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SYNTHESIS AND MASS SPECTROMETRY OF SOME METHOXYLATED PCB

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ABSTRACT

The syntheses of 46 methoxy-polychlorobiphenyls (MeO-CBs), containing 3 to 7 chlorine atoms, are described. The MeO-CBs were synthesized via the Cadogan diaryl coupling reaction of the appropriate polychloroaniline and polychloroanisole, or via the Ullmann coupling of a polychloroiodobenzene and 4-iodoanisole with subsequent chlorination of the isolated 4-MeO-CB product. The synthesized MeO-CBs were characterized by electron ionization (EI) mass spectrometry (MS) on an ion trap MS instrument and by EI and negative ion chemical ionization (NICI) on a quadrupole mass spectrometer.

Both instruments gave similar EI spectra but the fragments were in general more abundant relative to the molecular ion, in the spectra obtained from the ion trap instrument. Characteristic fragmentation patterns were obtained by EI for *ortho*-, *meta*- and *para*-MeO-CBs, respectively, depending on the position of the MeO-group, with the exception of three *meta*-substituted MeO-heptaCBs, with a 3-MeO-2,4,6-trichloro-substitution pattern, that gave an abundant $[M-15]^+$-fragment, similar to *para*-substituted MeO-CBs. MS(NICI) of *ortho*-, *meta*- and *para*-MeO-CBs did not give any characteristic fragmentation patterns depending on the position of the MeO-group, except for *ortho*-substituted MeO-CBs that showed abundant fragments at $[M-36]^-$. The MS(NICI) gave approximately 10-50 times higher response for MeO-tetraCBs - MeO-heptaCBs than the MS(EI). The ion trap instrument (ITS40) has a somewhat lower detection-limit than the quadrupole MS when operated in the EI-mode.

INTRODUCTION

Polychlorinated biphenyls (PCB), known as ubiquitous environmental contaminants, are transformed in mammals to both hydroxylated and sulphur-containing metabolites (1-3). Hydroxylated chlorobiphenyls (OH-CBs) are semi-polar metabolites of chlorinated biphenyls (CBs) that can be excreted as such or as e.g. sulphate or glucuronic acid conjugates (4). The formation and identification of OH-CBs, generally analyzed by gas chromatography (GC) or

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gas chromatography/mass spectrometry (GC/MS) after methylation to the corresponding methyl ethers (MeO-CBs), are frequently reported from analysis of urine and faeces of experimentally exposed animals (5-8). One early observations of formation of PCB metabolites was in made in excreta from guillemot and seal (9). More recently, studies have shown that certain OH-CBs bind to a thyroxin transporting protein (transthyretin) in blood serum (10-13). The observation of a strong and selective retention of OH-CBs in blood from environmentally exposed humans and grey seals, and in experimentally PCB-exposed rats, further confirm the observation that plasma proteins may bind certain OH-CBs (14). MeO-CBs are thus required as standards for the identification and quantification of methylated OH-CBs found in biota or in excreta.

Halogenated methoxy-biphenyls with 1 to 6 halogen atoms have previously been synthesized via the Cadogan diaryl coupling method and their EI mass spectrometric fragmentation properties described (15,16). Comparison of the EI induced mass spectra revealed typical fragmentation patterns depending on the position of the methoxy-group. Thus, *ortho*-MeO-CBs were characterized by an abundant fragment at $[M-50]^+$ due to the loss of (CH_3+Cl) and the formation of a fragment corresponding to a dibenzofuran ion. MeO-CBs with the methoxy-group in *meta*- or *para*-position were differentiated by an abundant ion $[M-15]^+$ for *para*-substituted MeO-CBs (15,16). These specific fragmentation patterns of the methyl derivatives are a valuable tool for the structure identification of hydroxy-CB metabolites. No reports, using NICI to describe the fragmentation pattern of MeO-CBs, have so far been published.

The aim of the present study was to determine whether characteristic fragmentation patterns depending on the position of the methoxy-group in the MeO-CBs could be obtained by MS(NICI) and to extend the knowledge on fragmentation patterns of MeO-CBs obtained by MS(EI). The preparation of 46 MeO-CBs is described, and their EI and NICI mass spectra are reported. In addition, the fragmentation of MeO-CBs by EI on an ion trap instrument is compared with that obtained from a quadrupole instrument.

MATERIAL AND METHODS

Chemicals

2,4-Dichloroaniline was obtained from Eastman Organic Chemicals (New York, USA) and 2,5-dichloroanisole and 2,5-dichloroaniline from Janssen Chimica (Geel, Belgium). 3,4-Dichloroaniline, 2,3,4- and 3,4,5-trichloroaniline were purchased from Fluka (Buchs, Switzerland). 4-Chloroanisole, 2,4,5-trichloro- and 2,3,4,5-tetrachloroaniline, 4-iodoanisole, 2,3-, 2,6-dichloroanisole, and 2,3,4-, 2,3,6- and 2,4,6-trichloroanisole and 2,3,5,6-tetrachloroanisole, 2,3,4,6-tetrachloroaniline and 2,3,5,6-tetrachloroaniline were all purchased from Aldrich (Steinheim, Germany), and used in diaryl coupling reactions and/or as precursors for the preparation of the corresponding polychloroiodo-benzenes. 3,4-Dichloroiodobenzene was purchased from Koch-Light Laboratories Ltd (Colnbrook Bucks, England), 3-Methyl butyl nitrite was obtained from Fluka AG (Buchs, Switzerland), methyl iodide and sodium chlorate from Merck (Darmstadt, Germany) and copper bronze from Carlfors bruk (Huskvarna, Sweden).

Dichloromethane (DCM) and *n*-hexane (Merck, Darmstadt, Germany) were of *p.a.* quality. 2,3,4,5,3',4',5'-Heptachlorobiphenyl was synthesized as described elsewhere (17).

Preparative separations of the synthesized compounds were performed by liquid chromatography on silica gel (Kieselgel 60, <0.063 mm and/or 0.063 - 0.200 mm) from Merck (Darmstadt, Germany). Thin layer chromatography (TLC) was performed on silica gel TLC plates (Kieselgel 60 F 254, Merck) with hexane:DCM (4:1, v/v) as the mobile phase.

Instruments

GC/MS was performed with a TSQ 700 quadrupole mass spectrometer from Finnigan MAT on line with a Varian 3400 gas chromatograph equipped with a DB-5 fused silica DB5 capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific Inc. (Folsom, CA, USA). The oven temperature was programmed as follows: 80°C (1 min) - 10°C/min - 300°C (30 min). Helium was used as the carrier gas. An autosampler (A200S, Finnigan) was used for injections in splitless mode of the split/splitless injector. The TSQ mass spectrometer was operated at an electron energy of 70 eV and with an ion source temperature of 150°C and a pressure of 4.5 torr. NICI was performed at an electron energy of 70 eV with methane (>99.995% pure, with an O_2 content of ≤ 5 ppm) as the reagent gas at a pressure of 4.5 torr. Both EI and NICI mass spectra were collected by scanning from 80-550 amu at a speed of 1scan/s.

GC/MS was also performed on an ion trap instrument, Finnigan ITS40, connected to a Varian 3400 gas chromatograph as above. The temperature program was: 80°C (1 min) - 10°C/min - 280°C (10 min) and with helium as the carrier gas. The injections were made in the splitless mode with an autosampler (cf. above). The ITS40 was operated with an EI energy of 70 eV and a ion source temperature of 220 °C. Mass spectra were collected at a speed of 1 scan/s, scanning for ions in the range of 150-500 amu.

Synthesis of MeO-CBs

The nomenclature of the MeO-CBs discussed in the present study is slightly modified in comparison to the IUPAC guidelines, to facilitate for the reader to compare structures. The structures of the MeO-CBs and a number for abbreviation are shown in Figure 1 (appearing after Table 4c). The chemical names of the prepared MeO-CBs, their numbers, their relative retention time to the internal standard I.S. 2,3,4,5,3',4',5'-heptachlorobiphenyl, the synthetic method used and isomeric MeO-CBs formed in the same reaction are given in Table 1. The compounds are presented in the same order in Tables 2-4. Some of the MeO-CBs described have been synthesized and at least partly characterized as reported elsewhere (7,8,15,16,18) and in these cases the references are given in Table 1. Compound numbers and major fragments of all MeO-CBs analyzed are shown in Tables 2-4. All MeO-CBs were synthesized either according to the Cadogan diaryl coupling (17,19) or the Ullmann diaryl coupling method (20-21).

Method I - the Cadogan diaryl coupling reaction (17,19): A typical reaction is described: 2,3,4,5-Tetra-chloroaniline (1.0 g, 4.33 mmol) was dissolved in 2,3,6-trichloroanisole (5 g, 23.6 mmol) at 60°C. The tetra-chloroaniline was diazotized by addition of an excess of 3-methyl butyl nitrite (2.0 ml, 15 mmol). The temperature was slowly increased to 120°C (30 min) and kept at this temperature for 1.5 h. The unreacted trichloroanisole was removed by vacuum distillation. After removal of polar byproducts by liquid chromatography on a silica gel (0.063-0.200 mm) column (2 cm i.d. x 30 cm) with hexane: DCM (9:1) as the mobile phase, the crude MeO-CB products were isolated. The two isomers obtained in this particular reaction, 4-MeO-2,3,5,2',3',4',5'-heptaCB and 3-MeO-2,4,5,2',3',4',5'-heptaCB, were finally separated on a second silica gel (<0.063 mm) column (4 cm x 75 cm) with hexane: DCM (9:1) as the mobile phase. The individual MeO-CB isomers isolated were recrystallized; suitable solvents for recrystallization were hexane or ethanol. The total yield of MeO-CB isomers in this type of Cadogan diaryl coupling reaction was generally 20-30% but the ratio between the different MeO-CB isomers formed in the reactions varied.

Method II - Ullmann diaryl coupling reaction (20-21): Polychloroiodobenzenes were commercially obtained or prepared as described elsewhere (21). A typical reaction is described: 2,3,4-Trichloroiodobenzene (3.0 g, 9.8 mmol) and 4-iodoanisole (3 g, 13 mmol) were mixed with copper bronze (6.0 g) in a glass tube. The Ullmann diaryl coupling reaction was carried out by heating the tube in a salt bath at 200-230°C for 1.5 h. The products formed, 2,3,4,2',3',4'-hexaCB, 4-MeO-2',3',4'-triCB and 4,4'-$(MeO)_2$-biphenyl, were purified from polar byproducts by liquid chromatography on silica gel (0.063-0.200 mm) packed in a column (3 x 40 cm). The three products were eluted with i: hexane, ii: hexane:DCM (5:1) and iii: DCM, respectively. The 4-MeO-2',3',4'-triCB was isolated in 34% yield (960 mg, 3.35 mmol).

Chlorination: The MeO-CBs synthesized via the Ullmann reaction were 4-MeO-3',4'-dichlorobiphenyl, 4-MeO-2',3',4'-trichlorobiphenyl, 4-MeO-2',4',5'-trichlorobiphenyl, 4-MeO-3',4',5'-trichlorobiphenyl and 4-MeO-2',3',4',5'-tetrachlorobiphenyl. Each of these products were chlorinated as exemplified by the following reaction: 4-MeO-2',3',4'-triCB (0.96 g, 3.35 mmol) was dissolved in acetic acid and conc. hydrochloric acid (5:1, 30 ml). The solution was heated to 80°C before aqueous sodium chlorate (5 ml, 3.4 M) was added dropwise while stirring. The chlorine gas generated was let to react with the 4-MeO-2',3',4'-triCB for 0.5 h after which potassium disulphite was added to stop the reaction. The solution was neutralized with sodium hydroxide (2 M) and the products were extracted with DCM (5 x 10 ml). The extract was filtered through glass wool, the solvent evaporated and the residue recrystallized from hexane. The yield of the desired 4-MeO-3,5,2',3',4'-pentaCB was 630 mg (52 %).

Demethylation: Some isomeric MeO-CBs, formed in the same reaction, were demethylated by boron tribromide prior to separation of the isomers (22). Briefly; the MeO-CBs were dissolved in DCM (1.5 mg/ml) and a boron tribromide solution (1 M in DCM, 0.5 ml) was added. The reaction mixture was refluxed for 2 h. and allowed to cool to room temperature. The OH-CBs were extracted with DCM after the addition of water (2 ml). To obtain the MeO-CBs, the compounds were methylated with diazomethane (23).

Standard solutions and mixtures for GC/MS analyses.

The retention times of all individual MeO-CBs were determined from the MS(EI) analysis on the TSQ instrument. All pure MeO-CBs were prepared in 1 mg/ml chloroform standard solutions. Twelve different mixtures of MeO-CBs were prepared. The mixtures were made so that individual MeO-CBs were well separated from other MeO-CBs when analyzed on the DB-5 capillary column. The mixtures of MeO-CBs for MS analysis were diluted to a concentration of 5 μg/ml of each MeO-CB congener.

The MS responses of some tetra- to heptachlorinated MeO-CBs (**4-6**, **5-10**, **6-6**, **7-1**, **7-7**, **7-11**) were determined by comparing the signal to noise ratio (S/N) of the compounds at a concentration of 0.5 μg/ml. No attempts were made to optimize the sensitivity - the comparison was made to get an approximate idea of the relative responses of MeO-CBs, with a varying chlorination degree, using the different ionization techniques (EI on ITS40, EI and NICI on the quadrupole instrument).

RESULTS

Fortysix MeO-CBs were prepared (Figure 1, Table 1a-c) and the majority of these compounds were isolated in a pure form after several chromatographic separation steps and/or recrystallizations. The pair **5-5/5-11** was demethylated prior to separation of the isomers, methylated and isolated in a pure form.

Mass spectra were recorded for all compounds using EI and NICI mass spectrometry. The relative abundance of the most important ions are shown in Tables 2-4. The fragmentation patterns of *ortho-*, *meta-* and *para*-substituted MeO-CBs as obtained by MS(EI) from a quadrupole mass spectrometer (TSQ 700) are shown in Tables 2a-c. Likewise, the relative abundance of the fragments of the same MeO-CBs in EI mode from the ion trap mass spectrometer are shown in Tables 3a-c. Finally, NICI mass spectral data from a quadrupole instrument for all MeO-CBs are shown in Table 4a-c.

The relative response of some tetra- to heptachlorinated MeO-CBs was determined by comparing S/N-ratios in all three mass spectrometric measurements. Using NICI, the S/N-ratio for the MeO-CB with 4 chlorine atoms was 13 but the ratio increased with the number of chlorine atoms and was 120 for MeO-pentaCB, 250 for MeO-hexaCB and 500 for MeO-heptaCB. The trichlorinated MeO-CB (**3-2**) was not included in this part of the study but it should be mentioned that to obtain a fair mass spectra of **3-2**, a higher concentration (20 μg/ml, cf 5 μg/ml for the other compounds) was needed. The variation in S/N-ratio between homologues were much smaller when using EI-mode on the TSQ, varying from 4 to 13 without any correlation to the number of chlorine atoms of the MeO-CBs. The ion-trap instrument (ITS40) gave for the same MeO-CBs under the same conditions S/N-ratios of 16-35 and the detection limit was thus somewhat lower than that of the quadrupole instrument (TSQ) operated in the EI-mode.

Table 1a. Chemical name for the 2-MeO-CBs (references to previously characterized compounds), their number (first number gives the number of chlorine atoms), relative retention time compared to IS, method for synthesis with isomeric MeO-CBs formed, are given.

Name	No.	Rel. ret. time	Method	Isomers
2-Methoxy-3,4,2',4'-tetraCB	**4-1**	0.577	I	**4-3, 4-5**
2-Methoxy-3,4,3',4'-tetraCB (7)	**4-2**	0.654	I	**4-2, 4-6**
2-Methoxy-3,4,2',3',4'-pentaCB (8,15)	**5-1**	0.742	I	**5-8, 5-12**
2-Methoxy-3,4,2',4',5'-pentaCB	**5-2**	0.690	I	**5-9, 5-14**
2-Methoxy-3,4,5,3',4'-pentaCB	**5-3**	0.797	I	**5-6**
2-Methoxy-3,4,3',4',5'-pentaCB	**5-4**	0,787	I	**5-10, 5-17**
2-Methoxy-3,4,2',3',4',5'-hexaCB	**6-1**	0.874	I	**6-4, 6-7**

Table 1b. Chemical name for the 3-MeO-CBs (references to previously characterized compounds), their number (first number gives the number of chlorine atoms), relative retention time compared to IS, method for synthesis with isomeric MeO-CBs formed, are given.

Name	No.	Rel. ret. time	Method	Isomers
3-Methoxy-6,2',5'-triCB	**3-1**	0.491	I	-
3-Methoxy-4,5,2',4'-tetraCB	**4-3**	0.686	I	**4-1, 4-5**
3-Methoxy-4,5,3',4'-tetraCB (7)	**4-4**	0.795	I	**4-2, 4-6**
3-Methoxy-2,4,5,3',4'-pentaCB	**5-5**	0.794	I	**5-11**
3-Methoxy-4,5,6,3',4'-pentaCB (8)	**5-6**	0.902	I	**5-3**
3-Methoxy-2,4,2',3',4'-pentaCB (8)	**5-7**	0.737	I	**5-13**
3-Methoxy-4,5,2',3',4'-pentaCB (8,15)	**5-8**	0.897	I	**5-1, 5-12**
3-Methoxy-4,5,2',4',5'-pentaCB	**5-9**	0.829	I	**5-2, 5-14**
3-Methoxy-4,5,3',4',5'-pentaCB	**5-10**	0.972	I	**5-4, 5-17**
3-Methoxy-2,4,5,2',3',4'-hexaCB	**6-2#**	0.892	I	**6-5**
3-Methoxy-2,4,5,2',4',5'-hexaCB	**6-3**	0.821	I	**6-6**
3-Methoxy-4,5,2',3',4',5'-hexaCB	**6-4**	1.077	I	**6-1, 6-7**
3-Methoxy-2,4,5,2',3',4',5'-heptaCB	**7-1**	1.056	I	**7-9**
3-Methoxy-2,4,6,2',3',4',5'-heptaCB	**7-2**	0.809	I	-
3-Methoxy-2,4,5,2',3',4',6'-heptaCB	**7-3**	0.927	I	**7-11**
3-Methoxy-2,4,6,2',3',4',6'-heptaCB	**7-4**	0.824	I	-
3-Methoxy-2,4,5,2',3',5',6'-heptaCB	**7-5**	0.914	I	**7-10**
3-Methoxy-2,4,6,2',3',5',6'-heptaCB	**7-6**	0.927	I	-

#Isomers present in a mixture.

Table 1c. Chemical name for the 4-MeO-CBs (references to previously characterized compounds), their number (first number gives the number of chlorine atoms), relative retention time compared to IS, method for synthesis with isomeric MeO-CBs formed, are given.

Name	No.	Rel. ret. time	Method	Isomers
4-Methoxy-3,3',4'-triCB (7)	**3-2**	0.659	II	-
4-Methoxy-2,3,2',4'-tetraCB	**4-5**	0.663	I	**4-1, 4-3**
4-Methoxy-2,3,3',4'-tetraCB	**4-6**	0.761	I	**4-2, 4-4**
4-Methoxy-3,5,3',4'-tetraCB (7)	**4-7**	0.714	II	-
4-Methoxy-2,3,5,3',4'-pentaCB (8)	**5-11**	0.795	I	**5-5**
4-Methoxy-2,3,2',3',4'-pentaCB (8)	**5-12**	0.863	I	**5-1, 5-8**
4-Methoxy-3,5,2',3',4'-pentaCB (8)	**5-13**	0.799	II	-
4-Methoxy-2,3,2',4',5'-pentaCB	**5-14**	0.802	I	**5-2, 5-9**
4-Methoxy-3,5,2',4',5'-pentaCB	**5-15**	0.741	II	-
4-Methoxy-2,5,2',4',5'-pentaCB (15,24)	**5-16**	0.736	I	-
4-Methoxy-2,3,3',4',5'-pentaCB	**5-17**	0.935	I	**5-4, 5-10**
4-Methoxy-3,5,3',4',5'-pentaCB	**5-18**	0.869	II	-
4-Methoxy-2,3,5,2',3',4'-hexaCB	**6-5#**	0.895	I	**6-2**
4-Methoxy-2,3,5,2',4',5'-hexaCB	**6-6**	0.825	I	**6-3**
4-Methoxy-2,3,2',3',4',5'-hexaCB	**6-7**	1.041	I	**6-1, 6-4**
4-Methoxy-3,5,2',3',4',5'-hexaCB	**6-8**	0.955	II	-
4-Methoxy-2,3,5,6,3',4',5'-heptaCB	**7-7**	1.097	I	-
4-Methoxy-2,3,5,6,2',4',5'-heptaCB	**7-8**	0.960	I	-
4-Methoxy-2,3,5,2',3',4',5'-heptaCB	**7-9**	1.064	I	**7-1**
4-Methoxy-2,3,5,2',3',5',6'-heptaCB	**7-10**	0.915	I	**7-5**
4-Methoxy-2,3,5,2',3',4',6'-heptaCB	**7-11**	0.929	I	**7-3**

Isomers present in a mixture.

Table 2a. Major fragments and relative abundance of *ortho*-substituted MeO-CBs as determined by electron ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-35	M-50	M-70	M-113
4-1	100	0	<5	95	20	40
4-2	95	<5	10	100	15	45
5-1	100	0	5	90	20	25
5-2	100	0	5	95	20	20
5-3	80	5	<5	100	0	25
5-4	80	<5	15	100	15	25
6-1	100	0	10	95	25	30

Table 2b. Major fragments and relative abundance of *meta*-substituted MeO-CBs as determined by electron ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-43	M-50	M-113	Other fragment#
3-1	100	0	20	10	35	M-79 (10)
4-3	100	0	25	<5	35	M-79 (5)
4-4	100	0	25	0	35	
5-5	100	<5	40	5	15	M-79 (5)
5-6	100	0	30	5	15	
5-7	100	15	35	5	25	
5-8	100	0	0	95	20	M-70 (20)
5-9	100	0	30	5	20	
5-10	100	0	25	0	20	M-79 (5)
6-2	100	5	35	10	20	M-79 (5)
6-3	100	5	40	10	25	M-79 (5)
6-4	100	0	25	<5	20	
7-1	100	<5	25	10	20	M-70 (5)
7-2	100	25	15	5	25	
7-3	100	5	25	5	25	M-70 (5)
7-4	100	40	20	5	25	
7-5	100	5	25	5	25	
7-6	100	35	15	10	25	

Abundance given in parenthesis.

Table 2c. Major fragments and relative abundance of *para*-substituted MeO-CBs as determined by electron ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-35	M-43	M-50	M-113	Other fragment#
3-2	100	50	<5	25	0	35	M-79 (15)
4-5	100	20	<5	20	10	35	M-79 (10)
4-6	100	30	0	25	<5	40	M-79 (5)
4-7	100	95	0	30	0	50	M-79 (5)
5-11	100	10	0	35	5	20	M-79 (5)
5-12	100	20	0	30	0	25	
5-13	100	60	0	25	0	25	
5-14	100	20	0	25	<5	20	
5-15	100	60	0	30	0	25	
5-16	100	15	<5	30	0	25	
5-17	100	30	0	25	0	25	
5-18	100	90	0	30	0	25	
6-5	100	40	5	40	<5	30	
6-6	100	25	0	20	0	20	M-70 (5)
6-7	100	15	0	25	0	25	M-79 (5)
6-8	100	50	0	25	0	25	M-79 (5)
7-7	100	15	0	25	0	20	M-70 (5)
7-8	100	10	0	30	0	25	
7-9	100	25	0	20	0	30	
7-10	100	20	0	25	0	30	
7-11	100	25	0	30	0	35	

Abundance given in parenthesis.

Table 3a. Major fragments and relative abundance of *ortho*-substituted MeO-CBs as determined by EI mass spectrometry (ion trap instrument).

MeO-CB	M	M-15	M-35	M-50	M -70	M-113
4-1	100	5	16	76	27	25
4-2	100	5	21	61	22	25
5-1	100	0	15	62	24	20
5-2	100	0	18	63	25	20
5-3	100	7	11	61	11	19
5-4	100	0	37	53	25	20
6-1	100	0	27	57	31	23

Table 3b. Major fragments and relative abundance of *meta*-substituted MeO-CBs as determined by EI mass spectrometry (ion trap instrument).

MeO-CB	M	M-15	M-43	M-50	M-79	M-113	Other fragment#
3-1	100	5	24	13	10	30	M-35 (14)
4-3	100	0	30	5	10	25	M-30 (10)
4-4	100	0	29	0	10	30	M-30 (10)
5-5	100	5	47	5	10	22	
5-6	100	0	39	0	10	20	
5-7	100	20	40	10	10	30	
5-8	100	0	27	0	5	25	M-30 (5)
5-9	100	0	28	0	5	19	M-30 (6)
5-10	100	0	28	0	5	20	M-30 (6)
6-2	100	6	47	5	5	30	
6-3	100	6	45	0	10	37	
6-4	100	0	25	0	5	23	M-30 (5)
7-1	100	7	47	0	5	33	
7-2	100	67	45	5	5	44	
7-3	100	15	47	5	5	33	
7-4	100	71	45	5	5	46	
7-5	100	14	43	0	0	34	
7-6	100	57	38	10	5	41	

Table 3c. Major fragments and relative abundance of *para*-substituted MeO-CBs as determined by EI mass spectrometry (ion trap instrument).

MeO-CB	M	M-15	M-43	M-79	M-113	Other fragment#
3-2	100	76	47	15	45	
4-5	100	30	35	10	35	
4-6	100	47	42	10	40	
4-7	72	100	40	10	40	
5-11	100	80	54	0	39	M-30 (5)
5-12	100	22	33	10	26	
5-13	100	90	44	10	36	
5-14	100	21	33	10	25	
5-15	100	90	46	10	33	
5-16	100	14	37	10	25	
5-17	100	36	36	10	28	
5-18	80	100	42	10	32	
6-5	100	49	46	5	38	
6-6	100	45	45	10	37	
6-7	100	16	32	5	30	M-70 (5)
6-8	100	74	44	10	40	M-70 (85)
7-7	100	33	54	10	37	M-70 (5)
7-8	100	15	60	5	35	M-70 (5)
7-9	100	44	47	10	42	M-70 (85)
7-10	100	38	51	10	40	M-70 (85)
7-11	100	32	46	7	42	

Table 4a. Major fragments and relative abundance of *ortho*-substituted MeO-CBs as determined by negative ion chemical ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-36	M-70
4-1	0	0	100	5
4-2	5	10	100	5
5-1	100	0	25	0
5-2	0	0	100	15
5-3	60	15	100	35
5-4	100	50	40	30
6-1	0	0	100	30

Table 4b. Major fragments and relative abundance of *meta*-substituted MeO-CBs as determined by negative ion chemical ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-34	M-36	M-68	M-68
3-1	100	0	0	0	0	0
4-3	100	0	15	0	0	0
4-4	100		30	0	0	0
5-5	5	<5	0	100	40	0
5-6	100	0	25	0	10	0
5-7	20	0	0	100	70	0
5-8	100	0	25	0	10	0
5-9	100	0	0	0	20	0
5-10	100	0	30	0	25	0
6-2	5	0	100	0	0	15
6-3	70	0	0	100	0	10
6-4	100	0	50	0	20	0
7-1	100	0	0	95	0	35
7-2	100	0	0	20	0	<5
7-3	100	0	15	0	0	5
7-4	100	0	0	25	0	5
7-5	100	0	20	0	5	0
7-6	85	0	0	100	0	20

Table 4c Major fragments and relative abundance of *para*-substituted MeO-CBs as determined by negative ion chemical ionization mass spectrometry (quadrupole instrument).

MeO-CB	M	M-15	M-34	M-36	M-50	M-68	M-70	Other fragment#
3-2*	40	100	0	40	0	0	0	M-49 (20)
4-5	15	0	100	0	0	0	5	
4-6	100	0	80	0	0	0	5	
4-7	80	100	75	0	0	0	0	
5-11	50	40	0	60	<5	100	0	
5-12	100	0	0	20	0	80	0	
5-13	100	10	0	10	0	35	0	M-49 (10)
5-14	30	0	0	15	0	100	0	
5-15	100	30	0	10	0	25	0	M-49 (5)
5-16	5	5	0	70	0	100	0	
5-17	100	5	25	0	0	80	0	
5-18	90	100	10	0	0	25	0	M-49 (10)
6-5	75	30	0	60	<5	0	10	
6-6	100	25	0	0	0	0	5	M-43 (20)
6-7	100	15	0	0	0	5	5	M-43 (25)
6-8	100	15	20	0	0	5	0	M-49 (10)
7-7	50	100	10	0	0	0	0	M-49 (10)
7-8	100	85	0	<5	<5	0	0	
7-9	100	60	30	0	0	0	0	M-49 (10)
7-10	100	15	15	0	0	0	0	
7-11	100	25	15	0	0	0	0	M-49 (5)

#Abundance is given in parenthesis.

*Analyzed at 20μg/ml concentration.

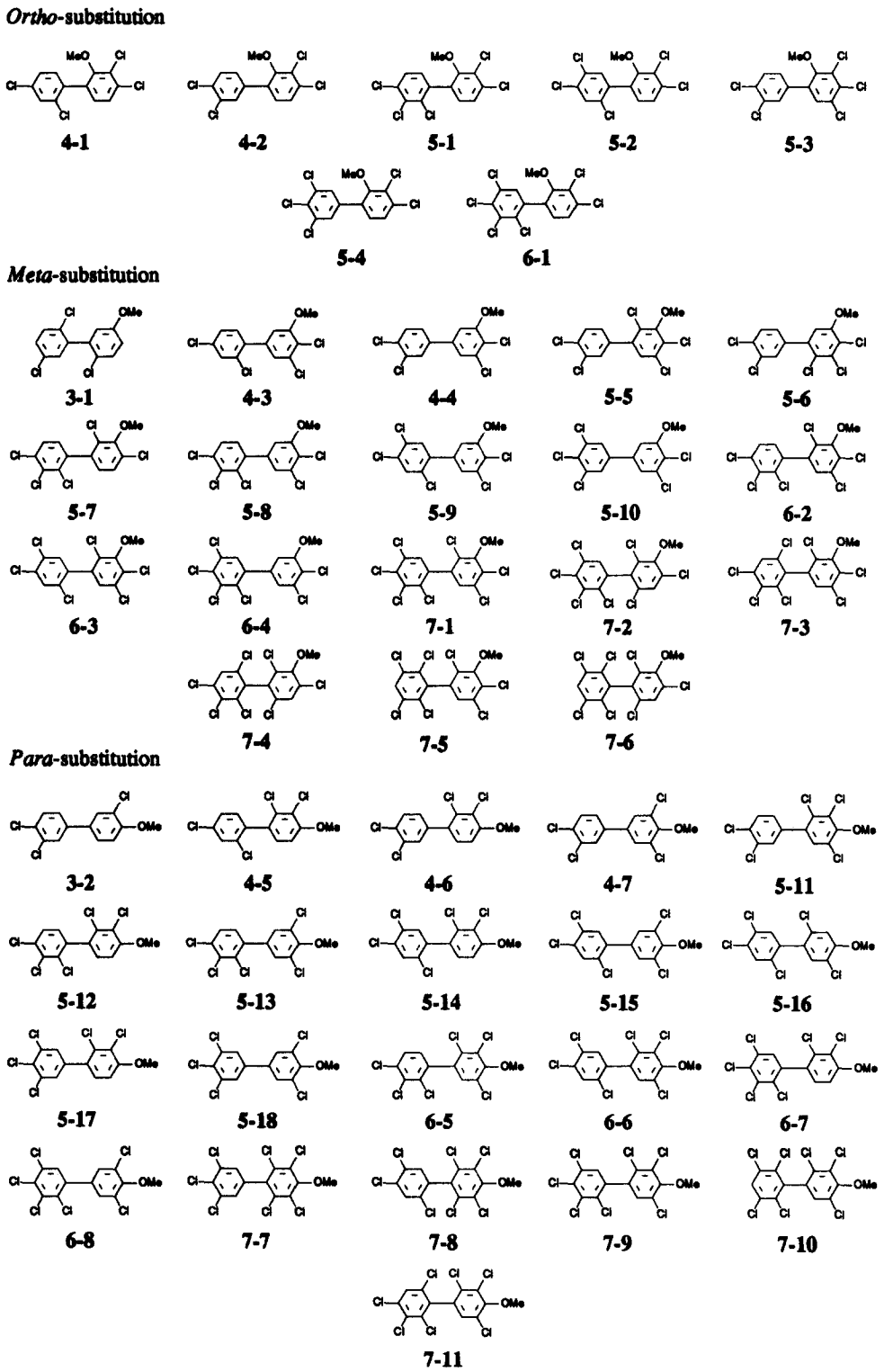

Figure 1. Structures of the MeO-CBs synthesized are shown with the abbreviation number used in this article.

DISCUSSION

The majority of the MeO-CBs described in the present paper were prepared via the Cadogan reaction. This reaction, where a chloroaniline is coupled with a chloroanisole, gives 2 or 3 isomeric MeO-CB products. These can easily be isolated in a pure form if the number of *ortho*-substituents differs but when this is not the case, it may be difficult to separate the MeO-CB isomers. Thus it was not possible to separate 3-MeO-2,4,5,3',4'-pentaCB (**5-5**) from 4-MeO-2,3,5,3',4'-pentaCB (**5-11**). However, after demethylation of the mixture of **5-5** and **5-11**, the corresponding OH-CBs were obtained and found possible to separate. After methylation with diazomethane, the pure **5-5** and **5-11**, respectively, were obtained. It is also possible to synthesize the OH-CBs directly by the Cadogan diaryl coupling by using a chlorinated phenol instead of anisole, as described by e.g. Mannila *et al* (24). The reported yield is however much lower (1-5%) than for the corresponding synthesis with anisole (20-30%).

To avoid mixtures of MeO-CB isomers that could be difficult to separate, some of the MeO-CBs (**3-2**, **4-8**, **5-6**, **5-13**, **5-18** and **6-8**) were synthesized via the Ullmann reaction. A disadvantage with this reaction is the lack of commercially available iodochlorobenzenes and iodochloroanisoles. It was found convenient to chlorinate the positions *ortho* to the MeO-substituent in the biphenyl, thus taking advantage of the *ortho*-directing properties of the MeO-group.

In MS(EI), the *ortho*-substituted MeO-CBs studied in the present work, show abundant ions at M^+ and $[M\text{-}CH_3Cl]^+$, the latter corresponding to a dibenzofuran type ion, similarly as previously reported (15,16). The MS(EI) fragmentations of *meta*- and *para*-substituted MeO-CBs confirm the previous reports (15,16). Thus, *meta*-substituted MeO-CBs show abundant fragments at $[M\text{-}43]^+$ corresponding to $[M\text{-}CH_3CO]^+$ whereas *para*-substituted MeO-CBs show abundant fragments at $[M\text{-}15]^+$ corresponding to $[M\text{-}CH_3]^+$ in addition to the $[M\text{-}43]^+$ fragment. In the present study, three *meta*-MeO-heptaCBs (**7-2**, **7-4** and **7-6**), all with a 3-MeO-2,4,6-trichlorinated phenyl ring, show abundant ions corresponding to the loss of a methyl group $[M\text{-}15]^+$, (cf. Table 2b and 3b). This observation has also been reported by Ariyoshi *et al* (25) for 3-MeO-2,4,6,2',4',6'-hexaCB - a MeO-CB with a corresponding substitution pattern. The loss of a methyl group is also observed, although much less abundant, for a few other *meta*-substituted MeO-CBs (**5-5**, **6-2**, **5-7**, **6-3**, **7-1**, **7-3** and **7-5**) with the MeO-group in 3-position in either of a 2,4-dichloro- or a 2,4,5-trichlorophenyl-ring. It is thus adviceable to be careful when assigning structures of higher chlorinated MeO-CBs only by mass spectra.

Para-substituted MeO-CBs with a 4-MeO-3,5-dichloro-substituted ring and no or only one chlorine atom in an *ortho*-position show a strongly abundant $[M\text{-}CH_3]^+$ fragment, (cf. Table 2c and 3c; compounds **4-7**, **5-13**, **5-15**, **5-18** and **6-8**), in comparison to *para*-substituted MeO-CBs with more chlorine atoms in the *ortho*-positions. Presumably, the ease to achieve a coplanar configuration, thus no or only one chlorine atom in an *ortho*-position, increases the probability to form a quinoid structure with a conjugated systems across the biphenyl bridge, and thereby the abundancy of the $[M\text{-}15]^+$ ion (15,16). In contrast, two or more chlorine atoms in *ortho*-positions gives

a lower probability to form the $[M-15]^+$ ion. A loss of $[M-30]^+$, probably corresponding to $[M-CH_2O]^+$, was found only for *meta*-substituted MeO-CBs with a 3-MeO-4,5-dichloro-substituted phenyl ring, *cf.* Table 2b and 3b, **4-3**, **4-4**, **5-8**, **5-9**, **5-10** and **6-4**.

The quadrupole mass spectrometer, operated in the EI-mode, and the ion trap mass spectrometer gave identical fragments of the MeO-CBs. However, slightly more abundant fragments, compared to the molecular ion, were generally obtained for the MeO-CBs analyzed by the ion trap mass spectrometer. An exception to this was the $[M-50]^+$ ions, corresponding to the loss of $(CH_3 + Cl)$, that were less abundant in mass spectra obtained from the ion trap instrument. Observed differences did not interfere with the interpretation of the mass spectra.

Virtually no structural information on the substitution pattern of the MeO-CBs is obtained by NICI mass spectrometry, except that all *ortho*-substituted MeO-CBs analyzed in this study gave a fragment at $[M-36]^-$, corresponding to $[M-HCl]^-$. The mass spectra of all *meta*- and *para*-substituted MeO-CBs are dominated by the molecular ion $[M]^-$. In contrast, some *ortho*-substituted MeO-CBs did not show any molecular ion.

In conclusion, MS(EI) is a preferable technique to MS(NICI) if structural information is required. The ion trap mass spectrometer and the quadrupole instrument both give similar mass spectra with characteristic fragmentation depending on position of the MeO-group. MS(NICI) always show a lower detection limit for the MeO-CBs with more than 4 chlorine atoms. The detection limit is improved (decreased) as the chlorination degree increases. The MS(NICI) gave approximately 50 times higher response for MeO-heptaCBs than the MS(EI). The ITS40 has a somewhat , approximately 4 times, lower detection-limit than the quadrupole EI.

The compounds synthesized and described in this study were primarily prepared to be used as standards for the identification of OH-CBs in blood from mammals (14). Several of those standards have been shown to correspond to metabolites of individual CBs (8,14,18). These specific MeO-CBs and their corresponding OH-CBs are thus of main interest for further analytical work on blood from individual wildlife species and also for toxicological evaluations.

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