The reaction sequence presented here provides a methodology for the homologation of α,β -unsaturated esters with simultaneous introduction of α -chlorine atom, though the limitation exists in the case of β,β -disubstituted **1** where hydrosilation failed, probably due to steric hindrance.⁵⁾

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Sulfur-containing Metabolites of 2,5,2',5'-Tetrachlorobiphenyl, a Major Component of Commercial PCB's

The excretion of four new metabolites of 2,5,2',5'-tetrachlorobiphenyl (TCB) in mice feces was revealed by gas chromatographic examination. Based on mass spectrometric data and syntheses, the structures of these metabolites were identified as 3- and 4-methylsulfonyl-2,5,2',5'-TCB (I and II, respectively), and 3- and 4-methylthio-2,5,2',5'-TCB (III and IV, respectively).

Since polychlorobiphenyls (PCB's) were recognized as one of the most widespread pollutants in the environment, a number of studies on the metabolic fate of individual chlorobiphenyl isomers have been undertaken. Hydroxylation is now well known as the major metabolic reaction among most of the chlorobiphenyls tested.¹⁾

During the course of a thorough gas chromatographic examination of the fecal excreta of mice given 2,5,2',5'-tetrachlorobiphenyl (TCB), we have found the presence of four nonpolar metabolites. This paper deals with the structural elucidation of these novel metabolites.

2,5,2',5'-TCB was dissolved in vegetable oil and intraperitoneally administered (8 mg/animal) to female dd strain mice weighing 19—21 g. The feces were collected for 6 days after administration of the material. The feces were dried and extracted with benzene in a Soxhlet apparatus. The benzene extracts were chromatographed on a silica gel dry column, and divided into three fractions: fraction 1, eluted with 10 ml of hexane; fraction 2, eluted with 6 ml of benzene; and fraction 3, eluted with further 10 ml of benzene. Each of the fractions was analysed using a gas chromatograph equipped with an electron capture detector and a combined gas chromatograph-mass spectrometer. Fraction 1 contained unchanged 2,5,2',5'-TCB. Both fractions 2 and 3 contained two metabolites, A and B, and C and D, respectively (Fig. 1).

The mass spectra of both metabolites C and D (Fig. 2) showed molecular ion at m/e 368, corresponding to an elemental composition $C_{13}H_8O_2Cl_4S$ (based on high-resolution measure-

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ments), with virtually identical fragment ions at m/e 353 ($M^+ - CH_3$), 305 ($M^+ - CH_3SO$, *via* rearrangement), 289 ($M^+ - CH_3SO_2$), 277 ($M^+ - CH_3SO - CO$), 254 ($M^+ - CH_3SO_2 - Cl$), and 219 ($M^+ - CH_3SO_2 - Cl_2$). These data suggested that metabolites C and D are isomeric methylsulfonyl TCB's. This suggestion was supported by the findings that both metabolites C and D were unaffected by the treatment with diazomethane, boiling ethanolic sodium hydroxide, or hydrogen peroxide in acetic acid at 70°. If we assume no migration of chlorine atoms on the metabolic pathways, the structures of metabolites C and D each must be 3-, 4-, or 6-methylsulfonyl-2,5,2',5'-TCB. Of the three possible isomers, 3-methylsulfonyl-2,5,2',5'-TCB (I), mp 148—149.5°, and 4-methylsulfonyl-2,5,2',5'-TCB (II), mp 155°, were synthesized, according to Cadogan,²⁾ by treating the respective methylsulfonyltetrachloroanilines with *p*-dichlorobenzene under the presence of amyl nitrite. Through comparison of gas and column chromatographic behavior and mass spectra, metabolites C and D were identified as I and II, respectively.

Fig. 1. Gas Chromatograms of the Fecal Extracts fractionated by Dry Column Chromatography:

(a) Fraction 1; (b) Fraction 2; (c) Fraction 3.
condition: column, 1% OV-1 on Chromosorb W, 1.5m x 3 mmφ; column temp., 180°; carrier gas, N₂, 0.8 kg/cm²

Fig. 2. Mass Spectra of Fecal Metabolites B and D, identified as IV and II, respectively

On the other hand, the mass spectra of both metabolites A and B (Fig. 2) showed molecular ion at m/e 336 corresponding to an elemental composition C₁₃H₈Cl₄S, with closely similar fragment ions at m/e 303 ($M^+ - SH$, *via* rearrangement), 286 ($M^+ - CH_3 - Cl$), and 251 ($M^+ - CH_3 - Cl_2$), which indicated the presence of the methylthio derivatives of TCB's. Fraction 2, when oxidized with hydrogen peroxide in acetic acid, readily yielded two products which were identified by gas chromatography and mass spectrometry to be metabolites C and D, respectively. On the basis of these data, it was suggested that the structures of metabolites A and B must be 3-methylthio-2,5,2',5'-TCB (III) and 4-methylthio-2,5,2',5'-TCB (IV), respectively or *vice versa*. The 4-methylthio isomer (IV), mp 82°, was prepared by coupling *p*-dichlorobenzene with 2,5-dichloro-4-methylthioaniline, and proved to be consistent with metabolite B. Therefore, metabolite A can be assigned to III.

The excretion rates of unchanged TCB and the metabolites, determined by gas chromatography, are shown in Table I.

TABLE I. Fecal Excretion of 2,5,2',5'-TCB and its Metabolites
after Intraperitoneal Administration of 2,5,2',5'-TCB
(8 mg/head) in Mice

Period (day)	2,5,2',5'-TCB	Amount excreted ^{a)} (μg/head) metabolite			
		A ^{b)}	B ^{b)}	C	D
0—1	35.0	0.15	0.06	<0.05	<0.05
1—2	45.1	0.69	0.07	0.87	0.26
2—3	57.6	2.50	0.34	0.37	0.18
3—4	35.7	3.41	0.48	1.84	0.58
4—5	21.4	2.24	0.32	1.75	0.61
5—6	16.9	1.74	0.43	1.50	0.61

a) mean value of four mice

b) determined after being converted to the corresponding methylsulfonyl derivative by oxidation with hydrogen peroxide in acetic acid

The methylthio type of metabolites have been isolated, although as alkaline-degradation products, from the liver of rats administered methylaminoazobenzene,³⁾ 2-acetylaminofluorene,⁴⁾ or other structurally related compounds.³⁻⁵⁾ By analogy with the mechanisms proposed for these instances, free or protein-bound methionine-S-yl derivatives of 2,5,2',5'-TCB could be suggested as possible intermediates in the formation of metabolites A and B. If this is the case, 2,5,2',5'-TCB would initially undergo microsomal epoxidation as suggested by Gardner, *et al.*,^{1c)} and this arene oxide, 2,5,2',5'-TCB-3,4-oxide, would be then opened by the nucleophilic attack of the sulfur atom of methionine residue at the 3 or 4 position to give two kinds of sulfonium intermediates, which eventually decompose to metabolites A and B, respectively. When mice were administered L-methionine-methyl-²H in addition to 2,5,2',5'-TCB, incorporations of the methyl-²H group into metabolites A and B were observed by mass spectrometry. These findings seem to support partly the above interpretation, but the whole suggestion must be considered as only a tentative one at the present time.

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