Pergamon

0045-6535(95)00162-X

*Chemosphere*, Vol. 31, No. 4, pp. 3017-3023, 1995
Elsevier Science Ltd
Printed in Great Britain

# SYNTHESIS AND CHARACTERIZATION OF HYDROXYLATED POLYCHLORINATED BIPHENYLS (PCBs) IDENTIFIED IN HUMAN SERUM

Stephen Safe, Kent Washburn, Timothy Zacharewski and Timothy Phillips

Departments of Veterinary Physiology & Pharmacology and
Veterinary Anatomy & Public Health, Texas A&M University,
College Station, TX 77843-4466, USA
and Department of Pharmacology & Toxicology, University of Western
Ontario, London, Ontario, Canada N6I 5C1

(Received in Germany 18 May 1995; accepted 12 June 1995)

## ABSTRACT

Hydroxylated polychlorinated biphenyls (PCBs) have been identified in wildlife and human samples. Most of these compounds are highly chlorinated (penta-hepatachloro) and contain a single meta- or para-hydroxyl group. Using the Cadogan coupling procedure, the following hydroxy-PCBs congeners were synthesized: 2,3,3',4',5-pentachloro-4-biphenylol, 2,3',4,4',5-pentachloro-3-biphenylol, 2',3,3',4',5-pentachloro-4-biphenylol, 2,2',3',4,4'-pentachloro-3-biphenylol, 2,2',3,3',4',5-pentachloro-4-biphenylol, 2,2',3',4,4',5-hexachloro-3-biphenylol, 2,2',3,4',5,5'-hexachloro-4-biphenylol, 2,2',3,3',4',5,5'-heptachloro-4-biphenylol, 2,2',3',4,4',5,5'-heptachloro-3-biphenylol, 2,2',3,4',5,5',6-heptachloro-4-biphenylol. Many of these compounds have been detected as residues in human serum and current studies are investigating their activities as agonists and antagonists for several endocrine-mediated responses.

Key Words: hydroxy-PCBs, synthesis, characterization

## INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial compounds which have been detected as environmental contaminants in almost every component of the global ecosystem including air, water, fish, wildlife and human adipose tissue, serum and breast milk (1-5). The production and use of PCBs have been banned in industrialized countries and environmental levels of these compounds are decreasing in many locations (6, 7). The distribution of PCB congeners in environmental samples is highly variable and depends on several factors including the dominant source of PCB contamination in specific locations or regions. In most fish, wildlife and human samples, the penta-

heptachlorobiphenyls are the major persistent congeners and this is due in part, to their relatively slow rate of oxidative metabolism. In contrast, most lower chlorinated biphenyls are more readily metabolized and therefore are present in low levels in most biological samples.

Hydroxylated PCB metabolites have also been detected in some wildlife samples and a recent study identified several higher chlorinated (penta-heptachloro) hydroxylated PCBs in human serum (8, 9). Although, the environmental and human health impacts of PCBs have been extensively investigated (10, 11), only a few studies have reported the biochemical and toxic effects of hydroxylated PCBs. Hydroxylated PCBs inhibit mitochondrial oxidative phosphorylation (12), exhibit estrogenic activity (13, 14), and bind to transthyretin (15-18). A comparison of the short term toxicity of PCB congeners and their metabolites indicates that for most responses, the parent compounds are more toxic (19). This study reports the synthesis and chemical characterization of the major hydroxy PCB congeners identified in human plasma (9); biochemical and toxic effects of these compounds are currently being investigated and will be reported separately.

## MATERIALS AND METHODS

***Chemical Synthesis.*** Nine of the major hydroxy-PCBs identified in human serum were synthesized by the Cadogan coupling of a chlorinated aniline with a chlorinated anisole followed by demethylation as previously described (21). A summary of the reactants are listed in Table 1. All of the reactants listed in the Table, boron tribromide and isoamyl nitrite were purchased from the Aldrich Chemical Co. (Milwaukee, WI). The compounds were synthesized using the following common reaction conditions. The chlorinated aniline (3.0 g) and chlorinated anisole (9.0 g) were heated to 140°C; isoamyl nitrite (3.0 ml) was added dropwise over a period of 30 min and allowed to stir for 16 to 20 hr at 140°C. The excess chlorinated anisole was removed from the reaction mixture; chloroform was added to dissolve the residue which was adsorbed on to silica gel (Davison Chemical, Baltimore, MD). The adsorbed material was layered on to the top of a silica gel column (4 x 20 cm)and eluted with 600 ml of petroleum spirit. The crude methoxy-PCB product was further purified by preparative thin-layer chromatography (tlc) on Kieselgel 60 $HF_{254}$ (EM Science, Gabbstown, NJ) using hexane as a solvent. The methoxy-PCB band was eluted from the silica gel, dissolved in methylene chloride (150 ml) and boron tribromide (1.0 ml) was added. After 24 to 72 hr, water was carefully added dropwise to the reaction mixture and the hydroxy-PCBs were isolated in the methylene chloride fraction. Separation of isomeric hydroxy-PCB mixtures was carried out by preparative tlc in mixtures of petroleum spirit:acetone (93:7) or petroleum spirit:chloroform (80:20). At least two preparative tlc separations were required for the separations of mixtures in which both isomers were formed. One reaction, namely the coupling of 2,4,5-trichloraniline with 2,3,6-trichloroanisole, gave the corresponding 4-biphenylol as the major reaction product and the 3-hydroxy isomer was not isolated. The compound isolated by preparative tlc were crystallized from petroleum spirit. The overall yields from the reactions varied from 120 to 450 mg of crystalline compound.

***Gas Chromatography - Mass Spectrometry (GC-MS).*** The hydroxy-PCBs were methylated by treatment with freshly distilled ethereal diazomethane and analyzed by GC-MS analysis using a Hewlett Packard 5970A spectrometer equipped with a Hewlett Packard 5890 gas chromatograph and a 9133XV data station. The gas chromatograph was fitted with a 50 m 5% crosslinked methyl silicone column (0.2 mm, i.d.); 2 $\mu$l of compound were injected (solvent delay, 5 min) and the column temperature was 245°C (isothermal).

***Nuclear Magnetic Resonance (NMR) Spectra.*** The purified hydroxy-PCB congeners (Table 1) were dissolved in deuterochloroform and NMR spectra were determined on a 400 mHz Varian NMR XL400 mass spectrometer housed in the Department of Chemistry, Texas A&M University.

## RESULTS AND DISCUSSION

Ten hydroxy-PCB congeners were synthesized by the Cadogan coupling procedure and their properties are summarized in Table 1. Most of the reactions gave 2 major coupling products and these were separated by preparative tlc. Several studies have demonstrated that GC-MS analysis of methoxy-PCBs gives highly characteristic fragmentation patterns which are dependent on the position of the methoxyl group (22, 23). In the MS for o-, m- and p-methoxy-PCBs, the molecular ion ($M^+$) is dominant for all 3 structural classes of compounds. Under electron impact, p-methoxy -PCBs give relatively intense M-15 ($-CH_3$) and M-43 ($-COCH_3$) ions; m-methoxy-PCBs also give an M-43 ion and a diagnostic M-50 ion ($-CH_3Cl$) whereas o-methoxy-PCBs give a diagnostic M-70 ($-Cl_2$) fragment ion. The GC-MS of the methoxy-PCBs prepared in this study readily distinguished between the p- and m-methoxy-PCB products and a typical isomer pair is illustrated in Figure 1. The electron impact-induced fragmentation pattern for the methoxy derivative of 2,3,3',4',5-pentachloro-4-biphenylol (Fig. 1, right) gave an intense $M^+$ and M-15 and

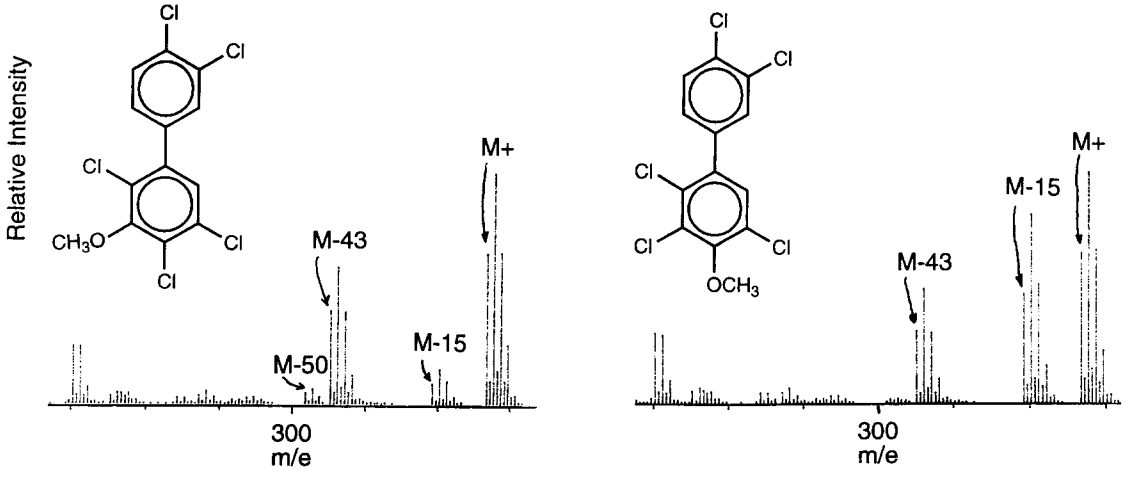


**Figure 1. Mass spectra of 3-methoxy-2,3',4,4',5-pentachlorobiphenyl (left) and 4-methoxy-2,3,3',4',5-pentachlorobiphenyl (right).**

Table 1. Synthesis and properties of hydroxylated PCBs identified in human serum.

| Compound (RRT) [1] | Precursors | Mol. Wt. ($M^+$, m/e) | NMR Spectra (ppm) |
|---|---|---|---|
| 2,3,3',4',5-pentachloro-4-biphenylol (15.6) | 3,4-dichloroaniline<br>2,3,6-trichloroanisole | 354 | 7.20 - 7.50 (H2', H5', H6' m), 7.25 (H6, s) |
| 2,3',4,4',5-pentachloro-3-biphenylol (15.6) | 3,4-dichloroaniline<br>2,3,6-trichloroanisole | 354 | 7.20 - 7.50 (H2', H5', H6', m), 7.04 (H6, s) |
| 2',3,3',4',5-pentachloro-4-biphenylol (15.7) | 2,3,4-trichloroaniline<br>2,6-dichloroanisole | 354 | 7.41 (H6', d, J = 8.2 Hz), 7.12 (H5', d, J - 8.2 Hz), 7.24 (H2,6, s) |
| 2,2',3',4,4'-pentachloro-3-biphenylol (13.2) | 2,3,4-trichloroaniline<br>2,6-dichloroanisole | 354 | 7.47 (H6', d, J = 8.2 Hz), 7.35 (H5', d, J = 8.2 Hz), 7.11 (H5', d, J = 8.2 Hz), 6.78 (H6, d, J = 8.2 Hz) |
| 2,2',3,3',4',5-hexachloro-4-biphenylol (24.3) | 2,3,4-trichloroaniline<br>2,3,6-trichloroanisole | 388 | 7.46 (H6', d, J = 8.2 Hz), , 7.20 (H6, s), 7.10 (H5', d, J = 8.2 Hz) |
| 2,2',3',4,4',5-hexachloro-3-biphenylol (22.0) | 2,3,4-trichloroaniline<br>2,3,6-trichloroanisole | 388 | 7.47 (H6', d, J = 8.2 Hz), 7.09 (H5', d, J = 8.2 Hz), 6.99 (H6,s) |
| 2,2',3,4',5,5'-hexachloro-4-biphenylol (16.8) | 2,4,5-trichloroaniline<br>2,3,6-trichloroanisole | 388 | 7.58 (H6', s), 7.32 (H3', s), 7.18 (H6, s) |
| 2,2',3,3',4',5,5'-heptachloro-4-biphenylol (28.9) | 2,3,4,5-tetrachloroaniline<br>2,3,6-trichloroanisole | 422 | 7.29 (H6', s), 7.17 (H6, s) |
| 2,2',3',4,4',5,5'-heptachloro-3-biphenylol (27.3) | 2,3,4,5-tetrachloroaniline<br>2,3,6-trichloroanisole | 422 | 7.28 (H6', s), 6.96 (H6, s) |
| 2,2',3,4',5,5',6-heptachloro-4-biphenylol (25.6) | 2,4,5-trichloroaniline<br>2,3,5,6-tetrachloroanisole | 422 | 7.64 (H6', s), 7.29 (H3', s) |

[1] RRT = relative retention time.

M-40 ions at m/e 354, 339 and 314, respectively. In contrast, the MS of 2,3',4,4',5-pentachloro-3-biphenylol gave a weaker M-15 fragment ion, an M-43 ion and an M-50 ion which was characteristically observed in the spectra of all 3-methoxy-PCBs (e.g. Fig. 1, left). Unlike lower chlorinated methoxy-PCBs, some of the m-methoxy isomers gave a more intense M-15 peak and the major difference between p- and m-methoxy isomers was associated with the diagnostic M-50 fragment ions in the spectra of m-methoxy PCBs.

The relative gas chromatographic retention times were also determined for the methoxy-PCBs and the NMR spectra of the hydroxy-PCBs were also determined. The relative retention times for these compounds were similar to those previously reported for methoxy PCBs in human serum (9). The NMR spectra are summarized in Table 1 and the chemical shift data and proton-proton coupling constants were consistent with their structures and complemented the fragmentation patterns observed in the MS. In the NMR spectra of the hydroxy-PCBs, the chemical shift values for protons on the hydrocarbon ring were similar to those previously reported by Mullin and coworkers (24). The chemical shifts for the proton at C-6 (H6) in the 4-hydroxy- and 3-hydroxy-substituted rings were highly diagnostic. For example, the chemical shift for H6 varied from 7.17 to 7.25 ppm for the 4-biphenylols whereas lower values (6.78 to 7.04 ppm) were observed for the 3-biphenylols.

In summary, this study describes the synthesis and properties of 10 hydroxy-PCB congeners and some of these compounds have recently been identified in human serum; moreover, many of these same compounds have been identified in wildlife samples. Current research in this laboratory is investigating the potential biochemical and toxic properties of these persistent hydroxy-PCBs.

## ACKNOWLEDGEMENTS

The financial assistance of the National Institutes of Health (P42-ES04917) and the Texas Agricultural Experiment Station is gratefully acknowledged. Dr. T. Zacharewski is supported by PMAC-HRF-MRC research award in medicine.

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