

Synthesis of ^{14}C -Labelled and Unlabelled Coplanar Polychlorinated Biphenyls (PCBs)

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A modified Ullman reaction is described for synthesis of the three most toxic coplanar PCBs, 3,3',4,4'-tetrachloro[^{14}C]biphenyl, 3,3',4,4',5-pentachloro[^{14}C]biphenyl and 3,3',4,4',5,5'-hexachloro[^{14}C]biphenyl. 3,3',4,5,5'-Pentachloro[^{14}C]biphenyl and one *ortho*-substituted PCB, 2,3,3',4,4'-pentachloro[^{14}C]biphenyl, were also prepared. Methods for the preparation of unlabelled 3,3',4,4',5-pentachlorobiphenyl and 3,3',4,4',5-pentachlorobiphenyl are presented. Three isomeric tetrachloro(nitro)biphenyls were isolated and characterized as intermediates in the synthesis of 3,3',4,4',5- and 2,3,3',4,4'-pentachlorobiphenyl via the Ponomarenko reaction. The Cadogan diaryl coupling method was also used for synthesis of 3,3',4,4',5-pentachlorobiphenyl.

Polychlorinated biphenyls (PCB), present in commercial products or in samples taken at any trophic level of our global environment, represent a large number of individual substances known as PCB congeners.¹ Of these, three minor constituents, 3,3',4,4'-tetrachlorobiphenyl (**1**), 3,3',4,4',5-pentachlorobiphenyl (**4**) and 3,3',4,4',5,5'-hexachlorobiphenyl (**6**), have been found to be the most toxic PCBs as determined from their acute toxicity, teratogenicity and enzyme induction properties.^{2,3} These compounds referred to as the three coplanar PCBs, exert toxic effects similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In environmental samples **1**, **4** and **6**, (see Fig. 1 for structures) have been found in concentration levels up to three orders of magnitude higher than the corresponding levels of the most toxic polychlorodibenzo-*p*-dioxins.^{4,5}

Furthermore, 3,3',4,4'-tetrachlorobiphenyl, previously synthesized uniformly labelled with carbon-14, is specifically localized in the offspring in mice as shown by whole body autoradiography.⁷ The metabolism of this tetrachlorobiphenyl has also been extensively investigated.^{8,9} So that additional studies regarding toxicity, tissue localization and metabolism could be carried out, all three coplanar PCB congeners were prepared. In the present work, a method for the synthesis of ^{14}C -labelled 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexa-chlorobiphenyls is described.

The synthesis of unlabelled **1**, **4** and **6** may be performed as described via an Ullman reaction (**1** and **6**) or a Cadogan reaction (**4**).¹⁰ The synthesis of ^{13}C -labelled **1**, **4** and **6** and

^{14}C -labelled **1** has previously been described.^{11,12} We now describe improved methods for the synthesis of **1**, **4** and **6** labelled with ^{14}C . Different routes for the preparation of the highly toxic **4** have been investigated and the results are discussed.

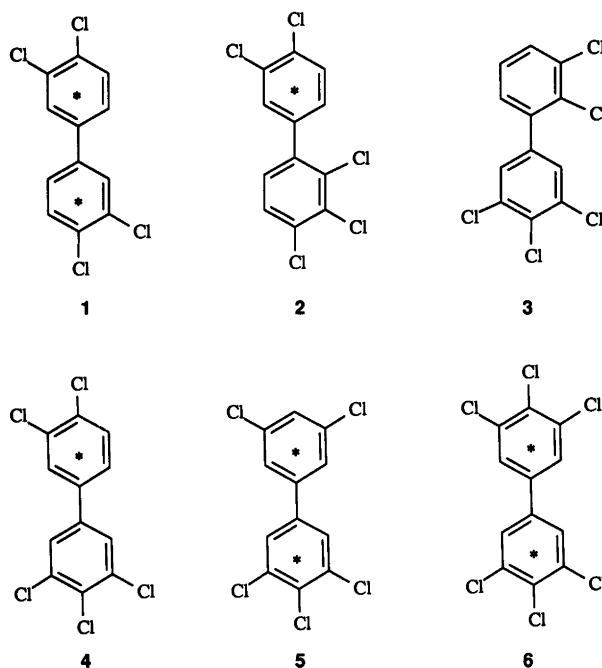


Fig. 1. Structures of the PCBs synthesized in the present study. ^{14}C -Labelled PCBs are marked with an asterisk (*) in the appropriate phenyl nucleus of the compound. The corresponding IUPAC numbers of the PCBs are: 77 (**1**); 105 (**2**); 122 (**3**); 126 (**4**); 127 (**5**); 169 (**6**).⁶

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Experimental

Chemicals. 3,4-Dichloro[U-¹⁴C]aniline (1.0 mCi, 12.2 mCi mmol⁻¹ and 2.5 mCi, 13.0 mCi mmol⁻¹) and 3,4,5-trichloro[U-¹⁴C]aniline (2.8 mCi, 15.8 mCi mmol⁻¹) were purchased from Sigma Radiochemicals (St. Louis, MO, USA). The following unlabelled chemicals were used: 3,4-dichloroaniline (Fluka AG), 3,4,5-trichloroaniline (Fluka AG), 2,3-dichloro(nitro)benzene (Aldrich), 3-methylbutyl nitrite (Aldrich), copper bronze (Karlors bruk) and 40% tetrabutylammonium hydroxide in water solution (Aldrich). Solvents for residue analysis were from Merck.

Thin layer chromatography was performed on silica gel plates (Merck, DC Fertigplatten, Kieselgel 60 F₂₅₄), RP-18 plates (Merck, RP18 F₂₅₄S) and on silica gel plates impregnated with a 0.8 M solution of tetrabutylammonium hydrogenphosphate.¹³ Liquid chromatography was performed on silica gel (Kieselgel 60, <0.063 mm). Larger quantities (>100 mg) of **4** were isolated after separation on activated charcoal (40 g, US Biochem. Corp., Decolorizing Carbon Norit A) put into a modified glass column.¹⁴ The charcoal was extracted three times with toluene and finally equilibrated with hexane (200 ml) prior to the separation of **3** and **4**.

Instruments. A homemade vertical steel furnace¹⁵ was used for all Ullman and Ponomarenko reactions.

GC-analysis was performed by means of a Shimadzu GC-9AM gas chromatograph equipped with a DB-5 fused silica capillary column, 30 m×0.25 mm, 0.25 μm film thickness (J&W Scientific, Inc, CA, USA) and a flame ionization detector (FID). Temperature program: 80°C, 1 min; 30°C; 10°C min⁻¹; 280°C. GC analysis of **4**, **5** and **6** were performed with Q DANI 8520 gas chromatograph equipped with an electron capture detector (ECD) operated in the splitless mode. The column was 50 m×0.25 mm (0.26 μm film) CP-SIL8-CB.

Gas chromatography/mass spectrometry (GC/MS) was performed on a Finnigan 4021 instrument upgraded with an 4500 ion source connected to a Finnigan Incos 2400 data system. The GC separations were performed under the conditions described above with helium as the carrier gas. GC/MS(EI) was performed with an ion source temperature of 140°C and an electron energy of 70 eV.

¹H NMR spectra were recorded on a Jeol 400 GX or a Bruker AM-500 instrument. Radioactivity was determined on a Packard TriCarb 460C liquid scintillation spectrophotometer.

Melting points were determined with a Kofler micro hot stage.

WARNING! 3,3',4,4',5-Pentachlorobiphenyl (**4**) and the other three coplanar PCBs **1**, **5** and **6** must be regarded as HIGHLY TOXIC compounds with toxicological properties similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. All handling must be performed in well ventilated fume hoods by trained personnel. Safety precautions similar those ap-

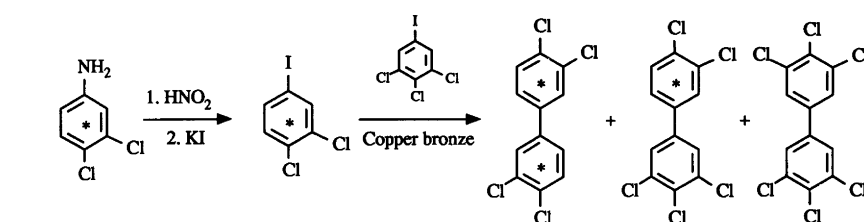
propriate to the handling of material labelled with radioactive isotopes should be adopted (cf. below).

Synthesis of radiolabelled compounds: general description.

Routes for the synthesis of radiolabelled compounds must be chosen with regard to available labelled starting materials. It is best to introduce the label as late as possible in the synthesis sequences to obtain a high isotopic yield and for safety reasons. The synthesis of radiolabelled compounds should only be started after appropriate pilot reactions have been performed, to identify major products and to determine the exact conditions for the reactions. A detailed protocol is then formulated taking into account the results from the pilot experiments and is followed for the synthesis of the labelled material. The radiolabelled material is controlled by liquid scintillation measurements after each major step during the synthesis. All work with ¹⁴C-labelled material is performed in a fume hood. Disposable glassware should be rinsed and disposed of in separate containers for radioactive contaminated material. Similarly all contaminated solvents to be discarded must be collected separately. All contaminated glassware should be carefully washed.

Non-volatile labelled compounds, as in the present case, can be transferred as solutions by use of Pasteur pipettes. In order to minimize the number of transfers, extractions are carried out in the reaction vessels. If water must be removed, the extract is passed through a column containing, e.g., Na₂SO₄. Evaporation of solvents is performed by passing a gentle stream of nitrogen over the solvent. The evaporated solvent is condensed in a trap cooled with ethanol and dry ice and the gas is finally passed through a flask containing ethanol. Both traps are checked for radioactivity after each evaporation. Vacuum distillations, with at least two cold traps, are performed when necessary. When working with radiolabelled compounds of high specific activity, the chemical amounts are often small and separations can therefore be performed on TLC. TLC bands containing labelled compounds are removed by vacuum into small glass containers (60 mm, 10 mm ID), narrow at both ends and supplied with a glass-wool plug. The container is then put in an upright position and the product is eluted from the gel with an appropriate solvent. The isolated products are compared with authentic unlabelled compounds on both straight phase and reversed phase (RP-18) TLC. The radioactive compounds are determined by autoradiography of the TLC plates. The labelled compounds are analyzed by GC-ECD when necessary.

3,3',4,4'-Tetrachloro[U-¹⁴C]biphenyl and *3,3',4,4',5-pentachloro[U-¹⁴C]biphenyl*. The synthesis of **1**, **4** and **6** is shown in Scheme 1. 3,4-Dichloro[¹⁴C]aniline (1.0 mCi, 12.2 mCi mmol⁻¹) dissolved in conc. H₂SO₄ (1 ml) was diazotized with NaNO₂ (8.2 mg, 0.12 mmol) in water (1 ml). Potassium iodide (19.6 mg, 0.12 mmol) in water was added to the diazonium salt as described.¹⁶ The product was extracted with dichloromethane (4×2 ml). The extract



Scheme 1.

was treated with a 10% $\text{Na}_2\text{S}_2\text{O}_5$ solution (3 ml) and dried by being passed through a small column filled with Na_2SO_4 . The eluate was collected and the volume of solvent was reduced to about 1 ml in an evaporator designed for handling radiolabelled material. 3,4-Dichloro(iodo)[U- ^{14}C]benzene (790 μCi) was obtained, yield 79%.

Dichloromethane solutions of 3,4,5-trichloro(iodo)benzene (33.5 mg, 0.11 mmol) and the labelled 3,4-dichloro(iodo)benzene (790 μCi , 0.065 mmol) were mixed and the solution was transferred to a tube containing copper bronze (100 mg). The solvent was carefully evaporated by means of a nitrogen gas flow and the residue was further dried in a desiccator for 15 min. The tube was sealed by use of a torch and after adjustment to room temperature, the ampoule was lowered into a vertical steel furnace of 13 mm diameter and kept at 220°C. The ampoule was removed from the oven after 1.5 h and opened, after being cooled in order to reduce internal pressure. Aliquots of chloroform were added to the opened glass ampoule and the products, together with copper bronze, were transferred to a Pyrex tube with a screw cap. The tube was centrifuged and the supernatant was transferred via a glass wool filter to a separate flask. The extraction procedure was repeated four times. The product mixture was separated on silica gel plates with impregnated tetrabutylammonium hydrogenphosphate with hexane as the mobile phase. The tetrachloro- and the pentachloro-[^{14}C]biphenyls were both further purified on RP-18 TLC plates with methanol as the mobile phase. The unlabelled 3,3',4,4',5,5'-hexachlorobiphenyl produced in the reaction was well separated from the ^{14}C -labelled **1** and **4** but was not isolated. According to TLC/autoradiography no other contaminants were detected in the final products. 3,3',4,4'-Tetrachloro[U- ^{14}C]biphenyl (67.1 μCi , 24.4 mCi mmol^{-1}), yield 4.2%, and 3,3',4,4',5-pentachloro[U-1',2',3',4',5',6'- ^{14}C]biphenyl (202.5 μCi , 12.2 mCi mmol^{-1}) yield 26%, were isolated.

3,3',4,4',5-Pentachloro[U-1',2',3',4',5',6'- ^{14}C]biphenyl and 2,3,3',4,4'-pentachloro[U-1',2',3',4',5',6'- ^{14}C]biphenyl. 3,4-Dichloro[U- ^{14}C]aniline (2.5 mCi, 13.0 mCi mmol^{-1}) was reacted with 1,2,3-trichlorobenzene according to the general Cadogan synthesis for preparation of ^{14}C -labelled PCBs.¹² The two ^{14}C -labelled products (**2** and **4**) were isolated after preparative TLC on tetrabutylammonium hydrogenphosphate impregnated silica gel plates. 3,3',4,4',5-Pentachloro[U-1',2',3',4',5',6'- ^{14}C]biphenyl (60 μCi ,

13.0 mCi mmol^{-1}) yield 2.4% and 2,3,3',4,4'-pentachloro[U-1',2',3',4',5',6'- ^{14}C]biphenyl (750 μCi , 13.0 mCi mmol^{-1}) yield 30%, were pure according to silica gel and RP-18 TLC/autoradiography.

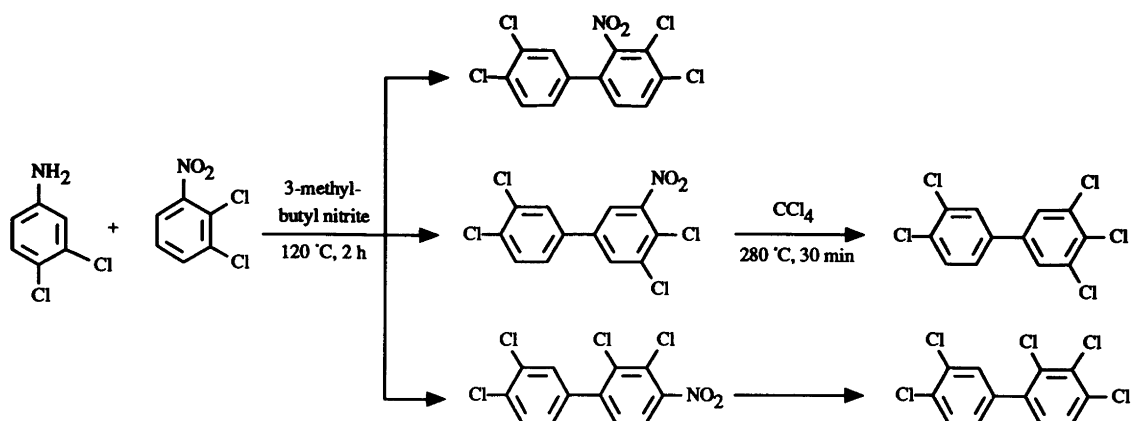
3,3',4,4',5,5'-Hexachloro[U- ^{14}C]biphenyl. 3,4,5-Trichloro[U- ^{14}C]aniline (36 mg, 2.8 mCi, 15.8 mCi mmol^{-1}) was dissolved in concentrated HCl (1 ml) and water (1 ml) and was diazotized with NaNO_2 (13.3 mg, 0.19 mmol) in water (1 ml) while being cooled in an ice bath. Potassium iodide (31.5 mg, 0.19 mmol) in cold water (1 ml) was slowly added to the diazonium salt and the resulting mixture was left gradually to reach room temperature. The product was extracted with hexane (4×2 ml) and passed through a small chromatography column consisting of an upper layer of Na_2SO_4 and a lower layer of neutral activated alumina, using hexane as the mobile phase. After solvent evaporation 33.6 mg (1.74 mCi, 0.11 mmol) of 3,4,5-trichloro(iodo)[U- ^{14}C]benzene (yield 60%) was obtained.

The labelled trichloro(iodo)benzene was again dissolved in hexane (5 ml) and transferred to a tube containing copper bronze (150 mg). The tube was put into an ultrasonic bath and the solvent carefully evaporated by means of a nitrogen gas flow. The residue was further dried in a desiccator for 1 h. The tube was sealed, placed in a vertical steel furnace of 15 mm diameter and kept at 220°C for 1.5 h. The reaction products were isolated as described above.

The crude mixture was initially purified by TLC on preparative silica gel plates with hexane as the mobile phase; the resulting bands were scraped off and extracted separately with hexane. Afterwards, two consecutive preparative TLC separations on 0.8 M tetrabutylammonium hydrogenphosphate impregnated silica gel plates were carried out on the extracts.

3,3',4,4',5,5'-Hexachloro[U- ^{14}C]biphenyl (**6**) (2.5 mg, 218 μCi , 31.6 mCi mmol^{-1}), 12% yield, was isolated in > 98% purity (GC-ECD). ^1H NMR 500 MHz (acetone- d_6): δ 7.53 (H2, H2', H6 and H6', s).

Two additional PCB congeners were also isolated as by-products of this reaction (purity > 95%, GC-ECD): 3,3',4,4',5-Pentachloro[U- ^{14}C]biphenyl (**4**) (0.9 mg, 88 μCi , 32 mCi mmol^{-1}), 5% yield. ^1H NMR 500 MHz (acetone- d_6), δ 7.68 (H5', d, J 8.4 Hz), 7.73 (H6', dd, J 8.4 and 2.2 Hz), 7.92 (H2 and H6, s), 7.87 (H2', d, J 2.2 Hz), and 3,3',4,5,5'-Pentachloro[U- ^{14}C]biphenyl (**5**) (1.8 mg, 179 μCi , 32 mCi mmol^{-1}), 10% yield. ^1H NMR 500 MHz



Scheme 2.

(acetone- d_6), δ 7.55 (H4', d, J 1.8 Hz), 7.77 (H2' and H6', d, J 1.8 Hz), 7.96 (H2 and H6, s).

3,3',4,4',5-Pentachlorobiphenyl via 3',4,4',5-tetrachloro-3-nitrobiphenyl. This synthesis is outlined in Scheme 2. 3,4-Dichloroaniline (2.0 g, 12.3 mmol) was dissolved in 2,3-dichloro(nitro)benzene (20 g, 104 mmol) at 80 °C. 3-Methylbutyl nitrite (4.0 ml) was added in the Cadogan coupling reaction as described elsewhere.¹⁷ The reaction mixture, after vacuum distillation of the dichloro(nitro)benzene, was separated on two successive silica gel columns, particle sizes 63–200 μm and < 63 μm , respectively. The following substituted biphenyl products were isolated in > 99% purity (GC and GC/MS).

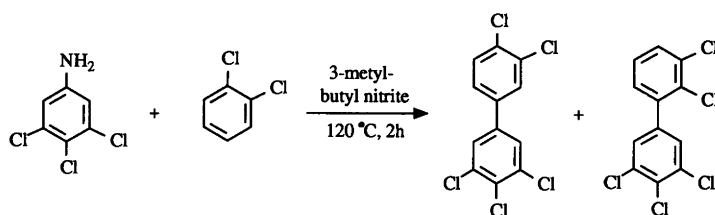
3,3',4,4'-Tetrachloro-2-nitrobiphenyl. Yield 146 mg (0.43 mmol) 3.5%, MS [EI 70 eV; % rel. abundance]: 335 (62, M^+), 337 [100, ($M+2$)⁺], 300 [27, ($M-\text{Cl}$)⁺], 270 {79, [$M-(\text{Cl}+\text{NO})$]⁺}, 254 {61, [$M-(\text{Cl}+\text{NO}_2)$]⁺}. M.p. 97.5–98 °C. ¹H NMR (400 MHz, acetone- d_6): δ 7.42 (H6', dd, J 8.3 Hz and 2.2 Hz), 7.66 (H2', d, J 2.2 Hz), 7.68 (H6, d, J 8.5 Hz), 7.73 (H5', d, J 8.3 Hz), 7.97 (H5, d, J 8.5 Hz). Found: C 42.70; H 1.50; N 4.10. Calc. for $\text{C}_{12}\text{H}_5\text{Cl}_4\text{NO}_2$: C 42.77; H 1.50; N 4.16.

3',4,4',5-Tetrachloro-3-nitrobiphenyl. Yield 119 mg (0.35 mmol) 2.8%, MS [EI 70 eV; % rel. abundance]: 335 (72, M^+), 337 [100, ($M+2$)⁺], 289 [5, ($M-\text{NO}_2$)⁺], 277 [10,

[$M-(\text{CO}+\text{NO})$]⁺}, 254 {80, [$M-(\text{Cl}+\text{NO}_2)$]⁺}. M.p. 141–142 °C. ¹H NMR (400 MHz, acetone- d_6): δ 7.74 (H5', d, J 8.3 Hz), 7.83 (H6', dd, J 8.3 Hz and 2.2 Hz), 8.09 (H2', d, J 2.2 Hz), 8.31 and 8.32 (H2 and H6, d, J 2.2 Hz), respectively. Found: C 42.80; H 1.50; N 4.20. Calc. for $\text{C}_{12}\text{H}_5\text{Cl}_4\text{NO}_2$: C 42.77; H 1.50; N 4.16.

2,3,3',4'-Tetrachloro-4-nitrobiphenyl. Yield 572 mg (1.70 mmol) 13.7%, MS [EI 70 eV; % rel. abundance]: 335 (73, M^+), 337 [100, ($M+2$)⁺], 305 [22, ($M-\text{NO}$)⁺], 277 [15, [$M+(\text{CO}+\text{NO})$]⁺]. M.p. 114–115 °C. ¹H NMR (400 MHz, acetone- d_6): δ 7.52 (H6', dd, J 8.3 Hz and 2.2 Hz), 7.70 (H5', d, J 8.3 Hz), 7.75 (H6, d, J 8.3 Hz), 7.76 (H2', d, J 2.2 Hz), 8.04 (H5, d, J 8.3 Hz). Found: C 42.60; H 1.55; N 4.10. Calc. for $\text{C}_{12}\text{H}_5\text{Cl}_4\text{NO}_2$: C 42.77; H 1.50; N 4.16.

3,3',4,4',5-Pentachlorobiphenyl (4). 3',4,4',5-Tetrachloro-3-nitrobiphenyl (60 mg, 0.18 mmol) was dissolved in tetrachloromethane (0.6 ml) and the solution transferred to a glass tube subsequently made into an ampoule. The ampoule was lowered into a vertical steel furnace kept at 280 °C. The oven was turned off after 30 min and left to cool for 3 h. (This is a safety precaution with regard to the internal pressure since the product formed is a highly toxic PCB). The Ponomarenko reaction product was isolated as described elsewhere.¹⁸ 3,3',4,4',5-Pentachlorobiphenyl was isolated in 60% yield, > 99% pure. The identity of the product was confirmed by comparison of GC and GC/MS



Scheme 3.

retention times and mass spectra of the appropriate compound **4**.

2,3,3',4,4'-Pentachlorobiphenyl 2. 3,3',4,4'-Tetrachloro-2-nitrobiphenyl was used as the starting material in a Ponomarenko synthesis of **2** as described for the synthesis of **4** above. The PCB congener was obtained in 86% yield. M.p. 116–117 °C (117–118.5 °C).¹⁹

3,3',4,4',5-Pentachlorobiphenyl and 2',3,3',4,5-pentachlorobiphenyl via Cadogan reaction, (Scheme 3). 3,4,5-Trichloroaniline (2.0 g, 10.2 mmol) was dissolved in 1,2-dichlorobenzene (12 ml) and the reaction was performed as described.¹⁷ The two PCB congeners were isolated as a mixture after removal of other by-products on a silica gel column with hexane as the mobile phase. The mixture was dissolved in 300 ml hexane–toluene (95:5) and slowly (10 ml h⁻¹) transferred to a charcoal column, prepared as described above. A further 300 ml of the same solvent was passed through the column at the same rate. Then the solvent was changed to hexane–toluene (1:3, 500 ml, 20 ml h⁻¹). More than 95% of the *ortho*-substituted PCB **3** was washed out from the charcoal with this solvent mixture. The glass column was subsequently connected to a 250 ml round-bottomed glass flask containing toluene (200 ml) and a modified Soxhlet extraction took place.¹⁴ The extraction was repeated once to ensure that all **4** was extracted.

Results and discussion

Different routes for the preparation of coplanar PCBs have been investigated. The majority of all radiolabelled PCBs synthesized were prepared via the Cadogan diaryl coupling reaction. Occasionally, the Ullman, Ponomarenko and Gomberg-Bachman methods were used for the preparation of ¹⁴C-labelled PCBs.

The Cadogan reaction between 3,4-dichloroaniline, a readily available ¹⁴C-labelled starting material, and 1,2,3-trichlorobenzene only give a low yield of **4**. Instead, formation of the isomeric **2**, is favored and results in a fair total yield of the two PCB isomers. This route may thus be used if primarily ¹⁴C-labelled **2** is required. It must, however, be emphasized that the separation of the two isomers obtained must be performed on silica gel plates impregnated with a tetrabutylammonium salt since no separation is obtained on conventional silica gel or RP-18 plates. If unlabelled **2** is to be synthesized, it is better to use 2,3,4-trichloroaniline and 1,2-dichlorobenzene in a Cadogan reaction since no difficulties are encountered upon the separation of **2** and 2,2',3,3',4-pentachlorobiphenyl, the other isomer formed in the reaction, on ordinary silica gel. Incidentally it is less difficult to separate isomeric PCB products substituted with chlorine atoms in one and two of the *ortho*-positions, respectively, compared with separate isomeric PCBs with only one chlorine or no chlorine atom in any of the *ortho*-positions.

It is shown, in the present study, that improved yields of the labelled coplanar PCBs may be obtained when synthesized via the Ullman reaction and compared with yields obtained in Cadogan reactions. This is also true for the preparation of the unsymmetrical component **4**. It is thus shown that the reaction can be performed on this semi-microscale after minor modifications of the general Ullman reaction procedure. The reaction between 3,4-dichloro-(iodo)[U-¹⁴C]benzene and 3,4,5-trichloro(iodo)benzene results in the formation of two labelled and one unlabelled PCB products (Scheme 1). Also, in this case, product separation must be carried out on impregnated TLC plates. In relation to the molar ratio of the two polychloro(iodo)benzenes in the reaction, a notably high yield of **4** was obtained. This may be due to the slightly higher reactivity of the trichloro(iodo)benzene. The low yield of the labelled **6** can be partly explained by accompanying products present in the starting material, 3,4,5-trichloro(iodo)-[U-¹⁴C]benzene. By comparison with authentic reference compounds, it was possible to identify two major labelled pentachlorobiphenyls formed in this reaction, namely **4** and **5** of which 3,3',4,5,5'-pentachloro[U-¹⁴C]biphenyl (**5**) has to our knowledge not previously been described. Even though **5** has not been identified in commercial PCB or in environmental samples, it is important to study the toxicity and tissue localizations of this pentachlorobiphenyl *in vivo*, since it does not contain any *ortho*-substituents.

In order to meet the requirements for larger quantities of unlabelled **4**, especially for toxicological investigations, methods for the safe preparation of **4** were investigated. Use of the Ullman reaction is not possible since no adequate preparative separation methods seem to be available. Also, due to the high toxicity of the three coplanar PCBs formed in the Ullman reaction, alternative methods must be considered. The two methods described in the present study both meet the safety requirements for the synthesis of toxic compounds as long as all glassware, solvent and chromatographic TLC plates or gels are treated with extreme care. The preparation of **4** via 3',4,4',5-tetrachloro-3-nitrobiphenyl is convenient and safe since the last step via the Ponomarenko reaction results in a pure product in high yield. Both isomeric tetrachloro(nitro)biphenyls formed in the initial Cadogan reaction can be used for the preparation of **2** via the Ponomarenko reaction. It is, however, also possible to use 3,4,5-trichloroaniline and 1,2-dichlorobenzene in a Cadogan reaction to obtain **4** in a mixture together with 2',3,3',4,5-pentachlorobiphenyl **3** in one step. The isomers **4** and **3** are formed in a molar ratio of approximately 2:3. Due to the strong retardation of **4** on the charcoal it is possible to isolate both isomers in a pure state.

The mass spectra of the isomeric 2-, 3- and 4-nitro(tetrachloro)biphenyl show some significant differences. Thus, the 4-NO₂-substituted PCB shows an abundant loss of NO (*m/z* = 305). The 2-NO₂-substituted PCB loses a chlorine (*m/z* = 300) more easily than the other isomers. All isomers have abundant fragments at *m/z* = 254 which represents

$[M-(Cl+NO_2)]^+$. Similar characteristics have been reported for some other of polychloro(nitro)biphenyls²⁰ and may be useful in structure elucidations for this type of compound.

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References

1. Ballschmiter, K., Rappe, C. and Buser, H. R. In: Kimbrough, R. D. and Jensen, A. A., Eds., *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*, Elsevier, Amsterdam 1989, Part 1, pp. 47–69.
2. Safe, S. *CRC Crit. Rev. Toxicol.* 13 (1984) 319.
3. Andersson, L., Nikolaidis, E., Brunström, B., Bergman, Å. and Dencker, L. *Toxicol. Appl. Pharmacol.* Submitted.
4. Tanabe, S., Kannan, N., Subramanian, A., Watanabe, S. and Tatsukawa, R. *Environ. Pollut.* 47 (1987) 147.
5. Norén, K., Lundén, Å., Sjövall, J. and Bergman, Å. *Chemosphere* 20 (1990). *In press.*
6. Ballschmiter, K., Schäfer, W. and Buchert, H. *Fresenius Z. Anal. Chem.* 326 (1987) 253.
7. Wehler, E. K., Bergman, Å., Kowalski, B. and Brandt, I. *Xenobiotica* 16 (1986) 295.
8. Wehler, E. K., Bergman, Å., Brandt, I., Darnerud, P. O. and Wachtmeister, C. A. *Drug Metab. Dispos.* 17 (1989) 441.
9. Koga, N., Beppu, M., Ishida, C. and Yoshimura, H. *Xenobiotica* 19 (1989) 1307.
10. Hutzinger, O., Safe, S. and Zitko, V. *The Chemistry of PCB's* CRC Press, Boca Raton 1974, p. 41.
11. Roth, R. W., Heys, J. R., Sangier, J. M., Chien, D. H. T., Rotert, G. A. and Erickson, M. D. *J. Labelled Comp. Radiopharm.* XXIV (1987) 185.
12. Bergman, Å., Bamford, I. and Wachtmeister, C. A. *J. Labelled Comp. Radiopharm.* XVIII (1981) 1023.
13. Bergman, Å., Göthe, R. and Wachtmeister, C. A. *J. Chromatogr.* 123 (1976) 231.
14. Jensen, S. and Athanasiadou, M. *Chemosphere*. Submitted.
15. Bergman, Å., Hagman, A., Jacobsson, S., Jansson, B. and Åhlman, M. *Chemosphere* 13 (1984) 237.
16. *Vogel's Textbook of Practical Organic Chemistry*, 4th ed., 1978, p. 695.
17. Sundström, G. *Acta Chem. Scand.* 27 (1973) 600.
18. Sundström, G. *Chemosphere* 5 (1976) 191.
19. Sundström, G. *Chem. Commun. Univ. Stockholm No. 10* (1974) 1. (Thesis).
20. Bakke, J. E., Feil, V. J. and Bergman, Å. *Xenobiotica* 13 (1983) 555.

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