THE SYNTHESIS OF ^{14}C-LABELLED (U) 2,4,2',4'-TETRACHLOROBIPHENYL.

B. Melvas.
The Institute of Hygiene, University of Gothenburg, Gothenburg, Sweden.
Received on February 13th 1974.

SUMMARY

It was possible to prepare ^{14}C-2,4,2',4'-tetrachlorobiphenyl with a purity of 98% by condensation of ^{14}C-1-chloro-2,4-dinitrobenzene to yield ^{14}C-2,4,2',4',-tetranitrobiphenyl and substitution of the nitro groups by chlorine with thionyl chloride. The synthesis must be carried out on a small scale for two reasons- the cost and the hazards with radioactive material. The synthesis described here was performed on a 10mg scale. Both of the chemical reactions were performed in sealed ampoules and the overall yield varied between 30 and 40%.

INTRODUCTION

Since polychlorinated biphenyls (PCB) are known as an environmental hazard, great effort has been made to discover their distribution in nature. Toxic effects on man, animals and environment have been investigated. Although most of the experiments have been performed with technical products (1, 2) some investigators e.g. Hutzinger (3), have worked with pure isomers of PCB. When this study was planned it was reasonable to suppose that biphenyls with different numbers of chlorine atoms and isomers with the same number of chlorine atoms would be distributed and metabolized differently in animals. Thus the goal of this work was to synthesize a pure ^{14}C-labelled tetrachloroisomer of biphenyl. We chose 2, 4, 2', 4'-tetrachlorobiphenyl, which was then being used in autoradiographic and metabolic studies (4). It was shown that tetrachlorobiphenyl was metabolized very fast in comparison with hexachlorobiphenyls prepared by Wachtmeister and Sund-

ström (5, 6). It is now clear that 2, 4, 2′, 4′-tetrachlorobiphenyl does not give an ordinary fat distribution picture, but gives an accumulation in other tissues as well, especially in the cortex of the pronephros, the liver and the gastrointestinal organs. The excretion products in faeces and urine are mainly hydroxylated metabolites. In this paper the synthesis of pure ^{14}C-labelled 2, 4, 2′, 4′-tetrachlorobiphenyl is described (fig. 1, synth. I) (4). Ullman (7) showed that it is possible to prepare 2, 4, 2′, 4′-tetrachlorobiphenyl by condensation of 1-iodo-2, 4-dichlorobenzene (fig. 1, synth. II).

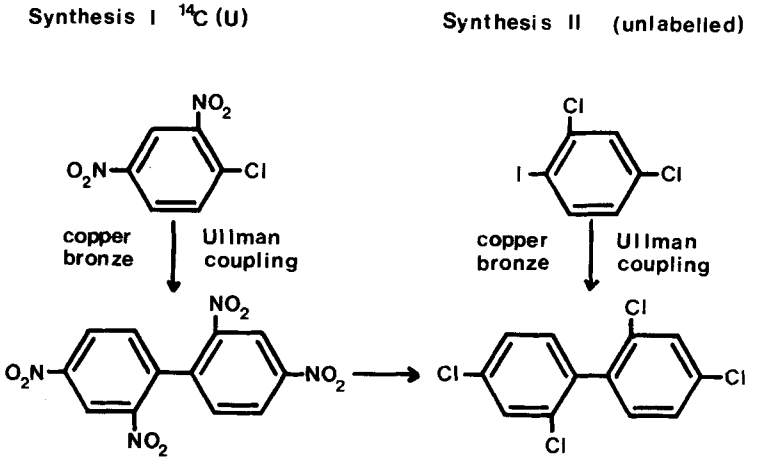


Fig. 1.
Synthetic scheme for the preparation of labelled and unlabelled 2,4,2′,4′-tetrachlorobiphenyl.

This method has been used for the preparation of larger amounts of unlabelled 2, 4, 2′, 4′-tetrachlorobiphenyl, but in order to prepare a labelled substance by this method on a milligram scale we had to start with commercial labelled aniline, and the synthesis involved several steps which lowered the yield.

Instead of with aniline it is possible to start with commercial ^{14}C-labelled 1-chloro-2, 4-dinitrobenzene, and by condensation according to Ullman (11) we obtained 2, 4, 2′, 4′-tetranitrobiphenyl. The nitro groups were then exchanged for chlorine by reaction with thionyl chloride (10), (fig. 1, synth. I).

The synthesis was performed on a 10 mg scale and the reaction product was identified by autoradiographic thin layer chromatography, gas chromatography and mass spectrometry. The results of the analyses of the labelled

compound were checked by comparison with equivalent analyses of unlabelled 2,4,2',4'-tetrachlorobiphenyl synthesized from 1-iodo-2,4-dichlorobenzene. The total yield was verified by liquid scintillation counting using a Hewlett-Packard Tri-Carb 2425.

THE SYNTHESIS OF 2,4,2',4'-TETRACHLOROBIPHENYL

Step I. - the synthesis of 2,4,2',4'-tetranitrobiphenyl.

The method of Ullman (11) for the preparation of 2,4,2',4'-tetranitrobiphenyl was originally performed on a 10 g scale, with nitrobenzene as solvent and a reaction time of one hour. This did not suit our purpose, and it was consequently necessary to gradually reduce the amount of starting material without lowering the yield.

This was possible by performing the reaction in a sealed test tube instead of in an open system. Nitrobenzene was used as a solvent because in the absence of a solvent a vigorous and exothermic reaction may occur. In addition to nitrobenzene, toluene, p-cumene, naphtalene, biphenyl and anthracene have been used as solvents (12). The use of nitrobenzene is a drawback if the reaction is carried out on a micro scale since it is difficult to separate nitrobenzene from the reaction product and at the same time achieve a good yield on a 10 mg scale. Kornblum and Kendall (13) used dimethylformamide as solvent, but our experiments with dimethylformamide did not give better results than those without solvent.

Activation of copper bronze was carried out as described by Fuson and Cleveland (8, 9). In order to obtain more homogenous results, the activation must be performed thoroughly. It is essential that drying of the activated copper bronze is carried out in a way prohibiting oxidation. Experiments have been done with drying the copper bronze in air, in desiccator or under nitrogen with heating. In the first ways the results varied between 5 and 40% total yield after substitution with chlorine, compared to 30-40% when the copper bronze was dried under nitrogen.

That tetranitrobiphenyl is the sole reaction product has been established by thin layer chromatography, gas chromatography and mass spectrometry. Mass spectrometry shows clear peaks for each nitro group even if the molecular peak is weak.

Step II - substitutions of nitro groups by chlorine.

The usual way to substitute an aromatic nitro group by chlorine is to reduce the nitro compound to the corresponding amine, which is then diazotized. The diazo group is replaced by a chlorine atom through a Sandmayer reaction. This reaction gave a very low yield on a micro scale, possibly depending on carbazole formation (7). Instead of by the traditional method, the displacement of nitro groups was carried out with thionyl chloride according to principles given by Meyer (10). He showed that 1-nitro-

2,4-dichlorobenzene and nitrobenzene, at a temperature of about 180-200°C are quantitatively converted to 1,2,4-trichlorobenzene and chlorobenzene, respectively.

We have repeated these experiments on a 1 and 10 mg scale. After analysing the products with gas chromatography and mass spectrometry, we could show that even on those scales it is possible to obtain a pure product. Guided by these first experiments, the substitution of nitro groups on a synthesized 2,4,2′,4′-tetranitrobiphenyl was carried out.

A certain temperature dependence of the reaction was noted. If the temperature rises above 190°C, the tetranitrobiphenyl was destroyed, but if the temperature goes below 170°C the yield of the displacement product decreases. Experimentally we found that the optimum temperature for the reaction was 186°C.

It should be noted that the reaction is very sensitive to the presence of moisture and methanol so the tetranitrobiphenyl must be carefully dried in a nitrogen atmosphere before the thionyl chloride is added.

FURTHER STUDIES OF THE REACTION WITH THIONYL CHLORIDE

Conversion of other nitrobiphenyls to chlorobiphenyls with thionyl chloride.

In attempt to synthesize the corresponding chlorobiphenyls from 2-nitrobiphenyl, 4-nitrobiphenyl, 2,4′-dinitrobiphenyl, 4,4′-dinitrobiphenyl and 2,4,6,2′,4′,6′-hexanitrobiphenyl in the same way as 2,4,2′,4′-tetrachlorobiphenyl, it was found that this was not possible at a temperature of 186°C and a reaction time of 24 hours. In contrast to 2,4,2′,4′-tetranitrobiphenyl, the nitrobiphenyls mentioned above gave a mixture of chlorinated biphenyls (see figures 2-6). (The numbers over the peaks in the gas chromatogram indicates the numbers of chlorine atoms in the biphenyls).

Direct chlorination of chlorobiphenyls with thionyl chloride.

In order to investigate this reaction further, 4-chlorobiphenyl and 4,4′-dichlorobiphenyl were treated with thionyl chloride in the same way as the nitrobiphenyls. From figures 7 and 8 it can be seen that a chlorination takes place although no nitro groups are present. This explains the fact that more than one chlorobiphenyl is obtained in the reaction with thionyl chloride. One should also bear in mind the fact that nitrobiphenyls are used as explosives and will disintergrate at higher temperatures. The aim of this last study was not to optimize the reaction and it might be possible to chose more suitable conditions favorizing substitution and minimizing the direct chlorination. In this way other pure chlorobiphenyls besides 2,4,2′,4′-tetrachlorobiphenyl might be prepared according to this method.

EXPERIMENTAL

Figure 2

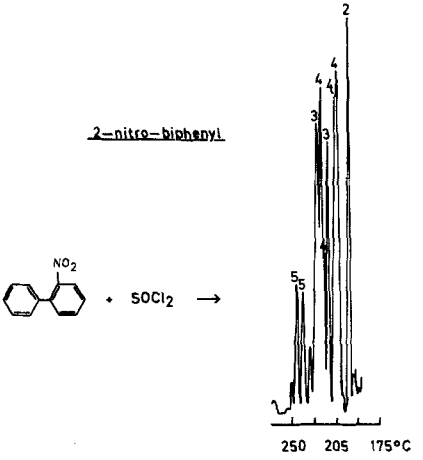


Figure 3

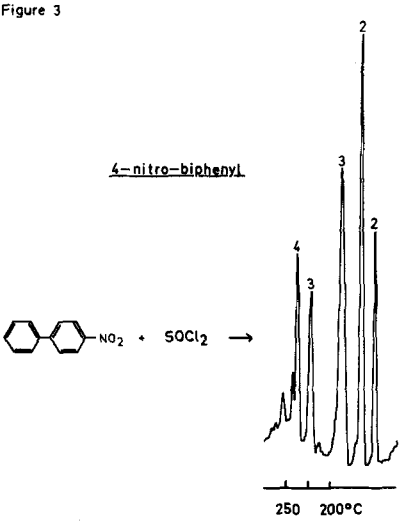


Figure 4

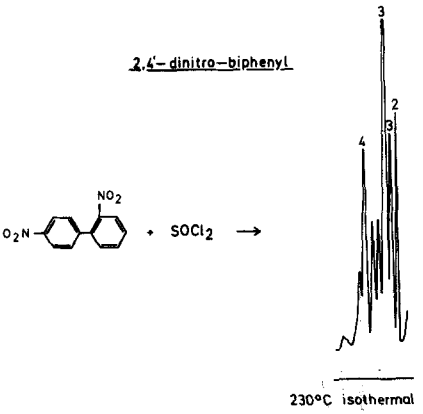


Figure 5

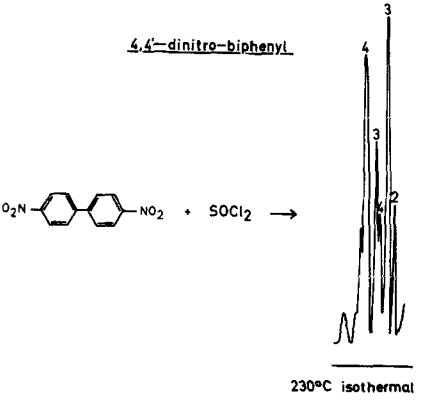


Figure 6

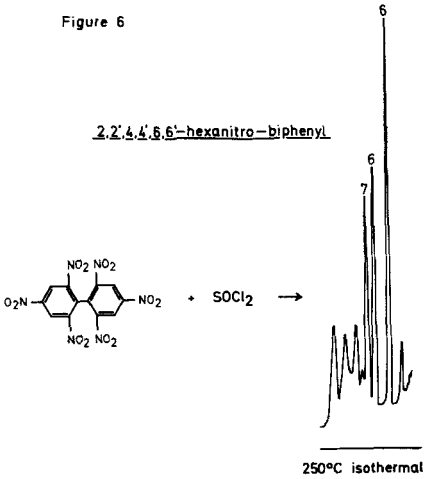


Fig. 2-6.
Substitution of nitro groups of nitrobiphenyls by chlorine with thionyl chloride. The separation of the reaction products are made on LKB-9000 gas chromatograph-mass spectrometer. The figures above the peaks indicates the number of chlorine atoms in the molecule according to mass spectra.

Fig. 7-8.
The gas chromatograms shows the possibility of direct chlorination of chlorinated biphenyls with thionyl chloride under conditions given in this paper. The figures indicates the number of chlorine atoms according to mass spectra.

Figure 7

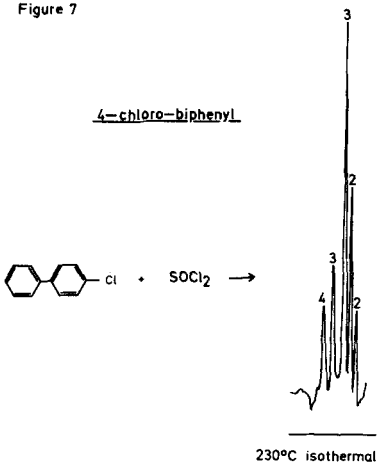
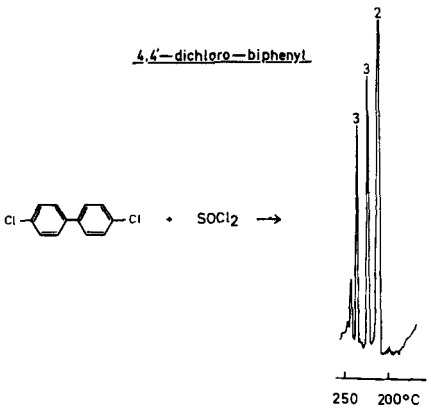


Figure 8



Activation and drying of the copper bronze.

2 g of copper bronze was activated (8, 9) by treatment with a 2% iodine solution in a 50 ml Erlenmeyer flask for 10 minutes, with occasional stirring.

The mixture was filtered through an OOH-filter in a Büchner funnel and washed 3 times with 10 ml of acetone: HCl (1:1). The resulting activated copper bronze cake was gently broken up without too much air exposure and dried under a stream of nitrogen in a heated (60°C) test tube for 2 hours.

The preparation of 2, 4, 2', 4'-tetranitrobiphenyl from 1-chloro-2, 4-dinitrobenzene.

10 mg of 1-chloro-2, 4-dinitrobenzene was dissolved in 100 µl of ether. This solution was used for dissolving 250 µCi of 1-chloro-2, 4-dinitrobenzene with a specific activity of 9.9 mCi/mmol. To a thick-walled test tube (120 mm in length, 10 mm OD, 7 mm ID) 10 mg of the activated copper bronze and the ether solution (100 µl) of 1-chloro-2, 4-dinitrobenzene were added.

The ether was evaporated under a nitrogen stream and the test tube was sealed. The test tube was put into an oven (186°C) in a rotating holder. After 24 hours it was taken out and eventual condensation was centrifuged down in the test tube before opening.

The reaction mixture was extracted 3 times with 2 ml of methanol (dried over molecular sieves). The methanol extraction was carried out at 40°C in an ultrasonic bath.

The three mixed methanol extracts were evaporated in a nitrogen stream in a new thick-walled test tube (120 mm in length, 10 mm OD, 7 mm ID), since traces of methanol or water will interfere with the synthesis.

To the residue 0.5 ml of thionyl chloride was added. The test tube was sealed and allowed to rotate in an oven at 186°C for 48 hours.
The reaction solution was slowly poured into water and when the excess thionyl chloride had been destroyed, the residue was extracted 3 times with ether and the combined ether extracts were dried over anhydrous sodium sulphate. The reaction product was purified by column chromatography. The ether solution was then evaporated to dryness. The residue was dissolved in pentane and placed on a dry column of silica gel (70-325 mesh, 110 mm x 5 mm). Pentane was used as an eluent. The first fractions contain mainly trichlorobenzenes and were well separated from the tetrachlorobiphenyl. The eluates were analysed by gas chromatography, mass spectrometry and autoradiographic thin layer chromatography.

Chemicals.

Hydrochloric acid, zur Analyse, Merck.
Acetone, für Analyse, Riedel de Haën.

Jodum resublimatum, pro analysi, Merck.
Copper bronze, for organic syntheses, BDH.
1-chloro-2,4-dinitrobenzene, AnalaR, BDH.
^{14}C-labelled 1-chloro-2,4-dinitrobenzene (U), Radiochemical Center, Amersham.
Ethyl Ether Anhydrous, Analytical Reagent, Mallinckrodt.
Methanol, zur Analyse, Merck.
Molekular sieve, Union carbide, Type 3A.
Thionyl chloride, zur Synthese, Merck.
Sodium sulphate anhydrous, AnalaR, BDH.

Unlabelled substance.

For the confirmation of the structure of the radioactive 2,4,2′,4′-tetrachlorobiphenyl, unlabelled 2,4,2′,4′-tetrachlorobiphenyl was synthesized by condensation of 1-iodo-2,4-dichlorobenzene according to Ullman (7), but with activation of the copper bronze. The activation of the copper bronze is described under the synthesis of labelled tetrachlorobiphenyl.

Gas chromatography.

The gas chromatographic separations were made on a Varian 204 gas chromatograph.
Column: 1.8 m x 1/8" stainless steel.
Support: Chromosorb W AW DMCS 80/100 mesh.
Liquid phase: 2% SE-30.
Carrier gas: Nitrogen.
Flow rate: 20 ml/min.
Detector: Flame ionisation.
Detector temperature: 270°C.
Injector temperature: 250°C.
Flame ionisation was preferred instead of electron capture detection in order to detect impurities other than those with high electron affinity.

Both isothermal and programmed modes were used. When programmed from 80°C to 260°C only one single peak could be seen on the chromatogram. The 2,4,2′,4′-tetrachlorobiphenyl was eluted at 179°C.

Mass spectrometry.

Labelled 2,4,2′,4′-tetrachlorobiphenyl was analysed by mass spectrometry and so was the unlabelled 2,4,2′,4′-tetrachlorobiphenyl synthetized from dichloro-iodobenzene. Both mass spectra were identical within the error of the instrument. (Figures 9 and 10).

Mass spectrometric conditions:
Ionisation voltage: 70eV.
Ionisation source temperature: 285°C.
Separator temperature: 250°C.
Column-liquid phase: OV 1.
He flow: 20 ml/min.

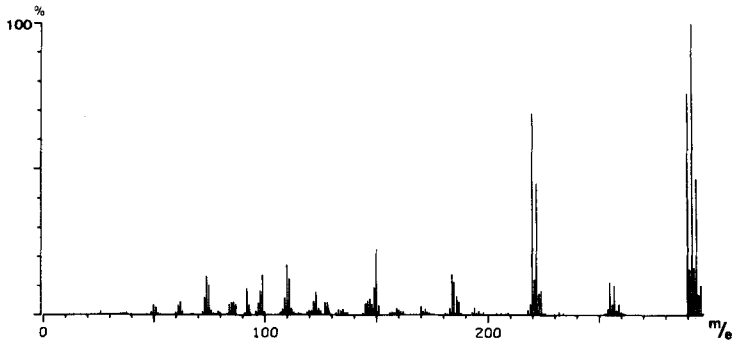


Figure 9

Mass spectra of
2,4,2' 4'-tetrachlorobiphenyl ^{14}C (U)
starting from 2,4-dinitrochlorobenzene ^{14}C (U)

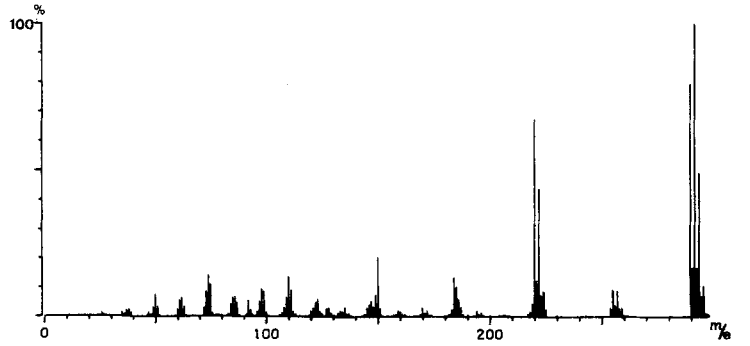


Figure 10

Mass spectra of
2,4,2',4'-tetrachlorobiphenyl (unlabelled)
starting from 1-iodo-2,4-dichlorobenzene

Program rate: 10°C/min; starting at 180°C.
The mass spectra were run on an LKB-GC-mass spectrometer at the crystallographic group, Institute of medical chemistry, University of Gothenburg.

Thin layer chromatography.

Pre-coated 0.25 mm silicagel TLC-plate with fluorescent indicator F-254, 10 x 20 cm (Merck 5729/0050) were used. With heptane as a moving phase the radioactive compound had an Rf-value of 46.

Autoradiography of the plate showed only one spot. No fluorescent spot could be detected in UV-light.

ACKNOWLEDGEMENTS

The author wishes to thank Professor Bengt Smith, Department of Technical Analytical Chemistry at the Lund Institute of Technology, for helpful and valuable discussions, Professor Maths Berlin, Department of Hygiene, University of Lund and Professor Bengt Mollstedt, Department of Hygiene, University of Gothenburg, for their kind interest, Docent Robert E. Carter for linguistic criticism and Miss Birgitta Börjesson for her skilful technical assistance.

Financial support for this investigation was provided by the Swedish Environmental Protection Board.

REFERENCES

1. Grant, D. L. et al., Bull. Env. Contam. Toxicol. 6:102 (1971).
2. Bagley, G. E. et al., J. A. O. A. C. 53:251 (1970).
3. Hutzinger, O., Science 178:312 (1972).
4. Melvås, B., Brandt, I., PCB-conference 1972. Publ. nr. 1973:4E, p. 87.
5. Wachtmeister, C. A., Sundström, G., PCB-conference 1972. Publ. nr. 1973:4E, p. 73.
6. Sundström, G., Wachtmeister, C. A., Acta Chem. Scand. 26 (1972). In press.
7. Ullman, S., Liebigs Ann. der Chemie. 332:38 (1904).
8. Fuson, R. C., Cleveland, E. A., Org. synth. 20:44 (1940).
9. Melvås, B., Nord. Hyg. tidskr. LII:40 (1971).
10. Meyer, H., Sitzb. d. mathem. naturw. Kl., Abt. IIb 124 Bd:339.
11. Ullman, S., Ber. 34:2177 (1901).
12. Fanta, P. E., Chem. Rev. 38:149 (1946).
13. Kornblum, N., Kendall, D. L., J. Am. Chem. Soc. 74:5782 (1952).