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METABOLISM OF 2,3,5,6-TETRACHLOROBIPHENYL-^{14}C AND 2,3,4,5,6-PENTACHLOROBIPHENYL-^{14}C IN THE RAT*

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In previous papers[1-2], we reported the metabolism of di- and trichlorobiphenyls. In this paper, we describe the metabolic behavior of higher chlorinated biphenyl compounds in the rat.

EXPERIMENTAL

Starting from (U-^{14}C) benzene (purchased from New England Nuclear, Boston, USA) and chloroanilines[3], ($^{14}C_{1',2',3',4',5',6'}$) 2,3,5,6-tetrachlorobiphenyl (2,3,5,6-tetraCB) (178×10^3 cpm/mg) and ($^{14}C_{1',2',3',4',5',6'}$) 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-pentaCB) (186×10^3 cpm/mg) were prepared. 2-Hydroxybiphenyl and 3-hydroxybiphenyl were purchased from Wako Pure Chemical Ind., Ltd., Tokyo. 4-Hydroxybiphenyl, 3,4-dihydroxybiphenyl and their chlorinated analogs were synthesized as described in the former publication[2]. Details on the syntheses are given in Table I.

Administration of ^{14}C-labeled compounds to the rat, collection of the urine and feces, extraction of polychlorobiphenyls and their conversion products from feces, urine and body were performed as described previously[2]. Three main radioactive fractions were isolated: Fraction I including the unchanged polychlorobiphenyl and its metabolites I - a, I - b and I -c; Fraction II containing metabolite II; and Fraction III containing conjugates. Further purifications of the metabolites, as well as dehalogenations, were achieved as described in the former publication[2].

*Ecological Chemistry V. For IV, see ref. 2

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TABLE I

SYNTHESES OF BIPHENYL COMPOUNDS

Biphenyls (no.)	Mp (°C)	Crystn solvent*	Yield %	Analyses
2,3,5,6-Tetrachloro-4'-nitro- (3)	257-258°	C_6H_6	p 26	CHNCl
2,3,4,5,6-Pentachloro-4'-nitro- (4)	220-222°	C_6H_6	p 26	CHNCl
2,3,5,6-Tetrachloro-4'-amino- (7)	169-170°	C_6H_6	p 62	CHNCl
2,3,4,5,6-Pentachloro-4'-amino- (8)	200-201.5°	C_6H_6	p 80	CHNCl
2,3,5,6-Tetrachloro-4'-hydroxy- (11)	158.5-160°	C_6H_6	n 41	CHCl
2,3,4,5,6-Pentachloro-4'-hydroxy-(12)	174.5-176°	C_6H_6	p 43	CHCl
2,3,5,6-Tetrachloro-3',4'-dihydroxy-(14)	163-165°	C_6H_6	n 23	CHCl
2,3,4,5,6-Pentachloro-3',4'-dihydroxy-(15) **	199.5-201°	C_6H_6	n 33	CHCl

*n: needles; p: pillar-shaped crystals.

**Analyses for the elements shown were within 0.4% of theoretical values.

Nmr (CD_3Cl) ppm: 7, 3.75 (2H, 4'-NH_2), 6.60 (2H, 3',5'-H), 6.88 (2H, 2', 6'-H) (J=9cps, $A_2B'_2$-type), 7.47 (1H, 4-H); 8, 3.75 (2H, 4'-NH_2), 6.62 (2H, 3',5'-H), 6.88 (2H, 2', 6'-H) (J=9cps, $A_2B'_2$-type); 15, solvent: CD_3Cl-$(CD_3)_2SO$-D_2O, 6.85 (1H, 5'-H, J=7.5 cps), 6.58 (1H, 1'-H) (J=1.8cps), 6.42 (1H, 6'-H) (J=7.5cps, 1.8 cps).

UV: 15, λ_{max}^{EtOH} 290 nm (ε 1.7X10^3), $\lambda_{max}^{EtOH-H_3BO_4-AcONa}$ 290 nm (ε1.7X10^3), 315 nm (ε1.27X10^3).

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RESULTS AND DISCUSSION

2,3,5,6-TetraCB and 2,3,4,5,6-pentaCB (^{14}C-labeled) were administered orally to the rats. Unchanged polychlorobiphenyls were excreted in the feces mostly in the first five days. The higher the compounds were chlorinated, the more the unchanged compounds were found in the feces. After 10 days, polychlorobiphenyls in the body were found only in a slight amount. Table II shows the excretion of unchanged compounds in feces and residues in the body and includes, for comparison, the results of 2,3-diCB and 2,4,6-triCB[2].

The metabolites found in the urine were exclusively those of conjugates (β-D-glucuronide and others). Most of the radioactivity was found in the feces.

TABLE II

EXCRETION OF POLYCHLOROBIPHENYLS IN FECES AND THE RESIDUES IN THE BODY (RAT)

Feces were collected during 10 days after feeding the radioactive compounds. For residue determinations, the rat was sacrificed 10 days after feeding the radioactive compounds. The amounts are given in % of total radioactivity fed to the rat.

Compound	Excretion of unchanged compds in feces	Residues in the body
2,3-DiCB	5.7	0.03
2,4,6-TriCB	9.2	0.23
2,3,5,6-TetraCB	7.0	0.12
2,3,4,5,6-PentaCB	23.2	0.02

Besides the unchanged PCB's, four metabolites (I-a, I-b, I-c, II) were detected in the excreta. Table III shows the excretion of the metabolites (I and II) and includes, for comparison, 2,4,6-triCB.

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TABLE III

EXCRETION OF THE METABOLITES OF POLYCHLOROBIPHENYLS IN FECES AND URINE (RAT)

Feces and urine were collected during 10 days after feeding the radioactive compounds. The ratios of the metabolites I and II are given in %. The ratios (%) of the conjugated metabolites hydrolyzed are given in the parentheses.

Compounds	Feces Metabolites I	Feces Metabolites II	Urine Metabolites I	Urine Metabolites II
2,4,6-TriCB	40(7.9)	11(17.8)	34(100)	15(100)
2,3,5,6-TetraCB	57(13.3)	31(40.1)	7(100)	5(100)
2,3,4,5,6-PentaCB	69(11.1)	26(17.5)	3(100)	2(100)

The structures of metabolites I-a of the two PCB's were determined by comparing the mass spectra with those of reference compounds; they were found to be derivatives hydroxylated at the 4'-position. On dehalogenation with RDB (sodium dihydroxy-bis (2-methoxy-ethoxy) aluminate), both compounds gave 4-hydroxybiphenyl; this was confirmed by mass spectrometry and by comparison with authentic material.

The mass spectra of the metabolites I-b derived from 2,3,5,6-tetraCB and 2,3,4,5,6-pentaCB gave molecular ions, M^+ 306 ($C_{12}H_6OCl_4$) and 340 ($C_{12}H_5OCl_5$), respectively. On dehalogenation with RDB both compounds gave 3-hydroxybiphenyl; this was identified by mass spectrometry (M^+ 170, $C_{12}H_{10}O$) and by comparison with authentic samples (TLC). Thus, the metabolites were proven to be 2,3,5,6-tetrachloro-3'-hydroxybiphenyl and 2,3,4,5,6-pentachloro-3'-hydroxybiphenyl.

By comparison of mass spectra before and after dehalogenation, the metabolites I-c were found to be derivatives with one hydroxy and one methoxy group in the 3'- and 4'-positions.

In a similar manner, the metabolites II were identified as the 3',4'-dihydroxyderivatives of the polychlorobiphenyls.

The Rf-values of different biphenyl compounds are given in Table IV.

The metabolic behavior of the polychlorobiphenyls which were discussed here and in the former publication[2] are summarized in Fig. 1. The metabolic patterns are very similar to those of unsubstituted biphenyl in the rabbit[4].

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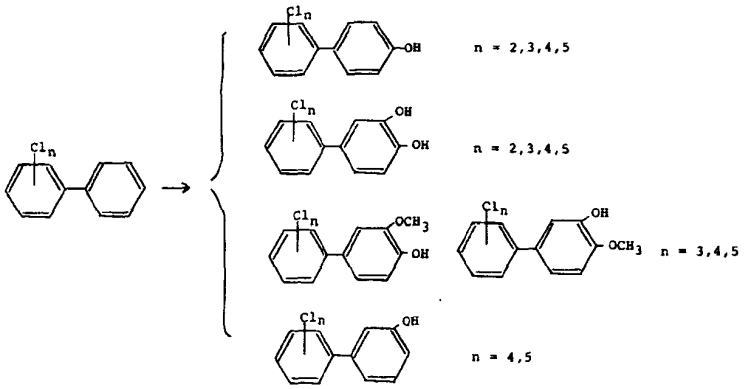

Fig. 1. Scheme for the metabolism of polychlorobiphenyls in the rat.

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TABLE IV

THIN-LAYER CHROMATOGRAPHY OF BIPHENYL COMPOUNDS

Solvents: I, benzene - ethyl acetate (12:1, v/v); II benzene - ethanol - formic acid (90:9:1, v/v/v); III, benzene - 2-propanol - conc. ammonia (70:25:5, v/v/v). Detection: 0.5% alcoholic $AgNO_3$ solution was sprayed and UV irradiated. Detection by Folin's reagent was also applied.

Compound	R_f values		
	I	II	III
2,3-DiCB	0.82	0.88	0.80
2,4,6-TriCB	0.82	0.88	0.84
2,3,5,6-TetraCB	0.82	0.89	0.83
2,3,4,5,6-PentaCB	0.82	0.89	0.84
4'-Hydroxy-2,3-diCB	0.56	0.56	0.62
4'-Hydroxy-2,4,6-triCB	0.58	0.55	0.64
4'-Hydroxy-2,3,5,6-tetraCB	0.58	0.56	0.64
4'-Hydroxy-2,3,4,5,6-pentaCB	0.58	0.57	0.64
4-Hydroxybiphenyl	0.45	0.51	0.63
3-Hydroxybiphenyl	0.48	0.55	0.68
2-Hydroxybiphenyl	0.72	0.70	0.74
3',4'-Dihydroxy-2,4,6-triCB	0.20	0.27	0.38
3',4'-Dihydroxy-2,3,5,6-tetraCB	0.20	0.27	0.40
3',4'-Dihydroxy-2,3,4,5,6-pentaCB	0.20	0.26	0.38
3,4-Dihydroxybiphenyl	0.18	0.20	0.32

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