Vol. 84, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 30, 1978 Pages 936-942

PURIFICATION AND STRUCTURAL CHARACTERIZATION OF POLYBROMINATED BIPHENYL CONGENERS

Robert W. Moore[1] and Steven D. Aust

Department of Biochemistry

Michigan State University

East Lansing, Michigan 48824

Received August 4,1978

SUMMARY

Only three congeners of the environmental contaminants polybrominated biphenyls (PBBs) have previously been identified. PBBs were fractionated and subjected to instrumental analysis. In order of their gas chromatographic elution, the additional congeners identified were 2,2',4,5,5'-penta-, 2,3',4,4',5-penta-, 2,2',3,4,4',5'-hexa-, 2,3',4,4',5,5'-hexa-, 2,2',3,3',4,4',5,5'-octa-, and 2,2',3,3',4,4',5,5',6-nonabromobiphenyl. Increasing ortho bromination caused a characteristic upfield shift of the ^{1}H-NMR signals from the remaining ortho protons.

INTRODUCTION

Polybrominated biphenyls (PBBs) were manufactured for use as flame retardants, but have been discovered as environmental contaminants (1,2). To date, only the structures of two major congeners of these complex mixtures (3-6) [plus decabromobiphenyl (7)] have been reported. A number of polar PBB contaminants have also been partially characterized (6). Because of the widespread interest in the environmental and potential human health effects of PBBs, and because the two major congeners (structures known) cannot account for all of the biological effects of the Firemaster PBB mixture (8,9), we have attempted to purify and characterize the remaining PBB components. The structures of six additional congeners have been elucidated, and are reported here.

METHODS

PBBs were assayed with a Hewlett Packard Model 402 GC equipped with a pulsed ^{63}Ni electron capture detector. The column, 3% OV-1 on Gas Chrom Q, 100-120 mesh, was maintained at 270°, using 95% Ar, 5% CH_4 for both carrier and purge gas. An LKB

[1]Present address: Department of Pathology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

0006-291X/78/0844-0936$01.00/0
Copyright © 1978 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Type 9000 GC-MS was used to determine the molecular weights of the congeners. Source temperature was 290°, a 3500 V accelerating voltage was used, and the column was 3% OV-1. 180 MHz ^{1}H-NMR spectra were obtained on a Bruker WP 180 spectrometer at ambient temperature. Dilute (< 3% w/v) solutions were prepared in $CDCl_3$, using Me_4 Si as the internal standard. The 15.08 MHz ^{13}C-NMR spectrum was taken at ambient temperature on a Bruker WP 60 spectrometer equipped with quadrature detection. C_6D_6 was the solvent, and Me_4 Si the internal standard. Melting points were determined with a Hoover Unimelt capillary melting point apparatus.

PBBs were fractionated by column chromatography in the presence of hexane. The hexane was prepurified by passage over alumina. Typically 1 g of PBBs and 110 g of activity grade I alumina were used. In most cases, repeated chromatography of the most highly enriched fractions was required. Recrystallization of partially purified fractions from hexane was highly effective at separating **10** and **11** from **8** and **12**; and **4** from **6**, but in other cases was of limited value. Only **5** behaved as an oil. Knowledge of which contaminants could be removed by recrystallization was a key factor in deciding which column fractions should be pooled. Elution volumes also increased as the amount of a given congener applied to a column was decreased, a factor which limited the ultimate purities of congeners which eluted after their major contaminant(s). Purification of minor components of a mixture therefore required prior chromatography to remove the major component(s). When it was desired to recover all remaining PBB components from a column, they could be rapidly eluted with $CHCl_3$, ethyl acetate, or acetonitrile.

RESULTS AND DISCUSSION

PBB congeners were purified from two commercial mixtures: Firemaster FF-1 (or BP-6), which averages six bromines per molecule, and an octabromobiphenyl mixture. The gas chromatographic profiles and numbering systems are shown in Figure 1. Chromatography on alumina with hexane was the only column technique found to fractionate the PBB mixtures, and in certain instances, recrystallization from hexane was a useful adjunct to this technique. While **4**, **6**, **8**, **12**, and **13** could be prepared to 95% or higher purity, the other congeners had to be identified from less pure mixtures, although each was enriched at least tenfold. Melting points for the recrystallized congeners were (compound number in parentheses): 159-160° (**4**), 165-166° (**6**), 165-166 (**8**), 232-233° (**12**) and 263-264 (decomposed) (**13**).

Molecular weights were determined by GC-MS analyses of both the crude mixtures and of partially purified fractions. **1** and **2** are penta-, **3** -**6** are hexa-, **7** - **9** are hepta-, **10** is undetermined, **11** and **12** are octa-, **13** is nona-, and **14** is 2,2',3,3',4,4',5,5',6,6'-decabromobiphenyl. The empirical formulas for these congeners agree with those reported elsewhere (5,7,10), and **10** has been reported to be an octabromobiphenyl (7).

Knowing these formulas, NMR spectroscopy was sufficient to identify the structures of **1**, **2**, **5**, **6**, **12** and **13** [**4** and **8** had previously been identified (3-6)]. Peak assignments, coupling constants, and structural formulas are given in Table 1. Structural assignments

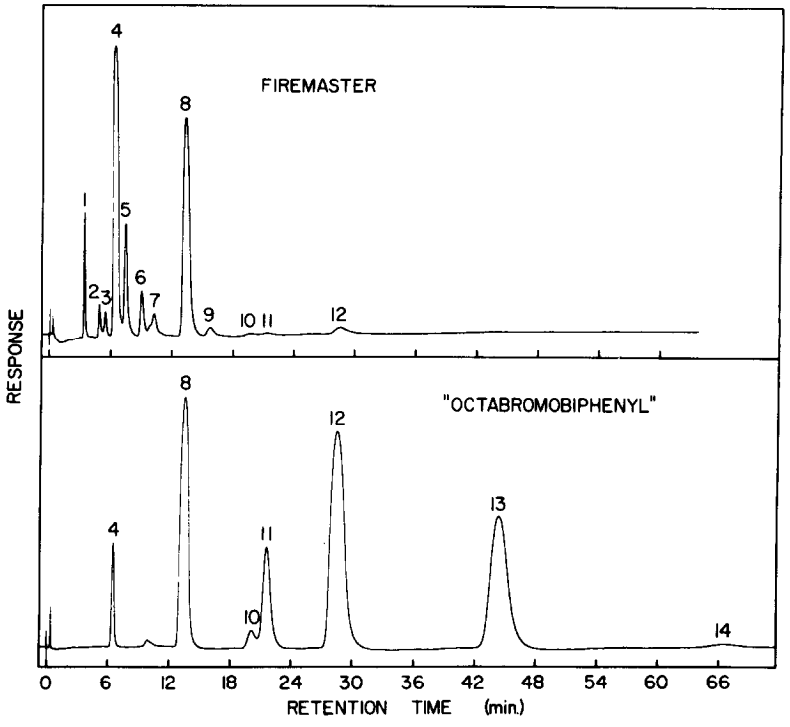

Figure 1. Gas chromatographic profiles and numbering system for the components of polybrominated biphenyl mixtures.

were made as described below, based on several assumptions. Coupling constants of 7-9 Hz, 2-3 Hz, and < 1 Hz result from protons ortho, meta, and para to each other, respectively. If no coupling is observed (J < 0.5 Hz), the protons are either para to each other or on opposite rings. Based largely on the known spectra of **4** and **8** (3,4), proton chemical shifts of 7.8-8.0 ppm, 7.3-7.7 ppm, and 6.9-7.2 ppm are indicative of protons adjacent to 2,1, and 0 bromines, respectively. Also, for biphenyls containing 3-7 bromines, two singlets (J < 1 Hz) necessarily mean that one ring has a 2,4,5-tribromo substitution pattern, since this is the only possible para proton arrangement.

Congener **1** has five protons, and the two singlets indicate that one ring is 2,4,5-tribrominated. The ortho + para, ortho + meta, and meta + para splitting pattern of the remaining three protons allows only three possible structures for the second ring, of which two require signals at 7.53 or 7.41 ppm to arise from a proton adjacent to no bromines. The second ring must therefore have bromines at the 2 and 5 positions. Congener **2** also

Table 1: Proton Chemical Shifts and Coupling Constants of Polybrominated Biphenyl Congeners

Proton	Chemical Shift (ppm)		Protons	Coupling Constants (Hz)
2,2',4,5,5'-Pentabromobiphenyl (**1**)				
3	7.932	(s)	J (3,6)	< 0.5
6	7.486	(s)	J (3',4')	8.4
3'	7.533	(q)	J (3',6')	0.4
4'	7.409	(q)	J (4',6')	2.4
6'	7.357	(q)		
2,3',4,4',5-Pentabromobiphenyl (**2**)				
3	7.932	(s)	J (3,6)	< 0.5
6	7.543	(s)	J (2',5')	< 0.5
2'	7.627	(d)	J (2',6')	2.2
5'	7.684	(d)	J (5',6')	8.4
6'	7.183	(q)		
2,2',4,4',5,5'-Hexabromobiphenyl (**4**)				
3,3'	7.931	(s)	J (3,6)	< 0.5
6,6'	7.468	(s)	J (3',6')	< 0.5
2,2',3,4,4',5'-Hexabromobiphenyl (**5**)				
5	7.675	(d)	J (5,6)	7.8
6	7.020	(d)	J (3',6')	< 0.5
3'	7.932	(s)		
6'	7.458	(s)		
2,3',4,4',5,5'-Hexabromobiphenyl (**6**)				
3	7.934	(s)	J (3,6)	< 0.5
6	7.536	(s)		
2',6'	7.590	(s)		
2,2',3,4,4',5,5'-Heptabromobiphenyl (**8**)				
6	7.473	(s)	J (3',6')	< 0.5
3'	7.934	(s)		
6'	7.451	(s)		
2,2',3,3',4,4',5,6' or 2,2',3,3',4,5,5',6'-Octabromobiphenyl (**11**)				
6	7.370	(s)		
4' or 5'	7.959	(s)		
2,2',3,3',4,4',5,5'-Octabromobiphenyl (**12**)				
6,6'	7.456	(s)		
2,2',3,3',4,4',5,5',6-Nonabromobiphenyl (**13**)				
6'	7.379	(s)		

has five protons and a 2,4,5-tribrominated ring. Since the remaining protons are split ortho, meta, and ortho + meta, only three structures can be postulated for the second ring. Two of these are incorrect because the proton at 7.18 ppm would have to be adjacent to a bromine. The bromines on the second ring are therefore at the 3 and 4 positions.

Congener **5**, with four protons (two singlets), has a 2,4,5-tribrominated ring. The other two protons are only split ortho, so they must occupy either the 4 and 5 or the 5 and 6 positions on the other ring. However, the former possibility requires a 7.02 ppm proton to be adjacent to a brominated carbon. The second ring therefore has bromines at the 2,3, and 4 positions. Congener **6**, with four protons, has only three singlets, and therefore has a 2,4,5-tribrominated ring. The two protons on the other ring are equivalent and can occupy either the ortho or meta positions. Since meta protons would have a shift of approximately 7.8 ppm, the observed shift of 7.59 ppm indicates that the second ring is 3,4,5-tribrominated.

The octabromobiphenyl **11** has not been completely identified. Four structures are possible for two unsplit signals, one of which is incorrect because it requires a chemical shift of 7.37 ppm to result from a proton adjacent to two bromines. The two more likely structures are presented in Table I, although the molecule might also be fully brominated on one ring and have a 2,4,5-substitution pattern on the other. It is clear, however, that **11** has three ortho bromines. Congener **12**, an octabromobiphenyl, has a single unsplit signal and therefore five possible structures. Three of these are incorrect because their protons are adjacent to two bromines each while the signal is only at 7.46 ppm. However, the two remaining possibilities, 2,2',3,3',4,4',5,5'- and 2,3,3',4,4',5,5',6-octabromobiphenyl, cannot be resolved by ^{1}H-NMR spectroscopy. Since the latter structure should have markedly different bridge carbons, ^{13}C-NMR spectroscopy was performed[2]. Only one bridge carbon signal was observed, at 142.7 ppm, which identifies the former structure for **12** as the correct one.

Only three nonabromobiphenyls can exist, and the chemical shift of the singlet indicates that the only proton in **13** is at an ortho position.

The 2,4,5 and 2,3,4,5-substitution patterns were found to be very common. The third and fourth ortho positions appear to be the most difficult to brominate, presumably because of steric hindrance. In all cases for which the structures are known or partially

[2] $CDCl_3$ could not be used because it is a poor solvent for **12**. While C_6D_6 obscured most of the ^{13}C-NMR signal resulting from **12**, the bridge carbon signal was the only one seen downfield of 133 ppm.

known at least one meta position on each ring is brominated, and with the exception of 1 (and possibly 11), all para positions are brominated.

The chemical shifts of protons adjacent to two bromines all fall in the range of 7.96 to 7.93 ppm. Shifts for protons adjacent to both a brominated carbon and a bridge carbon fall into three categories, depending upon the total number of ortho bromines. When only one ortho bromine is present (2 and 6), shifts for the ortho protons are between 7.54 and 7.59 ppm. With two ortho bromines (1, 4 , 5, 8 and 12), shifts all fall within the range of 7.49 to 7.45 ppm, except for 1 (at 7.36 ppm), but this may be because this proton is on the only known ring with a para proton. With three ortho bromines (11, 13), the proton signals are at 7.37 and 7.38 ppm. Only 2 and 5 have ortho protons adjacent to another unsubstituted carbon, but again the trend towards decreasing chemical shifts with increasing ortho bromination is observed. Welti and Sissons have noted the same trend among polychlorinated biphenyls (11).

These same investigators also showed that when polychlorinated biphenyls were subjected to either gas or column (alumina with hexane) chromatography, the congeners having a given substitution pattern on one ring eluted in a characteristic order when the structure of the other ring was held constant (12). Results to date with PBBs are consistent with these observations.

The biological effects of PBB congeners are a function of both the position and number of bromines. While 4,4'-dibromobiphenyl causes hepatic ultrastructural changes and increases in microsomal drug metabolizing enzymes (13), its 2,2'-isomer had no effect on either these enzymes or the histology of any tissue examined (9). 3,3',5,5'-Tetrabromobiphenyl is incapable of causing a 3-methylcholanthrene-type microsomal induction, while 3,3',4,4',5,5'-hexabromobiphenyl is a very potent member of this class of inducing agents (14). Its isomer 6 , with one ortho bromine, causes both a 3-methylcholanthrene- and a phenolbarbital-type response in the hepatic microsomal enzymes (15), and it appears to be highly toxic (unpublished). Congeners 4 (Br_6), 8 (Br_7), and 12 (Br_8), each with two ortho bromines, cause histological and enzymatic changes similar to those caused by phenobarbital, and are relatively non-toxic (8,9,16). It appears that para bromination is a requirement for PBB congeners to cause biological effects, and that the number of ortho bromines determines the qualitative nature of these effects.

ACKNOWLEDGEMENTS

We thank the Michigan State University Chemistry Department (Frank Bennis) for the ^{1}H-NMR spectra, John O'Connor for the ^{13}C-NMR spectrum, Joelle Andre´ of the Michigan State University Mass Spectrometry Facility for the GC-MS, and Fred Askari for his excellent assistance with the purification of several of these congeners. Supported in part by a grant from the Michigan Department of Agriculture. Michigan Agricultural Experiment Station Journal Article Number 8538.

REFERENCES

1. Carter, L. J. (1976) Science 192, 240-243.
2. Kay, K. (1977) Environ. Res. 13, 74-93.
3. Sundstrom, G., Hutzinger, O. and Safe, S. (1976) Chemosphere 5, 11-14.
4. Moore, R.W., O'Connor, J. V. and Aust, S.D. Bull. Environ. Contam. Toxicol., in press.
5. Jacobs, L. W., Chou, S.-F. and Tiedje, J. M. (1976) J. Agric. Food. Chem. 24, 1198-1201.
6. Hass, J. R., McConnell, E. E. and Harvan, D. J. (1978) J. Agric. Food Chem. 26, 94-99.
7. DeKok, J. J., DeKok, A., Brinkman, U.A. Th. and Kok, R. M. (1977) J. Chromatog. 142, 367-383.
8. Moore, R. W., Sleight, S. D. and Aust, S. D. (1978) Toxicol. Appl. Pharmacol. 44, 309-321.
9. Moore, R. W.,Sleight, S. D. and Aust, S. D., submitted.
10. Zitko, V. (1977) Bull. Environ. Contam. Toxicol. 17, 285-292.
11. Welti, D. and Sissons, D. (1972) Org. Magnetic Resonance 4, 309-319.
12. Sissons, D. and Welti, D. (1971) J. Chromatog. 60, 15-32.
13. Ecobichon, D. J., Hansell, M. M., and Safe, S. (1977) Toxicol. Appl. Pharmacol. 42, 359-366.
14. Poland, A., and Glover, E. (1977) Molec. Pharmacol. 13, 924-938.
15. Dannan, G.A., Besaw L. C., and Aust, S. D. (1978) The Pharmacologist, 20, 251.
16.Besaw, L. C., Moore, R. W., Dannan, G. A., and Aust, S. D. (1978) The Pharmacologist, 20, 251.