Sulfur-Containing Metabolites of Chlorobiphenyl Isomers, a Comparative Study

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After single intraperitoneal doses of 21 chlorobiphenyl isomers to mice, the feces were examined for the occurrence of chloro(methylthio)biphenyls and chloro(methylsulfonyl)biphenyls, new metabolic reactions previously found in 2,2′,5,5′-tetrachlorobiphenyl. The experiments included all the possible isomers of symmetrical di-, tetra-, and hexachlorobiphenyls and seven unsymmetrical isomers containing a 2,5-dichlorophenyl ring as a structural unit. 2,2′,4,4′-Tetrachlorobiphenyl and all of the unsymmetrical isomers tested, except for 2,2′,5-trichloro- and 2,2′,3,4,5,5′-hexachlorobiphenyls, gave the methylthio and methylsulfonyl metabolites. The presence of the sulfur-containing metabolites was confirmed based on both GLC–mass spectrometry and chemical derivatization; in some cases the metabolites were identified by GLC and mass spectral comparison with authentic samples.

In a previous work (Mio et al., 1976) we identified four sulfur-containing metabolites of 2,2′,5,5′-tetrachlorobiphenyl in mice feces: 2,2′5,5′-tetrachloro-3-(methylthio)biphenyl, 2,2′,5,5′-tetrachloro-4-(methylthio)biphenyl, 2,2′,5,5′-tetrachloro-3-(methylsulfonyl)biphenyl, and 2,2′,5,5′-tetrachloro-4-(methylsulfonyl)biphenyl. The detection of a series of methylsulfonyl derivatives of polychlorobiphenyls (PCB) in seal blubber from the Baltic has also been reported (Jensen and Jansson, 1976).

The primary objective of this study was to explore the generality of this type of metabolic reaction. With special regard to the formation of sulfur-containing metabolites, the metabolic behavior in mice of all the possible isomers of symmetrical di-, tetra-, and hexachlorobiphenyls and other structurally related isomers was examined in this study.

EXPERIMENTAL SECTION

Synthesis of Test Compounds. *Symmetrical Di-, Tetra-, and Hexachlorobiphenyls.* 2,2′-Dichlorobiphenyl (**1**), 3,3′-dichlorobiphenyl (**2**), 4,4′-dichlorobiphenyl (**3**), 2,2′,3,3′-tetrachlorobiphenyl (**7**), 2,2′,4,4′-tetrachlorobiphenyl (**8**), 2,2′,6,6′-tetrachlorobiphenyl (**9**), 3,3′,4,4′-tetrachlorobiphenyl (**10**), 3,3′,5,5′-tetrachlorobiphenyl (**11**), 2,2′,3,3′,4,4′-hexachlorobiphenyl (**15**), 2,2′,3,3′,5,5′-hexachlorobiphenyl (**16**), 2,2′,3,3′,6,6′-hexachlorobiphenyl (**17**), 2,2′,4,4′,5,5′-hexachlorobiphenyl (**18**), 2,2′,4,4′,6,6′-hexachlorobiphenyl (**19**), and 3,3′,4,4′,5,5′-hexachlorobiphenyl (**20**) were prepared by the Ullmann condensation of the appropriate mono-, di-, or trichloroiodobenzenes (Kornblum and Kendall, 1952).

Unsymmetrical Chlorobiphenyls. 2,2′,5-Trichlorobiphenyl (**4**), 2,3′,5-trichlorobiphenyl (**5**), 2,4′,5-trichlorobiphenyl (**6**), 2,3′,5,5′-tetrachlorobiphenyl (**12**), 2,2′,3,-4,5′-pentachlorobiphenyl (**13**), 2,2′,4,5,5′-pentachlorobiphenyl (**14**), and 2,2′,3,4,5,5′-hexachlorobiphenyl (**21**) were prepared by a modified Gomberg reaction (Weingarten, 1961) from the appropriate chloroanilines and *p*-dichlorobenzene.

All the above compounds were isolated from the crude products by distillation in vacuo and purified by column chromatography on silica gel (Merck) by using hexane as developing solvent. Final purification was achieved by recrystallization from ethanol and/or benzene. Each isomer prepared was homogeneous on a thin-layer chromatogram (silica gel F-254, Merck) and gave a single peak when analyzed by GLC on a 2% OV-1 column. All the isomers, except **21**, had been synthesized before (Hutzinger et al., 1974) and the structures of these isomers were verified by mass spectrometry and by comparison of their melting points with reported data. The chemical authenticity of **21** was confirmed by NMR and mass spectrometry: **21**, mp 91–92 °C; NMR δ 7.25 (s, 1 H), 7.32 (d, J = 8 Hz, 1 H), 7.38 (s, 1 H), 7.41 (d, J = 8 Hz, 1 H); mass spectrum m/e (rel intensity) 360 (100), 358 (60, M^+), 323 (8), 288 (67), 253 (7).

Synthesis of Compounds Used in Identification. *2,4′,5-Trichloro-3-(methylsulfonyl)biphenyl* (I). A precursor for the synthesis of I was prepared starting with 1,4-dichloro-2-(methylsulfonyl)benzene (Miller and Smiles, 1925). This compound (8.8 g) was nitrated at 100 °C for 2 h with 10 g of KNO_3 in 60 mL of H_2SO_4 to give 2,5-dichloro-3-(methylsulfonyl)-1-nitrobenzene: mp 111 °C; IR 1518, 1356, 1328, 1145 cm^{-1}; NMR δ 3.36 (s, 3 H), 8.04 (d, J = 2 Hz, 1 H), 8.42 (d, J = 2 Hz, 1 H); mass spectrum m/e (rel intensity) 269 (100, M^+), 254 (19), 207 (89). Hydrogenation of the nitro compound over platinum black catalyst in ethyl acetate gave the corresponding amino compound, 2,5-dichloro-3-(methylsulfonyl)aniline: mp 141 °C; IR 3455, 3360, 1300, 1140 cm^{-1}; mass spectrum m/e (rel intensity) 239 (100, M^+), 208 (6), 177 (21), 160 (52). The aniline was subsequently converted by the general procedure for the Sandmeyer reaction to 2,5-dichloro-1-iodo-3-(methylsulfonyl)benzene: mp 156-157 °C; IR 1305, 1148 cm^{-1}; NMR δ 3.30 (s, 3 H), 8.18 (s, 2 H); mass spectrum m/e (rel intensity) 350 (100, M^+), 335 (16), 288 (39), 271 (49). The Ullmann condensation of this intermediate with *p*-chloroiodobenzene followed by the separation by column chromatography on silica gel gave the three expected products, i.e., 4,4′-dichlorobiphenyl (mp 148–149 °C), 2,2′,5,5′-tetrachloro-3,3′-bis(methylsulfonyl)biphenyl [mp 242–244 °C; mass spectrum m/e (rel intensity) 448 (100), 446 (71, M^+), 411 (58), 384 (12)], and the desired compound I [mp 124–126 °C; NMR δ 3.33 (s, 3 H), 7.28 (s, 2 H), 7.37 (d, J = 6 Hz, 1 H), 7.45 (d, J = 6 Hz, 1 H), 7.59 (d, J = 2 Hz, 1 H), 8.21 (d, J = 2 Hz, 1 H); mass spectrum m/e (rel intensity) 334 (73, M^+), 272 (9), 243 (25), 220 (100)].

2,4′,5-Trichloro-4-(methylsulfonyl)biphenyl (II). The starting material, *N*-(2,5-dichloro-4-nitrophenyl)acetamide (Dey et al., 1947) was catalytically reduced over platinum black in ethyl acetate–acetic acid (1:1 v/v) to the corresponding amino compound: mp 129–130 °C; IR 3380, 3306, 3230, 1655 cm^{-1}; mass spectrum m/e (rel intensity) 218 (35, M^+), 176 (100), 148 (11), 141 (12). This amino compound (5 g) was diazotized in dilute H_2SO_4 in the usual manner. After neutralization with sodium acetate, the resulting diazonium salt solution was poured into a mixture of 2.8 g of NaOH, 4 g of copper powder, 7.5 mL of 20%

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sodium methanethiolate solution, and 50 mL of water. After stirring at room temperature for 1 h, the reaction mixture was heated at 80 °C for 30 min. The product was extracted with benzene and purified by column chromatography on silica gel to give *N*-[2,5-dichloro-4-(methylthio)phenyl]acetamide: mp 197–198 °C; IR 3260, 1665 cm^{-1}; mass spectrum *m/e* (rel intensity) 249 (61, M^+), 207 (100), 192 (80), 157 (15). Oxidation of the methylthio compound in acetic acid with excess 30% H_2O_2 for 4 h at 70 °C followed by deacetylation with HCl in ethanol yielded 2,5-dichloro-4-(methylsulfonyl)aniline: mp 133 °C; IR 3460, 3370, 1322, 1140 cm^{-1}; mass spectrum *m/e* (rel intensity) 239 (87, M^+), 224 (58), 176 (100), 160 (45). The aniline was then converted according to the general procedure for the Sandmeyer reaction to 2,5-dichloro-1-iodo-4-(methylsulfonyl)benzene: mp 156–157 °C; IR 1322, 1148 cm^{-1}; NMR δ 3.25 (s, 3 H), 8.06 (s, 1 H), 8.15 (s, 1 H); mass spectrum *m/e* (rel intensity) 350 (100, M^+), 335 (18), 287 (71), 271 (33). This intermediate was subsequently subjected to the Ullmann reaction with *p*-chloroiodobenzene to yield the three expected products, which were separated by column chromatography on silica gel, i.e., 4,4′-dichlorobiphenyl (mp 148–149 °C), 2,2′,5,5′-tetrachloro-4,4′-bis(methylsulfonyl)biphenyl [mp 265–267 °C; mass spectrum *m/e* (rel intensity) 448 (100), 446 (77, M^+), 412 (64), 383 (41)], and the desired compound II [mp 163–165 °C; IR 1340, 1310, 1155 cm^{-1}; NMR δ 3.33 (s, 3 H), 7.44 (s, 1 H), 7.46 (s, 1 H), 7.50 (s, 2 H), 7.57 (s, 1 H), 8.30 (s, 1 H); mass spectrum *m/e* (rel intensity) 334 (99, M^+), 319 (4), 271 (52), 243 (25), 220 (100)].

2,4′,5-Trichloro-2′-(methylsulfonyl)biphenyl (III). 4-Chloro-2-(methylthio)aniline (Hodgson and Handley, 1928) was coupled with *p*-dichlorobenzene by a modified procedure (Weingarten, 1961) for the Gomberg reaction to give 2,4′,5-trichloro-2′-(methylthio)biphenyl: mp 70–72 °C; NMR δ 2.40 (s, 3 H), 7.06–7.52 (m, 6 H); mass spectrum *m/e* (rel intensity) 302 (4, M^+), 269 (74), 267 (100), 252 (72). Oxidation of the methylthio compound in acetic acid with excess 30% H_2O_2 for 4 h at 70 °C followed by dilution with water and by extraction with benzene yielded III: mp 143–145 °C; IR 1310, 1150, 1133 cm^{-1}; NMR δ 2.88 (s, 3 H), 7.33 (d, J = 8 Hz, 1 H), 7.43 (s, 3 H), 7.70 (d of d, J = 2, 8 Hz, 1 H), 8.24 (d, J = 2 Hz, 1 H); mass spectrum *m/e* (rel intensity) 334 (14, M^+), 299 (100), 236 (65), 220 (48).

2,4′,5-Trichloro-3′-(methylsulfonyl)biphenyl (IV). 4-Chloro-3-(methylthio)aniline (Cutler and Schalit, 1966) was converted into the title compound via 2,4′,5-trichloro-3′-(methylthio)biphenyl by the same procedure as described above for the preparation of III. The intermediate, 2,4′,5-trichloro-3′-(methylthio)biphenyl: mp 65–66 °C; mass spectrum *m/e* (rel intensity) 302 (100, M^+), 287 (7), 269 (8), 252 (25). IV: mp 146–147 °C; IR 1308, 1155, 1140 cm^{-1}; NMR δ 3.34 (s, 3 H), 7.27 (d, J = 6 Hz, 1 H), 7.29 (s, 1 H), 7.38 (d, J = 6 Hz, 1 H), 7.69 (s, 2 H), 8.24 (s, 1 H); mass spectrum *m/e* (rel intensity) 336 (100), 334 (97, M^+), 272 (8), 271 (8), 243 (29), 220 (93).

Animal Experiments. A group of six female dd strain mice weighing 20–25 g was treated intraperitoneally with each chlorobiphenyl isomer dissolved in soybean oil at dosages of 20 mg/head for di- and trichlorobiphenyls and 10 mg/head for tetra-, penta-, and hexachlorobiphenyls. Feces were collected for 6 days, after which the mice were killed, and the liver was excised and stored at −20 °C for residue analysis.

Extraction Procedures. The feces, previously dried over P_2O_5, were extracted with benzene in a Soxhlet apparatus. The solvent was evaporated from the extract under reduced pressure by means of a Kuderna-Danish evaporator. The residue was treated with 1 N KOH in 70% ethanol at 80 °C for 1 h to remove lipids and probable phenolic metabolites. After dilution with an equal volume of water, the resulting solution was extracted with benzene. The extract was evaporated to a small volume in a Kuderna-Danish evaporator and, finally, to near dryness with a gentle air stream at room temperature and redissolved in hexane for column chromatography.

The liver samples were digested with 1 N KOH in 70% ethanol at 80 °C for 1 h. The remainder of this procedure was the same as that for the alkaline hydrolyzate of fecal extract.

Column Chromatography. The procedure for chromatographic fractionation of metabolites is essentially the same as that employed for the metabolites of 2,2′,5,5′-tetrachlorobiphenyl (Mio et al., 1976).

A 2-g portion of silica gel, Merck No. 7734, dried at 130 °C for 3 h and deactivated with 5% of water, was placed in a 10 mm i.d. × 20 cm column without slurrying with solvent. The crude extract of feces or liver, dissolved in hexane, was chromatographed on the column and divided into three fractions: fraction A, eluted with 10 mL of hexane; fraction B, eluted with 6 mL of benzene; and fraction C, eluted with another 10 mL of benzene. Each fraction was analyzed by GLC and then by mass spectrometry.

Derivatization of Metabolites. An aliquot (3 mL) of the fraction B described above was transferred into a test tube, the solvent was removed under a stream of dry air, and a mixture of 3 mL of acetic acid and 1 mL of 30% H_2O_2 was added. The tube was loosely stoppered and the mixture was heated in a water bath at 70 °C for 2 h with occasional shaking. The content was extracted with hexane. The extract was washed with $NaHSO_3$ solution and concentrated under a stream of dry air. The sample was analyzed by GLC and mass spectrometry.

Gas–Liquid Chromatography. GLC with an electron-capture detector was performed on a Shimadzu GC-3E gas chromatograph fitted with a 2 m × 3 mm glass column packed with Chromosorb W containing either 2% OV-1 or 2–0.5% DEGS–H_3PO_4. Isothermal GLC was conducted at the two different temperatures, 150 and 180 °C, and the carrier gas used was nitrogen at 2 kg/cm^2.

Spectrometric Analysis. The mass spectra were obtained by using a JEOL JMS-D100 mass spectrometer coupled to a JEOL JGC-20K gas chromatograph by means of a single-stage jet separator. The ionizing energy was 20 eV. The GLC column was 1 m × 2 mm glass packed with Chromosorb W containing 2% OV-1. Isothermal GLC was conducted at 200 °C and the carrier gas was helium at 1 kg/cm^2.

The NMR spectra were recorded on a JEOL JMN-MH-100 100 MHz spectrometer. Chemical shifts are reported as δ from tetramethylsilane internal standard in $CDCl_3$.

The IR spectra were measured on a JASCO IRA-2 spectrometer in KBr disk.

RESULTS AND DISCUSSION

Table I shows the distributions, examined by GLC, of unchanged isomers and metabolites in fractions A, B, and C from the chromatography of fecal extracts together with the GLC retention time of each component. In each case studied the unchanged chlorobiphenyl was always eluted in fraction A and identified based on the GLC retention time. Compounds **1–4**, **7**, **9–11**, and **15–21** have not been observed to give any detectable peak attributable to metabolite for any of the fractions. As previously reported

Table I. Components of Fecal Extracts in the Fractions from Silica Gel Chromatography and Their GLC Characteristics

Substrate		Component of fecal extract					
		Fraction A[a]		Fraction B[b]		Fraction C[c]	
No.	Structure	Peak code	t_R,[d] min	Peak code	t_R, min	Peak code	t_R, min
1	2,2′-Cl_2-BP[e]	1A	1.0				
2	3,3′-Cl_2-BP	2A	1.6				
3	4,4′-Cl_2-BP	3A	1.7				
4	2,2′,5-Cl_3-BP	4A	1.7				
5	2,3′,5-Cl_3-BP	5A	2.2	$5B_1$	6.0	$5C_1$	13.2
				$5B_2$	6.2	$5C_2$	14.6
6	2,4′,5-Cl_3-BP	6A	2.3	$6B_1$	6.5	$6C_1$	14.6
				$6B_2$	6.7	$6C_2$	16.0
7	2,2′,3,3′-Cl_4-BP	7A	3.4				
8	2,2′,4,4′-Cl_4-BP	8A	2.8	$8B_1$	7.7	$8C_1$	11.1
				$8B_2$	9.3	$8C_2$	18.9
9	2,2′,6,6′-Cl_4-BP	9A	2.0				
10	3,3′,4,4′-Cl_4-BP	10A	5.8				
11	3,3′,5,5′-Cl_4-BP	11A	4.0				
12	2,3′,5,5′-Cl_4-BP	12A	3.2	$12B_1$	9.9	$12C_1$	20.6
				$12B_2$	10.3	$12C_2$	22.5
13	2,2′,3,4,5′-Cl_5-BP	13A	5.4	$13B_1$	20.0	$13C_1$	40.1
				$13B_2$	22.1	$13C_2$	43.1
14	2,2′,4,5,5′-Cl_5-BP	14A	4.7	$14B_1$	17.4	$14C_1$	31.3
				$14B_2$	18.9	$14C_2$	34.0
15	2,2′,3,3′,4,4′-Cl_6-BP	15A	11.0				
16	2,2′,3,3′,5,5′-Cl_6-BP	16A	7.4				
17	2,2′,3,3′,6,6′-Cl_6-BP	17A	5.6				
18	2,2′,4,4′,5,5′-Cl_6-BP	18A	8.0				
19	2,2′,4,4′,6,6′-Cl_6-BP	19A	4.3				
20	3,3′,4,4′,5,5′-Cl_6-BP	20A	17.2				
21	2,2′,3,4,5,5′-Cl_6-BP	21A	8.6				

[a] All the compounds eluted in these fractions were identified as unchanged chlorobiphenyls by comparison of GLC data. [b] See Table II for identification of the compounds eluted in these fractions. [c] See Table III for identification of the compounds eluted in these fractions. [d] Retention time on OV-1 column. [e] BP = biphenyl.

Table II. Mass Spectral Data and Identification of the Metabolites Represented in Table I (Fraction B)

Peak code	Identification	Mass spectra, *m/e* (rel intensity)				
		Base peak	M^+	M^+ - Me	M^+ - SH	M^+ - Me - Cl
$5B_1$	2,3′,5-Cl_3-3-MeS-BP[a,b]	304	302 (96)	287 (4)	269 (10)	252 (28)
$5B_2$	2,3′,5-Cl_3-4-MeS-BP[b]	302	302 (100)	287 (18)	269 (5)	252 (39)
$6B_1$	2,4′,5-Cl_3-3-MeS-BP[b]	304	302 (97)	287 (5)	269 (11)	252 (29)
$6B_2$	2,4′,5-Cl_3-4-MeS-BP[b]	304	302 (92)	287 (18)	269 (4)	252 (43)
$8B_1$	2,2′,4,4′-Cl_4-6-MeS-BP	286	336 (51)	321 (22)	303 (6)	286 (100)
$8B_2$	2,2′,4,4′-Cl_4-5-MeS-BP	338	336 (73)	321 (9)	303 (5)	286 (29)
$12B_1$	2,3′,5,5′-Cl_4-3-MeS-BP[b]	338	336 (84)	321 (2)	303 (10)	286 (22)
$12B_2$	2,3′,5,5′-Cl_4-4-MeS-BP[b]	338	336 (65)	321 (10)	303 (7)	286 (21)
$13B_1$	2,2′,3,4,5′-Cl_5-3′-MeS-BP[b]	372	370 (60)	355 (5)	337 (8)	320 (18)
$13B_2$	2,2′,3,4,5′-Cl_5-4′-MeS-BP[b]	372	370 (58)	355 (8)	337 (5)	320 (20)
$14B_1$	2,2′,4,5,5′-Cl_5-3′-MeS-BP[b]	372	370 (61)	355 (3)	337 (8)	320 (15)
$14B_2$	2,2′,4,5,5′-Cl_5-4′-MeS-BP[b]	372	370 (62)	355 (6)	337 (4)	320 (17)

[a] BP = biphenyl. [b] Tentatively identified based on mass spectral evidence only.

for 2,2′,5,5′-tetrachlorobiphenyl (Mio et al., 1976), the methylsulfonyl metabolites are accumulated in the liver and are also excreted in feces. Therefore, the liver samples of mice given the above isomers were also examined, and the absence of metabolites was further confirmed. In each case of **5**, **6**, **8**, and **12**–**14** both fractions B and C contained two metabolites. The presence of metabolites indicated by the GLC analysis was confirmed by means of combined GLC–mass spectrometry. All the peaks observed by the GLC analysis gave mass spectra with ion clusters characteristic of chlorine-containing compounds.

Significant and diagnostic ions in the mass spectra of the metabolites eluted in fractions B and C are shown in Tables II and III, respectively. The most common feature of the mass spectra of all the metabolites in fractions B was the occurrence of a molecular ion 46 mass units heavier than that of the corresponding parent isomer with fragments at $M^+ - 15$ (loss of Me), $M^+ - 33$ (loss of SH), and $M^+ - 50$ (loss of Me and Cl). These data substantially agreed with those for 2,2′,5,5′-tetrachloro-3-(methylthio)biphenyl and 2,2′,5,5′-tetrachloro-4-(methylthio)biphenyl previously reported (Mio et al., 1976) and indicated the presence of isomeric methylthio derivatives of the parent isomer in each case. The two metabolites in each fraction B (**$5B_1$** and **$5B_2$**, **$6B_1$** and **$6B_2$**, **$8B_1$** and **$8B_2$**, **$12B_1$** and **$12B_2$**, **$13B_1$** and **$13B_2$**, and **$14B_1$** and **$14B_2$**), when oxidized with H_2O_2 in acetic acid, yielded two products which were confirmed by GLC and mass spectrometry to be consistent with the two metabolites in the corresponding fraction C (**$5C_1$** and **$5C_2$**, **$6C_1$** and **$6C_2$**, **$8C_1$** and **$8C_2$**, **$12C_1$** and **$12C_2$**, **$13C_1$** and **$13C_2$**, and **$14C_1$** and **$14C_2$**, respectively).

On the other hand, the mass spectra of all the metabolites in fractions C exhibited a molecular ion 78 mass units heavier than that of the corresponding parent isomer with fragments at either $M^+ - 62$ (loss of CH_2SO) or $M^+ - 63$ (loss of MeSO) and $M^+ - 114$ (loss of $MeSO_2$ and Cl). These data, combined with the results of oxidative derivatization described above, suggested that the two metabolites in each fraction C are isomeric chloro-

Table III. Mass Spectral Data and Identification of the Metabolites Represented in Table I (Fraction C)

Peak code	Identification	Mass spectra, m/e (rel intensity) Base peak	M^+	M^+ - Me	M^+ - CH_2SO	M^+ - MeSO	M^+ - MeSO - CO	M^+ - $MeSO_2$ - Cl
$5C_1$	2,3′,5-Cl_3-3-$MeSO_2$-BP[a,b]	220	334 (79)		272 (7)		243 (27)	220 (100)
$5C_2$	2,3′,5-Cl_3-4-$MeSO_2$-BP[b]	220	334 (83)	319 (5)		271 (39)	243 (27)	220 (100)
$6C_1$	2,4′,5-Cl_3-3-$MeSO_2$-BP	220	334 (79)		272 (7)		243 (27)	220 (100)
$6C_2$	2,4′,5-Cl_3-4-$MeSO_2$-BP	220	334 (83)	319 (5)		271 (39)	243 (27)	220 (100)
$8C_1$	2,2′,4,4′-Cl_4-6-$MeSO_2$-BP	335	368 (26)			305 (2)		254 (44)
$8C_2$	2,2′,4,4′-Cl_4-5-$MeSO_2$-BP	370	368 (77)	353 (5)		305 (17)	277 (17)	254 (69)
$12C_1$	2,3′,5,5′-Cl_4-3-$MeSO_2$-BP[b]	256	368 (78)		306 (12)			254 (87)
$12C_2$	2,3′,5,5′-Cl_4-4-$MeSO_2$-BP[b]	256	368 (71)	353 (6)		305 (39)	277 (21)	254 (97)
$13C_1$	2,2′,3,4,5′-Cl_5-3′-$MeSO_2$-BP[b]	290	402 (57)		340 (13)		311 (25)	254 (72)
$13C_2$	2,2′,3,4,5′-Cl_5-4′-$MeSO_2$-BP[b]	404	402 (63)	387 (5)		339 (26)	311 (22)	288 (74)
$14C_1$	2,2′,4,5,5′-Cl_5-3′-$MeSO_2$-BP[b]	404	402 (69)		340 (7)		311 (21)	288 (69)
$14C_2$	2,2′,4,5,5′-Cl_5-4′-$MeSO_2$-BP[b]	404	402 (63)	387 (3)		339 (18)	311 (18)	288 (60)

[a] BP = biphenyl. [b] Tentatively identified based on mass spectral evidence only.

(methylsulfonyl)biphenyls structurally related to the methylthio metabolites detected in the corresponding fraction B.

The experiments reported here and previously (Mio et al., 1976) include all the possible isomers of symmetrical chlorobiphenyls which bear up to six chlorine atoms. It was concluded that among these isomers the only two, which bear the 2,4- or 2,5-dichlorophenyl ring as a structural unit, could be metabolized to yield methylthio and methylsulfonyl derivatives.

If we assume the additivity of the structural contribution for determination of the mode of metabolic reaction, it would be expected, because of the presence of 2,5-dichlorophenyl moiety, that all the unsymmetrical isomers studied in this work would give the sulfur-containing metabolites. This was found to be the case for **5**, **6**, and **12**–**14**, but not for **4** and **21**. Although no conclusive interpretation is available at present, the sparsely and the densely substituted structures of **4** and **21**, respectively, may be responsible for this discrepancy.

Among the metabolites detected above, metabolites $8B_1$, $8B_2$, $8C_1$, and $8C_2$ were identified by comparison with the authentic standards prepared in our laboratory to be 2,2′,4,4′-tetrachloro-6-(methylthio)biphenyl, 2,2′,4,4′-tetrachloro-5-(methylthio)biphenyl, 2,2′,4,4′-tetrachloro-6-(methylsulfonyl)biphenyl, and 2,2′,4,4′-tetrachloro-5-(methylsulfonyl)biphenyl, respectively. The details of these results will be reported separately.

From the results for the symmetrical chlorobiphenyl isomers it is expected that, in each metabolite originated from unsymmetrical chlorobiphenyls **5**, **6**, and **12**–**14**, the methylthio and the methylsulfonyl groups are exclusively located at the 3 (or 3′) or 4 (or 4′) position in the 2,5- (or 2′,5′-)dichlorophenyl ring. To structurally characterize the methylsulfonyl metabolites from an unsymmetrical chlorobiphenyl, **6**, we synthesized four (I–IV) of the five possible isomers, and $6C_1$ and $6C_2$ were identified, in accord with the above expectation, as 2,4′,5-trichloro-3-(methylsulfonyl)biphenyl (I) and 2,4′,5-trichloro-4-(methylsulfonyl)biphenyl (II), respectively, by comparison of GLC and mass spectral data (Table IV).

In addition to the above knowledge, a certain mass spectral and GLC evidence enabled tentative assignment of structures for all the other methylthio and methylsulfonyl metabolites. The mass spectra of the two isomeric methylthio metabolites of 2,2′,5,5′-tetrachlorobiphenyl were qualitatively similar as previously reported (Mio et al., 1976), but exhibited an important difference: the (M^+ – SH) peak was more abundant than the (M^+ – Me) peak in the 3-methylthio isomer, and the reverse was the case for the 4-methylthio isomer, i.e., the (M^+ – Me) peak was more abundant than the (M^+ – SH) peak. The same difference in quantitative fragmentation behavior was also observed for each pair of the isomeric methylthio metabolites resulting from **5**, **6**, and **12**–**14**, and could be used as a criterion for the tentative differentiation between 3-(or 3′-)methylthio and 4- (or 4′-)methylthio isomers (Table II). The assignments based on this evidence were also supported by the facts that GLC retention times for each pair of the isomeric metabolites were in the order 4-methylthio isomer > 3-methylthio isomer, consistent with the case of the identified metabolites from 2,2′,5,5′-tetrachlorobiphenyl (Mio et al., 1976). Furthermore, similar relations between structure and both mass spectral fragmentation and GLC retention time have been observed in the series of chloromethoxybiphenyls, the oxygen analogues of methylthio metabolites, i.e., the 4-methoxy isomers give more abundant (M^+ – Me) peaks and longer retention times relative to the corresponding 3-methoxy isomers (Tulp et al., 1977).

Table IV. GLC Data for Synthetic and Metabolic 2,4′,5-Trichloro(methylsulfonyl)biphenyls

Compound	Retention time, min OV-1	DEGS-H_3PO_4
2,4′,5-Cl_3-3-$MeSO_2$-BP[a] (I)	25.2	156.4
Metabolite $6C_1$	25.2	156.4
2,4′,5-Cl_3-4-$MeSO_2$-BP (II)	27.6	177.6
Metabolite $6C_2$	27.5	177.6
2,4′,5-Cl_3-2′-$MeSO_2$-BP (III)	13.6	68.8
2,4′,5-Cl_3-3′-$MeSO_2$-BP (IV)	28.6	186.8

[a] BP = biphenyl.

The identified methylsulfonyl metabolites, $6C_1$ and 2,2′,5,5′-tetrachloro-3-(methylsulfonyl)biphenyl (Mio et al., 1976), which bear a 2,5-dichloro-3-(methylsulfonyl)phenyl ring in common, showed (M^+ – CH_2SO) rather than (M^+ – MeSO) peak and no (M^+ – Me) peak in the mass spectra. In contrast, both of the known 4-methylsulfonyl metabolites, $6C_2$ and 2,2′,5,5′-tetrachloro-4-(methylsulfonyl)biphenyl (Mio et al., 1976) showed (M^+ – MeSO) and (M^+ – Me) peaks. This remarkable feature of mass fragmentation could serve as a criterion for the tentative assignment of the structures of $5C_1$ and $5C_2$, $12C_1$ and $12C_2$, $13C_1$ and $13C_2$, and $14C_1$ and $14C_2$ (Table III). These assignments were also supported by the findings that the GLC retention times for each pair of the isomeric metabolites were in the order 4-methylsulfonyl isomer > 3-methylsulfonyl isomer, consistent with the cases of the known metabolites from **6** and 2,2′,5,5′-tetrachlorobiphenyl.

The present results make the occurrence of the methylthio and methylsulfonyl metabolites from biphenyls with more than six chlorine atoms rather unlikely. However, there remains the possibility that some of these chlorobiphenyls may form the sulfur-containing metabolites to a detectable extent only after long term exposure, since the metabolic rate of chlorobiphenyls tends to decrease with increasing number of chlorine atoms. This possibility is strengthened by the work of Jensen and Jansson (1976) who demonstrated the presence of methylsulfonyl metabolites of PCB containing from three to seven chlorine atoms in wild seal blubber.

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Volatiles of Corn Kernels and Husks: Possible Corn Ear Worm Attractants

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The vacuum steam volatile oils of corn husks and kernels have been analyzed by capillary GLC–mass spectrometry. A total of 56 compounds were identified in the corn husk volatile oil and 34 in the corn kernel volatile oil. Major components identified in the corn husk volatile oil included nonan-2-ol, nonanal, hex-*trans*-2-enal, hept-4-en-2-ol, and hexanol. Major components identified in the corn kernel volatile oil included nonan-2-ol, heptan-2-ol, hept-4-en-2-ol, and undecan-2-ol. Unusual components identified included hept-4-en-2-ol, hept-4-en-2-one, α-ylangene, geranylacetone, β-ionone, and deca-2,4,7-trienal.

In the United States the corn ear worm (*Heliothis zea*) has caused considerable damage (e.g., $75,000,000 in 1953, McMillian and Wiseman, 1972) to the nation's corn crop over the last 100 years or more. The female corn ear worm moth probably locates the corn plant to deposit her eggs at least partly by odor (cf. Kennedy, 1977). A knowledge of the volatile compounds responsible for the odor of the corn plant is then of considerable interest in the development of methods of controlling this pest. Some volatiles from cooked corn kernels have been examined previously (cf. Flora and Wiley, 1974), and methanethiol, acetaldehyde, ethanol, acetone, ethanethiol and dimethyl sulfide were identified. Volatiles involved in attracting the moth most probably include those from the (uncooked) corn kernels, the corn husk, and the corn silk. Studies of corn silk volatiles have been carried out by Flath (1977). Studies on corn bud volatiles have been carried out by Thompson et al. (1974). The present study was undertaken to identify the volatile components of the corn husk and kernel.

EXPERIMENTAL SECTION

Materials. Several varieties of corn (*Zea mays*) were studied. These included Jubilee, Stylepak, Vanguard, and Illini Xtra Sweet. Most of these were obtained from a commercial experimental field station and some (Vanguard) from local retail markets. The samples were generally examined within a few days of picking or purchase with storage at ca. 5 °C.

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Authentic chemical compounds were obtained from reliable commercial sources or synthesized using established procedures.

Hept-*trans*-4-en-3-one was synthesized by first condensing butanal with malonic acid in triethanolamine (Boxer and Linstead, 1931) to give hex-*trans*-3-enoic acid and then treating this acid with methyllithium to give hept-*trans*-4-en-3-one. Hept-*trans*-4-en-3-ol was synthesized by sodium borohydride reduction of the above ketone.

Isolation of Volatile Oil. With corn husks, the husks (1.5 kg) were first stripped from the corn ears and placed in a 12-L round-bottom flask together with 6 L of odor-free water. A Likens-Nickerson steam distillation continuous extraction head was then attached to the flask. Purified hexane (100 mL) was placed in a flask attached to the solvent arm of the head. The isolation was carried out under reduced pressure (80–100 mm) for 3 h with the corn husks at 45–50 °C. The condenser on the head was cooled with water–ethanol at 0 °C. After the isolation the hexane extract was dried over sodium sulfate, filtered, and concentrated using low hold-up distillation columns to give the corn husk volatile oil. With corn kernels, essentially the same procedure was used except that cobs of corn (3 kg) were cut into quarters longitudinally and placed in the 12-L flask. The corn was not removed from the cob to minimize damage to the corn. It was necessary to add a small amount of silicone antifoam (steam deodorized) to prevent foaming with the corn kernels.

Isolation using vacuum steam distillation continuous extraction was also carried out on a larger scale using a 90-L glass-lined steel container with a scaled up Likens-Nickerson head to give larger quantities of volatile oils.