A New Metabolite of 2,4,3′,4′-Tetrachlorobiphenyl in Rat Feces

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Metabolism *in vivo* of 2,4,3′,4′-tetrachlorobiphenyl (TCB) was further studied using male Wistar rats. When the extract of feces of rats given TCB with chloroform was methylated and applied to gas chromatography (GC)–mass spectrometry (MS), a new metabolite was detected. The structure of this new metabolite was 4-hydroxy-2,5,3′4′-TCB based on both its retention time in GC and comparison of the mass spectrum with that of the synthetic sample. 4-Hydroxy-2,5,3′,4′-TCB was assumed to be formed *via* a 4,5-oxide intermediate followed by NIH-shift of a chlorine atom at 4-position.

Keywords 2,4,3′,4′-tetrachlorobiphenyl; metabolism; GC-MS; NIH-shift; 4-hydroxy-2,5,3′,4′-tetrachlorobiphenyl

Introduction

2,4,3′,4′-Tetrachlorobiphenyl (TCB) is one of the major components of Kanechlor 400[1] which is the causal oil of Yusho occurred in the southwestern part of Japan in 1968.[2] Our earlier studies demonstrated that 2,4,3′,4′-TCB was mainly metabolized to 5-hydroxy-2,4,3′,4′-TCB, and slightly to 3-hydroxy-2,4,3′,4′-TCB and other unknown metabolites, which were isolated from rat feces,[3,4] and also revealed that the major metabolite, 5-hydroxy-2,4,3′,4′-TCB, had 5 times more potent toxicity than its parent compound in mice.[4] In this study, we re-examined the *in vivo* metabolism of 2,4,3′,4′-TCB in rats to clarify in more detail the metabolic pathways of this TCB from a toxicological point of view and found a new metabolite in the feces.

Experimental

Chemicals 2,4,3′,4′-TCB was synthesized by the method of Saeki *et al.*[1] 3-Hydroxy-2,4,3′,4′-TCB and 5-hydroxy-2,4,3′,4′-TCB were synthesized as described elsewhere.[4] 3,4-Dichloroaniline and 2,5-dichlorophenol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phenyltrimethylammonium hydroxide was purchased from GL Sciences Inc. (Tokyo, Japan).

Thin-Layer Chromatography (TLC) Thin layer plates (0.25 mm thick) of silica gel were prepared by coating glass plates with a mixture of Kieselgel 60G (Merck AG, Darmstadt, Germany) and Wakogel B-5FM containing a fluorescent indicator (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 1 : 1 (w/w). 2,4,3′,4′-TCB and its methylated metabolites were visualized under ultraviolet light (254 nm).

Synthesis of 4-Hydroxy-2,5,3′,4′-TCB 3,4-Dichloroaniline (1.3 g) and 2,5-dichlorophenol (3.26 g) were used as starting materials. 4-Hydroxy-2,5,3′,4′-TCB was synthesized analogously by the previous method.[5] Finally, 4-hydroxy-2,5,3′,4′-TCB, mp 116—118 °C (5 mg, 0.4%) and 3-hydroxy-2,5,3′,4′-TCB, mp 128 °C (7 mg, 0.5%) were obtained separately by a preparative TLC developed with *n*-hexane–ethyl acetate–acetic acid (40 : 10 : 1, v/v) as a solvent system. The methylation of the authentic samples was performed with 20% phenyltrimethylammonium hydroxide in methanol at 210 °C on the injection port of a gas chromatograph.

4-Hydroxy-2,5,3′,4′-TCB: MS *m/z*: 326 ($M^+ + 6$, 12), 324 ($M^+ + 4$, 54), 322 ($M^+ + 2$, 134), 320 (M^+, 100), 305 ($M^+ - CH_3$, 25), 277 ($M^+ - COCH_3$, 30). ^{1}H-NMR (in $CDCl_3$) δ: 7.17 (1H, s, H-3), 7.24 (1H, dd, $J = 8.24$, 1.98 Hz, H-6′), 7.28 (1H, s, H-6), 7.487 (1H, d, $J = 1.98$ Hz, H-2′), 7.490 (1H, d, $J = 8.24$ Hz, H-5′).

Isolation of Metabolites 2,4,3′,4′-TCB dissolved in corn oil was administered intraperitoneally to 7 male Wistar rats (body weight 200—220 g) three times at a dose of 100 mg/kg on days 1, 4 and 8. The feces were collected for 10 d following the first injection, dried at 80 °C overnight and then extracted continuously with chloroform for 10 h. The chloroform phase was evaporated to dryness. To detect the hydroxylated metabolites as methylated derivatives, the extract was dissolved in 10 ml of acetone and then methylated by heating with 1.0 g of dimethyl sulfate at 80 °C for 2 h in the presence of K_2CO_3 (1.0 g) as reported previously.[6,7] The methylated extract was applied to a silica gel column (Kiesel gel 60, Merck AG, 100 g) equilibrated with *n*-hexane to remove the endogenous materials and unchanged 2,4,3′,4′-TCB. After the elution of unchanged 2,4,3′,4′-TCB with 800 ml of *n*-hexane, the methylated metabolites described in this study were eluted with an additional 500 ml of *n*-hexane. This fraction was concentrated to a smaller volume and applied to preparative TLC using *n*-hexane–ethyl acetate–acetic acid (40 : 10 : 1, by vol.) as a developing solvent. A major fluorescent band visualized under ultraviolet light (*Rf* value, 0.51) was scraped off and extracted with chloroform. The extract was applied to gas chromatography (GC)–mass spectrometry (MS).

Analytical Methods GC-MS was carried out with a gas chromatograph HP 5890 Series II directly interfaced with a mass selective detector HP 5971A with electron impact mode. An HP-1 fused capillary column (12 m × 0.25 mm i.d. × 0.33 μm thickness) was used. The other conditions were as follows: carrier gas, helium with a flow rate of 1 ml/min; split injection; column temperature, 210 °C; injection port temperature, 250 °C; detector temperature, 250 °C; ionizing energy, 70 eV.

Results and Discussion

Figure 1 shows the gas chromatogram of the methylated metabolites obtained by preparative TLC as described under Experimentals. Two major peaks at the retention times of 8.42 and 8.89 min in the chromatogram were the methylated derivatives of the known metabolites 3-hydroxy- (M-1) and 5-hydroxy-2,4,3′,4′-TCB (M-2)[3,4] from their gas chromatographic and mass spectral properties (Table I). In addition, a minor peak was observed at the retention time of 9.04 min and the mass spectrum showed that it was a new metabolite (designated as methylated

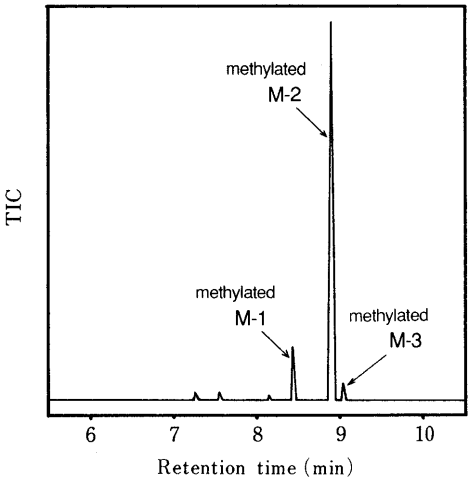

Fig. 1. Total Ion Chromatogram of the Methylated Metabolites of 2,4,3′,4′-TCB in Rat Feces

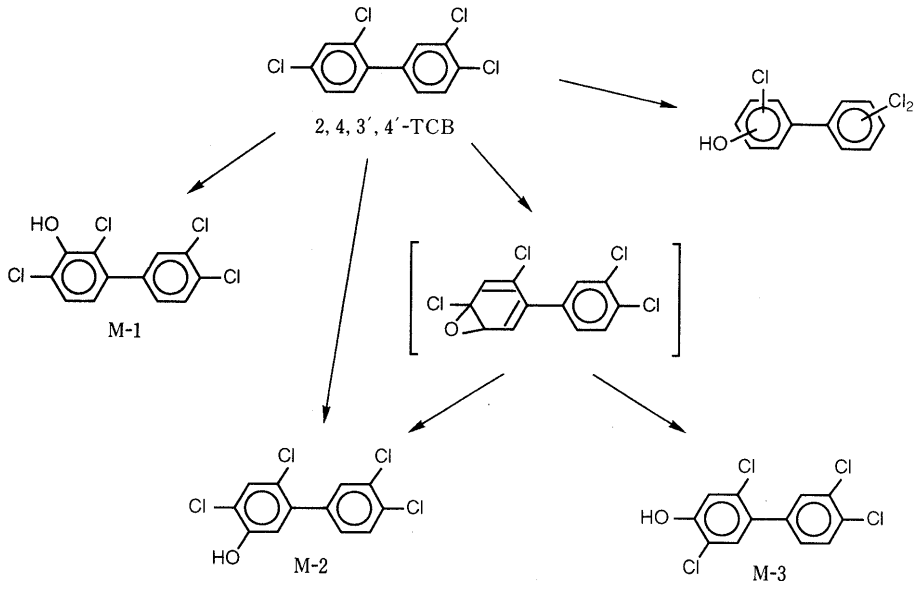

Fig. 2. The Postulated Metabolic Pathways of 2,4,3′,4′-TCB in Rats

TABLE I. Mass Spectral Data and Retention Times of Methylated Derivatives of Three Metabolites of 2,4,3′,4′-TCB in Rats

Compound	Molecular weight	Mass spectral data[a]			Retention time (min)
		$[M^+]$	$[M^+-15]$	$[M^+-43]$	
M-1	320	100	4	36	8.42
M-2	320	100	3	33	8.89
M-3	320	100	25	29	9.04
3-CH_3O-2,4,3′,4′-TCB	320	100	—	41	8.42
5-CH_3O-2,4,3′,4′-TCB	320	100	—	34	8.89
4-CH_3O-2,5,3′,4′-TCB	320	100	25	30	9.04

a) Values are percentages of molecular ion peak.

M-3) having the same molecular weight of 320 as methylated M-1 and M-2 (Table I). Moreover, a characteristic fragment ion peak $[M^+-15]$ in the spectrum suggests that a methoxy group is substituted at 4- or 4′-position in the biphenyl ring.[6,7] Therefore, we synthesized 4-methoxy-2,5,3′,4′-TCB and compared its retention time in GC and mass spectrum with those of the methylated M-3. As shown in Table I, the retention times and mass spectra of the two compounds were completely identical. From this evidence, M-3 was concluded to be 4-hydroxy-2,5,3′,4′-TCB. An alternative structure, 4′-hydroxy-2,4,3′,5′-TCB, has not yet been completely ruled out, although this metabolite seems more difficult to form metabolically.

In the total ion chromatogram (Fig. 1), three minor peaks were also observed at the retention times of 7.25, 7.55 and 8.17 min. Their mass spectra gave the same molecular ion peak at *m/z* 286 and the isotope ion peaks at *m/z* 288 and 290, suggesting the structure of monomethoxy-trichlorobiphenyl. The postulated metabolic pathways of 2,4,3′,4′-TCB in rats are illustrated in Fig. 2. This study found a new minor metabolite in rat feces and concluded that it was a methylated derivative of 4-hydroxy-2,5,3′,4′-TCB; it is suggested to be formed *via* a 4,5-epoxide intermediate and subsequent NIH-shift of a chlorine atom from 4-position to 5-position. Toxicological significance of the metabolism of 2,4,3′,4′-TCB will be discussed elsewhere in the near future.

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