Polychlorinated Biphenyls V. [+]Synthesis of ^{14}C-Labelled 2,2′, 4,4′, 5,5′-Hexachlorobiphenyl, a Major Component in PCB-Mixtures

by
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Structural investigations of the components in commercial PCB-mixtures have shown that 2,2′,4,4′,5,5′-hexachlorobiphenyl (I) is a major constituent in mixtures containing more than ca. 50% (w/w) chlorine (SISSONS and WELTI 1971, WEBB and McCALL 1972, TAS and DeVOS 1972). In extracts of the fatty tissues from higher animals - including man - I, among some other higher chlorinated biphenyls, is often found in high concentrations which indicates a very slow metabolism or excretion of this compound (JENSEN and SUNDSTRÖM). In a recent study on the metabolism of some structurally defined PCBs using unlabelled material, no metabolites were detected when I was given to trouts, pigeon and rats (HUTZINGER et al. 1972).

In order to study the turnover of I we required the ^{14}C-labelled compound and we now wish to report a convenient synthetic route to 2,2′,4,4′,5,5′-hexachlorobiphenyl-^{14}C. The synthesis is based on some of the reactions used in previously published work on ^{14}C-labelled polychlorobiphenyls (SUNDSTRÖM 1973a, MORON et al. 1972, SUNDSTRÖM and WACHTMEISTER 1972), and on the observation that nitration of 2,2′,4,5′-tetrachlorobiphenyl (II) (SUNDSTRÖM 1973b) gives a high yield of 4,5′-dinitro-2,2′,4′,5-tetrachlorobiphenyl (III). Hitherto published syntheses of I involves Ullmann or Sandmeyer reactions and are not easily applicable to ^{14}C-labelled material (WEBB and McCALL 1972, TAS and DeVOS 1972, SAFE and HUTZINGER 1972).

2,4-Dichloroacetanilide-^{14}C (MORON et al. 1972) was hydrolysed to give 2,4-dichloroaniline-^{14}C which was coupled with 1,4-dichlorobenzene by the general procedure described by Cado-

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[+] Part IV, SUNDSTRÖM (1973a).

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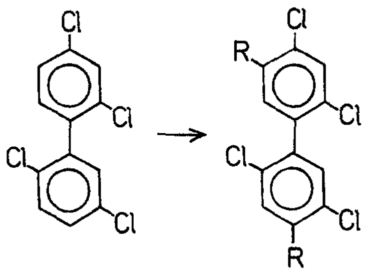
gan (SUNDSTRÖM 1973b, CADOGAN 1962). The product, 2,2',4,5'-tetrachlorobiphenyl-^{14}C upon nitration with fuming nitric acid gave one major and two minor di-nitrated compounds which were separated by preparative thin layer chromatography. On TLC a fourth band was also observed which contained several compounds and was not further investigated.

The major product, 4,5'-dinitro-2,2',4',5-tetrachlorobiphenyl-^{14}C (66%), was finally converted to 2,2',4,4',5,5'-hexachlorobiphenyl-^{14}C in almost quantitative yield by treatment with carbon tetrachloride at 280-290° for one hour (SUNDSTRÖM 1973a, PONOMARENKO 1962 a-b, PONOMARENKO 1963, PONOMARENKO and TSYBINA 1963).

Comparison of the hexachlorobiphenyl analogously prepared using unlabelled material with a sample of I synthesised by an Ullmann reaction (TAS and DeVOS 1972), showed that the products were identical (GLC, m.p., mixed m.p.). The structure of the dinitro compound III was also confirmed by its NMR spectrum, which showed four doublets ($J \sim 0.25$ Hz) in the region δ 8.00-8.38, consistent only with two pairs of protons in *para* positions to each other.

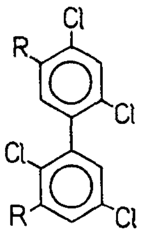
The two minor products obtained from the nitration of II were shown to be 3,5'-dinitro-2,2',4',5-tetrachlorobiphenyl (IV) and 2,5'-dinitro-2',3,4',6-tetrachlorobiphenyl (V) with reasonable certainty by exchange of the nitro groups for chlorine by the same procedure as for II. The chlorobiphenyls formed, VI and VII, respectively, were compared on GLC and TLC with samples unambigously synthesised (SUNDSTRÖM).



II

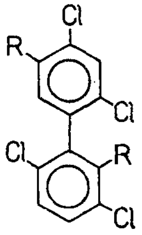
I R=Cl

III R=NO_2



IV R=NO_2

VI R=Cl



V R=NO_2

VII R=Cl

EXPERIMENTAL

Activities were measured with a Packard Tri-carb liquid scintillation spectrometer model 3320. Melting points were determined on a Kofler micro hot stage. Mass spectra were recorded on an LKB 9000 spectrometer. The NMR spectrum was obtained on a Varian A-60A instrument with tetramethylsilane as internal standard. All reagents used were of analytical grade unless otherwise stated.

Gas chromatography. The products were characterised by GLC using a Hewlett-Packard 7620A instrument fitted with an EC detector (Ni). Glass columns (0.20 x 160 cm) containing 4% (w/w) SF 96 on Chromosorb W A/W DMCS (100-120 mesh) at 180-210° were used. The gas flow (nitrogen) was about 30 ml/min.

Thin layer chromatography. The reactions were followed by TLC on 0.1 mm layers of silica gel (Kieselgel HF_{254}, Merck) using hexane or hexane-ethyl acetate (9:1) as solvent. Preparative TLC was performed on *ca.* 1 mm layers of the same adsorbent.

2,2',4,5'-Tetrachlorobiphenyl-^{14}C. 2,4-Dichloroacetanilide-^{14}C (MORON et al. 1972) (430 µCi, 10 Ci/mol) was heated with hydrochloric acid (12 M, 1 ml) and acetic acid (0.5 ml) at 90° for 2 h. The solution was cautiously evaporated to dryness in a vacuum dessiccator over sodium hydroxide and silica gel. To the residue was added 1,4-dichlorobenzene (Kebo AB, *purum*, 2 g) and the mixture was heated to *ca.* 70°. Isoamyl nitrite (Riedel-de Haen, *pure*, DAB. 6, 0.1 ml) was added with stirring which was continued for 2.5 h. After the addition of another portion of isoamyl nitrite (0.1 ml) the temperature was raised to *ca.* 100° for 2.5 h. Excess dichlorobenzene was evaporated *in vacuo* and collected in a cold trap chilled with dry ice (*cf.* SUNDSTRÖM and WACHTMEISTER 1972). The residue was dissolved in a small amount of chloroform-hexane (1:1) and added to a column (0.8 x 1 cm) of neutral alumina (Merck, activity grade I) which was eluted with hexane-ethyl acetate (9:1). The eluate was collected on a preparative TLC plate which was developed twice with hexane. The biphenyl zone was collected and eluted with chloroform. Evaporation of solvent gave 2,2',4,5'-tetrachlorobiphenyl-^{14}C, 115 µCi, 26%, as a crystalline residue.

4,5'-Dinitro-2,2',4',5-tetrachlorobiphenyl-^{14}C. To 2,2',4,5'-tetrachlorobiphenyl (115 µCi) was added cold fuming nitric acid (d=1.52, 1 ml). The mixture was stirred at 0^{o} for 1 h, diluted with ice water and extracted with chloroform (5 x 2 ml). The organic phase was dried by filtration through a small column of magnesium sulphate and applied to a preparative TLC plate. The layer was eluted continuously (cf. STAHL 1969) with hexane-ethyl acetate (95:5) for ca. 6 h. This procedure was necessary to separate the band containing the major product III from the slightly slower band with compound IV. Removal of the desired zone and elution with chloroform gave 4,5'-dinitro-2,2',4',5-tetrachlorobiphenyl-^{14}C as a slowly crystallising oil, 76 µCi, 66%.

The two minor products, IV and V (cf. below), were isolated in isotope yields of 6 and 8%, respectively, by elution of the appropriate bands from the TLC plate.

2,2',4,4',5,5'-Hexachlorobiphenyl-^{14}C. The above dinitro compound was transferred to an ampoule (Pyrex glass, volume 2.3 ml) with the aid of carbon tetrachloride (Riedel -de Haen, A.R.). The amount of carbon tetrachloride was adjusted to ca. 0.5 ml and the ampoule, placed in a steel autoclave, was heated in an electric oven at 280 - 290^{o} for 60 min. After cooling the solvent was evaporated and the residue dissolved in hexane and filtered through a column (0.5 x 1 cm) of neutral alumina which was eluted with hexane (10 ml). Evaporation of solvent gave chromatographically pure (TLC, GLC) 2,2',4,4',5,5'-hexachlorobiphenyl-^{14}C, 74 µCi, 97% (isotope yield from 2,4-dichloroacetanilide-^{14}C 17%).

4,5'-Dinitro-2,2',4',5-tetrachlorobiphenyl (III). 2,2',4,5'-Tetrachlorobiphenyl (SUNDSTRÖM 1973 b) (0.35 g) was added in small portions to nitric acid (d=1.52, 5 ml) at 0^{o}. The mixture was stirred for 1 h at 0^{o} and then poured on ice. Extraction with chloroform, drying ($MgSO_4$), and evaporation of solvent gave an oil which crystallised upon the addition of ethanol (0.33 g). Crystallisation from the same solvent gave pure 4,5'-dinitro-2,2',4',5-tetrachlorobiphenyl, m.p. 152.5 - 154^{o} (Found: C 38.1, H 0.7, N 7.3. M^{+} 380 m.u. (4 Cl) $C_{12}H_4N_2O_4Cl_4$ (382.0) requires C 37.7, H 1.1, N 7.3). NMR: (Acetone-d_6) δ 8.00 (d, J ~ 0.25), δ 8.12 (d, J ~ 0.25), δ 8.31 (d, J ~ 0.25) and δ 8.38 (d, J ~ 0.25).

GLC as above at 210° showed a retention time for compound III of 12.0 min.

Small amounts of the isomers IV and V (molecular ions 380 m.u. on MS) were isolated from the above mother liquor by preparative TLC using the same conditions as for the isolation of labelled III. The R_f values for compounds III, IV and V on TLC with hexane--ethyl acetate (9:1) as solvent were 0.42, 0.36 and 0.21, respectively.

Retention times on GLC at 210° were 10.9 and 7.8 min for compounds IV and V, respectively.

The conversions of IV to VI and V to VII were performed with 1 mg portions in 0.5 ml CCl_4 at 280-290° (Pyrex ampoule, volume 2.3 ml). Reaction times were _ca_. 2.5 and 1.5 h, respectively. The products were purified on TLC and injected on several GLC columns and compared with reference samples prepared by other routes (SUNDSTRÖM).

2,2',4,4',5,5'-Hexachlorobiphenyl (I). This compound was synthesised from 2,4,5-trichloroiodobenzene as described in Ref. 4 or by the same route as the labelled material. The products formed by the two methods, m.p. 103.5 - 104.5° (m.p. lit., 100 - 102°(WEBB and McCALL 1972); 102 - 103° (TAS and DeVOS 1972); 103 - 104° (SAFE and HUTZINGER 1972), were indistinguishable (TLC, GLC, m.p., mixed m.p.).

GLC as above at 210° showed a retention time of 4.3 min for this compound (13.4 min at 180°).

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