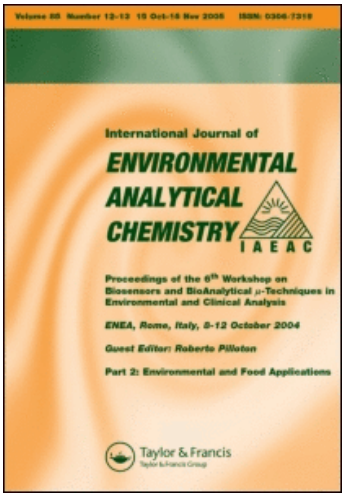

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Gas and Liquid Chromatography of Hydroxybiphenyls, Chlorinated Hydroxybiphenyls and Several Types of Halogenated Derivatives. I. Capillary Gas Chromatography and Mass Spectrometry

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To cite this Article De Kok, A. , Rijnierse, E. , Amoureus, M. , Geerdink, R. B. and Brinkman, U. A. Th.(1987) 'Gas and Liquid Chromatography of Hydroxybiphenyls, Chlorinated Hydroxybiphenyls and Several Types of Halogenated Derivatives. I. Capillary Gas Chromatography and Mass Spectrometry', International Journal of Environmental Analytical Chemistry, 29: 3, 227 — 247

To link to this Article: DOI: 10.1080/03067318708079838

URL: http://dx.doi.org/10.1080/03067318708079838

Intern. J. Environ. Anal. Chem., 1987, Vol. 29, pp. 227–247
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Printed in Great Britain

Gas and Liquid Chromatography of Hydroxybiphenyls, Chlorinated Hydroxybiphenyls and Several Types of Halogenated Derivatives. I. Capillary Gas Chromatography and Mass Spectrometry

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For 8 hydroxybiphenyls and 19 chlorinated hydroxybiphenyls derivatization reactions with 6 electrophilic reagents—viz., 4 perfluoro anhydrides, pentafluorobenzoyl chloride and tert.-butylpentafluorophenylmethylchlorosilane—have been studied to evaluate their potential for capillary gas chromatography with electron-capture detection. For some of the classes of derivatives, mass spectrometric data are presented.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are now generally recognized as one of the most ubiquitous and persistent types of environmental contaminants. In the past they have found widespread industrial use because of their physical and chemical stability and their dielectric properties. The total worldwide production of PCBs since 1929 is estimated[1] to be some 2 million tons. So-called open-end applications, inadequate waste disposal procedures, etc. have led to the release of PCBs into the environment where they have been routinely detected in soil, water and biota since the early sixties. Due to their toxicity, the PCBs are the subject of a still rapidly growing number of papers.

The initial view that PCBs are not significantly degraded in the environment has been replaced by a model involving both photochemical and microbial degradation. PCBs have also been shown to be metabolized in mammals and the major features of the pathways for PCB metabolism have been elucidated. Major metabolites in mammals[2–4] are the mono- and dihydroxylated PCBs; these compounds are also formed during microbial degradation,[5–8] viz. as intermediate products for the ultimate chlorinated benzoic acids. Photodegradation of PCBs results predominantly in dechlorinated products, but small amounts of hydroxylated PCBs have also been identified.[9,10] Recently, chlorohydroxybiphenyls have been detected[11,12] as products of the reaction between free chlorine and phenols during prechlorination in drinking water treatment. They have also been shown[12] to be formed during pyrolysis of PCBs.

Environmental concern about PCBs centers not only on the toxic effects of the compounds as such, but also on their hydroxylated metabolites, which in some cases have been shown[13,14] to be even more toxic than the parent compounds. Because of the significance of the chlorinated hydroxybiphenyls, analytical methods are required which are selective and sensitive because these metabolites appear in environmental samples in very low concentrations.

In the present study, two approaches have been followed to develop methods for the trace-level determination of non-chlorinated hydroxybiphenyls (BP-OHs) and chlorinated hydroxybiphenyls (PCB-OHs). In Part I (this paper), in order to fully utilize the potential of capillary gas chromatography with electron-capture

detection (GC-ECD), several derivatization reactions for (chlorinated) hydroxybiphenyls with electrophilic reagents were investigated in detail. For selected derivatives, their identity was confirmed by gas chromatography-mass spectrometry (GC-MS). In Part II (Ref. 15), the separation of the non-derivatized compounds was studied by reversed-phase column liquid chromatography (HPLC) on a C18 bonded phase, and normal-phase HPLC on bare silica as well as various polar bonded phases. Further, several of the above procedures were employed for the determination of biphenyl and PCB metabolites in extracts of liver microsomes and urine of rats administered with PCBs.

EXPERIMENTAL

Materials

All non-chlorinated and chlorinated hydroxybiphenyls were obtained from ULTRA Scientific (Hope, RI, U.S.A.; formerly RFR Corp.) and Analabs (North Haven, CT, U.S.A.). Some of the PCB-OHs were also received as free gifts from H. Pyysalo (Espoo, Finland), O. Hutzinger (Bayreuth, G.F.R.) and D. L. Stalling (Columbia, MO, U.S.A.). HPLC-grade hexane and analytical-reagent grade acetonitrile, ethyl acetate, toluene and all other reagents were obtained from Baker (Deventer, The Netherlands). Trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA) and pentadecafluorooctanoic anhydride (PFOA) were purchased from Aldrich (Beerse, Belgium), Pierce (Rotterdam, The Netherlands), Merck (Darmstadt, G.F.R.) and PCR (Gainesville, FL, U.S.A.), respectively. Pentafluorobenzoyl chloride (PFBC) and tert.-butylpentafluorophenylmethylchlorosilane (t-buflophemesyl chloride) were supplied by Ventron (Karlsruhe, G.F.R.). Triethylamine (TEA) was from Baker.

Apparatus

Capillary GC 25 m × 0.22 mm I.D. fused silica columns wall-coated with CP-Sil 5 (Chrompack, Middelburg, the Netherlands) were used, the gas chromatograph being a Pye Unicam (Philips, Eindhoven, the Netherlands) Model GCV apparatus equipped with a ^{63}Ni ECD. 1 μl injections were done with a moving needle injector. The

standard column temperature programs were from 110°C (1 or 5 min isothermal) to 250°C with a rate of 2.5 or 10°C min^{-1}; details are given in the legends to the figures and tables. The injector and detector temperatures were 275°C and 300°C, respectively. The nitrogen and purge-gas flow-rates were 1 and 30 ml min^{-1}, respectively.

GC-MS GC-MS was performed on a Finnigan (Sunnyvale, CA, U.S.A.) Model 9500 gas chromatograph connected with a Finnigan 3200 quadrupole mass spectrometer with electron impact at 70 eV, and an ion-source temperature of 230°C. GC conditions were the same as those given above.

HPLC For all experimental details about reversed-phase and normal-phase HPLC with, generally, UV absorbance detection, the reader is referred to the second paper in this series.[15]

Methods

Derivatization of BP-OHs and PCB-OHs with PFPA, TFAA, HFBA and PFOA 10 µl of a 10% solution of TEA in hexane is added to a solution of the compounds to be derivatized in 1 ml of hexane. After mixing, the addition of 10 µl of PFPA, and mixing for 1.5 min on a Whirli mixer, the mixture is allowed to stand at room temperature for 15 min. After completion of the reaction, 2 ml of a pH-6 phosphate buffer are added and mixing is carried out for 0.5 min. Next, a further 3 ml of hexane are added and the derivatives are extracted by shaking on a Whirli mixer. The hexane phase is now transferred to another tube and dried over anhydrous sodium sulphate.

The derivatization reactions with TFAA, HFBA and PFOA were performed in the same way. With PFOA, which has a melting point of 19°C, it was more convenient to have the reagent as a solution in hexane. Besides, centrifugation was necessary to separate the hexane and aqueous layers.

Derivatization of BP-OHs and PCB-OHs with PFBC The procedure is the same as that described by Renberg[16] for phenolic compounds in water. To a 15-ml test tube equipped with a PTFE-lined screw cap which contains the compounds in 8 ml of water, 1 ml of

1 M NaOH and 2 ml of hexane are added. The test tube is shaken for 2 min and the hexane phase, which contains neutral and basic compounds is discarded. Now 2 ml of 1 M $NaHCO_3$, 2 ml of hexane and 20 μl of a 10% solution of PFBC in toluene are added. After shaking for 0.5 min and subsequent phase separation, the aqueous phase is removed with the aid of a Pasteur pipette. After the addition of 10 ml of 1 M NaOH and 2-min shaking, the hexane phase is transferred to another tube and dried over anhydrous sodium sulphate.

RESULTS AND DISCUSSION

Introduction

Direct GC analysis of non-derivatized PCB-OHs has been reported[17] in the literature, but it requires special deactivation of the column to prevent tailing of the chromatographic peaks. Besides, when using an ECD, the response of each individual compound is strongly dependent on the number and substitution pattern of the chlorine atoms. Derivatization of the PCB-OHs with alkylation or silylation reagents eliminates[4,18] the problems of unsatisfactory chromatographic behaviour; however, the variation in ECD response of the derivatives remains. Besides, methylation reactions require working with the hazardous diazomethane and, as an additional disadvantage, methylation of the PCB-OHs makes discrimination between the derivatives and original chloromethoxybiphenyl metabolites impossible.

In order to improve both the chromatographic behaviour and the sensitivity towards an ECD (with uniformity of response for all derivatives) it lies at hand to use electrophilic reagents such as the perfluoro anhydrides trifluoroacetic (TFAA), pentafluoropropionic (PFPA) and heptafluorobutyric (HFBA) anhydride. Compared with silylation reagents, methyl iodide or acetic anhydride, TFAA[19] and HFBA[20] have been used only infrequently, and then only for the non-chlorinated biphenyls. We have focussed our attention on the development of PFPA derivatization for BP-OHs, as well as PCB-OHs. Derivatization procedures with some other reagents have been critically compared with the optimized PFPA procedure.

Derivatization with PFPA

For the optimization studies the entire group of standards was divided into three mixtures to avoid separation problems during GC analysis. Mixtures A and B contained 10 and 9 monohydroxy(chloro)biphenyls, respectively, while mixture C contained all 8 dihydroxy compounds (see Table I). The derivatization conditions were optimized starting from a preliminary procedure during which derivatization took place for 1 h at room temperature in hexane with 20 μl of PFPA and 10 μl of TEA. During this study the influence of reaction time and temperature, presence of catalyst, type of solvent and amount of reagent on the formation of the derivative, and the influence of the pH of the aqueous phase on the destruction of excess reagent were studied. Further, the stability of the derivatives, their chromatographic behaviour, and the repeatability and linearity of the procedure were tested.

Table I PCB-OHs and BP-OHs present in the mixtures A, B and C used in optimization studies

Mixture A			Mixture B		Mixture C	
Cl	OH	No.[a]	Cl	OH	Cl	OH
	2	(1)		4		2,2′
	3	(2)	5	2		2,5
3	2	(3)	2	5		3,4
2′,5′	2	(4)	3,5	2		3,3′
2	4	(5)	3	4		4,4′
4′	4	(6)	2,5,5′	2	4,4′	3,3′
2′,5′	3	(7)	3,5	4	3,3′	4,4′
2′,3,5′	2	(8)	3,4′	4	3,3′,5.5′	4,4′
2′,5′	4	(9)	2,3,3′,4′,5′	2		
3,4′,5	4	(10)				

[a]Peak numbers used in Figure 1.

Catalyst and reaction temperature Tertiary amines such as trimethylamine or TEA are widely used in, for example, the acylation of phenolic compounds. In our case, the presence of TEA also appeared to be necessary. All three test mixtures were derivatized at room temperature in the absence and presence of the amine. Without TEA,

yields were much lower and, often, less than 10% of the yield obtained in the presence of TEA (Figure 1). Increasing the reaction temperature to 50°C resulted in lower yields, comparable to those obtained in the absence of TEA.

The use of TEA causes the occurrence of a number of additional peaks in the GC chromatogram, which are due to impurities present in the reagent, or derivatives of contaminants contained in the sample solution. Interferences can be minimized by using distilled TEA, which should be kept in the refrigerator as a 10% solution in

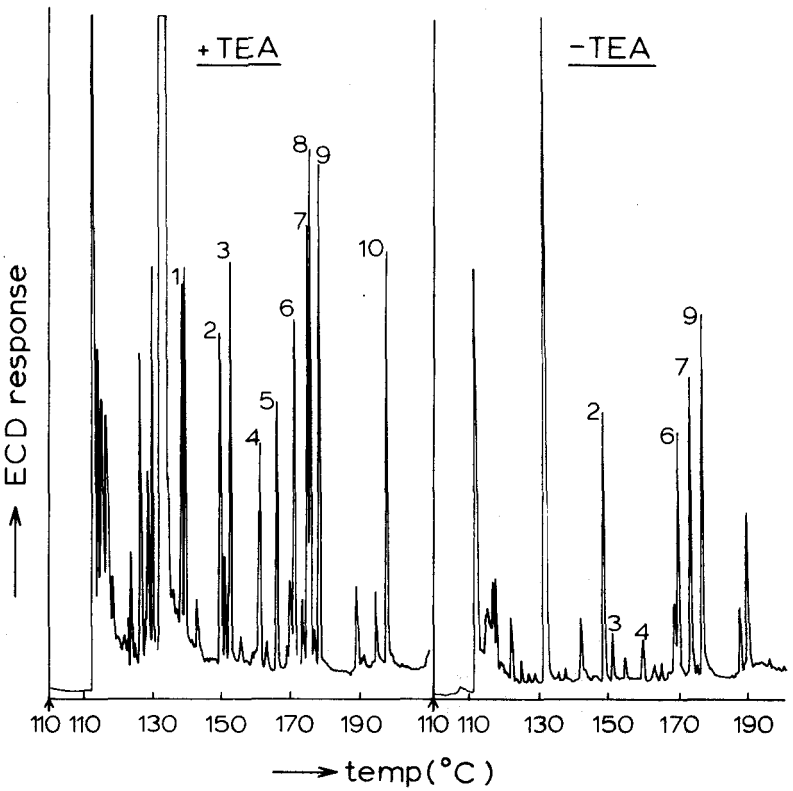


Figure 1 Influence of catalyst (TEA) on the yield of the derivatization of 10 PCB-OHs and BP-OHs (mixture A; for peak assignment, see Table I) with PFPA. Capillary GC conditions: CP-Sil 5; 110°C (1 min isothermal) to 250°C with a rate of 10°C min^{-1}.

hexane. In order to reduce interfering effects still further, the original amount of TEA was reduced from 10 μl to 1 μl, with no adverse effect on derivative yields.

Reaction time Using text mixtures B and C, the reaction time was varied between 2.5 and 60 min. Yields of 50–85% were obtained within 5 min, maximum values being reached between 10 and 20 min for the monohydroxy compounds, and after about 10 min for the dihydroxy compounds. A further increase of the reaction time occasionally was found to be detrimental; therefore, 15 min was selected as a good compromise.

Reaction medium In several series of experiments, hexane was replaced as solvent by toluene or ethyl acetate. In all such cases, reaction yields were distinctly lower than when using hexane; with the dihydroxy PCB-OHs, derivatives could not be observed at all. The unsatisfactory results for toluene and ethyl acetate are not caused by instability of the PFP derivatives in these solvents: derivatives prepared in hexane and redissolved, after evaporization of the hexane, in toluene or ethyl acetate, were quite stable. Obviously, therefore, it is the reaction conditions themselves which mar the results.

Reagent amount The amount of PFPA added was varied from 1 to 50 μl. Maximum yields were obtained at 10–50 μl. With 1 or 5 μl, significantly lower results were obtained with some monohydroxy and all dihydroxy compounds. An amount of 10 μl of PFPA was selected for all further work.

Aqueous-phase pH It is recommended to destroy the excess of PFPA, and simultaneously to introduce a simple clean-up step, by shaking the organic solution with an aqueous buffer after the derivatization has been completed. In view of the required stability of the PFP derivatives, it is important to select the proper pH value. Aqueous solutions studied ranged from 0.1 M HCl to 1 M NaOH. With the latter solution, no derivatives were detected at all in subsequent GC analysis, probably as a consequence of hydrolysis. For wash solutions with pH = 1–10 the yields relative to the maximum varied between 50 and 100% for the monohydroxy compounds (mixture B; maximum invariably at pH 6) and between 70 and 100% for the dihydroxy compounds (mixture C; maximum varying from

pH 4 to pH 8 depending on compound structure). Since, for mixture C, the relative yield at pH 6 invariably was at least 90%, a pH-6 buffer was used in all further work.

Derivative stability Solutions of the PFP derivatives in an anhydrous solvent must be stored in a refrigerator (+4°C) or a freezer (−18°C) to avoid rapid decomposition. The derivatives are only stable for 48 h at room temperature, and for 1 week at 4°C. Generally speaking, decomposition is more rapid for the dihydroxy as compared with the monohydroxy compounds.

In order to study the potential of clean-up over silica, the stability of the PFP derivatives on a small (1 g) silica column was tested first. After sample application and subsequent elution with hexane–ethyl acetate (50:50), 3 out of the 9 derivatives of mixture B (2-Cl-5-OH, 3-Cl-4-OH, 3,4′-Cl-4-OH) could not be detected. Probably, their decomposition is caused by hydrolysis which is stimulated by the slightly acidic nature of the silica surface. Obviously, clean-up over silica after derivatization can not be recommended.

Detection limits and linearity For the injection of standards, and when using the optimized procedure, the lower limit of detection of the PFP derivatives was between 0.5 and 2.0 pg (signal-to-noise ratio, 3:1). The strongest response to the ECD was shown by the PFP derivative of 2,2′-di-OH-BP, and the weakest by 3,4-di-OH-BP.

Calibration plots for various selected derivatives were found to be linear over the whole range tested (1–100 pg).

GC of PFP derivatives

Contrary to the parent compounds, the PFP derivatives display good chromatographic behaviour in capillary GC on an apolar stationary phase such as CP-Sil 5. Their (relative) retention times are recorded in Table II and a chromatogram of all 27 derivatives is shown in Figure 2. Only 20 peaks show up because seven pairs of derivatives were not separated. At this stage, no attempt was made to separate the unresolved pairs by using other stationary phases. We do mention, however, that the majority of the separation problems encountered here can be solved by means of HPLC (see Ref. 15). This indicates the usefulness of this technique for pre-separation with GC-ECD as the final determination step.

Table II Relative retention times (RRT; min)[a] and peak assignments[b] of PFP, PFO and PFBC derivatives of PCB-OHs and BP-OHs in capillary GC on CP Sil-5. Conditions: 110°C (1 min (PFBC) or 5 min (PFP and PFO) isothermal) to 250°C with a rate of 2.5°C min^{-1}

Analyte		PFP derivative		PFO derivative		PFBC derivative	
Cl	OH	RRT	No.	RRT	No.	RRT	No.
	2	1.00	1	1.00	1	1.00	1
	2,2′	1.07	2	1.42	6		
	3	1.32	3	1.22	2	1.35	3
	2,5	1.32	3	1.78	11		
	4	1.41	4	1.30	4	1.41	4
5	2	1.44	5	1.54	7	1.26	2
3	2	1.44	5	1.25	3	1.26	2
2	5	1.66	6	1.41	5	1.57	6
2′,5′	2	1.82	7	1.92	13	1.46	5
	3,4	1.83	8	2.06	15		
	3,3′	1.91	9	2.17	17		
3,5	2	1.91	9	1.25	3	1.57	6
2	4	1.98	10	1.67	8	1.70	7
3	4	1.98	10	1.67	8	1.70	7
4′	4	2.15	11	1.78	11	1.80	10
	4,4′	2.15	11	2.47	18		
2′,5′	3	2.31	12	1.81	12	1.92	11
2′,3,5′	2	2.33	13	1.76	10	1.73	8
2,5,5′	2	2.33	13	1.75	9	1.74	9
2′,5′	4	2.50	14	1.42	6	1.99	12
3,5	4	2.50	14	1.94	14	2.01	13
3,4′	4	2.74	15	2.15	16	2.15	14
4,4′	3,3′	3.10	16	2.86	20		
3,4′,5	4	3.34	17	2.49	19	2.35	16
3,3′	4,4′	3.40	18	3.17	21		
2,3,3′,4′,5′	2	3.71	19	2.49	19	2.32	15
3,3′,5,5′	4,4′	4.38	20	3.76	22		

[a]Absolute retention time of 2-hydroxybiphenyl as PFP, PFO and PFBC derivative: 6.9, 11.0 and 14.2 min, respectively.

[b]Numbers refer to peaks in Figure 2 (PFP), Figure 3 (PFO) and Figure 4 (PFBC), respectively.

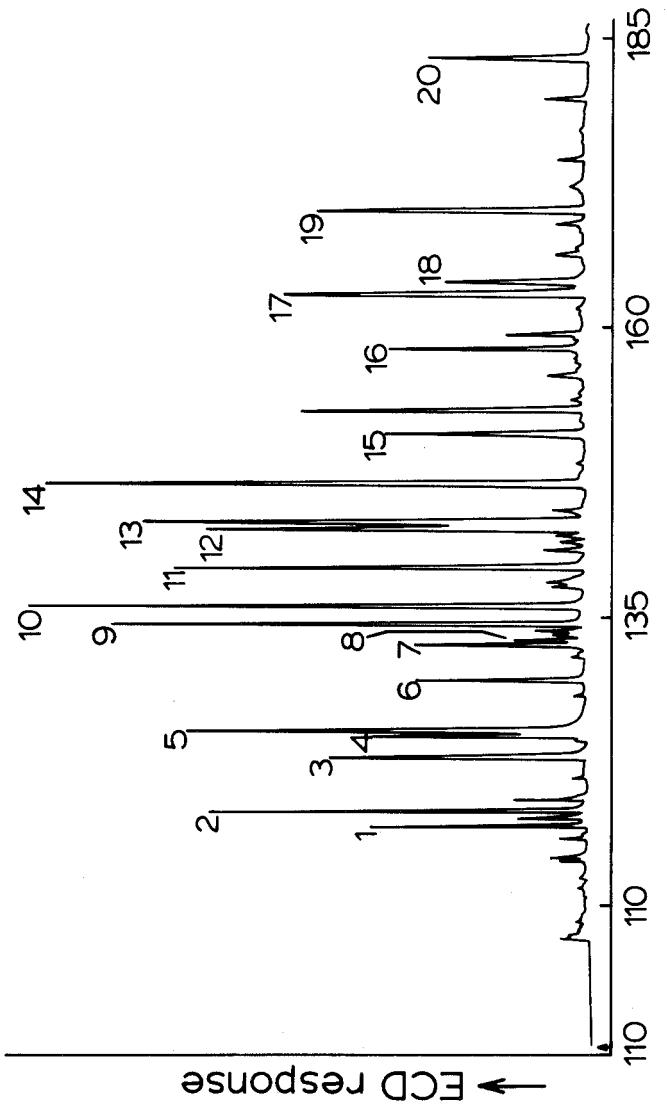


Figure 2 Capillary GC of all available PCB-OHs and BP-OHs as their PFP derivatives. For conditions and peak assignments, see Table II.

As regards the elution sequence of the PFP derivatives, retention appears to increase in the order 2-OH < 3-OH < 4-OH substitution. From the data available, no clear conclusions can be drawn regarding the dependence of retention on chlorine substitution patterns.

GC-MS of PFP derivatives

For 7 BP-OHs and 9 PCB-OHs, the mass spectra of their PFP derivatives were recorded. The main MS characteristics are given in Tables III and IV. All spectra show reasonably intense molecular ion peaks (rel. int. 5–76%) which, however, are never the base peak. For the 4-OH-substituted compounds this is mostly the $[M\text{-}147]^+$ peak caused by the rapid loss of the C_2F_5 radical and a CO molecule from the molecular ion; in the case of the 2-OH-substituted molecules this fragment ion is supposed to be stabilized by a benzofuran structure.[17,20,21] Subsequent losses of another CO and a C_2H_2 molecule yield prominent $[M\text{-}175]^+$ and $[M\text{-}201]^+$ ions, respectively. The $[M\text{-}C_2F_5\text{-}(CO)_2]^+$ ion is of importance with the non-chlorinated compounds, i.e., with the BP-OHs only, and it is the base peak for 3-hydroxybiphenyl. A very specific ion, $[M\text{-}C_2F_5]^+$, only encountered with the 2-OH compounds, may well be stabilized by the formation of a six ring (Scheme 1).

Further interesting conclusions to be drawn from the MS data are as follows. (1) Loss of chlorine atoms never occurs directly from the molecular ion, but only after the loss of the PFP group(s). (2) The $[M\text{-}C_2F_5\text{-}CO\text{-}Cl]^+$ ion is the base peak in the mass spectra of the PCB-OHs having the OH substituent in the 2 position and its presence, therefore, will provide rather specific information. (3) The relative intensity of the $C_2F_5^{+\cdot}$ peak increases with an increasing number of chlorine substituents. It is especially prominent with all dihydroxy compounds. (4) The PFP derivatives of the PCB-OHs and BP-OHs studied yield rather characteristic mass spectra. These will easily reveal the position of the hydroxy, though not those of the chlorine, substituents.

Derivatization with TFAA, HFBA and PFOA

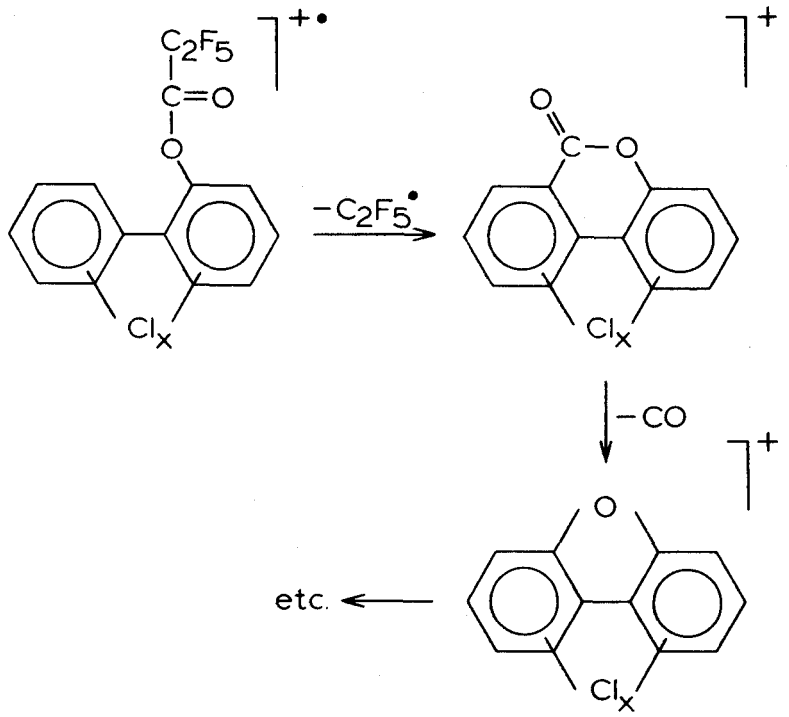
The use of PFPA as a first choice in the present study was more or less arbitrary. From the literature,[23] it is well known that other

Table III Main MS characteristics of PFP derivatives of monohydroxylated PCB-OHs and BP-OHs

Analyte			Relative intensity (%) of ion:										
			$M^{+\cdot}$	M-H	$M-C_2F_5$	$M-C_2F_5$ -CO	$M-C_2F_5$ $-CO_2$	$M-C_2F_5$ $-(CO)_2$	C_2F_5	$M-C_2F_5$ $-(CO)_2-C_2H_2$	$M-C_2F_5$ -CO-Cl	$M-C_2F_5-(CO)_2$ -HCl	$M-C_2F_5-(CO)_2$ $-Cl_2$
Cl	OH	M.W.		M-1	M-119	M-147	M-163	M-175	119	M-201	M-182	M-211	M-245
	2	316	64	22	*100*	74	24	62	14	59	—	—	—
	3	316	55	20	2	5	37	*100*	22	52	—	—	—
	4	316	76	25	—	*100*	8	62	12	46	—	—	—
5	2	350	8	5	48	6	1	3	11	7	*100*	—	—
2	4	350	25	13	—	*100*	3	44	21	13	2	51	—
3	4	350	35	19	—	*100*	2	43	18	12	3	45	—
4′	4	350	61	32	—	*100*	5	55	19	20	3	43	—
3,5	4	384	28	19	—	*100*	2	28	24	5	2	17	53
3,4′,5	4	418	26	18		*100*	2	23	32	2	3	11	49
2,5,5′	2	418	5	3	3	6	2	—	38	—	*100*	—	38
2,3,3′,4′,5′	2	486	14	13	4	2	2	—	88	—	*100*	—	33

Table IV Main MS characteristics of PFP derivatives of dihydroxylated BP-OHs and PCB-OHs

Analyte			Relative intensity (%) of ion:										
			$M^{+\cdot}$	$M-C_2F_5$ -CO	$M-C_2F_5$ $-(CO)_2$	C_2F_5	$M-C_2F_5$ $-(CO)_3$	$M-(C_2F_5)_2$ $-(CO)_2$	$M-(C_2F_5)_2$ $-CO_2H-CO$	$M-(C_2F_5)_2$ $-(CO)_3$	$M-(C_2F_5)_2$ $-CO_2-H(CO)_2$	$C_{10}H_8$	C_8H_6
Cl	OH	M.W.		M-147	M-175	119	M-203	M-294	M-310	M-322	M-339	128	102
	2,2′	478	30	10	1	36	—	17	*100*	9	75	25	17
	3,3′	478	22	42	5	62	50	6	5	25	17	*100*	29
	4,4′	478	45	1	62	*100*	15	2	22	5	47	63	18
	3,4	478	52	*100*	32	70	10	5	7	44	18	52	15
3,3′,5,5′	4,4′	614	5	12	—	*100*	—	10	—	8	—	—	—



Scheme 1 Proposed initial mass fragmentation of PFP derivatives of PCB-OHs having the OH substituent in the 2 position.

perfluoroacyl anhydrides may be superior with regard to speed of reaction (TFAA) and ECD response or stability of the derivatives (HFBA, PFOA). Improved separation of selected pairs of derivatives should also be considered.

Derivatization of the BP-OHs and PCB-OHs with TFAA, HFBA and PFOA was studied using the optimized procedure elaborated for PFPA. The GC behaviour of the TFA and HFB derivatives turned out to be markedly similar to that of the PFP derivatives. With the former reagent, the retention times were somewhat shorter, and with the latter reagent, slightly longer than with PFPA. More pronounced changes in elution time and elution order were observed in the case of PFOA. Relative retention times for the PFO derivatives of all test compounds have therefore been included in Table II, and a GC chromatogram is shown in Figure 3. It is especially noteworthy that

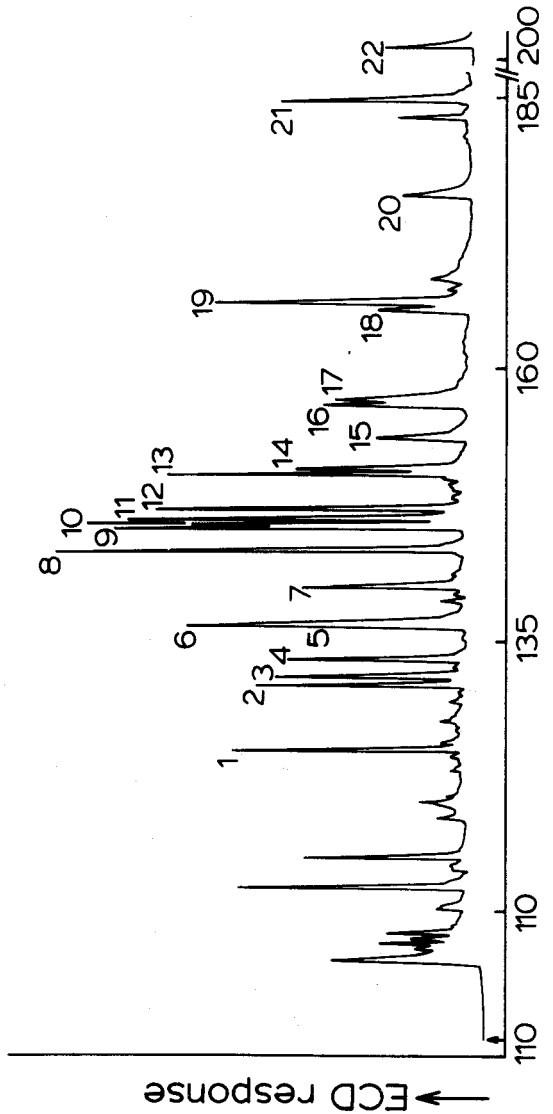


Figure 3 Capillary GC of all available PCB-OHs and BP-OHs as their PFO derivatives. For conditions and peak assignments, see Table II.

changing the nature of the perfluoroacyl group causes a distinct change in the order of elution: with PFOA as reagent, 5 pairs of derivatives can not be separated by GC on CP-Sil 5; with one exception (2-Cl-4-OH-BP and 3-4-OH-BP), however, these can all be separated as PFP derivatives. In other words, the combined use of PFOA and one of the other reagents will enhance the potential of identification by means of GC.

The responses of the three classes of derivatives towards the ECD were essentially the same as those of the PFP derivatives; in other words, detection limits again were from 0.5 to 2 pg. As regards the thermal stability of the various types of derivatives, mutual differences were small, with the PFO derivatives displaying somewhat better stability than the other classes of compounds.

Derivatization with t-buflophemesyl chloride

Trimethylchlorosilanes are well known reagents for the derivatization of, e.g., phenols and PCB-OHs to obtain thermostable and apolar reaction products. Recently several trialkylsilyl reagents other than trimethylchlorosilane have been propagated[24–27] for the chemical modification of various functional groups. The use of one such reagent, tert.-butylpentafluorophenylmethylchlorosilane has especially been recommended[26] because of the high ECD sensitivity and good thermal and hydrolytic stability of its derivatives. Besides, these display characteristic MS fragmentation patterns, which are useful in structure identification. In the present study, reactions were carried out in acetonitrile, using 20 μl of reagent, 27 μl of TEA as a catalyst, and a reaction time and temperature of 1 h and 60°C, respectively.

Derivatization of 200–400 μg of each of three monohydroxy and one dihydroxy compound(s) gave excellent results for all monohydroxylated analytes. No GC peak showed up, however, in the case of 3,3′,5,5′-tetrachloro-4,4′-dihydroxybiphenyl. Because HPLC studies revealed that a major portion of the starting product had disappeared after the reaction, the most probable explanation is that only one of the hydroxy groups reacted with t-buflophemesyl chloride.

In the next step, 40-μg instead of 200–400 μg amounts of 17 PCB-OHs and BP-OHs were derivatized. An excellent GC peak pattern was obtained with elution temperatures of between 250 and 300°C. Further experiments with monohydroxy compounds at the 1-μg level

were, however, a complete failure: not a single peak showed up in GC, and HPLC revealed that over 99% of the PCB-OHs and BP-OHs was still present in the reaction mixture. Many further attempts were made to obtain acceptable yields at low analyte concentrations. Notably, the ratio of reagent and/or catalyst over the analyte(s) was varied over a wide range. However, all attempts were essentially unsuccessful if the amount of analyte was below 30–40 μg: no or very small GC peaks showed up, and HPLC invariably revealed the presence of a large amount of the starting material.

On the basis of our combined results we conclude that t-buflophemesyl chloride is no suitable reagent for the trace-level determination of the analytes being studied. It is interesting to add that Poole and coworkers[26] used milligram amounts of test solutes in their original, and successful, experiments.

Derivatization with PFBC

The derivatization agents discussed so far share the characteristic that they have to be used in a non-polar and, preferably, anhydrous medium. Compounds such as chlorophenols, PCB-OHs, etc. often occur, however, in an aqueous medium, which implies that an extraction has to be carried out prior to derivatization. Derivatization directly in the aqueous phase has the advantage that the rather polar analytes are converted into fairly non-polar derivatives, which substantially facilitates the extraction procedure. We have therefore tested pentafluorobenzoyl chloride which is known to be, in alkaline medium, a reagent specific for phenolic compounds. Nose *et al.*[28] have used it for the micro GC determination of o-phenylphenol in citrus fruits, and Renberg[16] for the determination of various phenolic compounds in water.

In our study, we used the method of Renberg, which gave good results for all monohydroxy compounds (mixtures A and B). Changing the pH from Renberg's value of 9.9 to either 8.5 or 11 gave yields which were, at most, 5–10% different from those found at pH 9.9; i.e., the pH value during derivatization is not a very critical parameter. Derivatization of the dihydroxy compounds was unsuccessful, no matter what pH of the aqueous phase was used. To all probability, only one hydroxy group is derivatized, and the resulting derivative is either not extracted into the organic phase or, if extracted, is sorbed irreversibly onto the capillary GC column.

Characteristics of PFBC derivatives Compared with the PFP derivatives, the PFBC derivatives have some 15°C higher elution temperatures, as can be seen from the 130–185°C temperature range in Figure 4 as opposed to a 115–170°C range in Figure 2. Retention data are included in Table II. Obviously, there are only minor differences in retention order. On the other hand, combining the results of derivatization with PFPA *and* PFBC allows one to separate all PCB-OHs and BP-OHs from each other, with the exception of the pairs 3-Cl-2-OH/5-Cl-2-OH and 2-Cl-4-OH/3-Cl-4-OH.

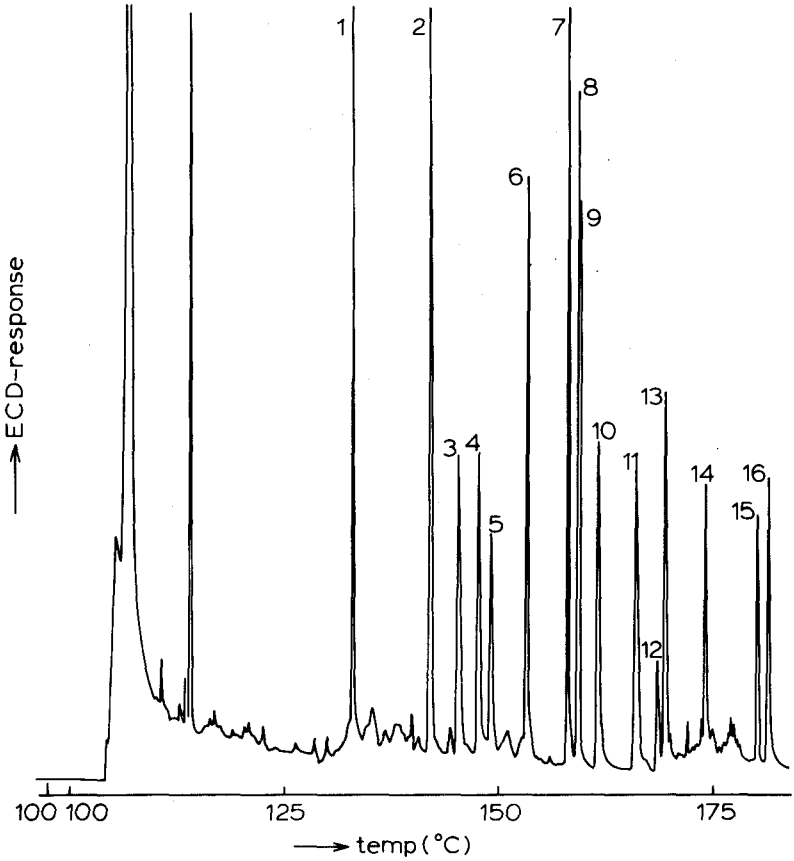


Figure 4 Capillary GC of all monohydroxylated PCB-OHs and BP-OHs as their PFBC derivatives. For conditions and peak assignments, see Table II.

On an average, the response of the PFBC derivatives to the ECD is 2-fold higher than that of the PFP derivatives (range, 1.1–3.7). The detection limits are all in the 0.25–2 pg range. The stability of the PFBC derivatives is excellent. They can be kept for several days at room temperature, and for at least 2 weeks in the refrigerator (+4°C). Derivatives kept at −18°C in a freezer showed less than 5% loss after one year.

The mass spectra of the PFBC derivatives are not very informative. The isomeric derivatives of the three monohydroxybiphenyls have virtually the same mass spectrum, viz. a molecular ion peak at m/z 364 (rel. int., ca. 20%) and a base peak at m/z 195 due to the $[C_6F_5CO]^{+\cdot}$ ion. The latter ion is invariably the base peak in the mass spectra of the PCB-OH derivatives, in which molecular ions are either present in low intensity or from which they are fully absent. From the fact that any other structure-relevant ions are also absent, it may be concluded that the PFBC derivatives do not possess good mass spectra for structure identification purposes. Better results can probably be expected in negative-ion chemical-ionization mass spectrometry.

CONCLUSIONS

Chlorinated and non-chlorinated hydroxybiphenyls are trace-level environmental contaminants, and at least some of them are the highly toxic degradation products of biphenyl and the notorious PCBs. Their determination in real samples requires the combined use of an efficient separation technique and an extremely sensitive method of detection. GC-ECD excellently serves these purposes, provided a suitable derivatization with, preferably, a perfluorinated reagent is included in the analysis. Pentafluoropropionic anhydride appears to be the most versatile reagent for both mono- and dihydroxy compounds. Pentafluorobenzoyl chloride is a good alternative for monohydroxy(chloro)biphenyls and can be applied directly in an aqueous medium. With both reagents the detection limits for all PCB-OHs and BP-OHs tested is on the order of 1 pg.

In cases where standards are not available or where definitive structure identification has to be made, GC-MS is an almost indispensable tool. As regards the present study, PFP derivatives

should certainly be preferred to their PFBC analogues. Firstly, PFP derivatives can be prepared also for the dihydroxy compounds and, secondly, their electron-impact mass spectra are highly informative. If GC-MS facilities are not available, the reader should consult one of our earlier papers,[29] in which a rather simple dechlorination procedure is described that can well serve as an interesting alternative for structure identification of unknown metabolites of PCBs.

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