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SYNTHESIS OF [^{14}C]BIPHENYL AND OF SOME CHLORINATED [^{14}C]BIPHENYLS CONTAINING 4-CHLORO-, 2,4-DICHLORO- AND 2,3,6-TRICHLOROPHENYL NUCLEI FROM THE CORRESPONDING LABELLED ANILINES

Å. Bergman and C.A. Wachtmeister

Environmental Toxicology Unit, Wallenberg Laboratory, University of Stockholm

S-106 91 Stockholm, Sweden

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ABSTRACT

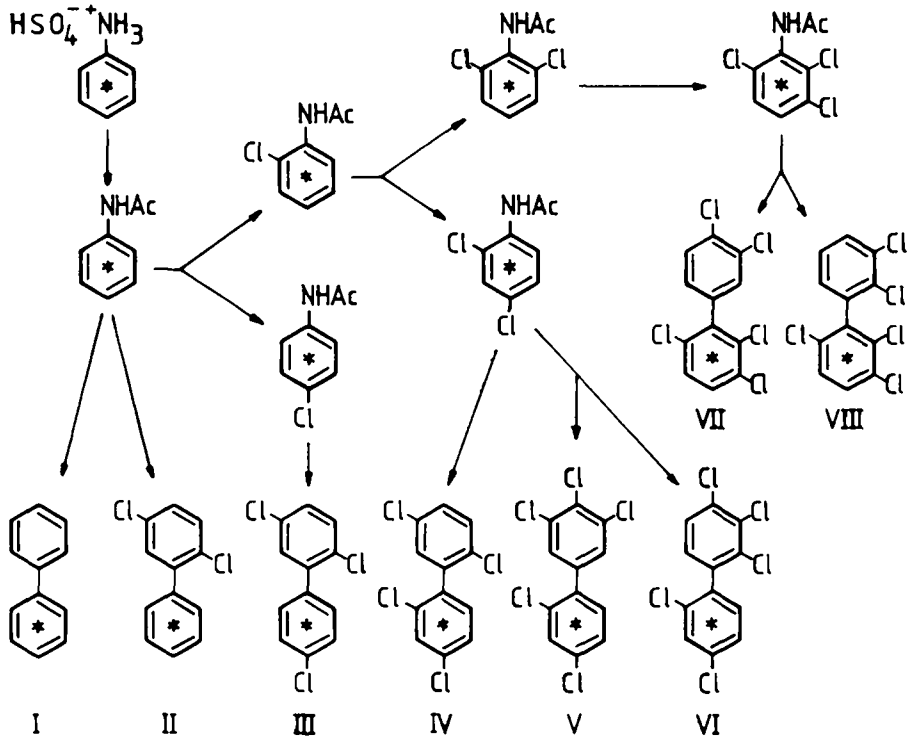
The preparation of ^{14}C-labelled biphenyl, 2,5-dichlorobiphenyl, 2,4',5-trichlorobiphenyl, 2,2',4,5'-tetrachlorobiphenyl, 2',3,4,4',5-pentachlorobiphenyl, 2,2',3,4,4'-pentachlorobiphenyl, 2,3,3',4',6-pentachlorobiphenyl and 2,2',3,3',6-pentachlorobiphenyl is described. [^{14}C]Aniline hydrogen sulfate used as a starting material was acetylated, chlorinated and deacetylated followed by coupling to benzene or an appropriate chlorobenzene to give the biphenyls labelled in the phenyl nuclei having chlorine atoms at the 4-, 2,4- or 2,3,6-positions, respectively. The structures of the labelled compounds were established by comparison with authentic samples among which 2',3,4,4',5- and 2,2',3,4,4'-pentachlorobiphenyl were not earlier described.

A simple method for the preparation of 2,3,6-trichloroacetanilide, unlabelled and labelled, was worked out. 2,6-Dichloroacetanilide in concentrated hydrochloric acid gave the meta substituted product when treated with chlorine.

An improved thin layer chromatographic technique utilizing plates impregnated with certain tetraalkylammonium salts was used for separation of some of the labelled compounds prepared.

INTRODUCTION

The preparation of pure isomers of polychlorinated biphenyls (PCB) has been necessary for analyses of components both in technical PCB mixtures and in biological material[1,2] as well as for studies of the metabolism of these compounds[3]. It has also been important to have radioactive



Scheme 1

labelled chlorinated biphenyls available for toxicological investigations and in this laboratory some ^{14}C-labelled biphenyls have been synthesized using [^{14}C]aniline hydrogen sulfate as starting material. The chlorine atoms were introduced by chlorination of [^{14}C]acetanilide to give chlorine at the 2,4- and 2,4,6-positions [4-6]. Nitration of [^{14}C]2,4,6-trichloroacetanilide and coupling of the corresponding aniline according to Shu Huang - Cadogan [7,8] to 1,2,3,4-tetrachlorobenzene, followed by displacement of the nitro group by chlorine gave 2,3,4,6-substitution in the labelled phenyl ring [9]. A hexachlorobiphenyl containing chlorine atoms at the 2,4,5-positions in both phenyl nuclei was finally prepared by nitration of [^{14}C]2,2',4,5'-tetrachlorobiphenyl prepared from [^{14}C]2,4-dichloroaniline and 1,4-dichlorobenzene [5]. Displacement of the two nitro groups by chlorine gave the desired substitution pattern of the biphenyl [5].

Autoradiography of mice treated with these biphenyls and with [^{14}C]2,2',4,5,5'-pentachlorobiphenyl, purchased from Mallinckrodt Co., St. Louis, U.S.A., indicated a highly specific accumulation of some of the biphenyls to the bronchial mucosa of the lungs [10-12]. In order to obtain more detailed knowledge about the nature of the bronchial accumulation some additional ^{14}C-labelled biphenyls were prepared and the preparation of these is presented in this paper (Scheme 1). From the results of autoradiographic investigations involving these biphenyls a hypothesis about certain structural requirements for the bronchial accumulation could be put forward [13,14]. In order to test the validity of the hypothesis a few additional labelled biphenyls were synthesized, the preparation of which is also described in the present paper (Scheme 1).

RESULTS AND DISCUSSION

An essential requirement for the preparation of the chlorobiphenyls (III-VIII) was the possibility to chlorinate [^{14}C]acetanilide at specific positions and in this work we have used three subsequent chlorination steps. N-Chlorosuccinimide was used in the first step to obtain a high yield of [^{14}C]4-chloroacetanilide [15,16], which was isolated by preparative thin layer chromatography (TLC). The [^{14}C]2-chloro- and 2,4-dichloroacetanilides also formed in this reaction were used as a mixture in the following step without further separation. In the second step chlorination was performed by the use of sodium chlorate [4], which under the conditions used gave [^{14}C]2,4-dichloroacetanilide as the major product and some 10% of the total yield as [^{14}C] 2,6-dichloroacetanilide.

The most common components of polychlorinated biphenyls in commercially produced PCB, with chlorine content between 50 and 60%, contain chlorine atoms in the 2,3,4-, 2,4,5- or 2,3,6-positions in at least one of the phenyl rings [2]. The synthesis of biphenyls containing 2,3,6-substitution was considered to be especially important for biological investigations because of the metabolic susceptibility of chlorinated biphenyls containing two vicinal hydrogens at the 4 and 5 positions [2,17,18]. A method earlier described by Attar *et al.* [16] for *meta* directed chlorination of [^{14}C]4-chloroaniline was considered unsuitable for a microscale preparation of the [^{14}C] 2,3,6-trichloroaniline required and other routes were tried. Thus a high yield of 2,3,6-trichloroacetanilide [19,20] was obtained when chlorine was passed through a solution of 2,6-dichloroacetanilide in concentrated hydrochloric acid. Acidic hydrolysis of the product gave a quantitative yield of 2,3,6-trichloroaniline [20,21], so far not commercially available. This method was readily applicable for the preparation of the labelled compound.

The preparation of 2,3,6-trichloroacetanilide is only successful if carried out in a sufficiently acidic medium, indicating that 2,6-dichloroacetanilide is basic enough to be practically completely protonated at least under the conditions used. This most probably reflects the inhibition of resonance caused by the two *ortho*-substituents [22]. The low rate of acidic hydrolysis of the 2,6-dichloro- and 2,3,6-trichloroacetanilides, a necessary prerequisite for the high yield observed in the reaction studied, should also reflect steric hindrance due to the *ortho*-substituents.

In order to make possible the isolation of the anilides in a pure state a new method, involving impregnation with ion pairs of silica gel thin layer plates was worked out [23]. Impregnation was done by dipping the plates into ethanol solutions of certain tetrabutylammonium salts of suitable concentrations. The silica gel was then regenerated at 100°C over night before the plates were used. Successful separation of labelled 2-chloro- and 2,4-dichloroacetanilides as well as of 2,6-dichloro- and 2,3,6-trichloroacetanilides was achieved when this method was used [23]. Also the separation of the biphenyls VII and VIII was improved when silica gel plates impregnated with tetrabutylammonium hydrogen phosphate were used.

The labelled acetanilides shown in Scheme 1 were hydrolyzed, the aniline salts formed were dried and then coupled to benzene or an appropriate chlorobenzene according to Shu Huang - Cadogan [7,8] with some modifications. The compounds prepared were [^{14}C]biphenyl (I) [24-28],

[^{14}C]2,5-dichlorobiphenyl (II)[29,30], [^{14}C]2,4',5-trichlorobiphenyl (III)[31], [^{14}C]2,2',4,5'-tetrachlorobiphenyl (IV)[5], [^{14}C]2,3,3',4',6-pentachlorobiphenyl (VII)[32] and [^{14}C]2,2',3,3',6-pentachlorobiphenyl (VIII)[32]. References 29-32 refer to the unlabelled compounds used for comparison with the labelled biphenyls prepared. Unlabelled 2',3,4,4',5- and 2,2',3,4,4'-pentachlorobiphenyls not previously described were prepared as well as their ^{14}C-labelled analogous (V and VI respectively).

The radiochemical yield of chlorobiphenyls in the coupling reactions ranged from high (~ 40%) of IV, V and VI prepared from [^{14}C]2,4-dichloroaniline hydrochloride, to more moderate yields (~ 20%) of I, II, III, VII and VIII prepared from the hydrochlorides of ^{14}C-labelled aniline, 4-chloroaniline or 2,3,6-trichloroaniline. The synthesis of biphenyl IV has earlier been described by Sundström[5] but with a slight modification made by us a marked increase of the isotope yield was obtained.

A number of pilot tests preceeded all the preparations of the labelled compounds here described in order to approach the optimal conditions for each reaction. This resulted in essential modifications of the experimental conditions for most preparations which makes a thorough description of each experiment necessary.

In connection with these studies several problems were encountered. It proved difficult to dissolve aniline- and 4-chloroaniline hydrochlorides in benzene and/or 1,4-dichlorobenzene at the conditions initially used resulting in very low yield of the corresponding biphenyls. These difficulties were at least partly overcome by suitable experimental techniques. No such problems were observed with the less polar 2,4-dichloro- and 2,3,6-trichloroaniline hydrochlorides. The latter salt, on the other hand, was very sensitive to the conditions during the evaporation procedure following the hydrolysis in concentrated hydrochloric acid. To prevent evaporation of 2,3,6-trichloroaniline one mole of sulfuric acid was added when the hydrolysis was completed. These observations might be explained by differences in basicity of the anilines in question[33]. The pK_a-value of 2,3,6-trichloroaniline is expected to fall between those of 2,6-dichloro- and 2,4,6-trichloroaniline, 0.42 and −0.03, respectively, which should be compared to that of aniline (4.60) and of 4-chloroaniline (3.98).

EXPERIMENTAL

Melting points were determined with a Kofler micro hot stage. UV-spectra were measured on a Beckman Model 24 spectrophotometer. Proton NMR spectra (100 MHz) were obtained on a Joel FX 100 instrument using tetramethylsilane as internal standard. Gas liquid chromatography (GLC) was performed on a HP 7620A gas chromatograph fitted with a ^{63}Ni detector. The glass column (1.8 m x 1/4") contained 3% SE-30 on Chromosorb W (AW, DMCS, 100-120 mesh). Gas chromatography-mass spectrometry (GC-MS) was performed on a HP 5700A gas chromatograph and a HP 5930A mass spectrometer. Thin layer chromatography (TLC) was performed on precoated silica gel plates (DC-Fertigplatten, Kieselgel 60 F-254, Merck) unless otherwise stated. All radioactivities were measured in 5 ml scintillation fluid (5 g PPO + 50 mg POPOP/l toluene) by an Intertechnique SL-30 liquid scintillation spectrometer. [^{14}C]Aniline hydrogen sulfate (66 Ci/mol,

98% radiochemical purity) was obtained from the Radiochemical Centre, Amersham, Great Britain. The identity of the labelled compounds prepared was confirmed by comparison (GLC, TLC) with authentic unlabelled compounds. The purity of the compounds prepared was established by GLC. All reaction temperatures mentioned below are bath temperature.

[^{14}C]Acetanilide. [^{14}C]Aniline hydrogen sulfate (5 mCi, 66 Ci/mol) was stirred with sodium hydrogen carbonate (6.4 mg) and water (25 µl) in dichloromethane (10 ml) for half an hour, followed by the addition of acetic anhydride (140 µl, 1.46 mmol) with the stirring continued over night at room temperature. This procedure was repeated twice to obtain a quantitative yield of [^{14}C]acetanilide. The [^{14}C]acetanilide obtained was diluted to a specific activity of 25 Ci/mol by the addition of acetanilide (16.8 mg).

[^{14}C]4-Chloroacetanilide. To the dry [^{14}C]acetanilide (5.1 mCi, 25 Ci/mol) was added a 0.1 M solution of N-chlorosuccinimide in acetic acid (3.8 ml) at 75°C. The temperature was raised to 100°C during 1 h and kept at this temperature for 2.5 h. After cooling aqueous solutions of sodium bisulfite (2.5 M, 2 ml) and sodium hydroxide (5 M, 5 ml) were added followed by chloroform extraction. The solution was evaporated to a volume of 1 ml and was subjected to preparative TLC with chloroform as eluent, to separate [^{14}C]4-chloroacetanilide (R_f = 0.09, 2.74 mCi) from a mixture of [^{14}C]2-chloro- and 2,4-dichloroacetanilides (R_f = 0.31, 2.49 mCi). The two anilides eluting together were used without further purification in the following chlorination step The relative amounts of [^{14}C]2-chloro- and 2,4-dichloroacetanilide was estimated after TLC separation on plates impregnated with a 0.4 M solution of tetrabutylammonium chloride in 50% ethanol and eluted with ether. From activity measurements of the extracts from spots with R_f-values of 0.22 and 0.12 the relative amounts of [^{14}C]2-chloro- and 2,4-dichloroacetanilide respectively was found to be 4:1.

[^{14}C]2,4-Dichloroacetanilide and [^{14}C]2,6-dichloroacetanilide. [^{14}C]2-Chloroacetanilide containing [^{14}C]2,4-dichloroacetanilide (2.49 mCi, 25 Ci/mol) from above was dissolved in a mixture of acetic acid (1.0 ml) and concentrated hydrochloric acid (1.8 ml). A 0.44 M solution of sodium chlorate (0.5 ml) was added and the mixture was stirred for 15 min at 19°C. The reaction was interrupted by the addition of sodium bisulfite and the anilides were extracted with chloroform. Evaporation of solvent and separation of the dichloroacetanilides by TLC with chloroform as an eluent gave [^{14}C]2,4-dichloroacetanilide (2.17 mCi) in a pure state (GLC) and [^{14}C]2,6-dichloroacetanilide (0.28 mCi) contaminated by traces of [^{14}C]2,4,6-trichloroacetanilide (GLC).

[^{14}C]2,3,6-Trichloroacetanilide. [^{14}C]2,6-Dichloroacetanilide (280 µCi, 12 Ci/mol) was chlorinated at 50°C in concentrated hydrochloric acid (2.5 ml) with dry chlorine gas (flow rate: 33 ml min^{-1}) during 15 h. After cooling to 0°C sodium bisulfite was added and the solution was neutralized with sodium hydroxide. Extraction with chloroform gave [^{14}C]trichloroacetanilide, which was purified on silica gel impregnated with a 0.4 M solution of tetrabutylammonium chloride in 50% ethanol and continuously eluted for 3 h with ether. [^{14}C]2,3,6-Trichloroacetanilide (126 µCi) containing traces of [^{14}C]2,3,4,6-tetrachloroacetanilide (GLC) was

isolated. No starting material was recovered.

2,3,6-Trichloroacetanilide. The unlabelled 2,3,6-trichloroacetanilide was synthesized by treatment of 2,6-dichloroacetanilide (1.0 g) in concentrated hydrochloric acid (100 ml) with chlorine as above. The reaction was terminated when almost all the starting material was consumed (GLC) (18 h). Any N-chloroacetanilides formed were reduced with sodium bisulfite. Extraction and evaporation of solvent gave a crystalline residue (0.96 g), which contained approximately 90% of 2,3,6-trichloroacetanilide (GLC). Purification of the anilide was carried out by repeated crystallisations from ethanol. The first recrystallisation gave a 97% pure product (0.52 g) and the second one gave 2,3,6-trichloroacetanilide of >99.9% purity, mp 174-175°C (lit.[20], 172-173°C). ^{1}H NMR [$(CD_3)_2CO$] : 7.48 (1 H, s), 7.50 (1 H, s), 9.00 (1 H) and 2.16 (3 H, s). MS (EE : 70 eV) M^+ : 237. UV (abs. EtOH (log ε)) 216 nm (4.38). (Found C : 40.3, H : 2.5, N : 5.8, $C_8H_6NOCl_3$ requires C : 40.3, H : 2.5, N : 5.9).

[^{14}C]Biphenyl. [^{14}C]Acetanilide (800 μCi, 25 Ci/mol) was treated with concentrated hydrochloric acid (1.8 ml) at 95°C for 3 h. The solution was evaporated ty dryness at room temperature in a desiccator (15 mm Hg). Benzene (1.0 ml) and potassium carbonate (2 mg) in water (10 μl) was added to the dry [^{14}C]aniline hydrochloride and the mixture was heated to 65°C. Isopentyl nitrite (40 μl) was added and the temperature was raised to 75°C. The reaction was completed in 2 h and the excess of benzene containing [^{14}C]benzene was carefully distilled off at a slight vacuum. The crude product was dissolved in hexane-chloroform (1:1) and transferred to a TLC plate which was developed twice with hexane. The appropriate band was removed and the [^{14}C]biphenyl was eluted with ethyl acetate. Activity measurements gave a yield of 21% of pure (TLC, GLC) [^{14}C]biphenyl (170 μCi, 25 Ci/mol).

[^{14}C]2,5-Dichlorobiphenyl. [^{14}C]Acetanilide (124 μCi, 12 Ci/mol) was hydrolyzed and evaporated to dryness as described above. The [^{14}C]aniline hydrochloride was dissolved in dimethyl sulfoxide (30 mg). 1,4-Dichlorobenzene (3 g) was added and the mixture was heated to 80°C. Acetic acid (10 μl) and isopentyl nitrite (50 μl) were added to the melt and the reaction mixture was stirred for 2 h at 80°C and finally for 1 h at 100°C. Excess 1,4-dichlorobenzene containing [^{14}C]benzene was carefully evaporated and the residue was chromatographed as described above. The total yield of [^{14}C]2,5-dichlorobiphenyl (> 98%, GLC) was 14% (17 μCi, 12 Ci/mol).

[^{14}C]2,4',5-Trichlorobiphenyl. [^{14}C]4-Chloroacetanilide (1700 μCi, 25 Ci/mol) was hydrolyzed as described using 4 h reaction time. The dry [^{14}C]4-chloroaniline hydrochloride was dissolved in 1,4-dichlorobenzene (6 g) at 60°C. Acetic acid (40 μl) and isopentyl nitrite (100 μl) were added and the solution was stirred for 2.5 h at this temperature and then for another 2.5 h at 100°C. The isolation and purification of the desired biphenyl was performed as described above. The total yield of [^{14}C]2,4',5-trichlorobiphenyl (> 98%, GLC) was 21% (250 μCi, 25 Ci/mol).

[^{14}C]2,2',4,5'-Tetrachlorobiphenyl. [^{14}C]2,4-Dichloroacetanilide (1570 μCi, 25 Ci/mol) was hydrolyzed as above. The dry hydrochloride was reacted with 1,4-dichlorobenzene (6 g) by the addition of acetic acid (54 μl) and isopentyl nitrite (100 μl) at 70°C. Another portion of isopentyl nitrite (100 μl) was added after 2.5 h and the temperature raised to 100°C and kept there for 2.5 h. Purification as above gave a 37% yield of [^{14}C]2,2',4,5'-tetrachlorobiphenyl (580 μCi, 25 Ci/mol, > 98% by GLC).

[^{14}C]2',3,4,4',5-Pentachlorobiphenyl and [^{14}C]2,2',3,4,4'-pentachlorobiphenyl. [^{14}C]2,4-Dichloroacetanilide (590 μCi, 25 Ci/mol) was hydrolyzed as described. To the dry [^{14}C]2,4-dichloroaniline hydrochloride was added 1,2,3-trichlorobenzene (3.5 g) which was heated to 70°C. Acetic acid (20 μl) and isopentyl nitrite (50 μl) was added in the same manner as for the synthesis of [^{14}C]2,2',4,5'-tetrachlorobiphenyl. Work up of the reaction mixture was performed as described above. The biphenyl fraction was separated by TLC into two bands with R_f = 0.36 containing [^{14}C]2,2',3,4,4'-pentachlorobiphenyl (170 μCi, 25 Ci/mol) > 99% pure by GLC and R_f = 0.40 containing [^{14}C]2',3,4,4',5-pentachlorobiphenyl (59 μCi, 25 Ci/mol) > 96% pure. The lower purity of the minor biphenyl was due to contamination by it's isomer. The total yield of the biphenyls formed in a ratio of 2.9:1 was 38%.

2',3,4,4',5-Pentachlorobiphenyl and 2,2',3,4,4'-pentachlorobiphenyl. 2,4-Dichloroaniline (1 g), was coupled as described by Sundström[34] with 1,2,3-trichlorobenzene (17 g). The coloured byproducts were removed on a small silica gel column (3 x 20 cm) eluted with hexane. Separation of the two pentachlorobiphenyls was achieved on a column (3.5 x 75 cm) of silica gel (Kiselgel 60, < 0.063 mm, Merck) eluted with hexane. The 2'3,4,4',5-pentachlorobiphenyl which eluted first was isolated and recrystallized from ethanol, m.p. 137-138°C. (Found: C: 44.2 H: 1.5; $C_{12}H_5Cl_5$ requires C: 44.2, H: 1.5). MS (EE: 70 eV): M^+ = 324. UV (abs. EtOH (log ε)): 254 (4.23) and 218 (4.63) nm. ^{1}H NMR ($CDCl_3$): 7.18 ($H_{6'}$, d, J 8.3 Hz), 7.30 ($H_{5'}$, dd, J 8.3 and 1.9 Hz), 7.40 ($H_{2,6'}$, s) and 7.47 ($H_{3'}$, d, J 1.9 Hz). The second biphenyl 2,2',3,4,4'-pentachlorobiphenyl was recrystallized from ethanol, m.p. 51-53°C. (Found: C: 44.4, H: 1.6; $C_{12}H_5Cl_5$ requires C: 44.2, H: 1.5). MS (EE: 70 eV): M^+ = 324. UV (abs. EtOH (log ε)): 218 (4.53) nm with a inflection point at 248 nm. ^{1}H NMR ($CDCl_3$): 7.07 (H_6, d, J 8.3 Hz), 7.13 ($H_{6'}$, d, J 8.3 Hz), 7.32 ($H_{5'}$, dd, J 8.3 and 2.0 Hz), 7.44 (H_5, d, J 8.3 Hz) and 7.50 ($H_{3'}$, d, J 2.0 Hz).

[^{14}C]2,3,3',4',6-Pentachlorobiphenyl and [^{14}C]2,2',3,3',6-pentachlorobiphenyl. [^{14}C]2,3,6-Trichloroacetanilide (126 μCi, 12 Ci/mol) was treated with concentrated hydrochloric acid (1.8 ml) at 95°C for 5 h. Before evaporation of the hydrochloric acid a 0.22 M solution of sulfuric acid (50 μl) was added, to avoid any evaporation of the aniline. To the dry [^{14}C]2,3,6-aniline hydrogen sulfate was added 1,2-dichlorobenzene (3 g) and the mixture was heated to 70°C. Isopentyl nitrite (50 μl) was added and 2.5 h later a new portion of the nitrite (50 μl) was added. After careful evaporation of unreacted dichlorobenzene the residue was dissolved in chloroform-hexane and transferred to a silica gel (0.5 g) column which was eluted with hexane. The chlorobiphenyl fraction was collected and the solvent evaporated. The two chlorobiphenyl isomers

were separated by the use of a silica gel plate impregnated with a 0.8 M solution of tetrabutylammonium hydroxide neutralized with phosphoric acid in 50% ethanol[23]. Elution with hexane gave [^{14}C]2,2',3,3',6-pentachlorobiphenyl with R_f = 0.32 (10.5 μCi, 12 Ci/mol, > 97% pure by GLC and [^{14}C]2,3,3',4',6-pentachlorobiphenyl R_f = 0.41 (9.6 μCi, 12 Ci/mol, > 97% pure by GLC in a total yield of 16%.

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