTION

Tricyclic polychlorinated C_{10} hydrocarbons are globally widespread environmental toxicants owing to their use as insecticides and their chemical persistence.[1,2] The identification of the components of chlordane (formerly one of the most widely used pesticides) and their metabolites and/or environmental degradation products is most often based on gas chromatographic–mass spectrometric techniques.[3] However, for their configuration and isomer-specific structure elucidation, ^{1}H NMR parameters are of predominant importance, as demonstrated in the case of *endo*- and *exo*-epoxides of heptachlor (**2**).[4] Further, the biological transformation of isomers, diasteremers and even enantiomers of toxicants can be (stereo)selective, and their uptake, metabolism and excretion can be very different.[3,4] In the literature there are some ^{1}H and ^{13}C NMR data on polychlorinated tricyclic C_{10} hydrocarbons with more or less incomplete spectral analyses and/or contradictory chemical shift assignments.[4–8] The aim of this study was to apply two-dimensional homonuclear NMR techniques, DQF COSY[9], NOESY[10,11] and INADEQUATE,[12] and proton detected, two-dimensional heteronuclear chemical shift correlation techniques, HMQC[13,14] and HMBC,[15] to ascertain the ^{1}H and ^{13}C NMR chemical shift assignments of **1–5** and their oxygen-containing derivatives. ^3J(H,H)s of these rigid tricyclic structures can be further utilized in refining the structural vs NMR spectral relationships. Solvent susceptibility matched micro-NMR tubes were tested for some of these compounds especially to determine their detection limits and to clarify the effect of resonance lineshape on their computer-assisted spectral analysis.

* *Correspondence to*: E. Kolehmainen, Department of Chemistry, University of Jyväskylä, P.O.B. 35, FIN-40351 Jyväskylä, Finland.
E-mail: ekolehma@cc.jyu.fi

EXPERIMENTAL

Compounds

Chlordene (**1**) (4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene), heptachlor (**2**) (1-*exo*,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene), *trans*-nonachlor (**3**) (1-*exo*,2-*endo*,3-*exo*,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), α-chlordene (**4**) (1,2,3,5,7,8-hexachloro-1,3a,4,5,6,6a-hexahydro-1,4-ethanopentalene), γ-chlordene (**5**) (2,3,3a,4,5,8-hexachloro-3a,6,7,7a-tetrahydro-1,6-methano-1*H*-indene) and heptachlor epoxide (**6**) (1-*exo*-4,5,6,7,8,8-heptachloro-2,3-*exo*-epoxy-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene) were commercial products: **1** (100%)

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from EPA, Research Triangle Park, NC, USA, **2** (99.5%) from Velsicol Chemical Manufacture Laboratory (Rosemont, IL) and **3** (98.3%), **4** (97.1%), **5** (100%) and **6** (100%) from EPA. Two derivatives which also can serve as model compounds, **7** (1-*exo*-hydroxy-4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene) and its acetate **8**, were synthesized by us. Compound **7** was formed from **1** by SeO_2 oxidation in aqueous 1,4-dioxane[18] and **8** was formed as a by-product (2% yield) of the SeO_2 oxidation of **1** in aqueous acetic acid.[17] Compound **7** was purified by column chromatography (silica gel–chloroform) and **8** by crystallization from *n*-pentane. The structure of the novel compound **8** was verified by hyberresolution mass and NMR spectrometry. The ^1H and ^{13}C NMR spectral parameters of **7** and **8** were determined for the first time in this work.

Spectroscopy

All NMR experiments were performed with a Bruker Avance DRX 500 NMR spectrometer equipped with a 5 mm diameter broad band probehead working at 500.13 MHz in ^1H and 125.77 MHz in ^{13}C. The chemical shift assignments are based on DQF COSY, HMQC and HMBC measurements. The ^1H NMR parameters were solved precisely by WIN-NMR and WIN-DAISY software.[18] For the ^{13}C NMR chemical shift assignment of heptachlor (**2**) a 2-D INADEQUATE measurement was also necessary. Owing to the insensitivity of this experiment (based on chemical shift correlations transmitted by the direct spin–spin couplings between adjacent carbon-13 nuclei with a natural abundance of 1.1%), the concentration of heptachlor had to be as high as possible in this experiment: *ca* 300 mg of heptachlor dissolved in $CDCl_3$, the total sample volume being 0.6 ml. In NOESY experiments, a 300 ms mixing time was used. Detailed lists of the acquisition and processing parameters used in all of these measurements are available on request.

The sensitivity tests were carried out using 5 mm diameter and 15 mm bottom length symmetrical NMR microtubes (Shigemi) which are magnetic susceptibility matched for $CDCl_3$. $CDCl_3$ was obtained from Eurisotop (water content <0.01%, 99.8% D).

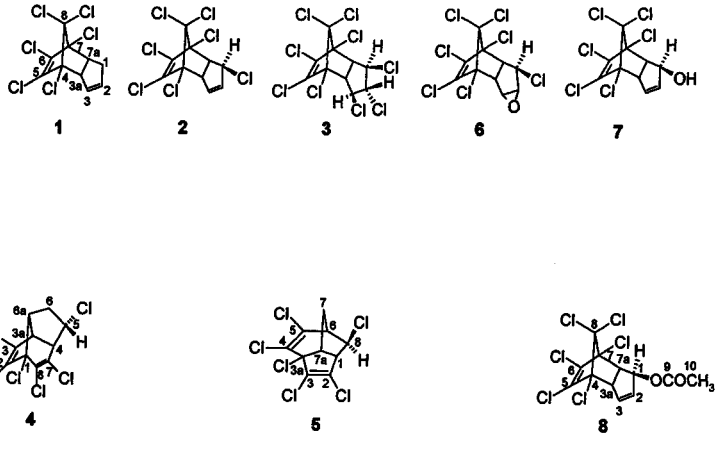
RESULTS AND DISCUSSION

Scheme 1 shows the structures of the compounds studied and their numbering. Tables 1–4 give the ^1H and ^{13}C NMR chemical shifts and spin–spin coupling constants for **1–8**. Figures 1 and 2 show the HMQC and INADEQUATE countour maps of heptachlor. In Fig. 3 are plotted an experimental (measured in a Shigemi micro-NMR tube) (top) and a calculated (WIN-DAISY) (bottom) 500 MHz ^1H NMR spectrum of heptachlor.

As mentioned before, a variety of NMR chemical shift assignments have been published for these compounds,[4–8] partly contradicting those obtained in this work. In order to obtain a reliable basis for our spectral assignments, a 2-D INADEQUATE (COSY-like[12b]) correlation map was recorded for **2**. This experiment reveals that (i) carbons 60.40 and 80.15 ppm are adjacent and that (ii) those with chemical shifts of 137.51, 130.95, 60.68 and 81.92 ppm are part of a linear system. An HMBC connectivity between H-3a and C-5 gives the final verification for the correct assignment for C-5 and C-6 of **2**. This assignment is also in agreement with that given by ApSimon *et al.*[5] On the other hand, the ^1H and ^{13}C NMR chemical shift assignments for **3**, **4** and **5** reported previously[6] have several deviations in comparison with our present results. Further, as far as we know, the ^nJ(H,H) data for **3** have not been reported previously.

Among the compounds studied, **1**, **2**, **7** and **8** have the same carbon skeleton, thus forming a basis for the comparison of substituent effects. Introduction of an electronegative substituent at position 1′ in **1** causes a clear downfield (deshielding) shift or α-effect on the proton H-1, being 2.46 ppm in **2** and 2.39 ppm in **7**. When the hydroxy substituent of **7** is substituted by an acetoxy moiety as in **8**, a 3.20 ppm downfield shift from the value in **1** is observed. The corresponding β-effects induced at protons H-2 and H-7a in **2**, **7** and **8** are much smaller, 0.22 ppm being the highest (at H-7a in **7**).

The same trends as observed in the ^1H NMR chemical shifts, joint with substitution, are also apparent in the ^{13}C NMR data (Table 2). Of course, the substituent chemical shifts (SCS) are much larger than in the case of protons because the ^{13}C NMR chemical shifts generally span a



Scheme 1

Table 1. ^{1}H NMR chemical shifts (ppm from $CHCl_3$, $\delta = 7.26$ ppm) of **1–8**

	$\delta(^1H)$ (ppm)							
Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	2.361	4.821	3.596	—	3.290	4.279	4.753	5.563
1′	2.499[a]	—	—	—	—	—	—	—
2	5.831	5.950	4.048	—	—	3.703	5.955	5.947
3	5.633	5.912	3.596	—	—	3.681	5.919	5.984
3a	3.860	4.073	3.544	3.808	—	3.672	3.986	3.985
4	—	—	—	3.110	—	—	—	—
5	—	—	—	4.732	—	—	—	—
6	—	—	—	2.577	3.139	—	—	—
6′	—	—	—	2.182[b]	—	—	—	—
6a	—	—	—	3.388	—	—	—	—
7	—	—	—	—	2.255	—	—	—
7′	—	—	—	—	2.388[c]	—	—	—
7a	3.459	3.643	3.544	—	3.724	3.298	3.241	3.282
8	—	—	—	—	4.057	—	—	—
10	—	—	—	—	—	—	—	2.036
OH	—	—	—	—	—	—	1.861	—

[a] H-1′ is *exo*.
[b] H-6′ is *syn* to Cl-5.
[c] H-7′ is *syn* to Cl-8.

Table 2. ^{13}C NMR chemical shifts (ppm from $CDCl_3$, $\delta = 77.0$ ppm) of **1–8**

	$\delta(^{13}C)$ (ppm)							
Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	32.28	60.00	59.06	78.34	61.32	55.82	75.24	76.82
2	135.96	137.51	69.64	127.65	136.05	62.59	138.36	135.33
3	126.22	130.95	59.06	138.04	133.95	59.09	131.45	133.22
3a	61.07	60.68	58.40	53.59	75.13	56.14	60.54	60.50
4	82.40	81.92	79.90	55.73	133.55	78.94	81.87	81.69
5	131.74	132.07	132.45	63.43	134.77	131.41	131.45	131.49
6	129.05	128.64	132.45	33.85	56.43	130.44	128.98	129.10
6a	—	—	—	59.79	—	—	—	—
7	81.81	80.15	79.90	134.03	29.96	80.51	80.11	79.77
7a	49.16	60.40	58.40	—	57.68	61.24	59.69	56.52
8	103.61	103.55	103.92	126.67	56.61	103.21	103.98	103.90
9	—	—	—	—	—	—	—	169.80
10	—	—	—	—	—	—	—	20.94

range more than 200 ppm whereas in the case of ^{1}H NMR the spread is generally less than 15 ppm.

The vinyl protons at C-2 in **1** and **2**, showing the two most deshielded chemical shift values among compounds **1–5**, can be useful in determining these congeners in their mixtures. Similarly, protonated double bond carbons 2 and 3 in **1** and **2** can be easily separated by HMQC because all the other olefinic carbons bear substituents in compounds **1–5**.

As in the case of chemical shifts, compounds **1**, **2**, **7** and **8** form a proper subgroup (owing to their structural similarity) to be used in estimating the substituent effects on the spin–spin coupling constants. As a general observation, it can be mentioned that introducing an electronegative substituent, chlorine or hydroxy, into position 1′ in chlordene (**1**) causes decreases in the absolute values of all spin–spin coupling constants. The most significant change takes place in ^4J(H-1,H-3) which contains a double bond in its coupling route. The vicinal coupling constant ^3J(H-1,H-7a) also exhibits great changes upon substitution at C-1.

Regarding the computer-based spectral analysis, the sign of the spin–spin coupling constant is also an important parameter which always has to be taken into account. A rule of thumb is the 'alternate change of the sign' depending on the number of bonds included in the coupling route: $^2J < 0$, $^3J > 0$, $^4J < 0$, etc. The best result (the smallest difference between the experimental and calculated spectra) in the iterative total line fitting is obtained with a correct sign combination, as shown in a previous paper.[19] Among scalar couplings, the most frequently used parameter in the structural analysis is the vicinal coupling, ^3J, exhibiting well known dihedral angle dependences.[20,21] However, substituent electronegativity also has an influence on these three-bond couplings, as can be seen, for example, in the values of ^3J(H-1,H-7a)

Table 3. ^{n}J(H,H) spin–spin coupling constants of **1**, **2**, **3**, **6**, **7** and **8**

	^{n}J(H,H) (Hz)					
Protons	**1**	**2**	**3**	**6**	**7**	**8**
1,1′	−18.44	—	—	—	—	—
1,2	2.37	2.25	10.30	2.37	2.24	2.36
1,3	−2.37	−1.33	0.11	0.49	−1.23	−1.26
1,3a	3.33	2.80	−0.51	0.53	2.67	2.73
1,7a	3.45	2.09	8.56	2.86	1.84	1.98
1,OH	—	—	—	—	6.46	—
1′,2	2.25	—	—	—	—	—
1′,3	−2.49	—	—	—	—	—
1′,3a	1.73	—	—	—	—	—
1′,7a	9.93	—	—	—	—	—
2,3	5.81	5.62	10.30	2.05	5.69	5.77
2,3a	−1.79	−1.77	0.18	0.70	−1.81	−1.86
2,7a	−0.15	−0.35	0.18	0.43	−0.47	0.00
2,OH	—	—	—	—	0.00	—
3,3a	2.12	2.18	8.56	0.00	2.16	2.20
3,7a	0.00	−0.12	−0.51	0.76	0.00	0.00
3,OH	—	—	—	—	0.00	—
3a,7a	8.23	7.46	10.37	7.54	7.35	7.34
3a,OH	—	—	—	—	0.00	—
7a,OH	—	—	—	—	0.00	—

of **1**, **2**, **7** and **8**. These findings can be further utilized in refining structure–NMR parameter relationships.

Sensitivity tests

Sensitivity tests were performed for heptachlor (**2**), α-chlordene (**4**) and γ-chlordene (**5**) (representing three different molecular structures included in this work) by using symmetrical, $CDCl_3$ matched, Shigemi micro-NMR tubes, a direct, 5 mm diameter multinuclear probehead in a 500 MHz (^{1}H) instrument and inverse (proton) detection in heteronuclear chemical shift correlation experiments, HMQC and HMBC. The concentrations of the samples were 4.39 mM for **2**, 3.10 mM for **4** and 3.07 mM for **5**. As measured in Shigemi micro-NMR tubes, this means that the amount of each sample was <0.5 mg. In proton, composite pulse, decoupled ^{13}C NMR experiments run overnight, all carbons of **4** and **5** were clearly detected whereas in **2** carbons 4, 6, 7 and 8 did not give reliable resonance lines and carbon 5 was only poorly visible. In the case of **2**, HMBC improved the situation, revealing the signals of carbons 6 and 7. In the case

Table 4. ^{n}J(H,H) spin–spin coupling constants of **4** and **5**

4		**5**	
Protons	^{n}J(H,H) (Hz)	Protons	^{n}J(H,H) (Hz)
3a,4	3.70	1,6	1.04
3a,5	0.00	1,7	0.64
3a,6	0.48	1,7′	0.00
3a,6a	5.95	1,7a	6.83
4,5	0.52	1,8	1.73
4,6	1.31	6,7	0.89
4,6′	1.21	6,7′	4.61
4,6a	1.02	6,7a	2.17
5,6	6.49	6,8	0.46
5,6′	0.69	7,7′	−12.69
5,6a	1.04	7,7a	0.92
6,6′	−16.23	7,8	1.41
6,6a	0.86	7′,7a	4.32
6′,6a	7.48	7′,8	0.22
—	—	7a,8	−0.14

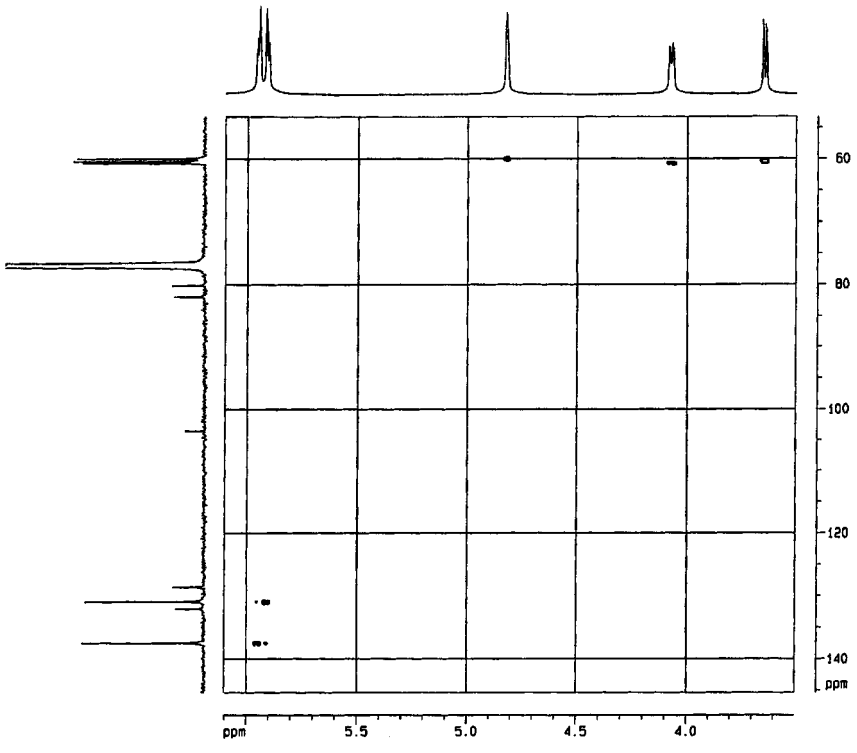


Figure 1. HMQC contour plot of heptachlor (**2**).

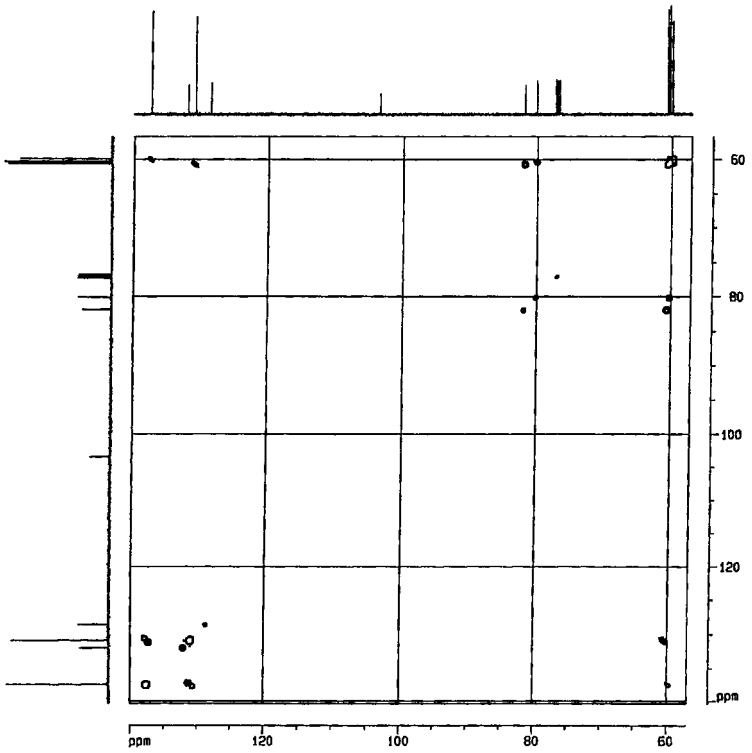


Figure 2. INADEQUATE contour plot of heptachlor (**2**).

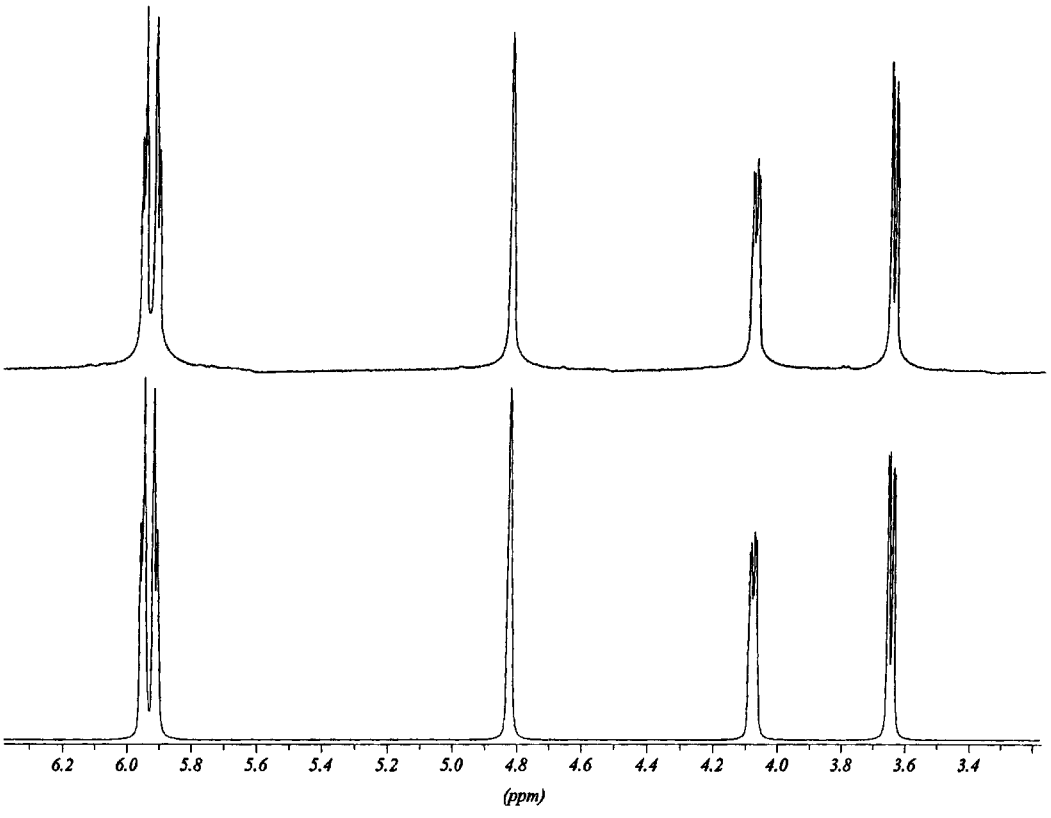


Figure 3. Experimental (measured in a Shigemi micro-NMR tube) (top) and calculated (WIN-DAISY)[18] (bottom) ^{1}H NMR spectra at 500 MHz of heptachlor (**2**).

of **4** and **5**, HMQC and HMBC gave complete chemical shift correlation data. By using an inverse, 5 mm broad band probehead, a reliable HMQC correlation map of **2** (4.39 mM) was produced in 3 h and of HMBC in 12 h. Unfortunately, an inverse probehead suffers from poor sensitivity in a direct ^{13}C measurement. This property is disadvantageous in cases where ^{13}C resolution is critical.[22] For polychlorinated hydrocarbons, the highest possible sensitivity in carbon detection is desirable. In our experience, the optimum production of both one-dimensional

^{1}H and ^{13}C NMR spectra and two-dimensional HMQC and HMBC correlation maps, needed for reliable spectral assignments and structure characterization of these tricyclic polychlorinated hydrocarbons, can be achieved with a direct probehead using solvent matched, micro-NMR tubes and inverse (proton) detection.

The ^{1}H NMR spectral lineshape measured in micro-NMR tubes differs from a pure Lorenzian line. This causes an increase in the *R*-factor (percentage difference between the experimental and calculated spectra) of a computer-based NMR spectral analysis performed by WIN-DAISY software. In the case of micro-NMR tubes, the *R*-values were $<3\%$ whereas in the case of normal, 5 mm diameter NMR tubes, the values were generally $<0.3\%$. According to the copyright owner of the WIN-DAISY software, *R*-values $<3\%$ are acceptable.[18] The main reason for increased *R*-values when using micro-NMR tubes is due to broad humps in spectral lines. Unfortunately, in spite of prolonged shimming, we were not able to remove totally these artefacts from the NMR spectral lines. Figure 3, describing an experimental (measured in a Shigemi micro-NMR-tube) (top) and a WIN-DAISY iterated (bottom) ^{1}H NMR spectrum of heptachlor (**2**), reveals that the result of computer analysis is satisfactory.

In conclusion, modern 2-D NMR measurement techniques provide a unique tool for isomer specific structure elucidation for polychlorinated hydrocarbons at submilligram levels. In addition to the structural data which are necessary for understanding the toxicity, metabolism and/or fate of these compounds, pushing the detection limits to concentrations as low as possible opens up new possibilities from an environmental analytical point of view.

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