Identification of Hydroxyhalobiphenyls as their Methyl Ethers by Gas Chromatography Mass Spectrometry

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The mass spectra and gas chromatographic properties of 17 synthetic fluoro-, chloro- and bromomethoxybiphenyls and 12 dichlorodimethoxybiphenyls have been examined. From this representative series it appears that the position of the methoxy group (*ortho, meta* and *para* to the biphenyl bond) in all monomethoxy compounds examined, and the positions of the two methoxy groups in most of the dimethoxy compounds, can be assigned unambiguously by their difference in fragmentation pattern. The value of this method was shown by metabolism experiments in which 4,4'-difluoro- and 4,4'-dibromobiphenyl were fed to rats and 4,4'-dichlorobiphenyl was administered to plants. All hydroxylated metabolites found were identified by gas chromatography mass spectrometry. Relationships between structure and gas chromatographic retention time of these compounds are discussed.

INTRODUCTION

Hydroxylated species are the main metabolic products of bromobiphenyls in the rabbit,[1] and of chlorobiphenyls in higher animals, plants and microorganisms. (For a review see Ref. 2.) Although the presence of the hydroxy group in a halobiphenyl can be demonstrated easily by m.s. on small samples, this method gives no information on the position of this group in the molecule, since most isomeric hydroxy halobiphenyls give similar mass spectra.[3] Therefore, for proper structure elucidation it was necessary either to obtain relatively large samples for n.m.r. analysis or to synthesize the appropriate standards.

Phenolic metabolites of halobiphenyls are often sensitive to air and show poor chromatographic properties. Therefore, derivatives such as methyl ethers,[4,5] trimethylsilyl ethers,[6,7] acetates[3,8] and dansyl esters[9,10] have been used during the purification procedure and analysis. In examining the mass spectra of a large series of methyl ethers of hydroxychlorobiphenyls, Sundström and co-workers[5,11] have recently shown that 2-, 3- and 4-methoxy chlorobiphenyls give characteristic fragmentations which allows unambiguous identification of these isomeric compounds. This is in contrast to the parent hydroxy compounds of which all isomers give identical mass spectra.[12]

We have now examined the mass spectra of the methyl ethers of a series of fluorobiphenylols, bromobiphenylols and chlorobiphenyldiols as well as of more chlorobiphenylols. Chlorobiphenyldiols have recently been shown to be important metabolites of chlorobiphenyls.[2] All other compounds are also of interest in metabolism studies.

EXPERIMENTAL

Gas chromatography

G.c. retention times were obtained using a Hewlett-Packard 5830A gas chromatograph connected with a Hewlett-Packard 18850A g.c. terminal. The column was a 0.15 × 300 cm glass column, packed with 3% OV 225 on Gaschrom Q 80/100 mesh. Temperature was programmed from 200 °C (2 min) to 240 °C at a rate of 8 °C min^{-1}. The injection port temperature was 270 °C, the flame ionization detector temperature was 300 °C and the helium flow rate was 22 ml min^{-1}.

Gas chromatography mass spectrometry

G.c.m.s. was performed on a Hewlett-Packard 5982A system. The g.c. conditions were similar to those mentioned above. Temperatures were: injector 250 °C; jet separator 350 °C; transfer line 280 °C; ion source 200 °C. Electron energy was 70 eV. Mass spectra were scanned from 50 to 400 a.m.u. with a speed of 80 a.m.u. s^{-1}.

Thin-layer chromatography

For preparative t.l.c. silica gel 60 F_{254} plates (Merck), 20 × 20 cm, layer thickness 0.25 mm were used. Solvents were hexane (repeated development; system A) and hexane + ethyl acetate 9 : 1 (system B).

Synthesis

Most compounds were synthesized by reacting an aniline with amyl nitrite in the presence of excess aroma-

Table 1. Synthesis procedure for methoxyhalobiphenyls

Methoxyhalobiphenyl prepared	Starting materials: Aniline	Starting materials: Aromatic reactant	T.l.c. purification system
1	2-Methoxyaniline	Benzene	B
6	2-Methoxyaniline	1,4-Dichlorobenzene	B
9, 10, 11	2,5-Dibromoaniline	1-Methoxybenzene	A and B
12, 13, 14	2,5-Difluoroaniline	1-Methoxybenzene	A and B
15, 16, 17	4-Fluoroaniline	1-Methoxybenzene	A and B
18, 19	2,5-Dichloroaniline	1,2-Dimethoxybenzene	A and B
20, 21, 22	2,5-Dichloroaniline	1,3-Dimethoxybenzene	A and B
26, 27	4-Chloro-2-methoxyaniline	1-Chloro-4-methoxybenzene	A and B
28, 29	2-Chloro-4-methoxyaniline	1-Chloro-4-methoxybenzene	A and B

tic reactant.[13] In all cases a molar ratio 1:2:10 for aniline, amyl nitrite and radical scavenger was used. After addition of the amyl nitrite the mixture was heated slowly to 100 °C and kept at that temperature for 2 h. Starting materials for these syntheses are given in Table 1. Structures of all methoxyhalobiphenyls prepared and used in this study are shown in Tables 2 and 3.

Compounds **2, 4, 5** and **24** are described in Ref. 3, and compounds **3** and **23** in Ref. 14. Compounds **7** and **8** were synthesized from the original rabbit metabolites (i.e. the corresponding hydroxy compounds, the structure of which was established by n.m.r. spectroscopy[1]) by methylation with diazomethane.

Metabolism experiments with rats

4,4′-Difluorobiphenyl and 4,4′-dibromobiphenyl were dissolved in *oleum arachidis* (peanut oil) and administered orally as a single dose to rats. Dosages were 57 mg kg^{-1} and 93 mg kg^{-1} (i.e. 6×10^{-5} M) respectively. Male Wistar rats (TNO, Holland) weighting an average of 200 g were housed in metabolic cages for ten days and supplied with water and food *ad libitum*. Faeces and urine were collected in 4 N sulphuric acid to prevent any microbial metabolism after excretion.

Metabolism experiments with plants

4,4′-Dichlorobiphenyl (111.6 mg) was dissolved in 10 ml acetone and mixed with 990 ml water to obtain 5×10^{-4} M suspension. In an aquarium, the surface (29×21 cm) of this suspension was covered with duck weed (*Lemnaceus minor* L). After 10 days the duck weed was removed and the water investigated for phenolic metabolites.

Isolation of metabolites and purification of samples

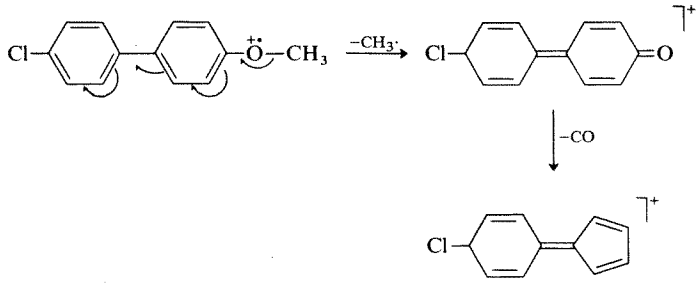
The acidic urine solution (pH *c.* 1) was heated at 80 °C for 2 h and extracted three times with ether + hexane 1:1. The two layers usually needed centrifugation for rapid separation. After evaporation of the solvent the residue was dissolved in acetone and methylated with an excess of methyl iodide and potassium carbonate at reflux temperature overnight. After centrifugation the acetone was evaporated and the residue taken up in hexane (100 ml) and extracted three times with 20 ml concentrated sulphuric acid. After centrifugation the clear hexane solution was reduced to 0.5 ml and investigated by g.c.m.s. The water from the plant experiment was extracted like urine. The organic solvent layer was reduced to *c.* 100 ml and extracted three times with an equal volume of 2 N potassium hydroxyde. The aqueous solution was separated from the organic solvent (containing the starting material, 4,4′-dichlorobiphenyl) and after addition of 100 ml hexane the pH was brought to 1.0 with sulphuric acid. After extraction the hexane solution was reduced to 0.5 ml and investigated by g.c.m.s. The sample was then methylated with methyl iodide (see above) and again investigated by g.c.m.s.

RESULTS AND DISCUSSION

Mass spectra

The aim of this study was to investigate the diagnostic value of the mass fragmentation pattern of methylated hydroxyhalobiphenyls. Mass spectral data for all compounds are given in Tables 2 and 3.

Chloromethoxybiphenyls. The fragmentation patterns of the compounds investigated were consistent with earlier results.[5,15] The position of the methoxy group relative to the biphenyl bond (*ortho*, *meta* or *para*) can be assigned unambiguously from the mass spectrum. 2-Methoxy groups give intense fragments for $[M-CH_3Cl]^{+\cdot}$ and $[M-Cl]^+$, 3-methoxy groups give one major fragment at $[M-CH_3CO]^+$ and 4-methoxy groups give fragments corresponding to both $[M-CH_3]^+$ and $[M-CH_3CO]^+$. Some of these fragments can be explained by plausible mechanisms: a *p*-methoxybiphenyl can lose $CH_3\bullet$ to give rise to a stable keto structure in which charge delocalization is possible (Scheme 1).



Scheme 1. Initial mass fragmentation of 4-chloro-4′-methoxybiphenyl.

Table 2. Mass spectral data and g.c. retention times of monomethoxyhalobiphenyls

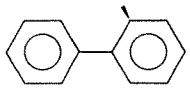
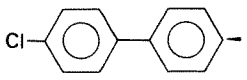
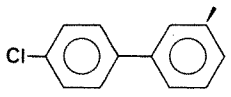
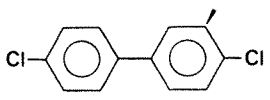
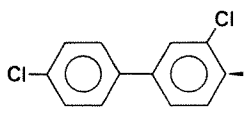
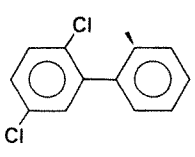
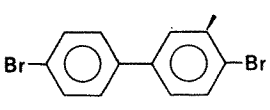
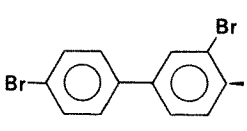
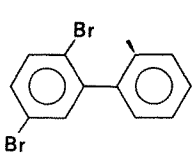
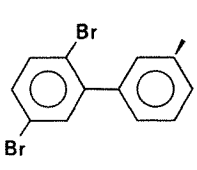
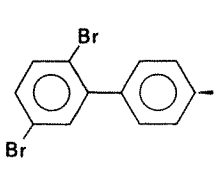
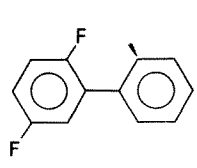
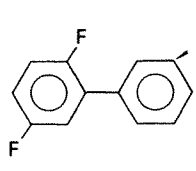
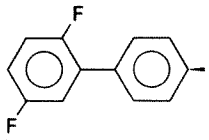
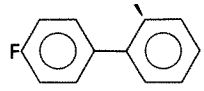
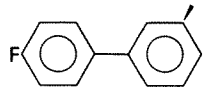
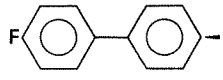
No.	Structure[a]	Molecular weight	Mass spectral data[b] (major fragments, *m/e*) $[M]^{+\cdot}$	$[M-CH_3]^+$	$[M-CH_3X]^{+\cdot d}$	$[M-X]^{+d}$	$[M-CH_3CO]^+$	Retention times[c]
1		184	100	52	16	25	45	1.2
2		218	100	55	—	—	38	3.6
3		218	100	—	—	—	32	3.2
4		252	100	—	—	—	35	5.7
5		252	100	76	—	—	35	6.1
6		252	73	—	100	24	—	2.7
7		340	100	—	—	—	30	8.0
8		340	100	86	—	—	40	8.4
9		340	46	—	100	24	—	4.4
10		340	100	—	—	—	10	5.3
11		340	100	19	—	—	14	5.8
12		220	100	25	8(H), 6(F)[e]	8(H), 6(F)[e]	40	1.1
13		220	100	—	—	—	31	1.4

Table 2 (continued)

No.	Structure[a]	Molecular weight	$[M]^{+\cdot}$	$[M-CH_3]^+$	Mass spectral data[b] (major fragments, *m/e*) $[M-CH_3X]^{+\cdot}$[d]	$[M-X]^{+}$[d]	$[M-CH_3CO]^+$	Retention times[c]
14		220	100	31	—	—	62	1.6
15		202	100	41	10(H), 3(F)[e]	9(H), e(F)[e]	25	1.2
16		202	100	—	—	—	26	1.7
17		202	100	38	—	—	29	1.8

[a] Arrow (►) indicates position of the methoxy group.
[b] Relative peak height in percent of base peak; fragments containing chlorine and bromine based on ^{35}Cl and ^{79}Br only.
[c] Retention time in minutes; for experimental conditions see the Experimental section.
[d] X=H, F, Cl or Br.
[e] The fragment formed by loss of a moiety containing hydrogen (H) or fluorine (F) is indicated in parentheses.

m-Methoxybiphenyls cannot give such a stabilized $[M-CH_3]^+$ fragment. The absence of this $[M-CH_3]^+$ peak from *o*-methoxybiphenyls is probably due to the facile loss of CH_3Cl which results in a stable fragment of the dibenzofurane structure.[5]

Bromomethoxybiphenyls. The fragmentation pattern of the bromomethoxybiphenyls is analogous to that of the corresponding chloro derivatives. The spectra of three typical bromomethoxybiphenyls are shown in Fig. 1 and further contrasted with the spectra of the corresponding hydroxy compounds.

Fluoromethoxybiphenyls. The fragmentation patterns of the fluoro derivatives are similar to the chloro and bromo compounds except for some differences in the *o*-methoxy compounds (see Table 1).

In addition to the usual fragments of *o*-methoxyhalobiphenyls (i.e. $[M-F]^+$ and $[M-CH_3F]^{+\cdot}$, *o*-methoxyfluorobiphenyls also give fragments corresponding to $[M-H]^+$, $[M-CH_3]^+$, $[M-CH_3H]^{+\cdot}$ and $[M-CH_3CO]^+$. The aromatic C–F bond is stronger than the aromatic C–H bond and therefore *o*-methoxyfluorobiphenyls behave similarly to *o*-methoxybiphenyl (**1**) and show only minor $[M-F]^+$ and $[M-CH_3F]^{+\cdot}$ fragments.

It is noteworthy that the relative intensities of fragments $[M-CH_3X]^{+\cdot}$ and $[M-X]^+$(X=F, H, Cl, Br) in the series of compounds **12, 1, 6** and **9** are inversely proportional to the aromatic C–X bond strengths (X= F: 125, H: 110, Cl: 95 and Br: 80 Kcal mol^{-1} at 298 K[16]).

Chlorodimethoxybiphenyls. Mass spectral data of 12 chlorodimethoxybiphenyls representing all possible dimethoxy substitution patterns are reported in Table 3. Similarly to monomethoxychlorobiphenyls, the frag-

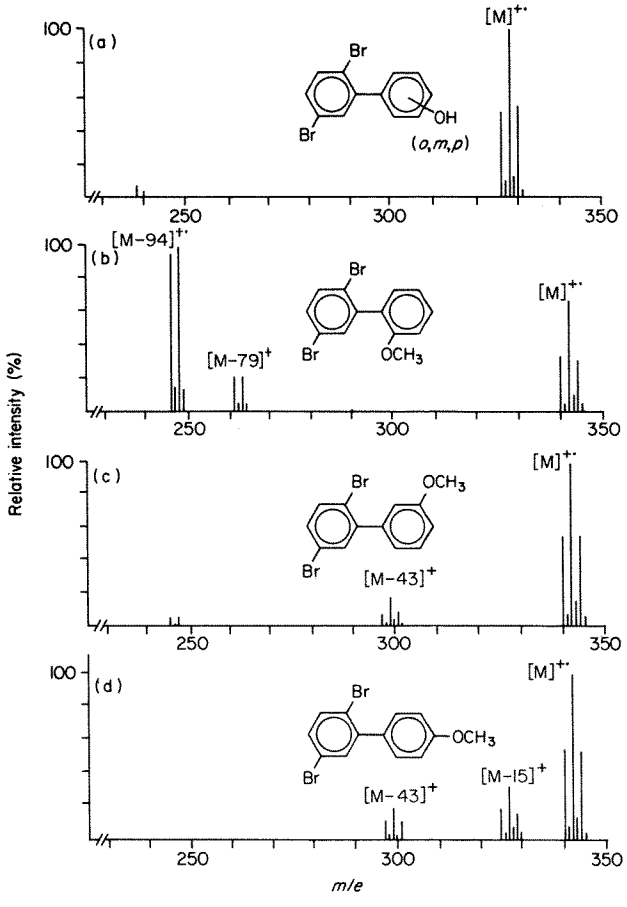
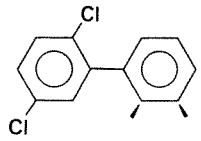
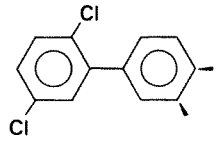
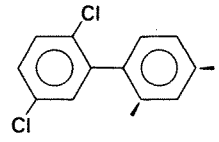
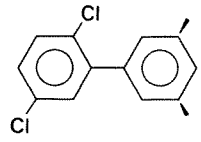
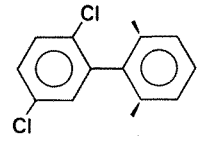
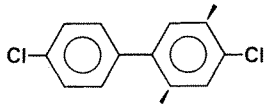
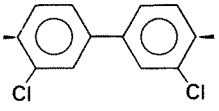
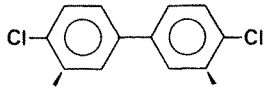
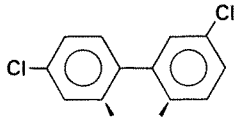
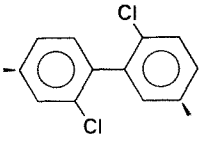
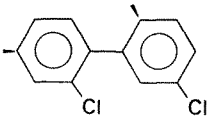
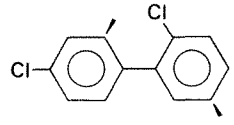


Figure 1. Partial mass spectra of: (a) the three isomeric 2',5'-dibromobiphenylols (all identical); (b) 2',5'-dibromo-2-methoxybiphenyl; (c) 2',5'-dibromo-3-methoxybiphenyl; (d) 2',5'-dibromo-4-methoxybiphenyl.

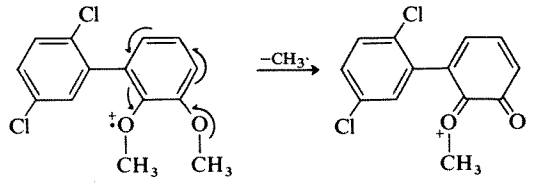
Table 3. Mass spectral data and g.c. retention times of dimethoxychlorobiphenyls

No.	Structure[a]	Mass spectral data[b] $[M]^+$	$[M-15]^+$	$[M-35]^+$	$[M-43]^+$	$[M-50]^{+\cdot}$	$[M-65]^+$	Retention times[c]
18		78	13	—	—	100	15	4.1
19		100	22	—	20	—	—	6.1
20		100	—	—	10	51	27	5.4
21		100	—	—	25	—	—	6.0
22		58	—	13	—	100	31	4.4
23		75	30	—	—	100	27	6.7
24		78	100	—	18	—	—	9.7
25		100	—	—	41	—	—	9.4
26		100	—	33	—	89	60	5.9
27		100	56	—	35	—	—	8.4
28		100	27	—	15	87	35	7.6
29		100	—	—	13	62	33	7.4

[a] Positions of methoxy groups are indicated by arrows (►).
[b] Figures in percent of base peak; fragments based on ^{35}Cl only.
[c] Retention time in minutes; for experimental conditions see the Experimental section.

mentation is determined solely by the positions of the methoxy groups and not by the substitution pattern of chlorine, e.g. 4,4-dichloro-2,5-dimethoxybiphenyl (**23**), 3',4-dichloro-2,5-dimethoxybiphenyl and 2',4-dichloro-2,5-dimethoxybiphenyl give identical spectra.

In contrast to monomethoxychlorobiphenyls in which an *o*-methoxy group always gives a significant $[M-35]^+(-Cl)$ fragment, with dimethoxychlorobiphenyls this fragment is observed only when both methoxy groups are in the *ortho* position (compounds **22** and **26**). However, with only one methoxy group in the *ortho* position the fragment $[M-65]^+$, $[M-CH_3ClCH_3]^+$, is always present. When the two methoxy groups are in different rings, the following specific fragments are observed: $[M-15]^+$, $[M-43]^+$ for *para*, $[M-43]^+$ for *meta* and $[M-50]^{+\cdot}$, $[M-65]^+$ for *ortho*. When both methoxy groups are in the same ring, the fragment $[M-15]^+$ is only present when methoxy groups are in either the *ortho* or *para* positions relative to each other (cf. Ref. 17) regardless of whether one of them is in the *para* position relative to the biphenyl bond. This can be explained by the formation of stable oxonium ions after elimination of a methyl radical from the molecular ion which is shown for an *o*-dimethoxy compound in Scheme 2. In contrast to monomethoxychlorobiphenyls,



Scheme 2. Initial mass fragmentation of 2,5-dichloro-2', 3'-dimethoxybiphenyl.

the fragmentation pattern of dimethoxychlorobiphenyls does not allow unambiguous assignment of position of the methoxy groups in all cases. However, by comparing significant fragments in the spectrum of an unknown with the data presented in Table 3, the number of possible methoxy substitution patterns is usually reduced to two. The knowledge of the chlorine substitution pattern and the relative intensities of the fragments are in most cases sufficient for structural proof.[14]

G.c. retention times

All other structural features kept equal, the retention times of halomethoxybiphenyls increase in the following series: (a) for different halogen substituents and hydrogen: F<H<Cl<Br; (b) for methoxy isomers: 2-CH_3O<3-CH_3O<4-CH_3O; (c) for isomers of the same halogen: 2-halo<3-halo<4-halo; (d) for methoxy groups in either one or two rings having the same position relative to the phenyl phenyl bond in each case: two CH_3O in one ring<two methoxy in two rings. In all cases, the effect of a methoxy group position is more pronounced than that of halogen with the columns used in this study.

Metabolism experiments

After feeding 4,4'-dibromobiphenyl to a rat, one urinary metabolite was observed. In the total ion chromatogram

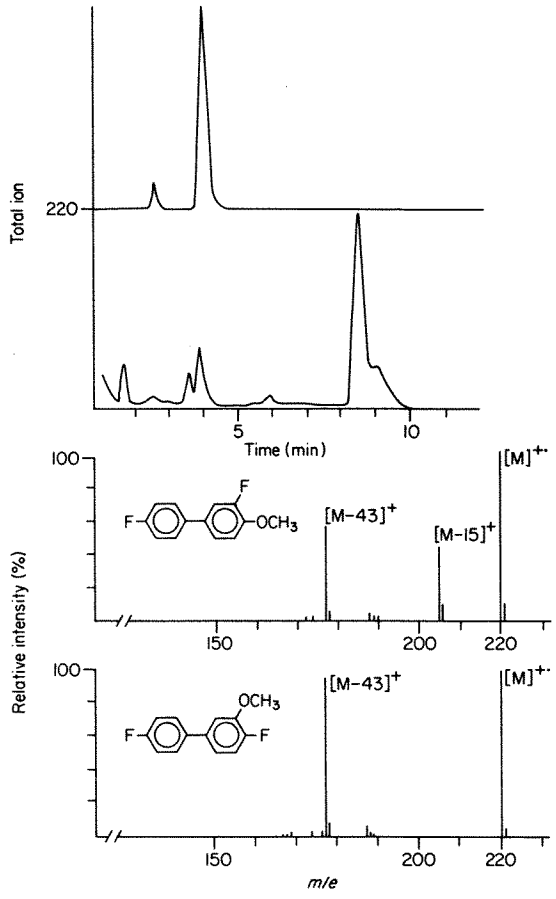


Figure 2. Mass and total ion chromatogram of purified and methylated extract of urine from a rat fed 4,4'-difluorobiphenyl. G.c. temperature programme: 150 °C, 2 min, rate 8 °C min^{-1} to 240 °C.
Partial mass spectra of: 3,4'-difluoro-4-methoxybiphenyl; 4,4'-difluoro-3-methoxybiphenyl.

of the purified and methylated urinary extract a peak with *m/e* 340 was detected. The mass spectrum of this compound was identical to that of compound **7** (Table 2): 4,4'-dibromo-3-methoxybiphenyl. After feeding 4,4'-difluorobiphenyl to a rat, two urinary metabolites were detected. The mass chromatogram of the purified and methylated urinary extract indicated that two peaks in the total ion chromatogram showed abundant *m/e* 220 ions, corresponding to monomethoxydifluorobiphenyls (Fig. 2). Based on the specific fragmentation patterns of the model compounds and by analogy with the corresponding chloro and bromo compounds, these methoxyfluorobiphenyls were identified as: 3,4'-difluoro-4-methoxybiphenyl and 4,4'-difluoro-3-methoxybiphenyl. 4,4'-Dichlorobiphenyl was administered to duck weed and the water investigated for phenolic metabolites. The total ion chromatogram of the purified, methylated extract gave a peak with mass *m/e* 252 corresponding to a dichloromethoxybiphenyl. The mass spectrum revealed a fragmentation pattern identical to synthetic 4,4'-dichloro-3-methoxybiphenyl (**4**).

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