from benzene–hexanes provided 48 mg (69%) of **3**: mp 161–162 °C. The ^{1}H NMR and IR spectra of this product were identical with those of **3**.

Methyl *trans*-2,5-Diphenyl-2-thiazoline-5-carboxylate (10). To a solution of 100 mg (0.35 mmol) of **8** in 10 mL of diethyl ether was added dropwise an 0.3 N ethereal solution of diazomethane until the evolution of N_2 ceased. A few drops of glacial acetic acid was added. The solution was extracted with 5% $NaHCO_3$ (3 × 10 mL) and saturated NaCl (2 × 5 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue obtained was purified by preparative TLC on a Whatman MK6F 20 × 10 cm plate, employing diethyl ether/hexanes (1:2) as eluant to provide 100 mg (88%) of **10** as a yellow oil: R_f[4] 0.66; ^{1}H NMR ($CDCl_3$) δ 8.10–7.84 (m, 2 H, ortho protons of 2-phenyl group), 7.60–7.18 (m, 8 H, Ar H), 5.42 and 5.31 (2 d, 2 H, CHCH), 3.76 (s, 3 H, CH_3); IR (NaCl, neat), 1743 (ester carbonyl), 1600 (C=N) cm^{-1}.

***N*-(*trans*-2,4-Diphenyl-2-thiazolin-5-ylcarbonyl)glycyl-proline (11).** To a chilled (0 °C) solution of 0.28 g (1.0 mmol) of **8** in 6 mL of dry THF was added 0.15 g (1.0 mmol) of HOBt and 0.23 g (1.1 mmol) of DCCI. The mixture was stirred for 0.5 h at 0 °C and 0.5 h at room temperature and filtered, and a solution of 0.27 g (1.5 mmol) of Gly-Pro-OH and 0.16 g (1.5 mmol) of Na_2CO_3 in 20 mL of water was added. The reaction mixture was stirred overnight. The THF was removed in vacuo, and the aqueous solution was extracted twice with 25-mL portions of diethyl ether, acidified to pH 2 (pH paper) with saturated $KHSO_4$, and extracted with chloroform (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to provide a light-yellow solid. Recrystallization from ethyl acetate provided 120 mg of **11**. The solid was dissolved in 1 mL of methanol and the solution applied to a 2.5 × 90 cm column of Sephadex LH-20, employing methanol as eluant at a flow rate of 5 mL/h. Fractions 70–85 were combined and concentrated in vacuo to dryness and the residue obtained was recrystallized from ethyl acetate to provide 82 mg (19%) of pure **11**: mp 173–174 °C dec, R_f[3] 0.67; $[\alpha]^{26}_D$ −19.2° (*c* 1.04, methanol); ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1 H, NH), 8.13–7.82 (m, 2 H, ortho protons of 2-phenyl group), 7.70 (m, 1 H, NH), 7.62–7.20 (m, 8 H, Ar H), 5.58 and 5.40 (2 d, 2 H, CHCH, J = 5 Hz), 4.54 (m, 1 H, Pro C_α-H), 4.10 (m, 2 H, Gly C_α-H), 3.53 (m, 2 H, Pro C_δ-H), 2.06 (m, 4 H, Pro C_β-H and C_γ-H); IR (KBr disk) 3350 (NH), 3600–2600 (CO_2H), 1740 (acid carbonyl), 1620 (amide I), 1600 (C=N), 1520 (amide II) cm^{-1}.

Anal. Calcd for $C_{23}H_{23}N_3O_4S$: C, 63.14; H, 5.30; N, 9.60; S, 7.33. Found: C, 63.06; H, 5.33; N, 9.59; S, 7.28.

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Registry No. L-(*Z*)-**2a**, 82865-27-8; (*Z*)-**3**, 17606-70-1; L-(*Z*)-**4**, 82865-28-9; (*Z*)-**6**, 82865-29-0; *trans*-**8**, 82865-30-3; **9**, 4371-55-5; *trans*-**10**, 82865-31-4; L-**11**, 82865-32-5; ACE, 9015-82-1; Gly-L-Pro-OH, 704-15-4; Gly-L-Pro-OMe, 53309-02-7; *Z*-Gly-L-Pro-OH, 1160-54-9; benzoyl chloride, 98-88-4.

Differentiation of Brominated Biphenyls by Carbon-13 Nuclear Magnetic Resonance and Gas Chromatography/Mass Spectrometry

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The chemical structures of specific brominated biphenyls were determined unambiguously from their ^{1}H NMR, ^{13}C NMR, and mass spectra. With these model compounds and the previously characterized components of a technical mixture, the existence of an ortho effect in the mass spectral fragmentation of brominated biphenyls was established. Comparisons of the relative abundances of the $[M - Br]^+$ fragment, the $[M - 2Br]^{+\cdot}$ fragment, and the molecular ion $[M]^{+\cdot}$ distinguish brominated biphenyls with 2,2′, 2,2′,6, or 2,2′,6,6′ substitution from biphenyls without ortho,ortho′ substitution and from biphenyls with 2,2′,3 substitution. The order of brominated biphenyl elution from nonpolar GC columns correlates with retention index measurements obtained for analogous chlorinated biphenyls. Use of the ortho effect combined with the order of GC elution appears to be a reliable method for the partial structural differentiation of brominated biphenyl isomers by standard GC/MS techniques.

Polybrominated biphenyls (PBBs) are of current interest because of their contribution to several instances of widespread environmental contamination, their established animal toxicity, their presence in human tissues, and thus their possible human health effects.[1-5] Investigations centering about PBB identification, partitioning in tissues, and biological effects have been numerous.[6-15] The metabolism and toxicity of PBBs vary with isomer structure.[16,17] Understanding the health effects of PBBs, therefore, clearly requires knowledge of the characteristics of specific brominated biphenyls.

Characterization of brominated biphenyls is complicated by the fact that there are 209 possible PBB congeners, excluding optical isomers that result from hindered rota-

(1) Dicarlo, F. J.; Seifter, J.; DeCarlo, V. J. *Environ. Health Perspect.* **1978**, *23*, 351.
(2) Carter, L. J. *Science* **1976**, *192*, 240.
(3) Kay, K. *Environ. Res.* **1977**, *13*, 74.
(4) DHEW: Final Report of the Subcommittee on the Health Effects of Polychlorinated Biphenyls and Polybrominated Biphenyls of the DHEW Committee to Coordinate Toxicology and Related Programs. *Environ. Health Perspect.* **1978**, *24*, 129.
(5) Bekesi, J. G.; Holland, J. F.; Fishbein, A. S.; Rom, W.; Wolff, M. S.; Selikoff, I. J. *Science* **1978**, *199*, 1207.
(6) Andersson, K.; Norström, A.; Rappe, C.; Rasmuson, B.; Swahlin, H. *Environ. Qual. Saf. Suppl.* 1975 **1978**, *3*, 798.
(7) Sundström, G.; Hutzinger, O.; Safe, S. *Chemosphere* **1976**, *1*, 11.
(8) Norström, A.; Andersson, K.; Rappe, C. *Chemosphere* **1976**, *1*, 255.
(9) Jacobs, L. W.; Chou, S.; Tiedje, J. M. *J. Agric. Food Chem.* **1976**, *24*, 1198.
(10) De Kok, J. J.; De Kok, A.; Brinkman, U. A. Th.; Kok, R. M. *J. Chromatogr.* **1977**, *142*, 367.
(11) Hass, J. R.; McConnell, E. E.; Harvan, D. J. *J. Agric. Food Chem.* **1978**, *26*, 94.
(12) Moore, R. W.; O'Connor, J. V.; Aust, S. D. *Bull. Environ. Contam. Toxicol.* **1978**, *20*, 478.
(13) Dannan, G. A.; Moore, R. W.; Aust, S. D. *Environ. Health Perspect.* **1978**, *23*, 51.
(14) Moore, R. W.; Dannan, G. A.; Aust, S. D. *Pharmacologist* **1978**, *20*, 251.
(15) Moore, R. W.; Aust, S. D. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 936.
(16) Domino, E. F.; Fivenson, D. P.; Domino, S. E. *Drug Metab. Dispos.* **1980**, *8*, 332.
(17) Moore, R. W.; Sleight, S. D.; Aust, S. D. *Toxicol. Appl. Pharmacol.* **1978**, *44*, 309.

tion of unsymmetrically substituted biphenyl rings.[18] About 40 of these compounds have been characterized fully.[19,20] Furthermore, although commercial analytical standard samples of the brominated biphenyls are available, several literature reports have questioned the identity and purity of some of these standards.[1,10,21]

Carbon-13 NMR techniques have been used to establish unambiguously the chemical structures of several polychlorinated biphenyls, terphenyls, naphthalenes, and naphthols.[22-24] Similar techniques are applied here to the characterization of brominated biphenyls.

Effects of ortho substitution on the physical properties of biphenyls have been recognized in cases of optical activity, UV spectra, dipole moments, and ^{13}C NMR chemical shifts.[24,25-27] Ortho effects on the GC and HPLC retention properties and UV spectra of brominated biphenyls have also been recognized.[10] A number of authors have reported ortho effects in the mass spectral fragmentation of substituted biphenyls and related compounds.[28-37] Of particular relevance is the report of Levy and Oswald[28] that the presence of three ortho chlorines in biphenyl (i.e., 2,2',6 substitution) results in a substantial increase in the abundance of the ion resulting from chlorine atom loss $[M - Cl]^+$, in contrast to biphenyls having fewer or more ortho chlorines. Therefore, we expected a similar ortho effect to occur in the electron-ionization positive-ion mass spectral fragmentation of ortho-brominated biphenyls. Such an effect would provide a method of obtaining structural information from the mass spectra of brominated biphenyls in addition to the usual information on molecular weight and number of bromines.

Our objectives in this study were fourfold: first, to obtain ^{13}C NMR parameters for individual well-characterized brominated biphenyls; second, to use these parameters to predict the NMR spectra of unknown brominated biphenyls; third, to identify correctly the commercial standards for use as model compounds; and fourth, to determine whether a mass spectrometric ortho effect occurs in brominated biphenyls.

Experimental Section

Materials. Most of the brominated biphenyls studied were purchased from Ultra Scientific Inc. (formerly RFR Corp.), Hope, RI. 4,4'-Dibromobiphenyl was purchased from Aldrich Chemical Co., Milwaukee, WI. The FireMaster BP-6 sample was obtained from Michigan Chemical Corp., Chicago, IL. All samples were screened for identity and purity by mass spectrometry. In questionable cases, GC and/or TLC analysis helped establish purity and identity.

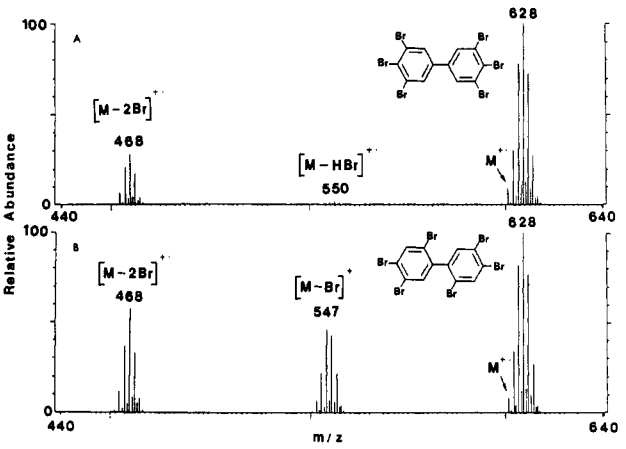

Figure 1. The mass spectra of (A) 3,3',4,4',5,5'-hexabromobiphenyl and (B) 2,2',4,4',5,5'-hexabromobiphenyl, showing the difference in the bromine-loss region of isomers with no ortho bromines and those with 2,2' bromine substitution.

^{13}C NMR Spectra. NMR spectra were obtained on 29–50-mg samples dissolved in 3.5 mL of chloroform-*d* or acetone-d_6 with $\simeq$2% tetramethylsilane (Me_4Si) added to provide an internal reference. A Varian XL-100/15 NMR spectrometer with a Nicolet TT-100A data system and MONA broad-band accessory was used. Sweep widths of ±3 kHz with quadrature phase detection, flip angles of $\simeq$15° (90° pulse) = 16 μs), pulse delays of 2–5 s, and 16K Fourier transforms were employed. Proton broad-band decoupling was furnished by a 100-Hz square-wave-modulated single frequency of 10 W centered on the ^1H spectrum. Assignments of the ^{13}C resonances were accomplished by standard techniques, employing selective ^1H decoupling, measurements of the ^1H–^{13}C spin–spin coupling constants, ^{13}C spin–lattice relaxation times, and nuclear Overhauser enhancements. The assumption of additivity of substituent effects[24,38,39] allowed prediction of the chemical shifts of the complex brominated biphenyls, using substituent effect parameters obtained from the simpler substituted biphenyls. No set of assignments for a given compound was based solely on additivity, however. The technique we used to assign ^{13}C spectra using additivity predictions has been discussed in detail elsewhere.[22]

Mass Spectrometry. Mass spectra were initially obtained by direct probe analysis with a Finnigan 4023 mass spectrometer with an Incos data system. The probe tip was loaded with 1–2 μg of the compound to be examined and was introduced into the ion source at 128 °C. The probe was then programmed from 128 to 300 °C at 10 °C/min. Samples were further examined by GC/MS analysis, using a 12-m × 0.2-mm OV-101 fused silica capillary GC column programmed from 60 to 280 °C at 8 °C/min. Spectra were obtained under standard 70-eV electron ionization conditions. Standard tuning procedures with perfluorotributylamine were used and high mass performance was verified[40] by use of tris(perfluoroheptyl)-*s*-triazine obtained from PCR Research Chemicals, Inc., Gainesville, FL.

Results and Discussion

Nuclear Magnetic Resonance Results. Values of the ^{13}C chemical shift for the brominated biphenyls are given

(18) Smith, D. H. *Anal. Chem.* **1975**, *47*, 1176.
(19) Sundström, G.; Hutzinger, O.; Safe, S.; Zitko, V. *Sci. Total Environ.* **1976**, *6*, 15.
(20) "Chemical Abstracts", 9th Decennial Index; Chemical Abstracts Service: Columbus, OH.
(21) Sovocool, G. W.; Wilson, N. K. *Chem. Eng. News*, **1979**, *57* July 30, 43.
(22) Wilson, N. K.; Zehr, R. D. *J. Org. Chem.* **1978**, *43*, 1768.
(23) Wilson, N. K.; Zehr, R. D. *J. Org. Chem.* **1982**, *47*, 1184.
(24) Wilson, N. K. *J. Am. Chem. Soc.* **1975**, *97*, 3574.
(25) Gilman, H., Ed. "Organic Chemistry: An Advanced Treatise"; Wiley: New York, 1943; Vol. 1, p 346.
(26) Adams, R.; Yuan, H. C. *Chem. Rev.* **1933**, *12*, 261.
(27) Eliel, E. L. "Stereochemistry of Carbon Compounds"; McGraw-Hill: New York, 1962; p 177.
(28) Levy, L. A.; Oswald, E. O. *Biomed. Mass Spectrom.* **1976**, *3*, 88.
(29) Oswald, E. O.; Levy, L.; Corbett, B. J.; Walker, M. P. *J. Chromatogr.* **1974**, *93*, 63.
(30) Hass, J. R.; Bursey, M. M.; Levy, L. A.; Harvan, D. J. *Org. Mass Spectrom.* **1979**, *14*, 319.
(31) Safe, S.; Hutzinger, O.; Jamieson, W. D. *Org. Mass Spectrom.* **1973**, *7*, 169.
(32) Hutzinger, O.; Safe, S.; Zitko, V. "The Chemistry of the PCB's"; Chemical Rubber Publishing Co.: Cleveland, 1974; p 41.
(33) Jansson, B.; Sundström, G. *Biomed. Mass Spectrom.* **1974**, *1*, 386.
(34) Tulp, M. Th. M.; Safe, S. H.; Boyd, R. K. *Biomed. Mass Spectrom.* **1980**, *7*, 109.
(35) Tulp, M. Th. M.; Hutzinger, O. *Biomed. Mass Spectrom.* **1978**, *5*, 224.
(36) Tulp, M. Th. M.; Olie, K.; Hutzinger, O. *Biomed. Mass Spectrom.* **1977**, *4*, 310.
(37) Sheley, C. F.; Patterson, R. T. *Org. Mass Spectrom.* **1974**, *9*, 731.
(38) Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley: New York, 1972; p 80.
(39) Wilson, N. K.; Anderson, M. In "Mass Spectrometry and NMR Spectroscopy in Pesticide Chemistry"; Haque, R., Biros, F. J., Eds.; Plenum: New York, 1974; p 197.
(40) Erickson, M. D.; Kelner, L.; Bursey, J. T.; Rosenthal, D.; Zweidinger, R. A.; Pellizzari, E. D. *Biomed. Mass Spectrom.* **1980**, *7*, 99.

in Table I. The effects of bromine substituents at various positions on the ^{13}C chemical shifts of biphenyl were obtained by subtraction of the biphenyl shifts from those of the monobrominated biphenyls. The substituent effect values, $\Delta\delta$, thus obtained were used to predict the ^{13}C chemical shifts of the PBBs by assuming additivity of substituent effects. Although deviations from the chemical shifts predicted by additivity, ones similar to those observed in chlorinated biphenyls,[24] did occur, it was possible to choose between two or more isomeric structures with the same degree of bromine substitution. The chosen structure was the one that gave the smallest overall standard deviation, σ, from the additivity predictions. In each case where ambiguities were not resolved by other techniques, for example by ^1H NMR, the difference between the predicted and experimental ^{13}C chemical shift values for the "best fit" isomeric structure had a σ smaller than that of alternative structures by a divisor of at least 2.5. As an illustration, in the worst case examined, that of 2,2′,3,3′,4,4′-hexabromobiphenyl, σ^2 values of 29.1, 28.2, 17.1, and 6.3 were obtained for the possible structures 2,2′,3,3′,5,5′-, 2,2′,4,4′,5,5′-, 2,2′,3,3′,6,6′, and 2,2′,3,3′,4,4′-hexabromobiphenyl, respectively. Thus, the 2,2′,3,3′,4,4′ substitution pattern is clearly established. Further, we are confident that the PBB congeners studied, however mislabeled they were originally, are now clearly and correctly identified.

Mass Spectrometry Results. Table II and Figure 1 show clearly that biphenyls having ortho bromines exhibit different mass spectral characteristics than those lacking such substitution. For example, the $[M - Br]^+$ fragment in the mass spectrum of 3,3′,4,4′,5,5′-hexabromobiphenyl (Figure 1A) is virtually nonexistent, while in the 2,2′,4,4′,5,5′-hexabromobiphenyl spectrum (Figure 1B) the $[M - Br]^+$ fragment is of comparable magnitude to the $[M - 2Br]^{+\cdot}$ fragment and is of an appreciable magnitude relative to the molecular ion $[M]^{+\cdot}$. Table II shows that the ratio of the abundance of the $[M - Br]^+$ fragment to the abundance of the $[M - 2Br]^{+\cdot}$ fragment is 0.02–0.06 in the nonortho-substituted cases. However, for brominated biphenyls with either two or three ortho substituents, at positions 2,2′ or 2,2′,6, this ratio is 0.5 to 1.4. Variability in these cases does not appear to be systematic and probably results from variations in the tuning of the quadrupole mass spectrometer, particularly at high mass.[40] The relative abundances in Table II were calculated with the largest component of the isotopic fragment or molecular ion cluster. The data support the existence of an ortho effect in the mass spectral fragmentation, which makes the relative abundances of the $[M - Br]^+$ and $[M - 2Br]^{+\cdot}$ fragments comparable for brominated biphenyls having two or three ortho bromines.

The only exceptions appear for the mono- and dibrominated biphenyls, which show loss of hydrogen bromide $[M - HBr]^+$, or the sequential loss of hydrogen and bromine $[M - (H + Br)]^{+\cdot}$ or $[M - (Br + H)]^{+\cdot}$. This loss also occurs in several of the more highly brominated biphenyls without ortho bromines. With two ortho bromines in the same ring, in 2,4,6-tribromobiphenyl, the abundance of the $[M - Br]^+$ fragment is not enhanced; this is another case where the absence of the ortho effect is characteristic of the absence of 2,2′ substitution.

The 2,2′,5,6′-tetrabromobiphenyl and 2,2′,4,5′,6-pentabromobiphenyl are cases of three ortho bromines and they exhibit the same ortho effect as 2,2′-substituted biphenyls. Two of the compounds studied possessed four ortho bromines: 2,2′,4,4′,6,6′-hexabromobiphenyl and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromobiphenyl. The

Table I. Carbon-13 NMR Chemical Shifts of Some Brominated Biphenyls

substitution	solvent	δ, ppm C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
nil	A[a]	141.95	127.74	129.68	128.15	129.68	127.74	141.95	127.74	129.68	128.15	129.68	127.74
	C	141.30	127.18	128.75	127.23	128.75	127.18	141.30	127.18	128.75	127.23	128.75	127.18
2-Br	A	143.37	122.88	132.15	130.06	128.38	129.81	141.88	128.78	130.01	128.49	130.01	128.78
3-Br	A	144.19	130.51	123.35	131.48	130.92	126.59	140.13	127.73	129.73	128.73	129.73	127.73
4-Br	C	139.98	(128.67)[b]	131.82	121.51	131.82	(128.67)	140.13	126.89	(128.84)	127.59	(128.84)	126.89
2,4-Br_2	C	141.62	123.32	135.40	121.54	130.54	132.24	140.05	128.11	129.20	127.91	129.20	128.11
	A	142.58	123.71	135.86	121.98	131.57	133.44	140.69	128.90	129.87	128.70	129.87	128.90
2,5-Br_2	A	145.39	121.77	135.56	134.60	121.94	132.73	140.56	128.96	129.92	128.96	129.92	128.96
2,6-Br_2	C	143.08	124.55	131.80	129.76	131.80	124.55	141.18	128.17	129.15	128.08	129.15	128.17
2,2′-Br_2	A	142.90	123.88	133.32	130.51	128.26	131.91	142.90	123.88	133.32	130.51	128.26	131.91
4,4′-Br_2	C	138.95	128.49	132.05	121.99	132.05	128.49	138.95	128.49	132.05	121.99	132.05	128.49
2,4,6-Br_3	A	143.31	125.63	135.10	122.50	135.10	125.63	141.17	129.25	129.81	129.25	129.81	129.25
2,2′,5-Br_3	A	144.84	121.45	135.15	134.51	123.09	133.46	141.56	123.59	133.54	131.03	128.49	131.82
2,3′,5-Br_3	A	143.83	121.82	135.70	134.63	121.94	133.37	142.72	131.03	122.36	132.84	132.02	129.10
2,4′,5-Br_3	A	144.22	121.80	135.72	134.55	121.92	133.20	139.75	(132.09)	(132.18)	122.89	(132.18)	(132.09)
2,2′,4′,5-Br_4	A	142.79	122.21	134.10	133.66	120.98	132.76	139.78	122.82	135.21	124.08	131.85	130.57
	C	143.81	122.91	135.21	134.43	121.57	133.87	140.88	123.29	135.60	124.66	133.28	131.71
2,2′,5,5′-Br_4	C	142.49	121.00	134.07	133.57	122.11	132.84	142.49	121.00	134.07	133.57	122.11	132.84
2,2′,5,6-Br_4	C	141.12	121.12	134.10	133.49	122.35	132.90	143.63	124.40	131.79	130.74	131.79	124.40
3,3′,5,5′-Br_4	C	141.95	129.01	123.61	133.96	123.61	129.01	141.95	129.01	123.61	133.96	123.61	129.01
2,2′,3,3′,4,4′-Br_6	A	138.88	127.85	128.85	128.06	130.69	126.94	138.88	127.85	128.85	128.06	130.69	126.94
2,2′,4,4′,5,5′-Br_6	A	141.65	123.41	137.55	126.28	124.34	136.12	141.65	123.41	137.55	126.28	124.34	136.12
3,3′,4,4′,5,5′-Br_6	C	138.87	130.69	126.92	128.03	126.92	130.69	138.87	130.69	126.92	128.03	126.92	130.69

[a] Samples were 2.5–6.7% w/v. A = acetone-d_6; C = chloroform-d. [b] Similar values in parentheses may be interchanged.

Table II. Mass Spectral Data for Brominated Biphenyls and Related Compounds

biphenyl substitution verified by ^{13}C NMR	% relative abundance[a]			[M − Br]$^+$
	[M]$^{+\cdot}$	[M − Br]$^+$	[M − 2Br]$^{+\cdot}$	[M − 2Br]$^{+\cdot}$
2-Br	100	79[b]	c	
3-Br	100	70[b]		
4-Br	100	67[b]		
2,4-Br_2	92	4[b]	100	0.04
2,5-Br_2	100	3[b]	77	0.04
2,6-Br_2	100	2[b]	87	0.02
2,2′-Br_2	42	51	100	0.5
4,4′-Br_2	100	1[b]	71	0.01
2,4,6-Br_3	100	1[b]	51	0.02
2,2′,5-Br_3	81	100	83	1.2
2,3′,5-Br_3	100	1[b]	51	0.02
2,4′,5-Br_3	100	1[b]	72	0.02
2,2′,4′,5-Br_4	100	58	65	0.8
2,2′,5,5′-Br_4	100	67	69	0.8
2,2′,5,6′-Br_4	100	57	59	1.0
3,3′,5,5′-Br_4	100	1[b]	36	0.03
2,2′,4,5′,6-Br_5	100	54	38	1.4
2,2′,4,4′,5,5′-Br_6	100	51	57	0.9
3,3′,4,4′,5,5′-Br_6	100	2[b]	28	0.06
2,2′,4,4′,6,6′-Br_6	100	8	39	0.2
2,2′,3,3′,4,4′,5,5′,6,6′-Br_{10}[d]	100	22	25	0.9
2,2′,3,3′,4,4′,5,5′,6,6′-$Br_{10}O$[d,e]	63	1	100	0.01
2,2′,3,3′,4,4′,5,5′,6,6′-Cl_{10}	100	14	80	0.2
2,2′,3,3′,4,4′,5,5′,6,6′-F_{10}[d]	100	11	10	1.1
2,2′,3,3′,4,4′,5,5′,6,6′-$Cl_{10}O$[d,e]	97	4	100	0.04

[a] Of the most abundant isotopic molecular or fragment ion. [b] [M − HBr]$^{+\cdot}$, if more abundant. [c] Not recorded due to low level. [d] Standard without ^{13}C NMR. [e] Diphenyl ether.

2,2′,4,4′,6,6′-hexabromobiphenyl yielded an [M − Br]$^+$ fragment about one-fifth as abundant relative to [M]$^{+\cdot}$ as did the 2,2′- and 2,2′,6-substituted compounds. The [M − 2Br]$^{+\cdot}$ abundance was also smaller for the hexabromobiphenyl. The fully brominated compound, decabromobiphenyl, likewise yielded about one-fifth abundance levels of the [M − Br]$^+$ fragment relative to the molecular ion; however, the ratio of [M − Br]$^+$ to [M − 2Br]$^{+\cdot}$ was 0.9, similar to that for biphenyls with two or three ortho bromines, whereas 2,2′,4,4′,6,6′-hexabromobiphenyl yielded a decidedly lower ratio of 0.2.

For comparison, the analogous set of fragments was examined for the fully chlorinated and fluorinated biphenyls. The values obtained were similar to those for decabromobiphenyl. The decachloro- and decabromodiphenyl ethers were also investigated; in both, the ortho effect was absent as expected.

Much effort has gone into the characterization of the technical brominated biphenyl mixture, FireMaster BP-6, because of its introduction into the environment.[6-15] GC/MS examination of this mixture allowed us to test the ortho effect for several unusual and mostly unsymmetrical brominated biphenyls, which are not yet available by synthesis.[41] Assignments of the components in the technical mixture were made on the basis of relative abundance and relative order of elution on a GC column of similar polarity to that used in previous characterizations; they are listed in Table III. Two of the previously assigned components of the mixture,[14,15,17] 2,3′4,4′,5-pentabromobiphenyl and 2,3′,4,4′,5,5′-hexabromobiphenyl, should show no ortho effect, with abundant [M − Br]$^+$ fragment signals in their mass spectra. This prediction was confirmed; these components were readily found and showed only small net loss of HBr in their mass spectra. The main component, 2,2′,4,4′,5,5′-hexabromobiphenyl, showed the expected ortho effect of 2,2′ substitution. The second most abundant component of the mixture, the known

(41) Pyle, J. L.; Shaffer, A. A.; Cantrell, J. S. *J. Org. Chem.* **1981**, *46*, 115.

Table III. Mass Spectral Data for Brominated Biphenyls in FireMaster BP-6

biphenyl substitution	% rel abundance			[M − Br]$^+$
	[M]$^{+\cdot}$	[M − Br]$^+$	[M − 2Br]$^{+\cdot}$	[M − 2Br]$^{+\cdot}$
2,2′,4,5,5′-Br_5	100	59	58	1.0
2,3′,4,4′,5-Br_5	100	1	19	0.05
2,2′,3,4′,5′,6-Br_6	86	100	60	1.6
2,2′,4,4′,5,5′-Br_6	100	64	71	0.9
(2,2′,3,3′,4,5′-Br_6)[a]	53	100	71	1.4
2,2′,3,4,4′,5′-Br_6	66	100	52	1.9
(2,3,3′,4′,5′,6-Br_6)	100	7[b]	27	0.3
(2,2′,3,3′,4,4′-Br_6)	58	100	27	3.7
2,3′,4,4′,5,5′-Br_6	100	2	25	0.08
(2,2′,3,3′,4,5,5′-Br_7)	40	100	25	4.0
2,2′,3,4,4′,5,5′-Br_7	76	100	57	1.8
2,2′,3,3′,4,4′,5-Br_7	54	100	35	2.9
(2,2′,3,4,4′,5,5′-Br_7)	100	14	c	

[a] Structures in parentheses represent tentative assignments for compounds not previously assigned. See text for basis of assignment. [b] 3.5% M − [HBr] present. [c] Not recorded due to low level.

2,2′,3,4,4′,5,5′-heptabromobiphenyl, was also readily observed. Examination of the mass spectra of the earliest eluting hexabromobiphenyl, the 2,2′,3,4,4′,5′ isomer, and of 2,2′,3,4′,5′,6-hexabromobiphenyl, known from its order of elution relative to the other components, led to the recognition of an additional ortho effect. Compounds possessing 2,2′,3 substitution yielded a diminished molecular ion abundance and an increased [M − Br]$^+$ abundance, relative to that of [M − 2Br]$^+$. This is similar to the enhanced ortho effect observed in 2,2′,3-substituted chlorinated biphenyls.

In the course of the examination of this technical mixture, several additional hexa- and heptabrominated isomers were detected. These are listed in Table III in the order of their elution, along with the previously identified components of FireMaster BP-6. An attempt was made to assign these on the basis of the following assumptions. It is known that bromine is ortho-para directing and weakly

deactivating and that a phenyl ring is ortho-para directing and activating to electrophilic aromatic bromination.[42] Furthermore, bromination by strong electrophiles is known to be irreversible.[43] It is possible to account for the known products of the bromination of biphenyl that are present in FireMaster BP-6 by a sequence of brominations starting with 4,4′-dibromobiphenyl and 2,4′-dibromobiphenyl, which are the known initial bromination products.[19] This sequential bromination is consistent with only one bromine being added ortho or para to an existing bromine on a ring. The ring is then deactivated to further bromination until the other, less brominated ring reaches an equal level of bromination. Thus, the prevalence of hexabrominated biphenyls with three bromines on each ring, pentabromobiphenyls with two and three bromines on each respective ring, and heptabromobiphenyls with three and four bromines on the respective rings is accounted for.

The order of elution of chlorinated biphenyls has been successfully correlated with a linear free-energy relationship to the position of ring substitution, using the retention index determined for model compounds relative to the elution of alkanes.[44,45] We assumed that the retention index of the chlorinated biphenyls would also correlate with the order of elution of brominated biphenyls. We tested this correlation for all the compounds of established structure in Tables II and III and found it to be reliable. With the asumptions in the preceding paragraph, the tested correlation with the retention properties of the chlorinated biphenyls, and the demonstrated validity of the ortho effect, it is possible to generate assignments for the previously unidentified components of the technical mixture, shown in parentheses in Table III. An example follows. There are 42 possible isomeric hexabromobiphenyls. However, only 21 isomers are possible with three bromines on each ring. Of these 21 possibilities, six should show no ortho effect, three should show the simple 2,2′-ortho effect, and 12 should show the 2,2′,3-ortho effect. The isomers that should elute between or near known isomeric components of the mixture were predicted. Only those isomers that had the expected elution properties and agreed with the experimental ortho effect were considered for assignment. The assignments are given in Table III; they are suggested as candidates for proof by synthesis. Unfortunately, model compounds possessing these ring substitutions were not available for further tests of the ortho effect and correlations with elution properties.

It is tempting to ascribe the origin of the ortho effect in the brominated biphenyls to the reduction of bromine–bromine steric interactions upon ionization and loss of Br· from the 2,2′-substituted biphenyls. However, Hass and co-workers,[30] in a study of the fragmentation of the chlorinated biphenyls, have pointed out how careful one must be in this argument. Their mass-analyzed ion kinetic energy spectrometric studies showed energy release in the chlorinated biphenyl series to be in the direction opposite to that expected from the release of steric interaction, as had been originally proposed by Levy and Oswald.[28,30] However, the conditions of metastable ion decomposition that were used in the studies of Hass and his group tend to focus only ions whose lifetimes are long on the metastable time scale. Such ions tend to be those undergoing rearrangement. The ion decompositions that occur in the ion sources of most mass spectrometers may often be the decompositions of more energetic ions on a faster time scale. Thus the results of metastable ion studies may not represent the main population of ions undergoing decomposition. The larger dimensions, greater polarizability, and weaker C–Br bond strength of bromine atoms make them useful probes in studies of these decompositions. Our results show the presence of an ortho,ortho′ bromine effect even in the case of 2,2′-dibromobiphenyl. The effect persists through 2,2′,6 substitution and is somewhat smaller for 2,2′,6,6′ substitution. An effect is also noted for 2,2′,3 substitution. These results argue, as in the chlorinated biphenyl case, against randomization of structures upon electron ionization, since no distinction would be expected between ortho- and nonortho-substituted biphenyls. The argument presented by Levy and Oswald would hold strictly only for the case of three ortho bromine atoms. However, 2,2′-dibromobiphenyl and other 2,2′-substituted biphenyls show the ortho effect. Like biphenyl, 2,2′-dibromobiphenyl is known to exist far from a planar conformation.[46–48] Electron diffraction studies of 2,2′-dibromobiphenyl in the gas phase show the average interring dihedral angle to be 75°, with the bromines in a syn conformation.[48] This conformational preference is probably due to London dipole attractive fources between the bromines. The massive nuclei may be considered to be immobile, on the time scale of electron removal, during the initial ionization process. Thus, the bromines will be in close proximity in the initially formed ion, most likely in a vibrationally or electronically excited state of the ion. Given the weaker bond strengths and longer bond lengths to be expected in the ion or its excited state, this close proximity of the bromines may enhance the expulsion to form the $[M - Br]^+$ ion.

Adding a third bromine to the ortho position does little further to influence the elimination. However, with four ortho bromines there is a decrease in the ortho effect. The ready generation of $[M - Br]^+$ from biphenyls with two or three, but not with four, ortho bromines may result from the formation of an energetically favorable daughter ion, presumably a bromonium ion, in which the phenyl rings lie in the same plane—possibly linked by a bromine bridge in the former case, but not in the latter, where coplanarity of the aromatic rings is prevented by the remaining syn bromines.

The presence of substituents in the 3-position appears to have an effect that may be rationalized as the tightening or buttressing of the motion of the bromines in the ortho positions. Again, this argues for integrity of the biphenyl ring and for bromine elimination from the 2- or 3-position. The completely brominated decabromobiphenyl, with four ortho bromines, does not exhibit an enhanced $[M - Br]^+$ fragment abundance; here the buttressing effect may be overridden by the inability of the system to form an energetically favorable planar daughter ion. The buttressing effect of an adjacent bromine in cases of 2,2′,3 substitution is analogous to similar buttressing by adjacent methyl groups in tetramethylphenanthrenes reported some time ago by Newman and his co-workers.[49]

Although the origin of the ortho effect is somewhat obscure, it lends itself to structural correlations helpful in distinguishing between brominated biphenyl isomers,

(42) Solomon, T. W. G. "Organic Chemistry"; Wiley: New York, 1976; p 469.
(43) Olah, G. A. "Friedel Crafts Chemistry"; Wiley: New York, 1973; p 463.
(44) Sissons, D.; Welti, D. *J. Chromatogr.* **1971**, *60*, 15.
(45) Albro, P. W.; Haseman, J. K.; Clemmer, T. A.; Corbett, B. J. *J. Chromatogr.* **1977**, *136*, 147.
(46) Ingraham, L. L. In "Steric Effects in Organic Chemistry"; Newman, M. S., Ed.; Wiley: New York, 1956; p 511.
(47) Karle, I. L.; Brockway, L. O. *J. Am. Chem. Soc.* **1944**, *66*, 1974.
(48) Bastiansen, O. *Acta Chem. Scand.* **1949**, *3*, 408.
(49) Karnes, H. A.; Kybett, B. D.; Wilson, M. H.; Margrave, J. L.; Newman, M. S. *J. Am. Chem. Soc.* **1965**, *87*, 5554.

particularly when combined with gas chromatographic considerations.

Conclusions

The chemical structures of specific brominated biphenyls were established from their ^{13}C NMR parameters. These compounds of known structure were then used to establish the occurrence of an ortho effect in the mass spectral fragmentation of brominated biphenyls having two or more ortho bromines on different rings. The ortho effect is seen as an increase in the abundance of the $[M - Br]^+$ fragment with respect to the $[M - 2Br]^{+\cdot}$ and $[M]^{+\cdot}$ ions. This effect allows one to establish the presence of 2,2′ substitution. It is analogous to that observed for three chlorines in the ortho chlorinated biphenyl series, yet persists for two, three, and to a lesser degree, for four ortho bromine substituents. Mass spectral differences brought about by the presence of 2,2′,3 substitution allow brominated biphenyls with this ring substitution to be distinguished from isomers without this substitution pattern.

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Registry No. Biphenyl, 92-52-4; 2-bromobiphenyl, 2052-07-5; 3-bromobiphenyl, 2113-57-7; 4-bromobiphenyl, 92-66-0; 2,4-dibromobiphenyl, 53592-10-2; 2,5-dibromobiphenyl, 57422-77-2; 2,6-dibromobiphenyl, 59080-32-9; 2,2′-dibromobiphenyl, 13029-09-9; 4,4′-dibromobiphenyl, 92-86-4; 2,4,6-tribromobiphenyl, 59080-33-0; 2,2′,5-tribromobiphenyl, 59080-34-1; 2,3′,5-tribromobiphenyl, 59080-35-2; 2,4′,5-tribromobiphenyl, 59080-36-3; 2,2′,4′,5-tetrabromobiphenyl, 60044-24-8; 2,2′,5,5′-tetrabromobiphenyl, 59080-37-4; 2,2′,5,6′-tetrabromobiphenyl, 60044-25-9; 3,3′,5,5′-tetrabromobiphenyl, 16400-50-3; 2,2′,4,5′,6-pentabromobiphenyl, 59080-39-6; 2,2′,3,3′,4,4′-hexabromobiphenyl, 82865-89-2; 2,2′,4,4′,5,5′-hexabromobiphenyl, 59080-40-9; 3,3′,4,4′,5,5′-hexabromobiphenyl, 60044-26-0; 2,2′,4,4′,6,6′-hexabromobiphenyl, 59261-08-4; 2,2′,3,3′,4,4′,5,5′,6,6′-decabromobiphenyl, 13654-09-6; 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether, 1163-19-5; 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl, 2051-24-3; 2,2′,3,3′,4,4′,5,5′,6,6′-decafluorobiphenyl, 434-90-2; 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorodiphenyl ether, 31710-30-2; 2,2′,4,5,5′-pentabromobiphenyl, 67888-96-4; 2,3′,4,4′,5-pentabromobiphenyl, 67888-97-5; 2,2′,3,4′,5′,6-hexabromobiphenyl, 69278-59-7; 2,2′,3,3′,4,5′-hexabromobiphenyl, 82865-90-5; 2,2′,3,4,4′,5′-hexabromobiphenyl, 67888-98-6; 2,3,3′,4′,5′,6-hexabromobiphenyl, 82865-91-6; 2,3′,4,4′,5,5′-hexabromobiphenyl, 67888-99-7; 2,2′,3,3′,4,5,5′-heptabromobiphenyl, 82865-92-7; 2,2′,3,4,4′,5,5′-heptabromobiphenyl, 67733-52-2; 2,2′,3,3′,4,4′,5-heptabromobiphenyl, 69278-60-0; Firemaster BP-6, 59536-65-1.

Preparative Resolution of Racemates on a Chiral Liquid Chromatography Column

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A 2 in. × 30 in. liquid chromatography column packed with a chiral stationary phase derived from (*R*)-phenylglycine has been found capable of resolving gram or larger samples of a variety of racemates. Nine such resolutions are presented. The racemates resolved include alcohols, lactams, lactones, sulfoxides, bi-β-naphthols, and hydantoins. Use of the column in an automated preparative chromatography system is demonstrated.

Since Prelog's partial resolution of Troger's base on lactose in 1944,[1] a number of attempts have been made to resolve useful quantities of enantiomers by chromatographing racemates upon chiral adsorbents. Typically, readily available naturally occurring materials (cellulose, wool, sugars, starches) have been used, and, while there have been sporadic successes, the usual result is incomplete separation of enantiomers.[2] Synthetically prepared chiral stationary phases (CSPs) have a somewhat similar record, notable exceptions being the polyacrylamide CSP of Blaschke, upon which complete separation of enantiomers was achieved for 530 mg of racemic chlorthalidone,[3] and the proline CSP of Jozefonvicz, upon which complete resolution was achieved for 160 mg of racemic proline by ligand-exchange chromatography.[4]

For some time we have been interested in the development of chromatographic methods for separating enantiomers. We recently described a high-performance liquid chromatographic column packed with 5-μm spherical silica particles to which is bonded CSP 1 upon which one can separate the enantiomers of a wide array of solutes.[5-8] Such columns are primarily intended to serve as analytical tools for the determination of enantiomeric purity and absolute configuration, even though they can also be used to resolve milligram quantities of enantiomers.[9] We now describe the use of a preparative version of this type of column and report a number of examples where gram-sized or larger samples of racemates have been successfully resolved.[10] We also describe the automated resolution of an alcohol useful as a starting material for a different type of CSP.

(1) Prelog, V.; Wieland, P. *Helv. Chim. Acta* **1944**, *27*, 1127.
(2) Many such instances are cited in the following reviews: Buss, D. R.; Vermeulen, T. *Ind. Eng. Chem.* **1968**, *60*, 12. Blaschke, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 13.
(3) Blaschke, G.; Markgraf, H. *Chem. Ber.* **1980**, *113*, 2031.
(4) Jozefonvicz, J.; Petit, M.; Szubarga, A. *J. Chromatogr.* **1978**, *147*, 177.
(5) Pirkle, W. H.; Finn, J. M.; Schreiner, J. L.; Hamper, B. C. *J. Am. Chem. Soc.* **1981**, *103*, 3964.
(6) Pirkle, W. H.; Finn, J. M. *J. Org. Chem.* **1981**, *46*, 2935.
(7) Pirkle, W. H.; Schreiner, J. L. *J. Org. Chem.* **1981**, *46*, 4988.
(8) Pirkle, W. H.; Finn, J. M.; Hamper, B. C.; Schreiner, J. L.; Pribish, J. *ACS Symp. Ser.* **1982**, *No. 185*, 245.
(9) Chiral HPLC columns of this type are available from the Regis Chemical Co., Morton Grove, IL, and from the J. T. Baker Chemical Co., Phillipsburg, NJ.
(10) A portion of this work was presented at the 179th National Meeting of the Americal Chemical Society, Houston, TX, 1980.

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