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SYNTHESIS OF ^{14}C-LABELLED DDD ISOMERS OF HIGH SPECIFIC ACTIVITY

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SUMMARY

Three major DDD isomers, o,p'-DDD, m,p'-DDD and p,p'-DDD, ^{14}C-labelled in the para-chlorophenyl ring, have been synthesized. The DDD products were obtained in isotopic yields ranging from 42% to 58% and were of high specific activities; 31 mCi/mmol (o,p'-DDD; m,p'-DDD) and 19,8 mCi/mmol (p,p'-DDD). A minor amount of ^{14}C-labelled o,m'-DDD was also isolated. 400 MHz ^{1}H NMR data of the three major DDD isomers and o,m'-DDD were obtained.

Key Words: o,p'-DDD, m,p'-DDD, p,p'-DDD, ^{14}C-DDD, DDT, NMR.

INTRODUCTION

o,p'-DDD[2] is a cytostatic agent (Mitotane) used for treatment of adrenal carcinoma and a major metabolite of o,p'-DDT[2]. In addition to the wellknown specific binding of o,p'-DDD to the adrenal cortex in the dog (1), Lund et al. (2) have shown a selective covalent binding of this compound to the lung alveolar region in mice. Furthermore, an unidentified metabolite of a radioiodinated analog to o,p'-DDD has been indicated in the adrenal cortex of rats (3).

In order to further investigate the lung-specific activation of o,p'-DDD, both

1. To whom correspondance should be addressed.
2. Abbreviations. DDT: 1,1,1-trichloro-2,2-bis(chlorophenyl)ethane,
DDD: 1,1-dichloro-2,2-bis(chlorophenyl)ethane,
DDE: 1,1-dichloro-2,2-bis(chlorophenyl)ethene.

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in vivo and in vitro, ^{14}C-labelled o,p'-DDD of high specific activity was needed. In addition to p,p'-DDE[2], p,p'-DDD is a major metabolite of p,p'-DDT in animals. Due to the global contamination of DDT, it is important to clearify if p,p'-DDD, similarly to o,p'-DDD, is activated and covalently bound in lung tissue of mice. Both p,p'-DDD and o,p'-DDD were thus synthesized, and for comparative reasons also m,p'-DDD was prepared. The procedure described by Counsell and Willette (4) was modified for semi-micro scale synthesis and improved yields were obtained.

RESULTS AND DISCUSSION

Synthesis of the three ^{14}C-labelled isomers, o,p'-DDD, m,p'-DDD and p,p'-DDD, has been performed according to Figure 1. It was possible to carry out the reactions in the original [^{14}C]chlorobenzene ampoules if a small amount of solvent was permitted for the transfer of the appropriate 2,2-dichloro-1-(chlorophenyl)ethanol to the ampoule. Ultrasonic mixing of the reactants significantly improved the yields of the DDD products, even though the scale of the reaction was only 1/100 of that earlier reported (4). It was however necessary to perform the reactions in this semi-micro scale, since a high specific activity is required in the biological work planned to be done with these DDD isomers.

In addition to the major DDD product also a minor isomeric compound was obtained in each reaction. As much as 13% of ^{14}C-o,p'-DDD and ^{14}C-o,m'-DDD was isolated in the synthesis of ^{14}C-p,p'-DDD and ^{14}C-m,p'-DDD, respectively. Traces of an isomeric DDD was observed in the product mixture from the synthesis of ^{14}C-o,p'-DDD, and confirmed by gas chromatography-mass spectrometry (GC-MS) on unlabelled material from a pilot synthesis. The total isotopic yields of ^{14}C-DDD isomers obtained after purification were 46-71%.

The major ^{14}C-DDD isomers synthesized were compared to unlabelled material prepared via the same pathway, and also to reference material obtained from other sources (except for m,p'-DDD). The major DDD isomers were compared to the reference compounds by TLC, gas chromatography and GC-MS. The four different DDD-isomers, isolated in a pure state, were further characterized by 400 MHz ^{1}H NMR (see Figure 3 and Materials and Methods)). The proton numbering system of the DDD isomers is shown in Figure 2. 100 MHz ^{1}H NMR of DDD isomers has earlier been reported by Keith et al (5) and Sharpless and Bradley (6). Chemical shifts and coupling constants of the para-substituted phenyl-ring, given in the present study, were calculated as for an AA'XX'-system. After resolution enhancement of 64K spectra, by a sine-bell function, additional splittings were observed in some signals. However, it has not been possible to verify the cause of this phenomena.

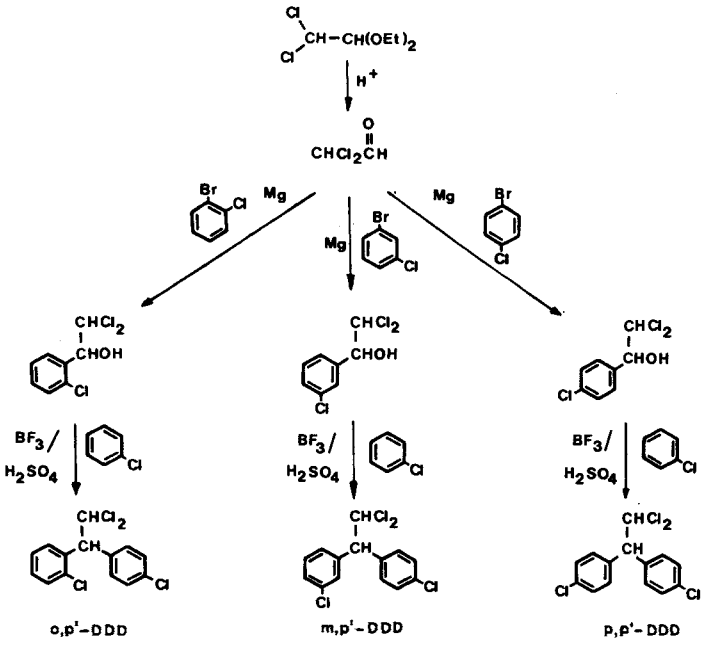

Figure 1. Pathway for synthesis of DDD isomers labelled with ^{14}C in the 4-chlorosubstituted phenyl ring. Only the major DDD-compound formed in each reaction is shown.

MATERIAL AND METHODS

Chemicals: Dichlorodiethylacetal (99%, Aldrich); benzoic anhydride (Merck); 2-bromochlorobenzene, 3-bromochlorobenzene and 4-bromochlorobenzene (98%, EGA-chemie). [U-^{14}C]-Chlorobenzene (Amersham International plc) delivered in P1 break-seal ampoules was obtained at three different occasions with the amounts of radioactivity and specific activities given below. All other reagents and solvents were of analytical grade. Unlabelled o,p'-DDD and p,p'-DDD were received for reference purposes as a kind gift from Dr. Bo Jansson (The Special Analytical Laboratory, The National Environmental Protection Board). m,p'-DDD was synthesized and characterized as described below.

Instruments: Purities and yields of the DDD isomers in the pilot preparations were determinated by gas chromatography on a Varian 3700 gas chromatograph fitted with a DB-1 megabore fused silica column (15m x 0.53mm ID) and operated with a temperature program: 200°C (5 min), 5°C/min, 250°C (20 min). The nitrogen gas flow rate was 12 ml/min.

The identity of the synthesized products were compared to the reference compounds by gas chromatography, and the DDD isomers were further

characterized by GC-MS and ^{1}H NMR. A Finnigan Model 4021 quadrupole mass spectrometer with a Finnigan Model 9610 gas chromatograph was used. Data were acquired with an Incos data system. The MS was operated in the electron impact mode. ^{1}H NMR was obtained at 25°C on a JEOL GX-400 spectrometer with a digital resolution of 0.1 Hz. The samples were dissolved in d_6-acetone with tetramethylsilane as internal standard.

Radioactivity was measured by a Packard TriCarb 460C liquid scintillation spectrometer. The samples were dissolved in toluene PPO/POPOP and the effectivity was calculated by use of an internal standard (^{14}C-standard capsules, LKB).

2,2-Dichloro-1-(2-/3- and 4-chlorophenyl)ethanol: Dichlorodiethylacetal (0.27 mol), benzoic anhydride (0.29 mol) and concentrated sulphuric acid (5.0 ml) was heated in an oil bath (190°C). A distilled fraction (88-90°C) was taken and the 2,2-dichloroacetaldehyde was kept in a desiccator at +4°C until used.

The Grignard reagent was prepared from magnesium (0,065 mol), a catalytic amount of iodine and the appropriate 2-, 3- or 4-bromochlorobenzene (0.068 mol) in diethyl ether (100 ml). After the addition of bromochlorobenzene was finished the mixture was refluxed until the reaction was completed. The 2,2-dichloroacetaldehyde (0.062 mol) was dissolved in diethyl ether and kept on ice in an inert atmosphere. About 1 ml of this solution was added at a time through a dropping funnel by means of a pasteur pipette to the Grignard reagent in the flask. The reaction was refluxed for 30 min before it was stopped by pouring it on a 2M H_2SO_4/ice-slurry (200 ml). Any excess of dichloroacetaldehyde was destructed by sodium bisulphite (10%) in water. The products were extracted three times with diethylether, washed and dried by use of sodium sulphate. The appropriate 2,2-dichloro-1-(chlorophenyl)ethanol prepared was purified on a silica gel (300 g) column and the products eluted with hexane:ethyl acetate (4:1). Typical yields were about 75% of the 2,2-dichloro-1-(chloro- phenyl)ethanol products.

^{14}C-o,p'-DDD: The [U-^{14}C]chlorobenzene (500 uCi, 31 mCi/mmol) was frozen with liquid nitrogen to the bottom of the ampoule in which the chlorobenzene was delivered. The liquid nitrogen was changed for dry ice in ethanol before the glass seal of the ampoule was broken with a glass rod. 2,2-Dichloro-1-(2-chlorophenyl)ethanol (8.9 mg, 39.3 umol) dissolved in dichloromethane (25 ul) and borontrifluoride saturated concentrated sulphuric acid (25 ul) were added by use of micropipettes to the bottom of the ampoule. A cap was put on the ampoule before it was transferred to an ultrasonic bath at 40-50°C and kept there for 3 h. After the reaction was finished the ampoule was cooled, the cap changed for a screw-cap with septum and hexane (1.5 ml)

plus water (1.5 ml) was added by means of a syringe. The products were extracted three times with hexane (1.5 ml) and two times with dichloromethane (1.5 ml). The organic phase was transferred by help of a nitrogen pressure via a needle to another flask. The radioactivity in this fraction was controlled before the solvent was evaporated. The residue was separated on a silica gel TLC plate (Kieselgel 60 F-254, 0.25mm, Merck) with hexane:ethyl acetate (9:1) as mobile phase. A major product, ^{14}C-o,p'-DDD, was isolated and further purified on a reversed phase TLC plate (RP-18 F-254S, Merck) with methanol as mobile phase. ^{14}C-o,p'-DDD (226 uCi, 31 mCi/mmol) was isolated in 42% yield. A minor isomeric product, most probably ^{14}C-o,o'-DDD, was also indicated (GC-MS). The ^{1}H NMR spectrum of o,p'-DDD determined is shown in Figure 3a and the shifts (ppm) and coupling constants were: 7.457 (H_3; $J_{3,4}$=7.9 Hz, $J_{3,5}$=1.5 Hz), 7.313 (H_4; $J_{4,3}$=7.9 Hz, $J_{4,5}$=7.4 Hz, $J_{4,6}$=1.6 Hz), 7.411 (H_5; $J_{5,6}$=7.9 Hz, $J_{5,4}$=7.4 Hz, $J_{5,3}$=1.5 Hz), 7.839 (H_6; $J_{6,5}$=7.9 Hz, $J_{6,4}$=1.6 Hz), 7.551 ($H_{2'}$; $H_{6'}$;$J_{2',3'}$=$J_{6',5'}$=8.3 Hz, $J_{2',5'}$=$J_{6',3'}$=0.5 Hz), 7.394 ($H_{3'}$; $H_{5'}$; $J_{3',2'}$=$J_{5',6'}$=8.3 Hz, $J_{3',6'}$=$J_{5',2'}$=0.5 Hz), 7.082 (H_a; $J_{a,b}$=10.1 Hz), 5.207 (H_b; $J_{b,a}$=10.1 Hz). Additional 0.4 Hz splittings were observed after resolution enhancement in signals from H_3; H_5; $H_{2'}$; $H_{6'}$ and 0.2 Hz splittings in signals from $H_{3'}$ and $H_{5'}$. Meta-couplings of the para-substituted phenyl-ring were not resolved.

^{14}C-m,p'-DDD: [U-^{14}C]Chlorobenzene (750 uCi, 31 mCi/mmol), 2,2-dichloro-1-(3-chlorophenyl)ethanol (8,7 mg, 38,8 umol) in dichloromethane (25 ul) and borontrifluoride saturated concentrated sulphuric acid (25 ul) were reacted as described above for o,p'-DDD. A major product, ^{14}C-m,p'-DDD (376 uCi, 31 mCi/mmol) was obtained in 50% yield after TLC on a silica gel plate and a reversed phase RP-18 plate eluted with hexane:ethyl acetate (9:1) and methanol respectively. A minor product, ^{14}C-o,m'-DDD (99 uCi), was also obtained. The ^{1}H spectrum of m,p'-DDD determined is shown in Figure 3b and the shifts (ppm) and coupling constants were: 7.609 (H_2; $J_{2,4}$=2.1 Hz, $J_{2,6}$=1.7 Hz, $J_{2,5}$=0.4 Hz), 7.306 (H_4; $J_{4,5}$=8.1 Hz, $J_{4,2}$=2.1 Hz, $J_{4,6}$=1.2 Hz), 7.383 (H_5; $J_{5,4}$=8.1 Hz, $J_{5,6}$=7.7 Hz, $J_{5,2}$=0.4 Hz), 7.516 (H_6; $J_{6,5}$=7.7 Hz, $J_{6,2}$=1.7 Hz, $J_{6,4}$=1.2 Hz), 7.585 ($H_{2'}$; $H_{6'}$; $J_{2',3'}$=$J_{6',5'}$=8.4 Hz, $J_{2',5'}$=$J_{6',3'}$=0.4 Hz), 7.399 ($H_{3'}$; $H_{5'}$; $J_{3',2'}$=$J_{5',6'}$=8.4 Hz, $J_{3',6'}$=$J_{5',2'}$=0.4 Hz), 7.076 (H_a; $J_{a,b}$=9.8 Hz), 4,799 (H_b; $J_{b,a}$=9.8 Hz). Additional 0.4 Hz splittings were observed after resolution enhancement in signals from H_2; H_6; $H_{2'}$; $H_{6'}$ and 0.2 Hz splittings in signals from $H_{3'}$ and $H_{5'}$. Meta-couplings of the para-substituted phenyl-ring were not resolved.

^{14}C-p,p'-DDD: [U-^{14}C]Chlorobenzene (470 uCi, 19.7 mCi/mmol), 2,2-dichloro-1-(4-chlorophenyl)ethanol (13.2 mg, 58.5 umol) in dichloromethane (25 ul) and borontrifluoride saturated concentrated sulphuric acid (25 ul) was

reacted and purified as described above. ^{14}C-p,p'-DDD (275 uCi, 19.7 mCi/mmol) was obtained in 58% yield. ^{14}C-o,p'-DDD (60 uCi) was also isolated from the reaction mixture. The ^{1}H NMR spectrum of m,p'-DDD determined is shown in Figure 3c and the shifts (ppm) and coupling constants were: 7.560 (H_2; $H_{2'}$; H_6; $H_{6'}$; $J_{2,3}=J_{2',3'}=J_{6,5}=J_{6',5'}$=8.4 Hz, $J_{2,5}$, $J_{2',5'}$, $J_{6,3}$, $J_{6',3'}$=0.5 Hz), 7.388 (H_3; $H_{3'}$; H_6; $H_{6'}$; $J_{3,2}=J_{3',2'}=J_{5,6}=J_{5',6'}$=8.4 Hz, $J_{3,6}=J_{3',6'}=J_{5,2}=J_{5',2'}$ =0.5 Hz), 7.029 (H_a; $J_{a,b}$=9.7 Hz), 4.786 (H_b; $J_{b,a}$=9.7 Hz). Additional 0.4 Hz splittings were observed after resolution enhancement in signals from H_2; $H_{2'}$; H_6; $H_{6'}$ and 0.2 Hz splittings in signals from H_3; $H_{3'}$; H_5 and $H_{5'}$. Meta-couplings were not resolved.

m,p'-DDD: Chlorobenzene (2,77 g, 24,6 mmol), 2,2-dichloro-1-(3-chlorophenyl)-ethanol (2,28 g, 10,1 mmol) and borontrifluoride saturated concentrated sulphuric acid (7,5 ml) was mixed according to Counsell and Willette (4). m,p'-DDD and o,m'-DDD was extracted with hexane and isolated from a silica gel (150 g) column eluted with hexane:ethyl acetate (9:1), recrystallized from diluted ethanol and characterized: m.p. 55-56°C (litt. 54°C (4)); mass spectrum (70 eV, EI) M^+: m/z=318 and as base peak m/z=235. For the ^{1}H NMR spectrum of m,p'-DDD cf. above. The ^{1}H NMR spectrum of o,m'-DDD determined is shown in Figure 3d and the shifts (ppm) and coupling constants were: 7.466 (H_3; $J_{3,4}$=7.9 Hz, $J_{3,5}$=1.4 Hz), 7.320 (H_4; $J_{4,3}$=7.9 Hz, $J_{4,5}$=7.4 Hz, $J_{4,6}$=1.6 Hz), 7.423 (H_5; $J_{5,6}$=7.9 Hz, $J_{5,4}$=7.4 Hz, $J_{5,3}$=1.4 Hz), 7.864 (H_6; $J_{6,5}$=7.9 Hz, $J_{6,4}$=1.6 Hz), 7.583 ($H_{2'}$; $J_{2',4'}$=2.0 Hz, $J_{2',6'}$=1.7 Hz, $J_{2',5'}$=0.4 Hz), 7.321 ($H_{4'}$; $J_{4',5'}$=8.0 Hz, $J_{4',2'}$=2.0 Hz, $J_{4',6'}$=1.2 Hz), 7.389 ($H_{5'}$; $J_{5'4'}$=8.0 Hz, $J_{5',6'}$=7.7 Hz, $J_{5',2'}$=0.4 Hz), 7.508 ($H_{6'}$; $J_{6',5'}$=7.7 Hz, $J_{6',2'}$=1.7 Hz, $J_{6',4'}$=1.2 Hz), 7.129 (H_a; $J_{a,b}$=10.2 Hz), 5.224 (H_b; $J_{b,a}$=10.2 Hz).

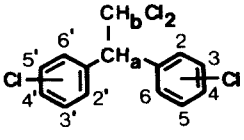

Figure 2. Proton numbering system used for ^{1}H NMR of the DDD isomers

Additional 0.4 Hz splittings were observed after resolution enhancement in signals H_3; H_5; H_2, and $H_{6'}$.

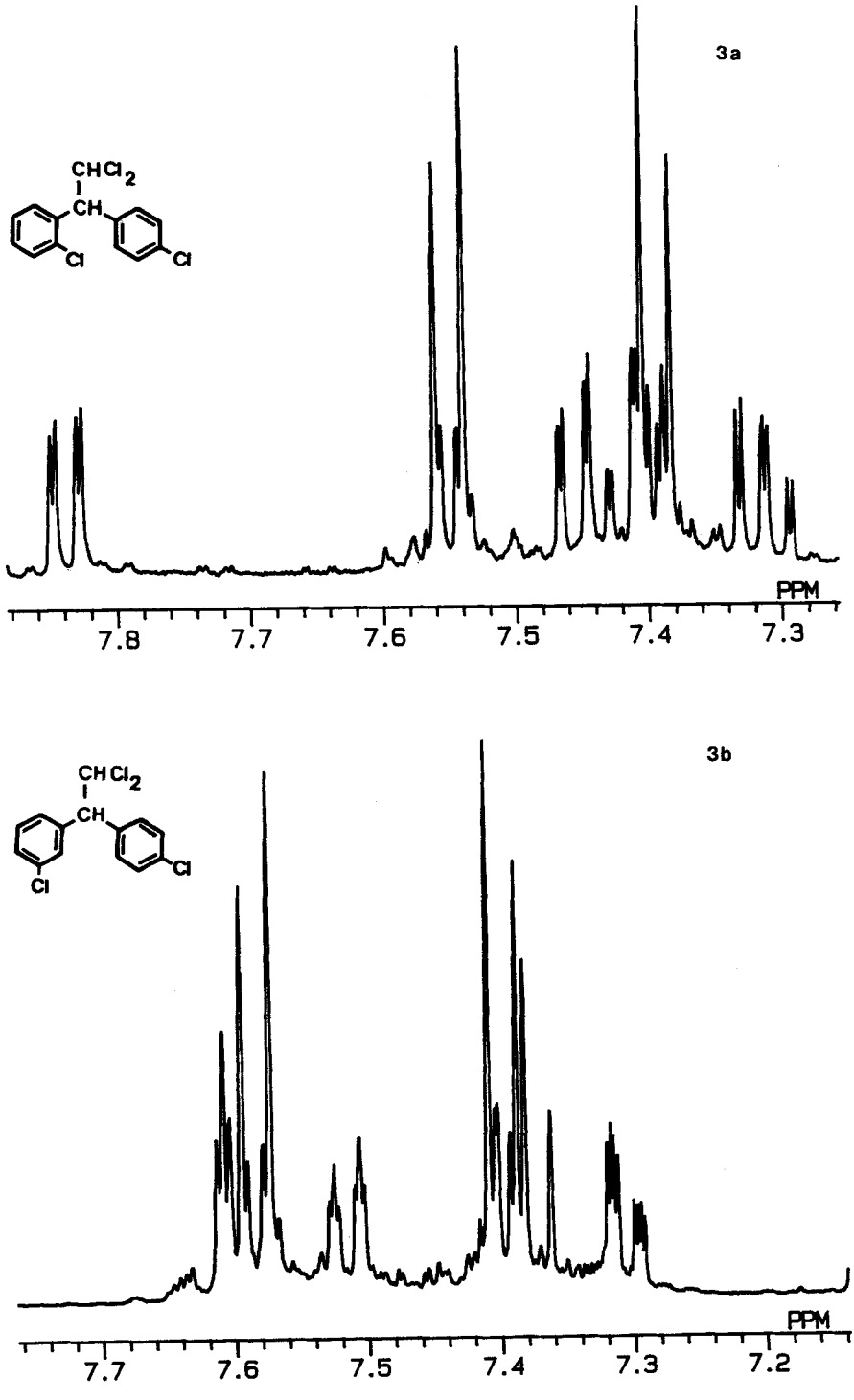

Figure 3. ^{1}H NMR (400 MHz), aromatic region, of o,p'-DDD (3a) and of m,p'-DDD (3b).

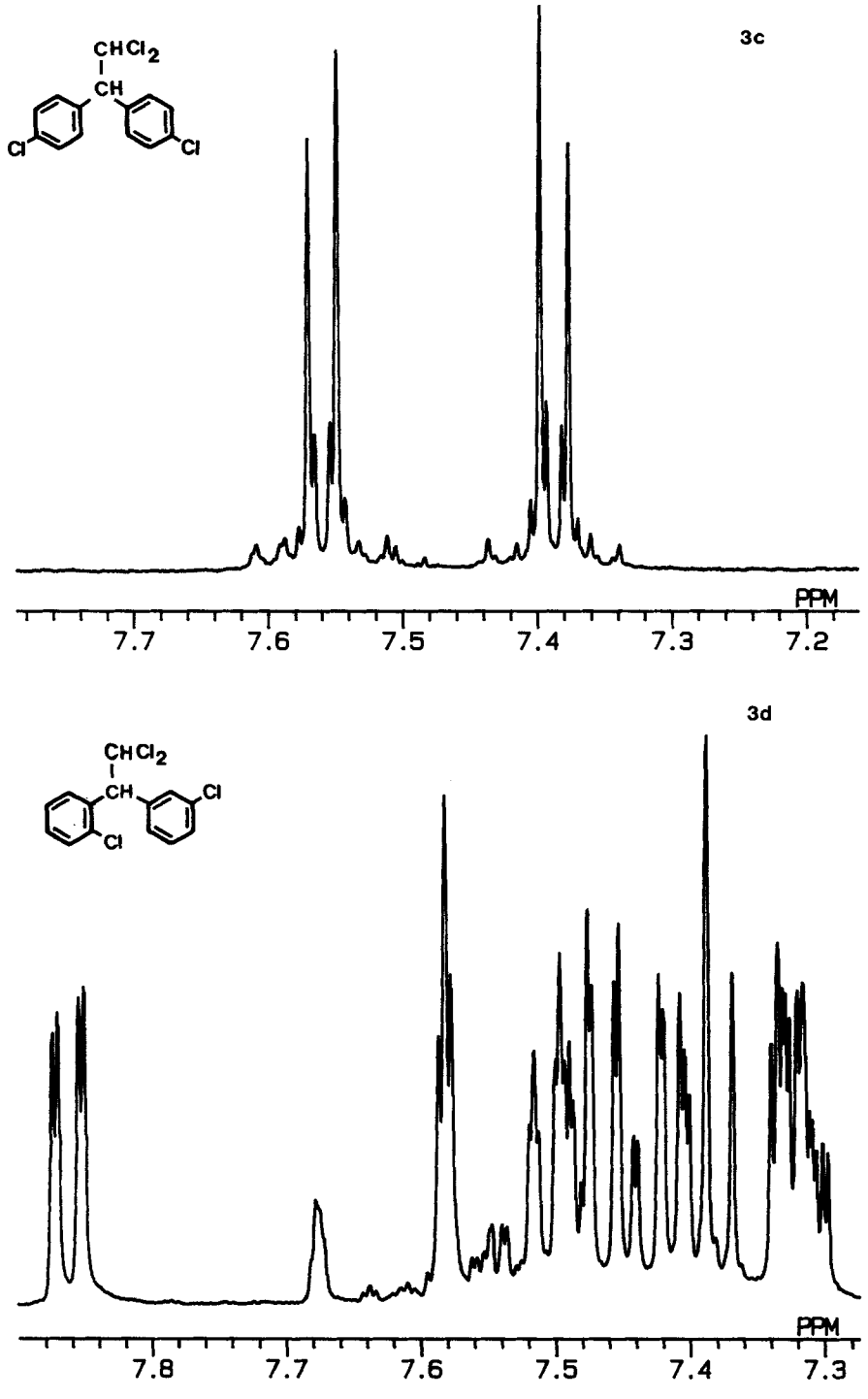

Figure 3 (cont.). ^{1}H NMR (400 MHz), aromatic region, of p,p'-DDD (3c) and of o,m'-DDD (3d).

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