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Preparation, Separation and Identification of TMS Derivatives of Hydroxylated PCBS and Chlorophenols

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INTRODUCTION

Chlorophenols are used as preservatives, molluscicides, and slimicides, and are synthetic intermediates in processes for the production of some pesticides and preservatives (THE MERCK INDEX, 1960). Chlorinated phenolic residues are found in water (BENVENUE et al., 1972; CHAU and COBURN, 1974), fish (ZITKO et al., 1974), other aquatic organisms (FOX et al., 1975), their eggs (ZITKO et al., 1974), and droppings (JANSSON et al., 1975). Hydroxylated metabolites of polychlorinated biphenyls (PCBs) are chemically similar to chlorophenols and have been found in a number of organisms (HUTZINGER et al., 1974). Thin-layer chromatography (TLC), gas chromatography (GC), charcoal column chromatography, derivatization with various reagents, colorimetric and ultraviolet analyses, and mass spectroscopic (MS) analyses have all been used to isolate and identify various PCB isomers, hydroxylated PCB metabolites, and chlorophenols.

Because of its specificity and sensitivity, gas chromatography/mass spectrometry (GC/MS) is often used for analysis of organochlorine compounds, including hydroxylated PCBs and chlorophenols. GC is used to separate closely related isomers (JENSEN and SUNDSTROM, 1974), and when combined with MS, unequivocal identification of individual compounds can be achieved. However, hydroxylated PCBs and chlorophenols are generally derivatized to alter their polarity and volatility so that they can be more readily resolved by GC.

Several chemical derivatives of chlorinated phenolic compounds have been prepared by other investigators. For instance, the mass spectral characteristics of the dansyl derivative of a dihydroxy metabolite of 4,4'-dichlorobiphenyl was reported by SUNDSTROM et al., 1976. These authors also reviewed the preparation and use of derivatives (methyl ethers, acetates and trimethylsilyl ethers) of chlorobiphenylols used in conjunction with GC.

This report resulted from an investigation to determine which derivative was most suitable for GC/MS examination of hydroxylated PCB metabolites. While examining TMS derivatives of chlorophenols and chlorobiphenylols, we observed ion fragments (m/e 93/95) in spectra of these compounds which in conjunction with the $(M-15)^+$

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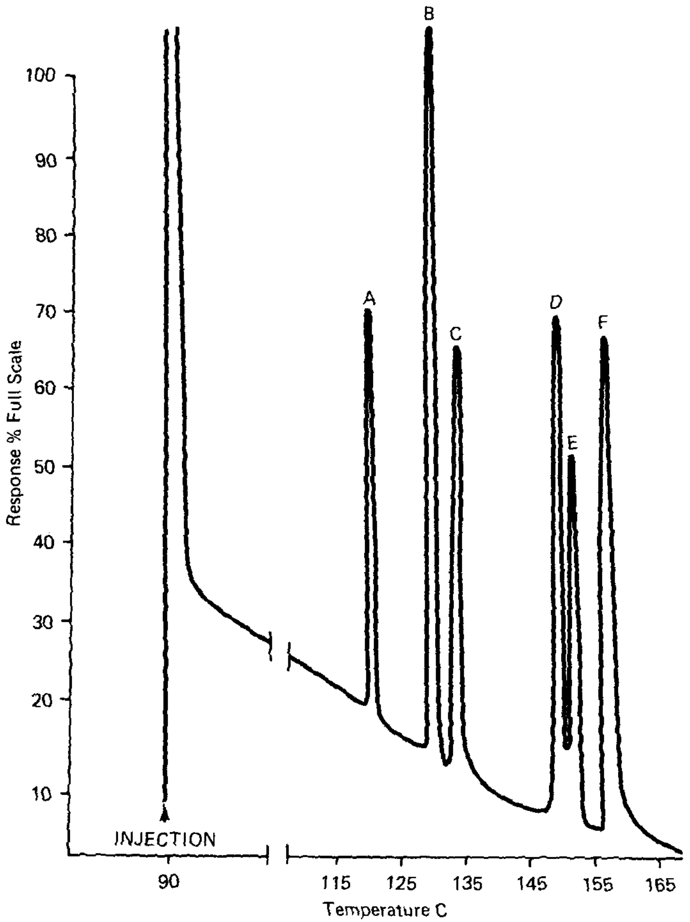

Figure 1. Gas chromatographic analysis of the TMS *derivatives of a mixture* of chlorophenols: A) unreacted BSTFA, B) *o*-chlorophenol, C) *p*-chlorophenol, D) 2,5-dichlorophenol, E) 2,4-dichlorophenol, and F) 3,4-dichlorophenol.

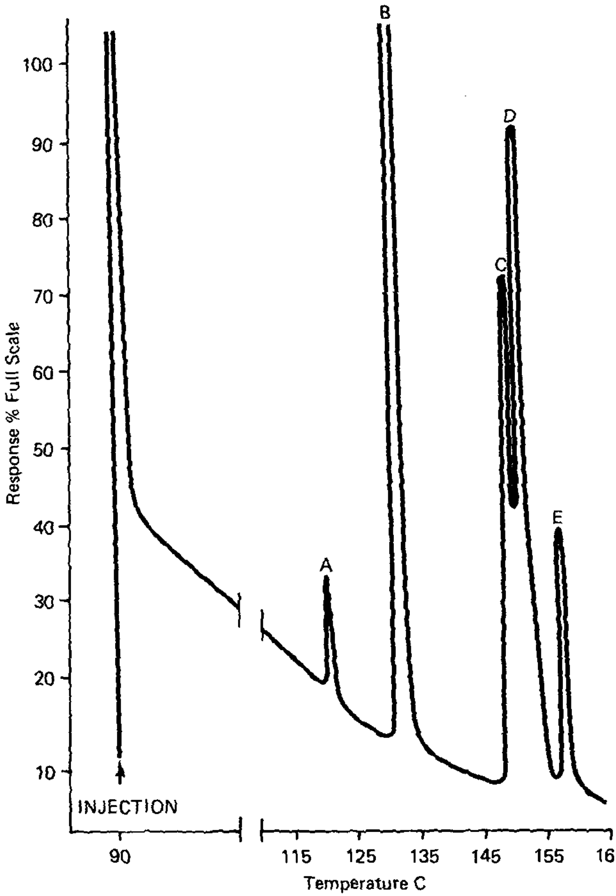

Figure 2. Gas chromatographic analysis of the TMS derivatives of a mixture of chlorophenols: A) *unreacted* BSTFA, B) *m-chlorophenol*, C) 3,5-dichlorophenol, D) 2,3-dichlorophenol, and E) 2,6-dichloro*phenol.*

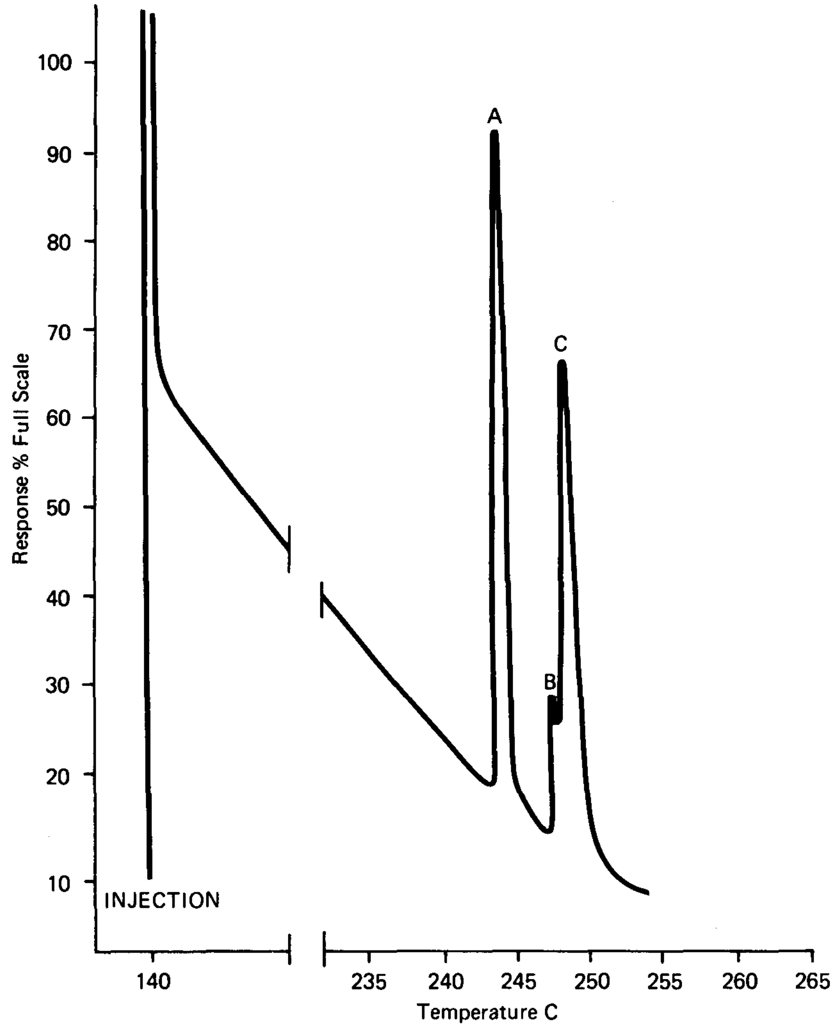

Figure 3. Gas chromatographic analysis of the TMS derivatives of a mixture of three hydroxylated tetrachlorobiphenyls: A) 6-hydroxy-2,5,2',5'-tetrachlorobiphenyl, B) 4-hydroxy-2,5,2',5'-tetrachlorobiphenyl, and C) 3-hydroxy-2,5,2',5',-tetrachlorobiphenyl.

ion can be used to determine the presence of a chlorine atom ortho to the phenolic hydroxyl group. TMS derivatives offer an important advantage for detecting chlorinated phenolic compounds in environmental samples, especially when using multiple ion monitoring GC/MS.

EXPERIMENTAL

Mass spectra were obtained using a Perkin-Elmer Model 270-B GC/MS equipped with a 1 m x 2 mm glass column packed with 3% OV-7 on Chromosorb W-HP (w/w). All scans were taken at an ionization energy of 70 electron volts. The GC column temperature was programmed from 90 to 265 C at 5/min. with 20 psi of helium as the carrier gas. A PDP-12 LDP computer was interfaced with the GC/MS. A computer program initiated and acquired spectra every 8 sec with a scan duration of 4 sec. Perfluorokerosene was used for calibration of the MS. A more detailed description of the GC/MS computer system was reported by STALLING et al. 1973.

TMS derivatives of hydroxylated PCBs and chlorophenols were formed by reaction with N-O- bistrimethylsilytrifluoroacetamide (BSTFA, Regis Chemical Co.) in acetonitrile at 60 C for 15 min. The one exception to this procedure was 3-hydroxy-2,5,2',5'-tetrachlorobiphenyl, which was very difficult to derivatize. We prepared the TMS derivative of this hydroxylated PCB by using BSTFA with 1% trimethylchlorosilane (TMCS) and heating at 100 C for 1 hr. All reactions were conducted in 5 ml culture tubes sealed with Teflon® lined caps.

RESULTS AND DISCUSSION

The TMS derivatives of the chlorophenols and hydroxylated PCBs produced excellent GC peaks (Figures 1, 2, 3). Use of BSTFA containing 1% TMCS was required to obtain conversion of 3-hydroxy-2,5,2',5'-tetrachlorobiphenyl. Mass spectra of the compounds examined had base peaks of either m/e $(M-15)^+$ or 93. The intensity of the molecular ion peak (M^+) ranged from 8 to 35 percent of the base peak and the spectra are relatively simple (Figure 4, 5). We observed that the ratio of m/e 93:$(M-15)^+$ fragments was indicative of the presence of a chlorine atom ortho to the O-TMS. The ion ratios for ortho-chloro phenols ranged from 0.71 to 6.3 while non-ortho chloro phenols fall in the range 0.17 to 0.29 (Table I). We suggest a structure of $^{35}ClSi^+ (CH_3)_2$ and $^{37}ClSi^+ (CH_3)_2$ for the m/e ion fragments 93 and 95. These ion fragments could arise from the migration of an ortho chlorine atom to the TMS group with subsequent rearrangement of the TMS methyl groups prior to fragmentation. The ion at m/e 93 is a good fragment to use for GC/MS ion monitoring in conjunction with the $(M-15)^+$ ion for each chlorinated phenolic homologous series (i.e. chlorophenols:m/e 185, Table II). Thus, the TMS derivatives of chlorinated phenolics have considerable utility in analysis for environmental contaminants such as chlorinated phenols and chlorinated phenoxyphenols (predioxins) (RAPPE and NILSSON, 1972) and metabolites of PCBs.

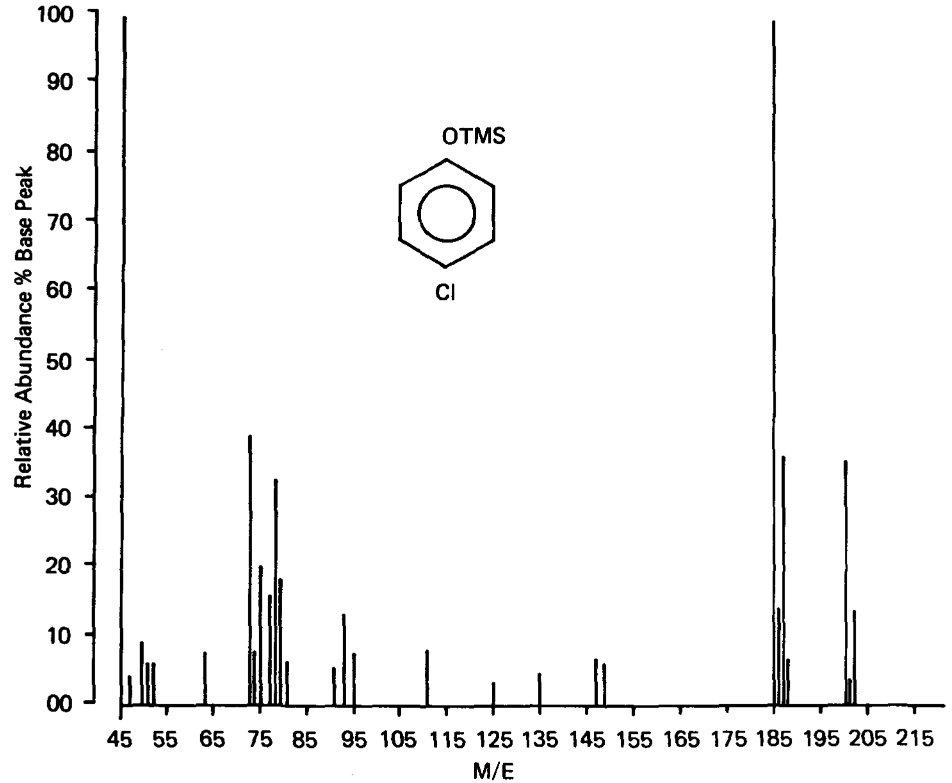

Figure 4. Mass spectra of the TMS derivative of *p*-chlorophenol.

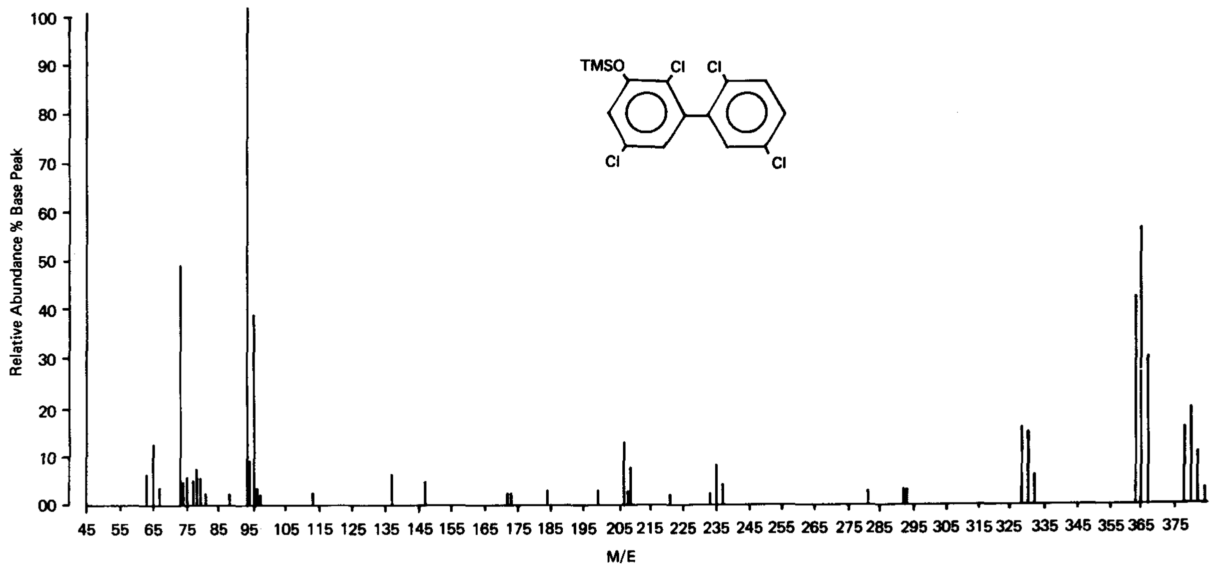

Figure 5. Mass spectra of the TMS derivative 3-hydroxy-2,5,2'5'-tetrachlorbiphenyl

TABLE I

Mass spectral characteristics of TMS derivatives of hydroxylated polychlorinated biphenyls and chlorophenols.

Compound	Derivative GFW	Base peak	ion intensity as % of base peak					93/(M-15)$^+$ ratio
			m/e M$^+$	73	147	93	(M-15)$^+$	
3-hydroxy-2,5,2',5'-tetra-chlorobiphenyl	378	93	15	47	5	100	42	2.4
4-hydroxy-2,5,2',5'-tetra-chlorobiphenyl	378	93	15	50	7	100	26	3.8
6-hydroxy-2,5,2',5'-tetra-chlorobiphenyl	378	93	8	37	6	100	16	6.3
o-chlorophenol	200	93	28	32	5	100	83	1.2
m-chlorophenol	200	185	28	20	5	17	100	0.17
p-chlorophenol	200	185	33	37	7	13	100	0.13
2,3-dichlorophenol	234	93	14	26	9	100	53	1.9
2,4-dichlorophenol	234	93	23	46	4	100	65	1.5
2,5-dichlorophenol	234	93	16	33	9	100	55	1.8
2,6-dichlorophenol	234	219	24	38	15	71	100	0.71
3,4-dichlorophenol	234	219	35	52	11	28	100	0.28
3,5-dichlorophenol	234	219	31	42	11	29	100	0.29

TABLE II

Fragment ion series for monitoring TMS derivatives of chlorophenols and chlorobiphenylols

#Cl	formula	m/e[1]	formula	m/e[1]
0	$C_8H_{11}OSi$	151	$C_{14}H_{15}OSi$	227
1	$C_8H_{10}Cl_1OSi$	185	$C_{14}H_{14}Cl_1OSi$	261
2	$C_8H_9Cl_2OSi$	219	$C_{14}H_{13}Cl_2OSi$	295
3	$C_8H_8Cl_3OSi$	253	$C_{14}H_{12}Cl_3OSi$	329
4	$C_8H_7Cl_4OSi$	287	$C_{14}H_{11}Cl_4OSi$	363
5	$C_8H_6Cl_5OSi$	321	$C_{14}H_{10}Cl_5OSi$	397
6			$C_{14}H_9Cl_6OSi$	431
7			$C_{14}H_8Cl_7OSi$	465
8			$C_{14}H_7Cl_8OSi$	499
			$C_{14}H_6Cl_9OSi$	533

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[1] m/e of first ion in ^{35}Cl-^{37}Cl isotope cluster

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