Experience in Isolation and Identification of Toxaphene Congeners and Prospects of Congener-specific Analysis of Environmental Samples

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1. Isolation of Chlorinated Bornanes (CBs)

The process in general was described by us previously[1]. It includes :
- chlorination of camphene to 2-exo,10,10-Trichlorobornane (TrCB-2049)[2]
- chlorination of TrCB-2049 to a mixture of CBs with desired percent of Cl
- separation of a mixture on silica gel/hexane column
- purification by successive crystallisations and structure elucidation by NMR

Use of TrCB-2049 as a precursor gives three important advantages :
- it yields simplified mixtures, containing only CBs with 2 Cl at C-10 (odd numbers in binary-decimal nomenclature[2]), which makes isolation easier
- it simplifies structure elucidation of isolated compounds
- it allows to monitor the separation process and to perform precise quantitation of congeners in mixtures by proton NMR

2. Isolated compounds

Below is the list CBs, isolated by our group in amounts of at least 20mg (usually > 50 - 500mg) , with purity of at least 95%, and with reliable structure elucidation. Literature references for proton NMR are given for previously described compounds, and proton NMR data in $CDCl_3$ are given for the new ones.

1. HpCB-2439 : 2-*exo*,5,5,9,9,10,10-Heptachlorobornane [6.87s, broad; 6.40s; 4.34dd(8.68, 4.48Hz); 3.68d(15.94); 3.28dd(15.84, 8.76); 2.92d(4.36); 2.79d(15.98); 2.31ddd(15.85, 5, 5); 1.85s(3H)].
2. HpCB-2453 : 2-*exo*,5,5,8,9,10,10-Heptachlorobornane [6.57s; 4.59dd(11.92, 2.40); 4.55d(12.10); 4.33dd(8.58, 5.38); 4.18d(12); 4.18dd(12, 2.5); 3.62d(16.25); 3.25dd(15.73, 8.81); 3.09d(4.49); 2.83d(16.17); 2.30ddd(15.75, 5, 4.9)].
3. HpCB-2837 : 2-*exo*,3-*exo*,5-*endo*,8,9,10,10-Heptachlorobornane [in C_6D_6 : 6.41s; 4.83dd(12.49; 2.78); 4.60d(8.27); 4.00d(12.58); 3.81ddd(10.74, 5, 5); 3.75d(12.59); 3.71d(8.31); 2.70dd(12.58, 2.75); 2.45d(4.14); 2.29dd(15.29, 10.80); 1.10dd(15.39, 5.32)]
4. HpCB-3157 : 2-*exo*,3-*endo*,6-*endo*,8,9,10,10-Heptachlorobornane[1]

5. HpCB-3207 : 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-Heptachlorobornane [6.75s, broad; 6.29s; 4.78dd(9.01, 5.17); 4.62dd(4.81, 4.72); 4.07d(4.60); 3.01dd(15.01, 5.14); 2.72d(4.52); 2.63dd(15.00, 9.05); 1.87s(3H)].
6. HpCB-3221 : 2-*exo*,3-*endo*,5-*exo*,8,9,10,10-Heptachlorobornane[1)]
7. HpCB-4661 : 2-*endo*,3-*exo*,6-*exo*,8,9,10,10-Heptachlorobornane[1)]
8. HpCB-4785 : 2-*endo*,3-*exo*,5-*exo*,6-*exo*,8,10,10-Heptachlorobornane [6.69s; 5.13d(5.26); 4.67d(5.40); 4.58d(10); 4.57d(10); 4.21d(12.82); 3.58d(12.82); 2.69s; 1.90s(3H)]
9. HpCB-6293 : 2,2,5-*exo*,8,9,10,10-Heptachlorobornane [6.78s, 4.57d(10.87); 4.29-4.35m(2H); 4.20d(12.54); 4.14d(8.51, 5.34); 3.34dd(15.38, 3.69); 3.14dd(15.78, 4.73); 2.83dd(15.46, 5.58); 2.75d(4.70); 2.66d(15.67)].
10. HpCB-6452 : 2,2,5-*endo*,6-*exo*,8,9,10,-Heptachlorobornane[3,4,5)]
11. HpCB-6533 : 2,2,5,5,9,9,10,10-Heptachlorobornane[5)]
12. OCB-2455 : 2-*exo*,5,5,8,9,9,10,10-Octachlorobornane[5)]
13. OCB-2969 : 2-*exo*,3-*exo*,5,5,8,8,10,10-Octachlorobornane [7.23s, broad; 6.51s; 5.50d(8.58); 4.59d(8.57); 3.73d(16.26); 3.16s; 2.81d(16.28);1.87s(3H)].
14. OCB-4917 : 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-Octachlorobornane[5)]
15. OCB-4921 : 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-Octachlorobornane[5-7)]
16. OCB-6535 : 2,2,5,5,9,9,10,10-Octachlorobornane[1,5)]
17. NCB-4925 : 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-Nonachlorobornane[4-8)]
18. NCB-6551 : 2,2,5,5,8,9,9,10,10-Nonachlorobornane[4,5)]
19. NCB-7047 : 2,2,3-*exo*,5,5,9,9,10,10-Nonachlorobornane[1)]
20. NCB-7061 : 2,2,3-*exo*,5,5,8,9,10,10-Nonachlorobornane[5)]
21. DCB-3799 : 2-*exo*,3,3,5-*exo*,6-*endo*,8,9,9,10,10-Decachlorobornane[1)]
22. DCB-6583 : 2,2,5-*exo*,6,6,8,9,9,10,10-Decachlorobornane[4,5)]

23. DCB-6967 : 2,2,3-*exo*,5-*endo*,6-*exo*,8,9,9,10,10-Decachlorobornane[4,5)]
24. DCB-7063 : 2,2,3-*exo*,5,5,8,9,9,10,10-Decachlorobornane[4,5)]

3. Structure - behaviour relationship for CBs

We made two important conclusions, based on the analysis of proton NMR :
1) For the compounds with two Cl at C-10, chemical shift of H-10 (singlet) lies between 6.0 and 6.9 ppm. No other proton appears as a singlet in this region.
2) For the compounds containing one chloromethyl and one dichloromethyl group "on the top" (C-8 and C-9), one can select the right position of the two groups using simple rule : if there is an exo-proton at C-2 or C-6, "under" chloromethyl group, doublet of doublets from chloromethyl group has lower chemical shift, than doublet. If doublet has lower chemical shift, there is no exo-proton "under" chloromethyl group.

Unlike Parlar et.al.[5)], we failed to isolate or even detect any chlorocamphenes. Taking into account, that our mixtures have been irradiated for 24 hours prior to separation, it is likely, that chlorocamphenes are not the products of Wagner-Meerwein rearrangement of CBs only, but the products of successive chlorination of camphene.

We did not pay much attention to positioning of the protons and chlorines in chloro- and dichloromethyl groups. Available NMR data are not sufficient to prove, that there is no rotation. In any case, only after

corresponding isomers, differing only in conformation of rotating group(s), are isolated or detected, this problem might become important.

Toxaphene congeners demonstrate different stability not only to UV-light, but to acid or alkaline treatment as well. Therefore, special experiments are necessary, to confirm, that all Toxaphene goes through sample preparation safe and unchanged.

4. Proton NMR analysis of Toxaphenic mixtures

As mentioned above, NMR spectra of all CBs with 2-Cl at C-10 have one and only one singlet between 6.0 and 6.9 ppm. It allowed us, along with isolation of several CBs, to carry out semi-quantitative analysis of our mixture (containing an average of 7 - 7.5 Cl in a molecule), by quantifying the singlets between 6.0 and 6.9 ppm in each fraction from silica gel/hexane separation.

Experimental
20 g of CB-mixture with an average 7 - 7.5 Cl in a molecule was separated on 2m high, 5cm i.d. column, filled with specially prepared silica[1]. Using hexane as eluent, total of 55 fractions have been collected. Fractions were different in eluent volume, but contained nearly equal amount of CBs by weight. After hexane was evaporated off, a portion of each fraction was dissolved in $CDCl_3$ and proton NMR recorded on Bruker AMX-500 (500MHz). An area between 6.0 and 6.9 ppm was investigated. In very few cases we observed overlapping of the signals from different CBs. Therefore, we assumed, that each signal corresponds to its CB.

Discussion
29 compounds appeared to be major components in at least one fraction. These 29 represent at least 1% of a mixture each and 55% together.
141 compound, which appeared in at least one fraction in amount not less than 10% of major component of the fraction, represent at least 0.2% each and 41% together.
171 other compound have been detected, representing about 0.02% each and about 4% together.
This semi-quantitative analysis of artificial mixture is done by NMR only. The sensitivity of NMR is not sufficient for environmental samples, therefore it is necessary to correlate the results of NMR and EI GC/MS for each fraction. This work is currently in progress.

5. Directions of future research

After successful isolation of individual CBs from Heptachloromixture and Decachloromixture, we realised, that such approach has one important disadvantage : you isolate something first, and then you try to find it in the environmental samples. The more new compounds you have isolated, the less is a probability to find the last one in real samples.
There are three possible direction for the future:

1. To continue isolation of pure congeners from "chemical" mixtures, like technical Toxaphene, CB-standard, chlorination mixtures of DiCB-2048(2-*exo*,10-Dichlorobornane), TrCB-2049(2-*exo*,10,10-Trichlorobornane) or other precursors - camphenic, tricyclenic, pinenic, etc. and search for them in environmental samples.

2. To isolate Toxaphene congeners from environmental samples of different origin.

3. Not to isolate, but to separate and identify all Toxaphene congeners by combination of LC, NMR, HRGC/EIMS.

The first two are traditional[1,3-8] methods. The third one is to be discussed. At present we see this work including the following stages :

- Preparation of "the best mixture" - a mixture of Toxaphene, CB-standard, acid- and base-treated Toxaphenes, perhaps, ferment-treated Toxaphene.
- Separation of such mixture into a great number of fractions
- Proton NMR of each fraction
- GC/EIMS of each fraction, MS-library for all Toxaphene congeners
- Selection of characteristic ions for each Toxaphene congener
- Correlation of NMR signals and GC peaks
- Quantitation, based on NMR with internal standard (any proton-containing substance)
- Calculation of absolute response factors in SIM-mode for each Toxaphene congener
- Check on starting mixture
- Analyses of real samples

6. References

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