Contributions to Ecological Chemistry CXV[1] Metabolism of 2, 5, 4,′—Trichlorobiphenyl—^{14}C and 2, 4, 6, 2′, 4′—Pentachlorobiphenyl—^{14}C in the Marsh Plant *Veronica beccabunga*

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Hydroxylation of polychlorinated biphenyls upon metabolism in diverse organisms like birds (HUTZINGER et al. 1972), mammals (BLOCK and CORNISH 1959; HUTZINGER et al. 1972; YOSHIMURA et al. 1973; GARDNER et al. 1973; LAY et al. 1975; GREB et al. 1975 a and b; KAMAL 1975), microorganisms (WALLNÖFER et al. 1973) and higher plants (MOZA et al. 1973 and 1974) has been shown. The fact that 2, 2' -dichlorobiphenyl (a representative of the lower chlorinated group) is metabolized to hydroxy compounds in a marsh plant (MOZA et al. 1973) led us to investigate the fate of higher chlorinated isomers of PCBs in the same marsh plant in an effort to get comparative data on the metabolic behaviour of PCB isomers with different chlorine content. Results of the conversion of 2, 5, 4' -trichlorobiphenyl-^{14}C and 2, 4, 6, 2' , 4' -pentachlorobiphenyl-^{14}C in the marsh plant Veronica beccabunga are recorded in this work.

MATERIALS AND METHODS

Plant Growing and Working up

Veronica beccabunga, a plant which grows on river banks, was collected and recultured in glass jars, one for the experiment with trichlorobiphenyl and the other for pentachlorobiphenyl,

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under phytotron conditions (day and night cycle 18/6 hrs., temp. $25^{o}/8^{o}$ C, relative humidity 65/95%). The plants were rooted in soil (2 cm from the bottom of the jars which was covered with a 2 cm layer of small pebbles and a 2.5 cm layer of water). The water level in the experiment jars was kept constant, and the jars were cooled with flowing tap water. After one week, 2, 5, 4' -trichlorobiphenyl-^{14}C (spec. activity 0.66 mC/mMol, radiochemical purity 99%) and 2, 4, 6, 2', 4' -pentachlorobiphenyl-^{14}C (spec. activity 1.82 mC/mMol, radiochemical purity 99.6%), both synthesized in this Institute (SANDROCK and ATTAR, unpublished), dissolved separately in a minimum quantity of acetone, were applied dropwise on the leaves with a micro syringe (76 ppm of 2, 5, 4' -trichlorobiphenyl and 133 ppm of 2, 4, 6, 2', 4' -pentachlorobiphenyl, on the basis of the fresh plant weight at the end of the experiment).

Six weeks after application of the compounds, water, plants and soils were analysed. The plants were homogenized and plants and soils were extracted in a soxhlet with hot methanol for 48 hours. The water, after acidification to pH 2, was extracted several times with ether in the case of trichlorobiphenyl and with dichloromethane in the case of pentachlorobiphenyl.

The radioactivity of the water and of the extracts was determined by liquid scintillation counting (Packard, Tri-Carb 3380 and 3375) with external standardization (scintillator based on dioxane). Unextractable residues were determined by automatic combustion (Oxymat, Intertechnique) and liquid scintillation counting; a toluene-based scintillator containing phenethylamine was used for counting $^{14}CO_2$. Thin-layer-chromatography (TLC) plates were scanned for radioactive substances on a scanner supplied by Berthold-Frieseke GmbH, Karlsruhe. For the

determination of 2, 5, 4' -trichlorobiphenyl, 2, 4, 6, 2', 4' -pentachlorobiphenyl and their conversion products, the individual extracts were concentrated in a rotary evaporator. The concentrates were subjected to TLC (thin-layer-chromatography) analysis on silicagel plates, solvent benzene/ethyl acetate (9/1). Zones of 1 cm were removed from the plates and the radioactivity of each zone was counted in a liquid scintillation counter (scintillator based on dioxane).

Isolation of Metabolites of 2, 5, 4' -Trichlorobiphenyl

The methanolic plant extract was resolved into four radioactive zones (Table 2) on a TLC plate using benzene/ethyl acetate (9/1). Zone IV which moved to the front was found to be 2, 5, 4' -trichlorobiphenyl upon TLC and GLC comparison with an authentic sample. The combined zones II and III were separated into several radioactive substances on a TLC plate (plate run first with 25% benzene in n-hexane and then with benzene). The main metabolite (Rf 0.47), after repeated purifications, was methylated with freshly prepared diazomethane. The methylated substance (Rf 0.59 on silicagel plate developed with 25% benzene in n-hexane) was subjected to GLC/MS (combination gaschromatography/mass spectrometry) analysis after further purification. Another substance in this zone (Rf 0.03) was also methylated with ethereal solution of diazomethane and subjected to GLC/MS analysis.

The polar fraction (zone I, Table 2) was hydrolysed with 9N H_2SO_4 for 8 hours, diluted with water, and extracted with ether. The ether extract on TLC examination was found to be a mixture of radioactive components. The major one (Rf 0.47, plate run first with 25% benzene in n-hexane and then with benzene) was purified by repeated TLC, methylated with diazomethane and subjected to GLC/MS analysis. The other substance

(Rf 0.03) was also methylated after several TLC purifications.

The methanol extract of soil and the extract of water contained inadequate amounts of conversion products for further investigations.

Identification

A Packard unit, series 7400, with ECD and FID, fitted with a glass column (diameter 4 mm, length 2.0 m) packed with 1% OV_1 on chromosorb W-AW-DMCS 80-100 mesh was used for gas-liquid-chromatography (GLC); column temperature: 160° C. Nitrogen was used as a carrier gas. The radioactive substances were collected in anthracene tubes with the aid of an auxiliary Packard fraction collector 852. The mass spectra were taken with a gaschromatograph/mass spectrometer LKB 9000 from LKB-Produkter, Bromma, Sweden.

RESULTS AND DISCUSSION

Distribution of Radioactivity

Six weeks after application of 2, 5, 4' -trichlorobiphenyl and 2, 4, 6, 2', 4' -pentachlorobiphenyl on the leaves of Veronica beccabunga, 3.7% of the applied activity could be accounted for upon analyzing the plants, water and soil from the experiment with the trichlorobiphenyl and 18.3% from the experiment with the pentachlorobiphenyl. These figures suggest that the evaporation of trichlorobiphenyl and its metabolites is more rapid as compared to pentachlorobiphenyl. The sum of the unextracted activity amounts to about 0.2% for both experiments. The distribution of radioactivity in plants, soil and water is given in Table 1. The maximum radioactivity in both experiments was found in the methanolic plant extracts.

TABLE 1

Radioactivity Recovered in Plants, Soil and Water (in % of the radioactivity applied) after Application of ^{14}C-labeled Chlorobiphenyls to the Leaves

Substance applied	Plant extract	Soil extract	Water extract	Unextracted radioactivity	Total
2, 5, 4' -Trichlorobiphenyl	3.2	0.3	<0.1	0.2	3.7
2, 4, 6, 2', 4' -Pentachlorobiphenyl	17.5	0.6	<0.1	0.2	18.3

Metabolism Rate

Thin-layer-chromatography of the individual extracts (silicagel, benzene/ethyl acetate, 9/1) showed four radioactive zones. The percentage of each zone is given in Tables 2 and 3. By comparing the Tables, it is evident that total metabolism of 2, 5, 4' -trichlorobiphenyl (in plants, soil and water, 45.0% of residues) is higher than with 2, 4, 6, 2', 4' -pentachlorobiphenyl (2.1%). The lower metabolic conversion of pentachlorobiphenyl can be attributed to the higher chlorine content in the molecule; this is in good agreement with other studies which report that higher chlorinated biphenyls are more resistant to metabolism in mammals than lower ones (GREB et al. 1975 b). The low percentage conversion of pentachlorobiphenyl in plants did not warrant isolation and identification of conversion products. The radioactivity that enters the water through roots and soil consisted of only 37.5% PCB; the remaining activity was due to hydrophilic conversion products. However, the amount of conversion products was insufficient for further analysis.

TABLE 2

Conversion of 2, 5, 4' -Trichlorobiphenyl-^{14}C in Plants (Veronica beccabunga), Soil and Water 6 Weeks after Application to Leaves (in % of the radioactivity of each sample), TLC (benzene/ethyl acetate, 9/1)

Extract	Zone I (Conjugates)	Zone II (Phenols)	Zone III (Phenols)	Zone IV (PCB)
Methanol extract of plants	41.0	2.2	4.9	51.9
Methanol extract of soil	3.5	1.2	6.1	89.2
Ether extract of water	34.8	17.9	21.9	25.4

TABLE 3

Conversion of 2, 4, 6, 2', 4' -Pentachlorobiphenyl-^{14}C in Plants (Veronica beccabunga), Soil and Water 6 Weeks after Application to Leaves (in % of the radioactivity of each sample), TLC (benzene/ethyl acetate, 9/1)

Extract	Zone I	Zone II	Zone III	Zone IV (PCB)
Methanol extract of plants	1.1	0.1	0.6	98.2
Methanol extract of soil	2.4	0.9	2.4	94.3
CH_2Cl_2 extract of water	40.0	14.6	7.9	37.5

Identification of Metabolites of 2, 5, 4' -Trichlorobiphenyl

The combined zones II and III (Table 2) contained a major component which was identified by gas-liquid-chromatography/mass spectrometry (GLC/MS) after methylation (metabolite A). The mass spectrum (Fig. 1) of this compound showed a characteristic isotope distribution pattern for 3 chlorine atoms at a molecular ion at m/e 286 ($C_{13}H_9OCl_3$, 100%). The fragments are at 271 (M^+-CH_3, 32.5%), 243 (M^+-CH_3-CO, 32.5%), 221 (M^+-OCH_3-Cl+H, 4.2%), 207 (M^+-CH_3-CO-HCl, 10.9%), 173 (M^+-CH_3-CO-2Cl, 33%), 138 (M^+-CH_3-CO-3Cl, 8.7%) and at 137 (M^+-CH_3-CO-2Cl-HCl, 11.5%). These fragments suggest that one oxygen atom was introduced into the molecule giving a hydroxy product which is methylated by diazomethane to methylether.

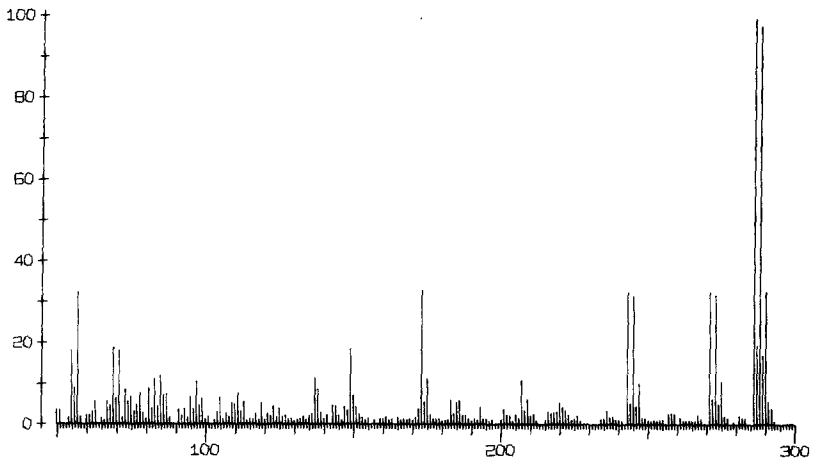

Fig. 1 Mass Spectrum of Metabolite A of 2, 5, 4' -Trichlorobiphenyl in Veronica beccabunga, after methylation

The second substance (metabolite B) in these zones, after methylation, on GLC/MS analysis gave a mol peak at M^+ 286 ($C_{13}H_9OCl_3$) and fragments at 271 (M^+-CH_3), 243 (M^+-CH_3-CO) and a fragment at 221 (M^+-OCH_3-Cl+H, 66.6%). The fragment at m/e 221 was only 4.2% in metabolite A. The peak intensities of these two substances were not identical with the peak intensities of 4 synthetic isomers (3, 6, 4, 3') of methoxy derivatives of 2, 5, 4'-trichlorobiphenyl reported by JANSSON and SUNDSTRÖM (1974). The difference in R_f values on TLC plates and in retention times in GLC (R_t 9.8 min for metabolite A and R_t 12.2 min for metabolite B) suggests that they are isomers.

The hydrolysates of the polar fraction (zone I, Table 2) was found to be a mixture of radioactive substances on TLC examination. A major substance (50%) was identified, on GLC/MS analysis after methylation, as a methyl ether of a phenol. The mass spectrum was identical to the mass spectrum of metabolite A. The further similarity of these compounds was confirmed by GLC analysis. Another substance in the hydrolysate was found to be identical with metabolite B by TLC, GLC and mass spectrometry. The third substance could not be methylated with CH_2N_2. In the mass spectrum, the mol peak at M^+ 272 with 3 chlorine atoms was observed with no clear fragments. From the molecular peak, it is obvious that one oxygen atom was introduced into the molecule. Further analysis of this product was impossible because of insufficient quantity.

Conclusions

Under the experimental conditions used, 2, 4, 6, 2', 4'-pentachlorobiphenyl is more persistent than 2, 5, 4'-trichlorobiphenyl. 2, 5, 4'-Trichlorobiphenyl is metabolized to at least two monohydroxy derivatives which occur in free form as well as conjugated in the plant tissues (Fig. 2).

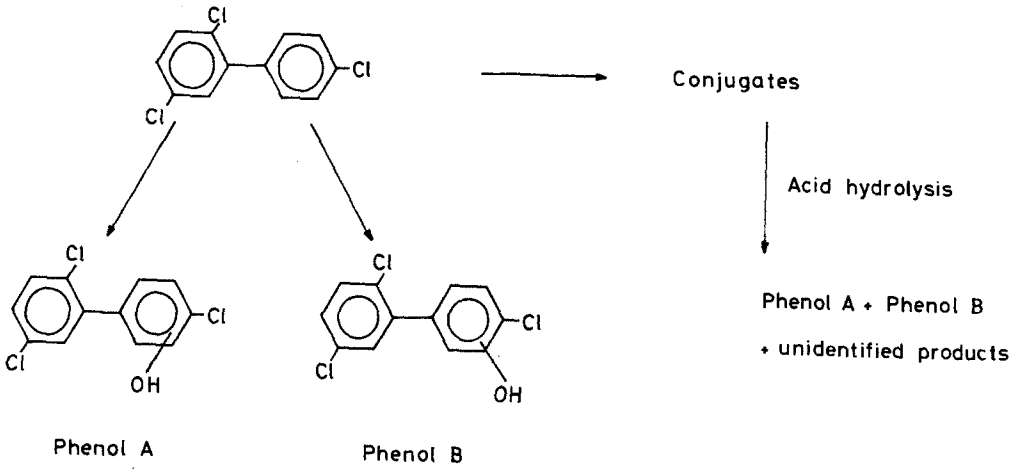

Fig. 2 Metabolism of 2, 5, 4' -Trichlorobiphenyl in Veronica beccabunga

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