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ScienceDirect
Chemosphere 67 (2007) 1695–1700
CHEMOSPHERE
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Toxaphene chemistry: Separation and characterisation of selected enantiomers of the Polychloropinene mixtures

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Accepted 26 May 2006
Available online 10 January 2007

Abstract

The primary goal for the study presented here was the preparation and characterisation of enantiomeric pure chlorobornane standards (Toxaphene®). In this context, we partially modeled the procedure for Polychloropinene production in the former USSR. The initial reaction was ionic addition of hydrogen chloride to (1*S*)-α-pinene resulting predominantly in (1*S*)-2-*endo*-chlorobornane. Further photochlorination gave mixtures of chlorinated terpenes with different average content of Cl per molecule. The resulting mixtures were separated on a silica-gel column and a number of known hepta to decachlorobornanes were identified in fractions with the help of NMR and GC (using electron capture and mass spectrometric detection) – but in very unusual ratios as compared to the technical Toxaphene® mixture formerly produced by Hercules (USA). Also several previously unknown congeners were isolated or detected. Three of the isolated congeners were obtained in crystalline state and X-ray crystallography showed their enantiomeric purity.

Keywords: Polychloroterpenes; Pinene photochlorination; Enantiomeric composition; X-ray analysis; NMR characterisation

1. Introduction

Toxaphene is an insecticidal mixture (average formula $C_{10}H_{10}Cl_8$), produced as technical product by Hercules Agrochemical Inc. (Passaic, NJ, USA) through the controlled photochlorination of camphene (Saleh, 1991), Fig. 1.

In the USSR, a similar insecticide – Polychloropinene (average formula $C_{10}H_{10.5}Cl_{7.5}$) was produced by chlorination of α-pinene with AIBN (azobis*iso*butyronitrile) as initiator with preliminary hydrochlorination in the cyclic reactor; hydrogen chloride evolved through the chlorination is returned to the reactor for hydrochlorination, Fig. 1 (Nikiforov, 2002).

Natural camphene occurs in a number of essential oils in (+)-form as well as (−)-form (Windholz et al., 1976). Production of camphene starts from natural α-pinene, which is also chiral. All types of camphene were used for Toxaphene® synthesis by different manufacturers. Some congeners in selected samples of technical Toxaphene were shown to have small deviations from racemic distribution (Buser and Müller, 1994). Nevertheless, there is no information about any enantiomerically pure congeners of Toxaphene, although deviations from the racemic distribution were reported for technical Melipax® mixtures, a similar products as Toxaphene® produced in the former German Democratic Republic (Vetter et al., 1997).

The amount of Polychloropinene produced in the USSR is estimated to be 160000 tons, which is a significant contribution to the total global production. There is no information on composition of Soviet Polychloropinene and unfortunately, there is no traceable sample of

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doi:10.1016/j.chemosphere.2006.05.082

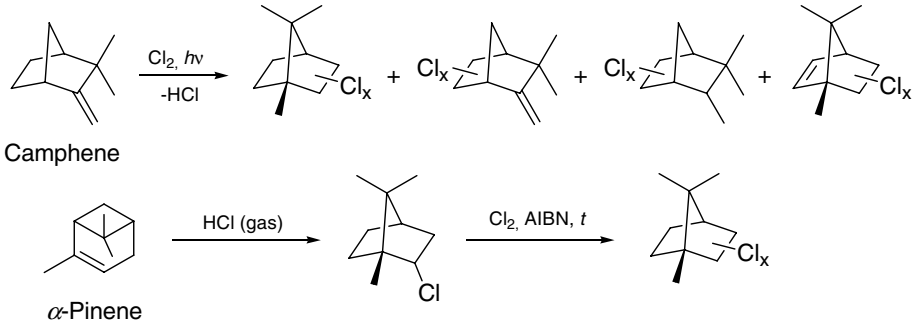


Fig. 1. Preparation of Toxaphene by chlorination of camphene and preparation of Soviet Polychloropinene by hydrochlorination and chlorination of α-pinene.

Polychloropinene available. In the present work, we report on the preparation of model Polychloropinene mixtures with different content of chlorine in an attempt to find the difference between Toxaphene and Soviet Polychloropinene.

At the same time it was an attempt to prepare pure enantiomers of important Toxaphene congeners from optically active pinene, to establish their configuration and thus to provide more opportunities for enantiomer-specific environmental analysis of Toxaphene residues.

2. Materials and methods

(1*S*)-α-pinene (Aldrich, St. Louis, MO, USA) used for this study has an enantiomeric excess of 81+%. Gaseous hydrogen chloride was prepared from sodium chloride and concentrated sulfuric acid with subsequent scrubbing with concentrated sulfuric acid. Chlorine gas in cylinder was obtained from a commercial source. Photochlorination experiments were carried out with a 400 W medium-pressure mercury arc lamp. GC equipment used for monitoring: Fisons MEGA with ECD (Fisons, Milan, IT), chiral capillary column based on β-cyclodextrin (BGB 172 capillary column, 15 m length, 0.25 mm i.d., 0.25 mm film thickness, BGB Analytics, Boeckten, Switzerland). Helium (5.0 quality) was used as carrier gas (Hydro gas, Porsgrunn, Norway). An aliquot of 2 μl was injected on-column in the enantioselective GC column. The ECD detector was held at 300 °C. The following temperature programme was used for separation: initial temperature: 60 °C (isothermal 2 min) − temperature rate 3 °C/min to 180 °C × Temperature rate 5 °C/min to 280 °C (isothermal 5 min) × 15 °C/min to 300 °C (final temperature).

2.1. Syntheses

2.1.1. Hydrochlorination of α-pinene

Dry hydrogen chloride was bubbled through the solution of 28 g (0.2 mole) (1*S*)-α-pinene ($[\alpha]_D$ −47°, neat) in 50 ml of *n*-hexane for 6 h at ambient temperature. The resulting solution was washed with 10% aqueous sodium hydrocarbonate, dried with anhydrous sodium sulfate and solvent evaporated. The solid was then recrystallised from a minimum amount of *n*-hexane to give 20 g of crude (1*S*)-2-*endo*-chlorobornane ($[\alpha]_D$ −26° to −32°, Et_2O, $c = 2$).

2.1.2. Preparation of "7–8" mixture

Crude (1*S*)-2-*endo*-chlorobornane (8.5 g) was added to 1000 ml of 0.4 M solution of Cl_2 in CCl_4. The solution was irradiated with a UV-lamp until the yellow color disappeared. GC analysis confirmed that the product contains mainly hepta and octachloroterpenes. After evaporation of the solution, 20 g of colorless oil was obtained.

2.1.3. Preparation of "9–10" mixture

Seven grams of crude (1*S*)-2-*endo*-chlorobornane was dissolved in 350 ml of carbon tetrachloride. Resulting solution was saturated with chlorine for 10 min and then irradiated with UV-lamp until until the yellow color disappeared. This saturation–irradiation cycle was repeated four times. After evaporation of the solution 16 g of colorless oil was obtained.

2.1.4. Separation of the polychloroterpenes mixture

Separation of the prepared mixtures (16 g of "9–10" mixture or 20 g of "7–8" mixture) was carried out on a "home-made" silica column: i.d. 4.7 cm; height – 100 cm; eluent – *n*-hexane. Each fraction was examined by ^1H NMR. Selected fractions were then purified by additional silica column or through multiple crystallisations.

2.1.5. Dechlorination of polychloroterpenes with copper

The combined fractions from separation of mixture "9–10" where Parlar no. 50 (**7**, Fig. 3) was enriched, were evaporated to dryness to yield ca. 400 mg of semi-solid residue. Fifty milliliters of glacial acetic acid and 1 g of Cu powder were added to it and the mixture was refluxed for 10 h. The mixture was allowed to cool, diluted with 200 ml of water, the solid collected and extracted with 3 × 10 ml of boiling *n*-hexane. The combined extracts were separated on a 60 cm long, 2 cm i.d. silica column with *n*-hexane as eluent. In addition to Parlar no. 50, four previously unknown polychlorobornenes (**3**–**6**, Fig. 3) were isolated.

2.2. X-ray crystallography

Data collection: Enraf-Nonius CAD-4 diffractometer, CAD-4-PC Software (Enraf-Nonius, 1992). Cell refinement: CELDIM in CAD-4-PC Software. Data reduction: XCAD4 (McArdle and Higgins, 1995). Program used to solve and refine structure: SHELXS97 (Sheldrick, 1997). Molecular graphics: ORTEX (McArdle, 1995).

2.3. NMR spectroscopy

All NMR experiments were performed on dilute $CDCl_3$ solutions at 30 °C. For the new compound **11** ^1H and ^{13}C DEPT spectra were recorded in the temperature range −30 to +24 °C, at intervals of ca. 10 °C. In addition to measurements at −30 °C, NOESY-EXSY and EXSY spectra for the mentioned compound were recorded. The NMR spectrometer used for serial analysis and the chemical shift assignments was a Varian Mercury 400 MHz equipped with a *z*-gradient accessory, low temperature measurements were carried out with a Bruker DPX-300 (Bruker, Karlsruhe, Germany).

The chemical shift assignments for new compounds are based on two-dimensional ^1H–^1H COSY and ^1H–^{13}C COSY.

3. Results and discussion

3.1. Preparation of Polychloropinene-like mixtures

The initial reaction was ionic addition of hydrogen chloride to (1*S*)-α-pinene ($[\alpha]_D$ −47°, neat) resulting in a mixture of (1*S*)-2-*endo*-chlorobornane and 2-*endo*-chlorofenchane in ca. 10:1 ratio (Fig. 2). The crude product was found to be optically active ($[\alpha]_D$ −26° to −32°, Et_2O, $c = 2$); we concluded that enantiomeric purity is retained, though we had no data for specific rotation of chlorofenchane. Formation of two different intermediate ion pairs with non-classical carbocations was postulated to explain the composition of the product mixture. Rearrangement includes 1,2-shift of either methylene or isopropylidene bridge with formation of less strained norbornane carbon sceleton.

The crude 2-*endo*-chlorobornane (bornyl chloride) was then used for chlorination without further purification. Chlorination was conducted in light-induced conditions in contrast to industrial synthetic procedure (thermal initiation with AIBN). Two different mixtures were prepared. "7–8" contained mainly hepta and octachlorinated compounds, and "9–10" was obtained by exhaustive chlorination and contained nona and decachloroterpenes. Both mixtures of chlorinated terpenes were separated on silica-gel column with *n*-hexane as eluent according to a previously developed procedure (Nikiforov et al., 1995).

3.2. Composition of Polychloropinene-like mixtures

On GC/ECD (gas chromatography with electron capture detection) all mixtures had peak patterns different from those on chromatograms of mixtures of chlorinated camphene with similar content of chlorine.

A number of known hexa to decachlorobornanes were identified in fractions with help of NMR and GC (Fig. 3). By the time of writing, 11 polychlorobornanes

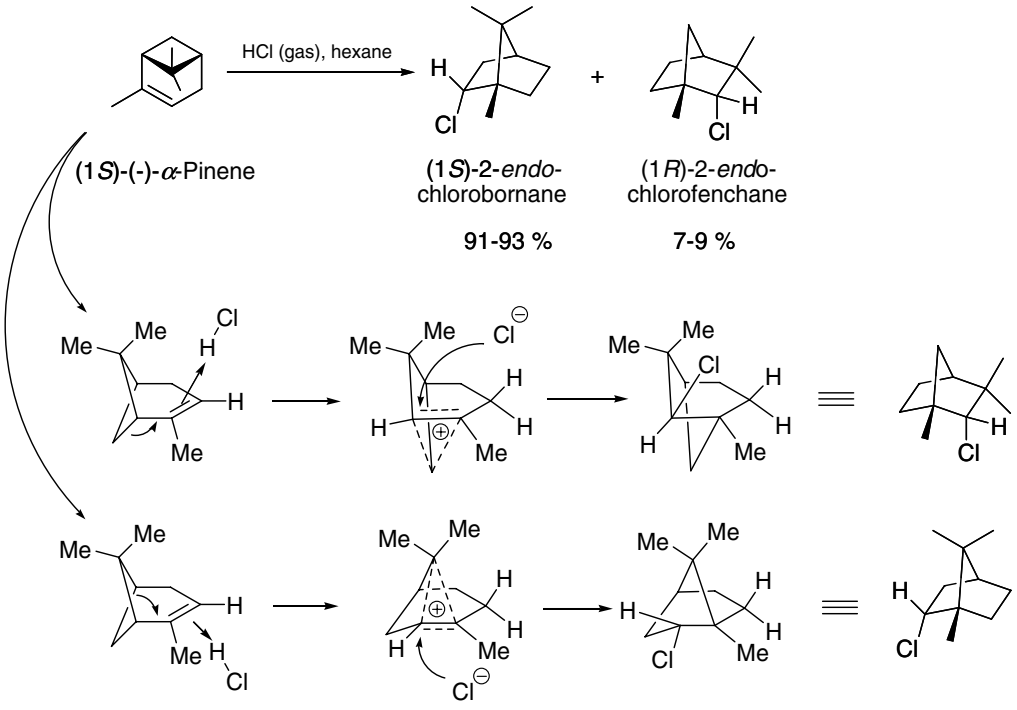


Fig. 2. Hydrochlorination of optically active α-pinene and its schematic mechanism.

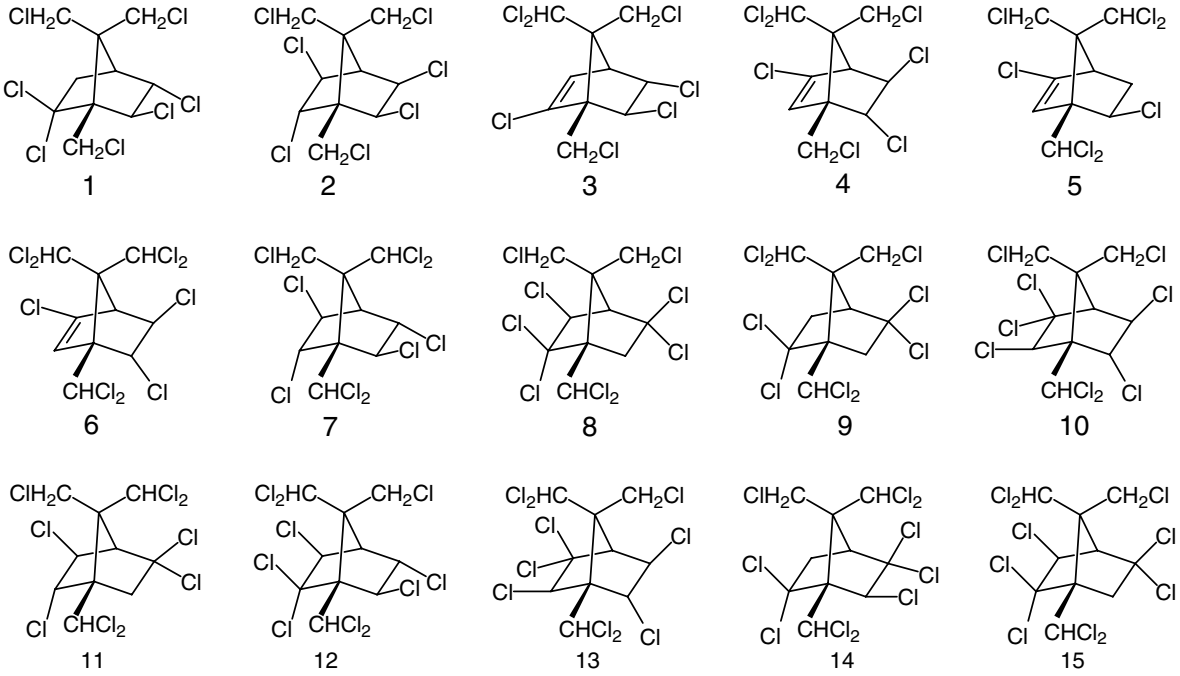


Fig. 3. Structures of polychloroterpenes obtained from α-pinene.

and 4 polychlorobornenes (after treatment of fractions with Cu in boiling acetic acid) were isolated in sufficiently pure state to establish or confirm their structures by NMR.

Compounds **1**, **7**–**10**, **12**–**15** are known chlorination products of camphene. Compounds **2**–**6** and **11** were previously unknown.

From "7–8" mixture, two compounds were isolated: the well-known Toxicant B (**1**; Parlar 32) and previously unknown 2-*endo*,3-*exo*,5-*exo*,6-*exo*,8,9,10-heptachlorobornane (**2**). Ring substitution pattern of the latter: 2-*endo*, 3-*exo*,5-*exo*,6-*exo* – has never been observed in polychlorobornanes prepared from camphene. Theoretically, this heptachlorobornane can be obtained by chlorination of camphene, but its presence in environmental samples would likely point to Polychloropinene as a source of pollution. None of the typical Toxaphene® congeners (except Toxicant B) were major components in fractions. Several other new compounds were identified, their structure elucidation is in progress.

Mixture "9–10" contained the same main components as perchlorinated camphene, 2-*exo*,10-dichlorobornane, 2-*exo*,10,10-trichlorobornane or Toxaphene – two nonachlorobornanes (**7**, **9**) and four decachlorobornanes – **12**–**15**. **13** is a major component of the mixture "9–10". This is completely different from what we see in perchlorinated camphene or Toxaphene®, where **13** is the smallest of the mentioned six congeners (Table 1). Also the ratio of Parlar 50 (**7**) and Parlar 62 (**9**) is opposite for the two mixtures ("9–10" and perchlorinated Toxaphene®, respectively).

Two compounds, the first and last major eluting components from the silica column, were purified to >99% by successive crystallisations from enriched fractions. The first eluting compound was the well-known decachlorobornane **12**, the last eluting was the previously unknown 2-*endo*, 3-*exo*,5,5,8,8,9,10,10-nonachlorobornane (**11**). Its structure was established by NMR and then confirmed by X-ray crystallography (Hansen et al., 2004). The compound has no chlorine atoms in positions 2-*exo* or 6-*exo*. The ring substitution pattern "2-*endo*,3-*exo*,5,5-" has never been observed in polychlorobornanes prepared from camphene. Thus, it is confirmed that Polychloropinene-specific polychlorobornanes exist and can serve as indicator compounds for source elucidation in the environment.

Table 1
Composition of perchlorinated bornyl chloride (Mixture "9–10") and perchlorinated Toxaphene

	Relative content of selected polychlorobornanes[a]					
	7	**9**	**12**	**13**	**14**	**15**
Mixture "9–10"	2.8	2.0	2.0	3.7	1	2.0
Perchlorinated Toxaphene	2.3	4.8	2.4	0.4	1	2.0

[a] Determined by NMR spectra.

3.3. Structural differences and similarities of Toxaphene and Polychloropinene

- All polychlorobornanes isolated from chlorination products of pinene contain at least one Cl in 2-*endo* or 6-*endo* positions.
- Environmentally relevant Toxaphene® congeners 2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane (Hx-Sed), 2-*exo*,3-*endo*,5-*exo*,9,9,10,10-heptachlorobornane (B7-

Table 2
^{1}H chemical shifts and ^{1}H–^{1}H coupling constants of new polychloroterpenes (at 300 MHz)

	^{1}H chemical shifts, δ, ppm											
	2	2-*exo*	3	3-*endo*	4	5-*endo*	5-*exo*	6-*endo*	6-*exo*	8	9	10
2	–	3.95d	–	5.07d	2.98s	4.36d	–	5.04d	–	3.67d, 4.92dd	4.21dd, 4.65d	4.18d, 4.54d
3	–	–	6.38d	–	3.64dd	–	4.82dd	4.26d	–	4.37d, 4.62dd	6.27d	3.69d, 3.96d
4	5.86s	–	–	–	3.60s	4.92d	–	–	4.09d	4.24d, 4.74d	6.35s	3.88d, 3.96d
5	6.24s	–	–	–	3.47d	2.48dd	2.26ddd	4.34dd	–	6.91d	4.31d, 4.45dd	6.44s
6	6.15d	–	–	–	3.71s	4.95d	–	–	4.07d	4.26dd, 4.85d	6.57d	6.22s
11	–	5.17dd	–	5.32d	3.61s	–	–	3.09bd	3.73bd	6.56d	4.28dd, 4.89bd	6.34bs

	^{1}H–^{1}H coupling constants, *J*, Hz											
	2-*exo*/3-*endo*	3/4	4/5-*exo*	5-*endo*/5-*exo*	5-*endo*/6-*endo*	5-*exo*/6-*endo*	5-*endo*/6-*exo*	6-*endo*/6-*exo*	8a/8b	8a/9a	9a/9b	10a/10b
2	4.5	–	–	–	8.2	–	–	–	12.6	2.1	12.1	12.5
3	–	3.3	3.6	–	–	3.7	–	–	13.5	1.5	–	12.4
4	–	–	–	–	–	–	3.3	–	12.8	–	–	12.4
5	–	–	3.7	14.6	8.4	3.7	–	–	–	1.5	12.8	–
6	–	–	–	–	–	–	3.3	–	12.6	1.4	–	–
11	3.9	–	–	–	–	–	–	16.8	–	2.4	13.5	–

1000), 2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10-octachlorobornane (Parlar no. 41), 2-*exo*,5,5,8,9,9,10,10-octachlorobornane (Parlar no. 44) have not been detected among chlorination products of pinene.

- No chlorobornanes with unsubstituted bridgehead methyl group have yet been detected among chlorination products of pinene.
- Ratios of common polychlorobornanes must have been different in Toxaphene® and Polychloropinene, because the ratios of major nona and decachlorobornanes are different in their respective perchlorination products.

3.4. Characterisation of selected polychloroterpenes

The main method for identification of known polychloroterpenes was NMR. NMR data for new compounds with provisional assignments is listed in Table 2.

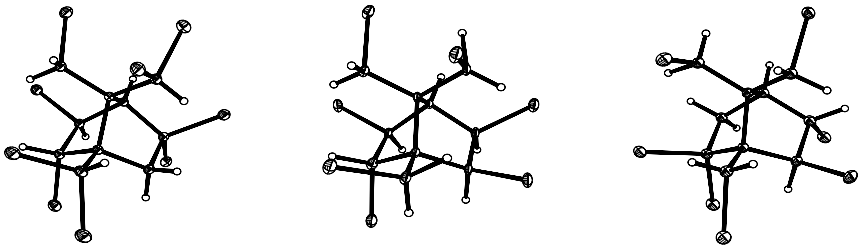
A number of ^{1}H-signals in NMR spectrum of **11** are broad. NMR investigation of **11** at different temperatures revealed the molecule has slow internal rotation of the C10-dichloromethyl moiety. This effect causes broadening of ^{1}H NMR signals of protons in its proximity (Koivisto et al., 2001). At −40 °C, all signals are sharp and the structure corresponds to the most stable conformation found in the crystalline state.

3.5. Enantioselectivity during preparation of polychlorobornanes from optically active α-pinene

One of the goals of our project was development of a general enantioselective synthetic method for polychloroterpenes. We had the technical means to use three methods for observation of the enantiomeric purity of isolated compounds:

- measurement of optical rotation;
- GC analysis on chiral phases;
- direct resolution of structure by X-ray crystallography.

Fig. 4. Chiral-GC/ECD profiles of **7** (Parlar 50): (a) standard solution, Promochem GmbH and (b) part of a chromatogram of "9–10" mixture.



(1*R*,2*S*,3*S*,4*R*,7*S*)-2-*endo*,3-*exo*,5,5,8,8,9,10,10-nonachlorobornane (**11**)

(1*S*,2*S*,3*S*,4*S*,5*R*,6*S*)-2-*endo*,3-*exo*,5-*exo*,6-*exo*,8,9,10-heptachlorobornane (**2**)

(1*S*,4*S*,5*S*,6*S*)-2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (**1**, Parlar no. 32)

Fig. 5. ORTEP plots for X-ray investigated polychlorobornanes.

Considering minuscule amounts of isolated pure compounds (milligrams or tens of milligrams) and low solubility in organic solvents (1–5%), measurement of optical rotation required a sensitive instrument, which was available to us only at the very beginning of the project. Therefore we managed to measure optical rotation for just one compound – isolated from mixture "9–10" congener **12**: $[\alpha]_D$ 10° ± 2 (CCl_4, $c = 1$ mg cm^{-3}).

A number of isolated compounds were tested on chiral GC/ECD using *t*-butyldimethylsilylated β-cyclodextrin as chiral selector (BGB 172 capillary column, 15 m length, 0.25 mm i.d., 0.25 mm film thickness, BGB Analytics, Boeckten, Switzerland). In all cases we observed single peaks. Minor peaks, identified as enantiomers or isomeric impurities were usually <10% compared to the main enantiomer signal in the chromatogram. At least, in one case we achieved confirmation of a single enantiomer (compound **7**, Parlar 50). For this compound we had a racemic standard, which gave well-resolved enantiomers on chiral GC/ECD chromatogram. The comparison of compound **7**, isolated from mixture "9–10" and from standard solution confirmed the enantiomeric purity (Fig. 4).

Three of the isolated congeners yielded crystals of sufficient quality for direct X-ray determination of structure – **1**, **2** from mixture "7–8" and **11** from mixture "9–10". All three congeners were found to be single enantiomers with preservation of the configuration of starting (1*R*)-*endo*-2-chlorobornane (Fig. 5).

Based upon the above information, we concluded that the three independent methods used in our study confirm the preservation of the enantiomeric purity during hydrochlorination and subsequent chlorination of optically active α-pinene. No evidence of racemization was found during our experiments.

This implies that the strategy of enantioselective preparation of lower chlorinated terpene and its further free-radical chlorination, followed by column chromatography was the right strategy for the preparation of enantiomerically pure chlorobornane materials. The general pathway in the synthesis of enantiomeric pure chloroterpenes was identified as follows: optically active α-pinene → optically active bornyl chloride → enantiomers of polychlorobornanes.

With regard to differences and similarities in structures and ratios of polychloroterpenes related to Toxaphene® and to Polychloropinene, our findings suggest that the earlier Soviet Polychloropinene, unlike Toxaphene contained non-racemic polychloroterpenes. Therefore observed deviations from racemic composition in the environment may in some samples be due to partial input of a non-racemic source, and not only to enantioselective degradation or metabolism.

Acknowledgements

This work was generously supported by Norwegian Research Council, project number 152806/730. We gratefully thank Arnfinn Kvarsnes from the University of Tromsø, Norway and Stanislav Selivanov from St. Petersburg State University, Russia for their invaluable help with NMR experiments.

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