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Identification of Sulfur-Containing Metabolites of 2,4,2′,4′-Tetrachlorobiphenyl in Mice

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INTRODUCTION

In an earlier paper (MIO et al. 1976) dealing with the metabolic fate of 2,5,2',5'-tetrachlorobiphenyl (TCB), a major component of commercial polychlorobiphenyls (PCB's), it was shown that this compound was metabolized to the sulfur-containing products: 3- and 4-methylthio-2,5,2',5'-TCB's and 3- and 4-methylsulfonyl-2,5,2',5'-TCB's in mice and these metabolites were excreted in feces along with the unchanged compound. During the course of a comparative study (MIZUTANI et al. 1975) on the metabolism of PCB isomers with special regard to sulfur-containing metabolites, among a variety of isomers 2,4,2',4'-TCB was also revealed to give sulfur-containing metabolites. The investigation reported here is an extension of this study and deals with the structural elucidation of four sulfur-containing metabolites of 2,4,2',4'-TCB in mice.

MATERIALS AND METHODS

2,4,2',4'-TCB, mp 39-40°, was synthesized from 2,4-dichloroiodobenzene by a modified Ullmann condensation procedure (KORNBLUM and KENDALL 1952).

Animal Experiments

For quantitative study five female dd strain mice (20-22 g) were each given intraperitoneally 4 mg of 2,4,2',4'-TCB dissolved in 0.2 ml of soybean oil. Feces were collected at 48-hr intervals and pooled. After 6 days, the mice were killed and the liver was excised for residue analysis.

Extraction of Metabolites

The feces were dried and extracted with benzene in a Soxhlet apparatus. The solvent was removed from the extract under reduced pressure. The residue was treated with 1N ethanolic KOH at 80° for 1 hr to remove

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lipids and probable phenolic metabolites. The solution was then diluted with an equal volume of water and extracted with benzene.

The liver samples were digested with 1N ethanolic KOH at 80° for 1 hr and, after dilution with water, the digest was extracted with benzene.

Fractionation of Metabolites

The benzene extract of feces or liver was evaporated to near dryness and redissolved in hexane. The solution was placed in a 10 mm i.d. X 20 cm column packed with 3 g of dry silica gel (deactivated with 5% of water) which was prepared by the described method (HOLDEN and MARSDEN 1969), and divided into fractions 1, 2, 3, and 4 by successive elutions with 10, 15, and 15 ml of hexane, and 30 ml of benzene, respectively.

Derivatization of Metabolites

i) Oxidation: After evaporation to near dryness, a 5-ml aliquot of fraction 2 or 3 obtained from the silica gel chromatography was treated with a mixture of 3 ml of HOAc and 1 ml of 30% H_2O_2 at 70° for 2 hr. The mixture was extracted with hexane. The extract was washed with aqueous $NaHSO_3$, concentrated, and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

ii) Desulfurization: A 5-ml aliquot of fraction 2 or 3 was evaporated to 1 ml, and the resulting solution was added to a suspension of 200 mg of freshly prepared Raney nickel in 2 ml of acetone. The mixture was refluxed for 30 min, and the organic solution separated from the catalyst was analysed by GC.

Synthesis of Compounds Used in Identification

i) 6-Methylsulfonyl-2,4,2',4'-TCB (I): Four grams of 2,4-dichloroaniline added in a mixture of 12 ml of HCl and 6 ml of water was diazotized with a solution of 2.1 g of $NaNO_2$ in 6 ml of water in the usual manner. After neutralization with NaOAc and filtration, the resulting diazonium salt solution was poured into a solution of 12 g of 3,5-dichlorothioanisole in 10 ml of CCl_4 and the mixture was vigorously stirred at room temperature for 48 hr. The organic layer was separated, washed with water, then with aqueous NaOH, and dried over Na_2SO_4. The solvent and the excess of 3,5-dichlorothioanisole were removed under reduced pressure and the residue was distilled in vacuo to give a viscous oil (bp 160-180° at 0.5 mm). The viscous distillate was oxidized in HOAc with excess 30% H_2O_2 for 4 hr at 70° and the product was extracted with benzene. Separation of the extract by silica gel chromatography by using benzene as eluting solvent gave the two expected isomers: 6-methylsulfonyl-2,4,2',4'-TCB (I), mp 95-97°; Mass Spectrum m/e 368 (M^+); NMR ($CDCl_3$) δ 8.22 (d, H-5, J=2 Hz), 7.82 (d, H-3, J=2 Hz), and 2.85 (s, SO_2CH_3), and 4-methylsulfonyl-2,6,2',4'-TCB, mp 155-157°; Mass Spectrum m/e 368 (M^+); NMR ($CDCl_3$) δ 8.02 (s, H-3,5) and 3.18 (s, SO_2CH_3).

ii) 5-Methylsulfonyl-2,4,2',4'-TCB (II): Coupling of 2,4-dichloroaniline with 2,4-dichlorothioanisole, oxidation of the products, and the subsequent separation of the resulting mixture of isomeric methylsulfonyl-TCB's were carried out by the essentially identical methods with those described above for the preparation of 6-methylsulfonyl-2,4,2',4'-TCB. Two of the three expected products were obtained: 5-methylsulfonyl-2,4,2',4'-TCB (II), mp 136-137°; Mass Spectrum m/e 368 (M^+); NMR ($CDCl_3$) δ 8.10 (s, H-6), 7.78 (s, H-3), and 3.27 (s, SO_2CH_3), and 2-methylsulfonyl-3,5,2',4'-TCB, mp 176-178°; Mass Spectrum m/e 368 (M^+); NMR ($CDCl_3$) δ 7.72 (d, H-4, J=2 Hz), 7.50 (d, H-6, J=2 Hz), and 3.31 (s, SO_2CH_3).

Instrumentation

The gas chromatograph was a Shimadzu Model GC-3E equipped with tritium electron capture detector. The column was 2 m X 3 mm i.d. glass packed with either 2% OV-1 or 2%-0.5% diethylene glycol succinate-phosphoric acid (DEGS-PA) on Chromosorb W. The column was operated isothermally at 180° using nitrogen (2 kg/cm^2) as carrier gas.

Mass spectra were obtained at 20 eV by using a JEOL JMS-D100 mass spectrometer coupled to a JEOL JGC-20K gas chromatograph.

RESULTS AND DISCUSSION

Figure 1 shows the results of typical GC analyses

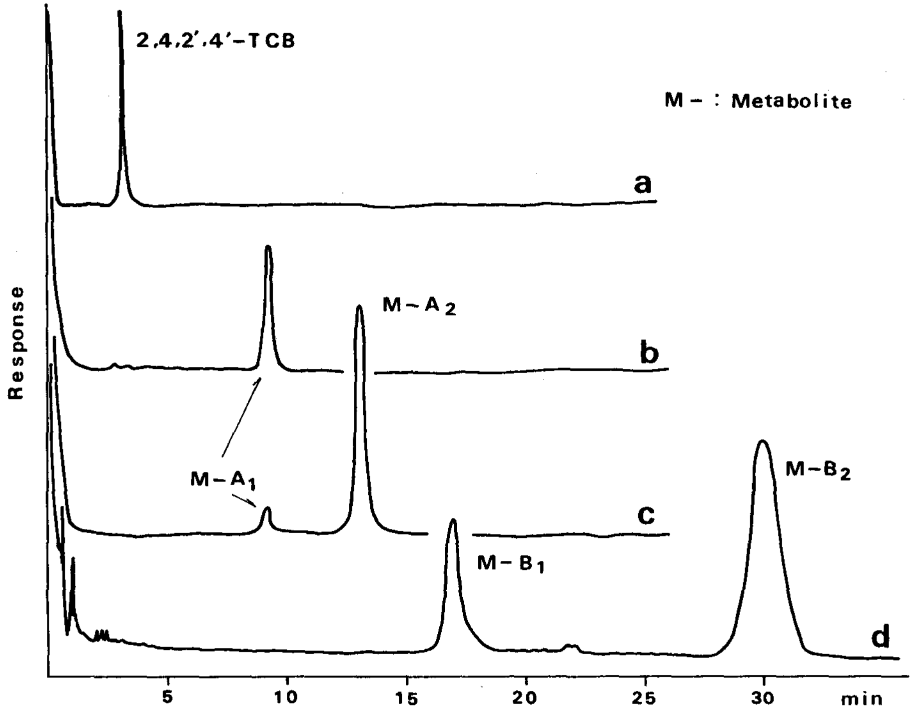

Figure 1. Gas chromatograms of the fecal extracts fractionated by silica gel chromatography: (a) fraction 1; (b) fraction 2; (c) fraction 3; (d) fraction 4. Column: 2% OV-1 on Chromosorb W.

of fractions 1, 2, 3, and 4 obtained from the fecal extract. The component present in fraction 1 was identified as the parent material, 2,4,2',4'-TCB, by gas chromatographic and column chromatographic comparison with the reference standard. Fractions 2, 3, and 4 mainly contained metabolites A_1, A_2, and B_1 and B_2, respectively. Fraction 3 also contained a small amount of metabolite A_1 as well as the major component A_2. The amounts of these metabolites were so minute that no attempt was made to isolate the metabolites, each of which was examined by GC-MS.

The mass spectrum (Figure 2a) of metabolite A_1 showed molecular ion at m/e 336, corresponding to an elemental composition $C_{13}H_8Cl_4S$, and fragment ions

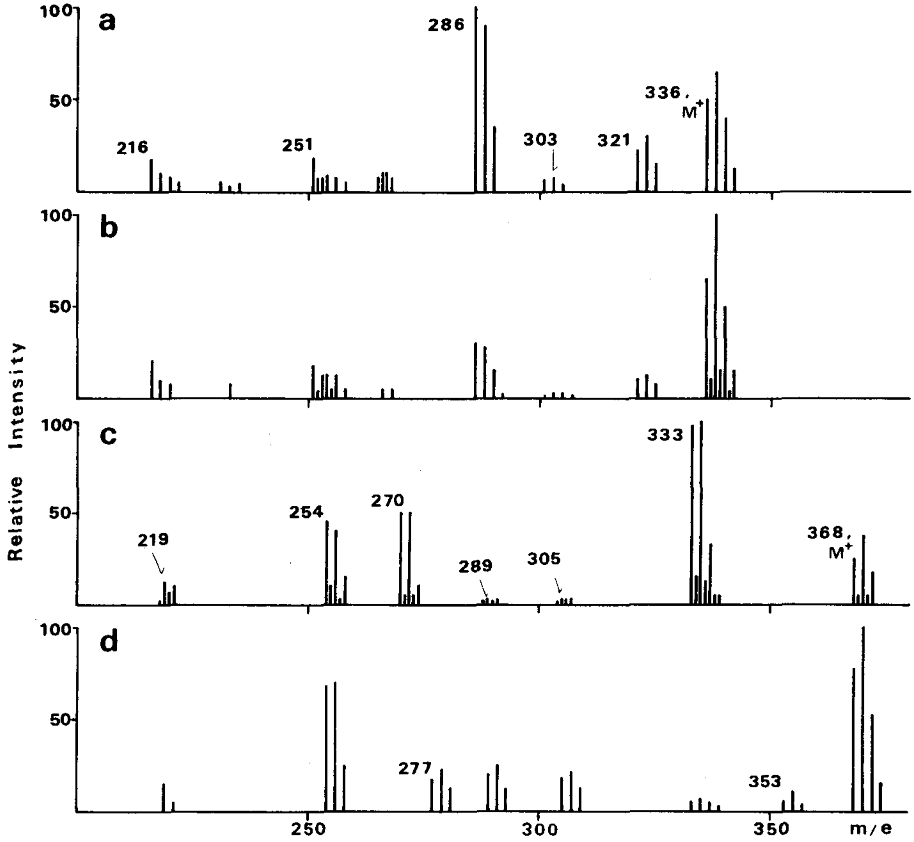

Figure 2. Mass spectra of fecal metabolites: (a) metabolite A_1; (b) metabolite A_2; (c) metabolite B_1; (d) metabolite B_2.

at m/e 321 (M^+-CH_3), 303 (M^+-SH, via rearrangement), 286 (M^+-CH_3-Cl), 251 ($M^+-CH_3-Cl_2$), and 216 ($M^+-CH_3-Cl_3$), indicating the presence of methylthio derivative of TCB. The mass spectrum (Figure 2b) of metabolite A_2 was qualitatively identical with that of metabolite A_1, also indicating the presence of methylthio-TCB. However, the relative abundance values of specific peaks, m/e 336 and 286, unequivocally distinguished these two metabolites from one another. The mass spectra (Figure 2, c and d) of both metabolites B_1 and B_2 showed molecular ions at m/e 368, corresponding to an elemental composition $C_{13}H_8O_2Cl_4S$, and common fragment ions at m/e 333 (M^+-Cl), 305 (M^+-CH_3SO, via rearrangement), 289 (M^+-CH_3SO_2), and 254 (M^+-CH_3SO_2-Cl). Although metabolites B_1 and B_2 could be spectrometrically distinguished by the relative abundance values of specific pesks, m/e 368 and 333, and by the presence of additional peaks at m/e 270 (M^+-CH_3SO-Cl) in the case of metabolite B_1 and at m/e 353 (M^+-CH_3) and 277 (M^+-CH_3SO-CO) in the case of metabolite B_2, the whole data indicated that both metabolites are isomeric methylsulfonyl-TCB's.

When oxidized with H_2O_2 in HOAc as described under Materials and Methods, fraction 2 containing metabolite A_1 and fraction 3 predominantly containing metabolite A_2 gave the products which were shown by GC and GC-MS to be consistent with metabolites B_1 and B_2, respectively. On the other hand, fraction 2 and 3, when treated with Raney nickel, yielded 2,4,2',4'-TCB as the sole product. From these results it was concluded that metabolites A_1 and A_2, and hence metabolites B_1 and B_2 are identical with the parent compound, 2,4,2',4'-TCB, with respect to the location of chlorines. Therefore, metabolites B_1 and B_2 each must be 3-, 5-, or 6-methylsulfonyl-2,4,2',

TABLE 1

Gas Chromatographic Data of Synthetic and Metabolic Methylsulfonyl-2,4,2',4'-TCB's

Compound	t_R*	
	OV-1	DEGS-PA
6-Methylsulfonyl-2,4,2',4'-TCB	0.28	2.84
Metabolite B_1	0.28	2.84
5-Methylsulfonyl-2,4,2',4'-TCB	0.47	6.87
Metabolite B_2	0.47	6.86

*Retention time relative to decachlorobiphenyl

TABLE 2

Fecal Excretion of 2,4,2',4'-TCB and its Metabolites after i.p. Administration of 2,4,2',4'-TCB (4 mg/head) in Mice

Period (day)	Amount excreted, µg/head				
	2,4,2'4'-TCB	Metabolite			
		A_1	A_2	B_1	B_2
0 - 2	95.0	0.29	0.31	nd	nd
2 - 4	86.0	0.63	1.86	0.15	0.12
4 - 6	70.0	0.53	1.33	0.10	0.29

TABLE 3

Concentrations of 2,4,2',4'-TCB and its Metabolites in Liver of Mice at 6 Days after i.p. Administration of 2,4,2',4'-TCB (4 mg/head)

Residue	Concentration*, µg/g wet tissue
2,4,2',4'-TCB	199.0 ± 67.8
Metabolite A_1	nd
Metabolite A_2	nd
Metabolite B_1	0.80 ± 0.20
Metabolite B_2	1.78 ± 0.38

*Values are means ± S.E. for 5 mice.

4'-TCB. Of these three possible isomers, two probable isomers, 6-methylsulfonyl-2,4,2',4'-TCB (I) and 5-methylsulfonyl-2,4,2',4'-TCB (II) were synthesized, and metabolites B_1 and B_2 were identified by comparison of GC and GC-MS data as to be I and II, respectively. Table 1 compares the chromatographic data. Since the structural correlations between metabolites A_1 and B_1 and between A_2 and B_2 were established on the basis of the oxidative derivatization, metabolites A_1 and A_2 can also be assigned to 6-methylthio-2,4,2',4'-TCB and 5-methylthio-2,4,2',4'-TCB, respectively.

A common feature of the metabolic reactions for

2,4,2',4'-TCB and 2,5,2',5'-TCB (MIO et al. 1976) is the substitution by methylthio (or methylsulfonyl) group at either of the two adjacent positions in the phenyl ring giving the two positionally isomeric metabolites. These observations may be rationalized by assuming the arene oxide intermediates involved in these metabolic reactions.

The levels of fecal excretion for 2,4,2',4'-TCB and its metabolites are shown in Table 2. Metabolites A_1 and A_2 were determined after being converted to the corresponding methylsulfonyl derivatives by the procedure described under Materials and Methods. Although a continued excretion seems to be expected beyond the experimental period, the four metabolites excreted during 6 days accounted for only 0.12% of the dose.

Table 3 presents the residual concentrations in the liver of unchanged TCB and its metabolites at the end of the excetion study. There existed no persistent residues of the methylthio-TCB's in the liver. Rapid conversion to the corresponding methylsulfonyl metabolites by microsomal oxidation is a possible explanation for the lack of accumulation of the methylthio metabolites in the liver.

Although the toxicological significance of the sulfur-containing metabolites is not known at the present time, it is of deep interest from the environmental point of view that some chlorobiphenyl isomers can be metabolized to give nonpolar products such as methylsulfonyl-TCB's which tend to be accumulated in animal tissue.

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