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METABOLISM OF 2,3-DICHLOROBIPHENYL-^{14}C AND 2,4,6-TRICHLOROBIPHENYL-^{14}C IN THE RAT*

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Different authors have reported on the metabolic behavior of polychlorinated biphenyls in the rat[1,2,3,4], rabbit[5], and microorganisms[6,7,8]. More extensive studies on the metabolic conversion of each chlorinated biphenyl in animals, plants, and microorganisms are desired. In the previous paper[9], we reported on the hydroxylations of dichlorobiphenyls. In this paper we report the metabolic behavior of 2,3-dichlorobiphenyl-^{14}C and 2,4,6-trichlorobiphenyl-^{14}C in the rat.

EXPERIMENTAL

The following compounds were prepared starting from (U-^{14}C) benzene (purchased from New England Nuclear, Boston, USA) and chloroanilines[10]: (^{14}C$_{1',2',3',4',5',6'}$) 2,3-dichlorobiphenyl (2,3-diCB) (340X10^{3}cpm/mg) and (^{14}C$_{1',2',3',4',5',6'}$) 2,4,6-trichlorobiphenyl (2,4,6-triCB) (139X10^{3}cpm/mg). 2-Hydroxybiphenyl and 3-hydroxybiphenyl were purchased from Wako Pure Chemical Ind., Ltd., Tokyo. 4-Hydroxybiphenyl[11] and 3,4-dihydroxybiphenyl[12] were synthesized analogous to the methods described below for their chlorinated derivatives.

Polychloro-4'-nitrobiphenyl: A mixture of 75 ml of acetic acid and 75 ml of nitric acid (d 1.52) was added slowly to a solution of polychlorobiphenyl (45 mmole) in acetic acid (20 ml) at 50-55°C over a period of 30 min. The mixture was stirred for 2 h at 50-55°C and then poured on 2 l of water; the precipitate was collected by filtration. The product was washed with water and crystallized from the solvent given in Table I.

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Polychloro-4'-aminobiphenyl: Polychloro-4'-nitrobiphenyl (12 mmole) in benzene (100 ml) was hydrogenated over PtO_2 (200 mg) at room temperature and under atmospheric pressure. After the theoretical amount of hydrogen was absorbed, the solution was filtered and the filtrate concentrated to dryness in vacuo. The product was crystallized from the solvent given in Table I.

Polychloro-4'-hydroxybiphenyl: To a solution of $NaNO_2$ (1.0 g) in conc. H_2SO_4 (22 ml) polychloro-4'-aminobiphenyl (9 mmole) was added during 10 min. During the addition the temperature was kept at 30-35°C. The mixture was stirred for 2 h at 30-35°C and then added slowly to aqueous $CuSO_4$ (50%, 22 ml) (40°C). This mixture was refluxed for 1 h. Water (50 ml) was added and the product extracted twice with benzene (200 ml and 100 ml). The benzene extracts were combined and washed with water (100 ml). The solvent was removed by evaporation and the compound purified on a silica gel column (30X200 mm)(developer: benzene - ethyl acetate 12:1, v/v). The purified compound was crystallized from the solvent given in Table I.

Trichloro-3',4'-dihydroxybiphenyl: To a solution of trichloro-4'-hydroxybiphenyl (2 mmole) in chloroform (7 ml) bromine (320 mg) was added slowly from a separatory funnel at room temperature. The mixture was stirred for 10 min at room temperature and then for 15 min at 45°C. After evaporating the solvent, the product was added to a mixture of Cu_2O (80 mg) and 1 N NaOH (15 ml) and the whole heated in a sealed tube for 1.5 h at 180-200°C. After cooling, the pH of the solution was adjusted to 3 with conc. HCl and the product extracted twice with chloroform (100 ml and 50 ml). The chloroform solutions were combined, washed with 5% $NaHCO_3$ (50 ml) and water (50 ml) and then concentrated to dryness in vacuo. The product was crystallized from the solvent in Table I.

Administration of ^{14}C-labeled compounds to the rat and collection of the urine and feces: ^{14}C-labeled compound (0.09 mmole) in olive oil (0.5 ml) was given orally to the rat which had not been fed for 12 h (each group: 5 male JCL-SD strain rats; 5 weeks old, average weight: 54 g). The rat was then fed with a standard diet for 10 days. The feces and the urine were collected in the first and second five days after administration of the radioactive compounds.

Extraction of polychlorobiphenyls and their conversion products from feces, urine, and body: From feces: The feces were dried at 40-45°C and pulverized. Polychlorobiphenyls were extracted with ether for 24 h using a Soxhlet apparatus. The feces (10 g) were then hydrolyzed with 4 N H_2SO_4 (200 ml) by refluxing for 1 h. The compounds were extracted with ether for 12 h using a continuous extractor. From urine: The urine was acidified with conc. H_2SO_4 (final pH: 1) and a part of the conjugated metabolites was extracted con-

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tinuously with ether for 24 h. Conc. H_2SO_4 was added to the urine (final concentration: 4 N) which was then refluxed for 1 h. Hydrolysis products were extracted with ether for 24 h using a continuous extractor. From body: A rat was put in 30% NaOH (200 ml) and heated at 70-80°C for 4 h. The resulting solution was acidified with conc. HCl (final pH: 1) and the compounds extracted with ether for 24 h using a continuous extractor. Total radioactivity was determined using a dioxane scintillator and an Aloka LSC-653 liquid scintillation counter (correction was made by an internal standard method). Component ratios were calculated from the radioactivities on thin-layer chromatograms (plate: Merck Kieselgel 60; developer: benzene - ethyl acetate (12:1, v/v); apparatus: Aloka TRM-IB thin-layer radiochromatogram scanner).

TABLE I

SYNTHESES OF BIPHENYL COMPOUNDS

Biphenyls (no.)	Mp (°C)	Crystn solvent	Yield %	Analyses
2,3-Dichloro-4'-nitro- (1)	180.5-181°	EtOH	n 49	CHNCl
2,4,6-Trichloro-4'-nitro- (2)	-	-	oil	-
2,3-Dichloro-4'-amino- (5)	51-53°	EtOH-H_2O	n 73	CHNCl
2,4,6-Trichloro-4'-amino- (6)	107.5-108°	n-C_6H_{14}	n 80	CHNCl
2,3-Dichloro-4'-hydroxy- (9)	99-99.5°	Petrol-Ether	p 30	CHCl
2,4,6-Trichloro-4'-hydroxy- (10)	130-131°	n-C_6H_{14}	n 29	CHCl
2,4,6-Trichloro-3', 4'-dihydroxy- (13)	177-179°	C_6H_6	n 20	CHCl

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

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

Nmr (CD_3Cl) ppm: 1, 8.25 (2H, 3', 5'-H), 7.50 (2H, 2', 6'-H) (J=9cps, $A_2'B_2'$-type), 7.12-7.51 (3H, 4,5,6-H, multiple); 6, 3.60(2H, 4'-NH_2), 6.62 (2H, 3', 5'-H), 6.92 (2H, 2',6'-H) (J=9cps, $A_2'B_2'$-type), 7.29(2H, 3,5-H).

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Isolation and degradation of the conversion products: The ether extracts of the feces and urine from each rat were combined and evaporated to dryness in vacuo. The residue was dissolved in 1 N NaOH and the products were extracted with ether for 24 h using a continuous extractor (Fraction I: this includes th unchanged polychlorobiphenyl and its metabolites I-a and I-c). The alkaline solution was neutralized with conc. HCl (final pH: 5.0) and the metabolites we again extracted continuously with ether (Fraction II: this contains metabolite II). The solution was further acidified with conc. HCl (final pH: 1) and again extracted with ether (Fraction III: this contains conjugated compounds). The compounds in Fraction I were purified on an alumina column (30X230 mm); the unchanged compound was eluted first using benzene and the metabolites with the mixed solvent, benzene - ethanol (5:1, v/v). Further purification of these metabolites and metabolite II was carried out by thin-layer chromatography (TLC) using silica gel as adsorbent and the following solvents: A, benzene - ethyl acetate (12:1, v/v); B, petroleum ether - methylene chloride - ethanol (5 45:5, v/v/v); C, methylene chloride - methanol - 28% ammonia (94:5:1, v/v/v). The conjugated compounds were hydrolyzed by refluxing in 4 N H_2SO_4 for 1 h and the product extracted with ether for 24 h (a continuous extractor).

Dehalogenation of the metabolites: The metabolite in a mixture of xylene (4 ml) and RDB (o.5 g sodium dihydroxy-bis(2-methoxyethoxy) aluminate) was heated by refluxing for 2 h. After water (3 ml) was added, the mixture was acidified with conc. H_2SO_4. Dehalogenated compounds were extracted with ether for 12 h (a continuous extractor). Further purification was achieved by thin-layer chromatography (silica gel; solvents: benzene - ethyl acetate (12:1, v/v); ethyl acetate; and petroleum ether - methylene chloride - ethanol (50:45:5, v/v/v).

RESULTS AND DISCUSSION

The following compounds (^{14}C-labeled)were administered orally to the rat: 2,3-diCB and 2,4,6-triCB. 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 collected during 10 days (for 2,3-diCB, 5.7% of total radioactivity fed to the rat; for 2,4,6-triCB, 9.2%). After 10 days polychlorobiphenyls in the body were found only in a slight amount.

The excretion pattern of both chlorobiphenyls in the urine and feces were very similar to each other.

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The metabolites found in the urine were exclusively those of conjugates (β-D-glucuronide and others). In the case where 2,4,6-triCB was given to the rat, 51% of the total radioactivity was found in the feces and 49% in the urine. In the case where 2,3-diCB was applied, only one metabolite (I-a) was detected. After application of 2,4,6-triCB, two main metabolite fractions were detected in urine and feces extracts: I, consisting of I-a and I-c, and II. In the case of 2,4,6-triCB, metabolite group I amounted to 40%, of which 7.9% were conjugated; metabolite II amounted to 11%, of which 17.8% were conjugated. In urine, metabolites I were 34% and II was 15%; both occurred only as conjugates.

The structures of these metabolites were determined as follows: The mass spectra of the metabolites I-a derived from 2,3-diCB and 2,4,6-triCB gave molecular peaks M^+ 238 ($C_{12}H_8OCl_2$) and 272 ($C_{12}H_7OCl_3$), respectively. On dehalogenation with RDB, both compounds gave 4-hydroxybiphenyl; this was confirmed by mass spectrometry (M^+ 170, $C_{12}H_{10}O$) and by comparison with authentic material (TLC). It was thus clarified that the hydroxylation of the polychlorobiphenyls had occurred on the 4'-position. 4'-Hydroxy-2,3-diCB and 4'-hydroxy-2,4,6-triCB were synthesized and the identities with these metabolites were confirmed.

The mass spectra of the metabolite I-c derived from 2,4,6-triCB gave a molecular ion M^+ 302 ($C_{13}H_9O_2Cl_3$). This spectrum shows the introduction of one hydroxy and one methoxy group into the trichlorobiphenyl. On dehalogenation with RDB the metabolite gave 3,4-dihydroxybiphenyl; this was identified by mass spectra and by comparison with a synthetic sample (TLC). These evidences show that two hydroxy groups were introduced, i.e. in the 3'- and 4'-positions of the trichlorobiphenyl, and one hydroxy group was methylated. This kind of metabolite was not obtained by giving 2,3-diCB to the rat in a similar way.

The mass spectrum of the metabolite II derived from 2,4,6-triCB gave a molecular peak M^+ 288 ($C_{12}H_7O_2Cl_3$). On dehalogenation with RDB, this compound gave 3,4-dihydroxybiphenyl, as identified by mass spectra and by comparison with authentic sample (TLC). 3',4'-Dihydroxy-2,4,6-triCB was synthesized and the identity with this metabolite was confirmed by thin-layer chromatography.

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