

SYNTHESIS OF CARBON-14 LABELED POLYCHLORINATED BIPHENYLS

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SUMMARY

Starting with benzene-U-¹⁴C, biphenyl-U-¹⁴C was synthesized in two steps in approximately 65 % yield. Then two kinds of polychlorinated biphenyls-U-¹⁴C mixtures were prepared by the direct chlorination of biphenyl-U-¹⁴C. The yield was more than 35 % based on benzene-U-¹⁴C consumed.

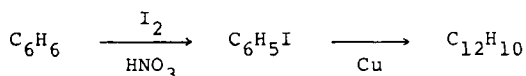
INTRODUCTION

Polychlorinated biphenyl (PCB) pollution in environment has brought about many problems to be solved. Radioactive PCB will be helpful for many biological investigations concerning distribution, metabolism and excretion of PCB in vivo.

Tritiated⁽¹⁾ and ³⁶Cl-labeled PCB⁽²⁾ have been prepared with the appropriate methods and utilized. Carbon-14 labeled PCB has been much required because of its high radiochemical stability. To our knowledge, however, it has not been available. Present work is directed to obtain two types of ¹⁴C-labeled PCB on a small scale. They have a similar composition to those of the commercial PCB. Kanechlor-400 and -600, which were chosen as the subject of our synthesis, had been produced and sold by Kanegafuchi Chemical

Industry Co. Ltd. until 1972. The main components of Kanechlor-400 and -600 are tetra- and hexachlorobiphenyls respectively, and these two types of PCB are abbreviated as KC-400 and KC-600 in the present paper.

Since benzene-U-¹⁴C has been synthesized in a high yield in our laboratory according to the method of Pichat and Baret⁽³⁾, this was used as a starting substance. Some synthetic routes for biphenyl-U-¹⁴C have been reported^(4,5,6), but all of them consist of many reaction steps. In order to improve the yield and to simplify the handling procedures, the following route was examined by modifying the known methods for non-labeled compounds^(7,8).



Then biphenyl-U-¹⁴C was chlorinated with chlorine gas in the presence of ferric chloride⁽¹⁾. The radiochemical purity of the product was determined by radio-gas chromatography.

DISCUSSION

Iodobenzene-U-¹⁴C was obtained with the yield of approximately 80 % without formation of by-products when equimolar amounts of benzene-U-¹⁴C and iodine were allowed to react in the presence of nitric acid (specific gravity: 1.28) at 85-90°C for 1.5 hr. The reaction was achieved in a Pyrex glass ampoule. The use of concentrated nitric acid (specific gravity: 1.4) or higher reaction temperature gave some by-products. Removal of the by-products is troublesome in the synthesis of a few millimole scale.

Crude iodobenzene-U-¹⁴C was converted to biphenyl-U-¹⁴C by the Ullmann reaction⁽⁸⁾. Using the copper catalyst treated with

iodine⁽⁹⁾, the satisfactory result (70-75 % yield) for a micro synthesis was obtained at 280-290°C for 3 hr.

Chlorination of biphenyl-U-¹⁴C was carried out according to a similar procedure to that by Ikegami, et al.⁽¹⁾ The reaction temperature was kept below 150°C to avoid a reactant loss due to the vaporization into the chlorine stream.

The rate and degree of chlorination were determined by the gas chromatographic analysis. The composition of the reaction mixture was very complicated at every stage of the chlorination. From a series of the experimental results, it was observed that the elution pattern of the gas chromatogram of the products changed with a certain regularity as the reaction proceeded. Samples for the examination were taken out successively with an appropriate interval and analyzed. This procedure was continued until the desired distribution pattern was obtained in the gas chromatogram. KC-400 or -600 analog could be obtained in every experiment by ceasing the chlorination at the desired stage.

The resulting reaction mixture was charged on a column filled with activated alumina and eluted with *n*-hexane. By this procedure, the almost colorless product was obtained from the black resinous mixture containing the catalyst. The overall yields based on benzene-U-¹⁴C consumed were 43 % for KC-400-U-¹⁴C and 35 % for KC-600-U-¹⁴C.

It can be seen from Figs. 1 and 2 that the distribution pattern due to mass signals on the gas chromatograms of KC-400-U-¹⁴C and KC-600-U-¹⁴C are almost identical to those of the corresponding commercial PCB and the radioactivity is distributed among all components in proportion to the mass peaks.

EXPERIMENTAL

Iodobenzene-U-¹⁴C. The mixture of 90 μ l of benzene-U-¹⁴C (1 mmol, 42.0 mCi), 140 mg of iodine and 1.5 ml of nitric acid (specific gravity: 1.28) in a sealed tube (Pyrex glass: 12 mm x 300 mm) was heated with shaking in an oil bath for 1.5 hr. The reaction temperature was maintained at 85-90°C. After cooling, the upper end of the ampoule was opened and the acidic content was neutralized with 10 % potassium hydroxide solution. Then the ampoule was connected to the vacuum line and evacuated under cooling with liquid nitrogen. Iodobenzene-U-¹⁴C was transferred together with other volatile components into a receiver by vacuum distillation. The receiver was detached from the vacuum line and the content was centrifuged. Iodobenzene layer was withdrawn with a syringe. The yield was 105 μ l, 41.5 mCi. The radiochemical purity determined by radio-gas chromatography was about 80 %. The impurity was benzene-U-¹⁴C alone. Crude iodobenzene-U-¹⁴C was used for the subsequent reaction without further purification.

Biphenyl-U-¹⁴C. A copper net of about 3 g was dipped in acetone containing 0.5 g of iodine until the brown color of iodine was discharged and then introduced into hydrochloric acid. Next the copper was washed with water and acetone, and then dried. The copper net and 95 μ l of crude iodobenzene-U-¹⁴C were sealed in a Pyrex glass ampoule (10 mm x 200 mm). The ampoule was placed in an electric furnace preheated at $210 \pm 10^\circ\text{C}$. The reaction temperature was raised to 280-290°C within one hour and was kept for 3 hr. After allowing to cool to room temperature, the ampoule was connected to the vacuum line and the upper end was opened with the conventional means. Unreacted benzene-U-¹⁴C was distilled in vac-

uum into a receiver cooled with liquid nitrogen. Then the ampoule was removed from the vacuum line and non-volatile components remaining in the ampoule were dissolved with petroleum ether. The petroleum ether solution was poured into a column filled with silica gel (10 mm x 300 mm). Biphenyl-U-¹⁴C was eluted with about 300 ml of petroleum ether. The radioactivity of the eluate was detected with the GM-detector. After the evaporation of petroleum ether, colorless crystalline biphenyl-U-¹⁴C was obtained. The yield was 42.4 mg, 22.4 mCi (53.3 % based on benzene-U-¹⁴C employed). Recovered benzene-U-¹⁴C was 7.4 mCi. Consequently, the radiochemical yield based on benzene-U-¹⁴C consumed was 64.7 %. The radio-gas chromatographic analysis showed that the product was radiochemically pure. Biphenyl-U-¹⁴C was diluted with the carrier to a suitable specific activity for the tracer experiment prior to chlorination.

KC-400-U-¹⁴C. Six hundred and sixteen milligrams (4 mmol, 20.4 mCi) of biphenyl-U-¹⁴C and 10 mg of anhydrous ferric chloride were placed in a 50 ml three necked pear-shaped flask, equipped with a capillary delivery tube, a reflux condenser (the upper end lead to an outlet) and a thermometer. This was heated in an oil bath at 80-90°C and then chlorine gas dried with concentrated sulfuric acid was bubbled into the molten biphenyl-U-¹⁴C at the rate of 60-80 ml/min. Chlorination was continued for 20 min with shaking. The reaction mixture became black and viscous. A small portion of the reaction mixture was sampled with a long fine pipette and dissolved in 1 ml of n-hexane. After filtration, one microliter of the solution was analyzed by the gas chromatography. This procedure was repeated with the 3 min interval until the

desired elution pattern was observed on the gas chromatogram. At the third analysis, in this case, the distribution pattern became almost identical to that of KC-400. After cooling, the reaction mixture was dissolved with 10 ml of n-hexane and purified on a column filled with the alkaline activated alumina (12 mm x 400 mm) using about 250 ml of n-hexane as an eluent. After evaporation of n-hexane, almost colorless KC-400-U-¹⁴C was obtained. The yield was 960 mg, 13.0 mCi (66.7 % based on biphenyl-U-¹⁴C). Indirect identification was made for non-labeled KC-400 synthesized under the conditions as described above.

Synthesized KC-400 : b.p. 155-170°C (3 mmHg), n_D^{20} 1.6305

Commercial KC-400 : b.p. 155-170°C (3 mmHg), n_D^{20} 1.6340

Each component of the synthesized KC-400 was also identified with the GC-MS. The number of chlorine atoms in each molecule corresponding to the major peaks on the gas chromatogram were identical to those of commercial KC-400 as shown in parentheses in Fig. 1.

KC-600-U-¹⁴C. Four hundred and sixty three milligrams of biphenyl-U-¹⁴C (3 mmol, 9.2 mCi) was chlorinated in the presence of 10 mg of anhydrous ferric chloride for 30 min under the same conditions as described for KC-400-U-¹⁴C. Then the flow rate of chlorine gas was increased to 110-120 ml/min and the reaction temperature was raised to 140-150°C. Chlorination was continued for further 25 min under these conditions. The flow of chlorine was stopped when the gas chromatogram of the reaction product showed the desired elution pattern. Purification was carried out with the same procedure as that for KC-400-U-¹⁴C. The sticky and almost colorless product was obtained. The yield was 618 mg, 5.0 mCi (55 % based on biphenyl-U-¹⁴C). Chlorine number in each molecule

corresponding to the major peaks of non-labeled KC-600 synthesized were identical to those of commercial KC-600.

Radio-gas chromatography(RGC). Two sets of the gas chromatographic apparatus, differing in the detecting method, were used for the radiochemical assay. One was equipped with a flame ionization detector (FID), and the other with radioactivity detector system consisting of a combustion train, a drying tube filled with magnesium perchlorate and a Geiger-Müller counting cell (4π, thin-window). Stainless steel columns (4 mm x 2 m) packed with "Silicone OV-17" were used for both gas chromatographs. The column-packing was 60-80 mesh Chromosorb W coated with Silicone oil OV-17. The ratio of support to column liquid was 100/20.

Both signals from the FID and the ratemeter were registered on a two-channel recorder. Helium was used as a carrier gas at the flow rate of 60 ml/min and was used as a scavenger in radioactivity assay too. The temperatures at the gas chromatographic column and FID-cell were 210°C and 250°C respectively. By adjusting the fine conditions of both gas chromatographs, close agreement between mass peaks and radioactivity peaks was obtained.

The radio-gas chromatogram of KC-400-U-¹⁴C is shown in Fig. 1. In this determination, one microliter of the sample solution, which was a solution of 20 mg of KC-400-U-¹⁴C in 1 ml of n-hexane, was used for mass assay and 3 μl for radioactivity assay. Figure 2 was obtained by the use of the n-hexane solution of KC-600-U-¹⁴C (55 mg/ml), 0.8 and 2 μl of which were used for mass assay and radioactivity assay respectively.

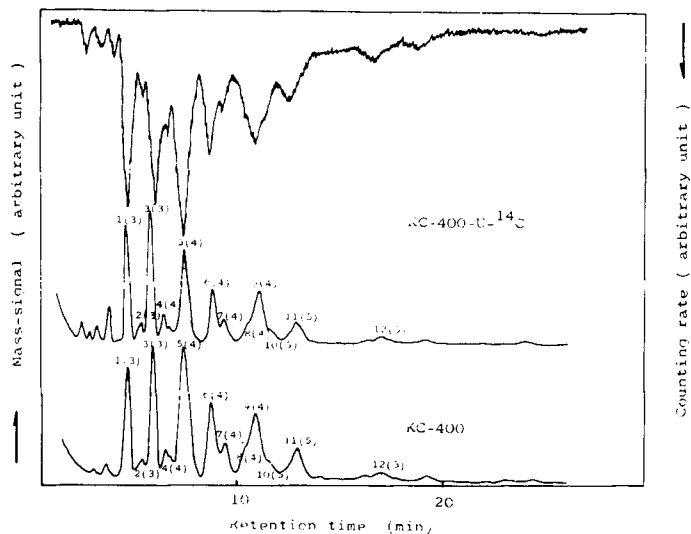


Fig. 1. The distribution pattern of the components in the synthesized KC-400-U- ^{14}C . The number in parentheses attached to the peak number indicates the number of chlorine atoms in a molecule determined by GC-MS.

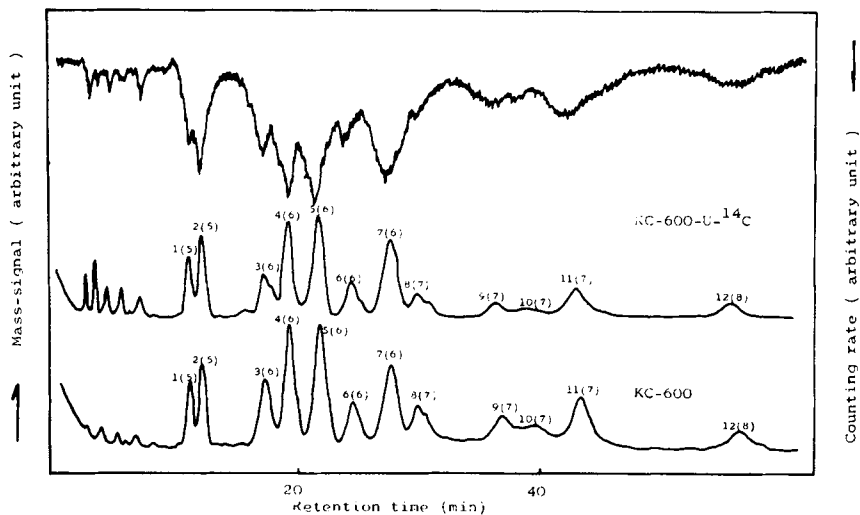


Fig. 2. The distribution pattern of the components in the synthesized KC-600-U- ^{14}C . The number in parentheses attached to the peak number indicates the number of chlorine atoms in a molecule determined by GC-MS.

Gas chromatography and mass spectrometry (GC-MS).

For identifying each component of PCB, the GC-MS, from Japan Electron Optics Lab. (Type; JOEL GC-JGC-1100), at Miyagi University of Education was used. Non-radioactive KC-400 and KC-600 synthesized under the same condition as described in each experimental section of this paper were used for the determination with the GC-MS. There were the resemblances between the signal from the total-ion monitor of the mass spectrometer and the signal from the FID, used for the mass assay in the RGC.

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