Chlorinated Derivatives of Dibenzo-*p*-dioxin and Related Compounds for Use as Reference Compounds in Method Development and Environmental Toxicology

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Analytical reference standards are not readily available for many of the chlorinated aromatic compounds that have been identified as environmental contaminants. We used sulfuryl chloride preparations to chlorinate aromatic compounds thereby obtaining the congeners needed as reference standards for gas chromatography/mass spectrometry analyses. We used commercially available vialed reagents and small vessels (reaction vials) for the production of milligram quantities of the toxicants. With biphenyl, dibenzofuran, and dibenzo-*p*-dioxin, the major products were polychloro derivatives of the respective starting material. With biphenylene and pyrene, polychloro-substitution products and addition products were also obtained. Polar constituents of the reaction mixtures were removed on silica gel, and the products were separated by high-performance liquid chromatography and identified by gas chromatography/mass spectrometry, nuclear magnetic resonance spectrometry, and infrared spectrometry.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are well-known environmental contaminants. These highly toxic compounds have been found in the fly ash of municipal incinerators in many countries (*1*). The polychlorobiphenylenes (PCBPs) are closely related to the PCDDs and PCDFs. The 2,3,6,7-TCBP isomer was found to be equipotent with 2,3,7,8-TCDD in microsomal enzyme induction (*2*). Rappe et al. reported that PCDFs and another series of chlorinated compounds were products of polychlorobiphenyl (PCB) capacitor fires investigated in Sweden (*1*). The molecular weights of the series indicated that they were polychlorinated pyrenes (PCPYs). The detection of PCBPs and PCPYs in these fires could not be validated because of lack of synthetic standards. Moreover, Williams et al. (*3*) described results from four PCB fires in which the PCBPs were present at concentrations 10 times higher than that of the PCDFs. In fact, PCBPs were the major chlorinated species present, excluding the PCBs. The chlorinated pyrenes were the most abundant chlorinated polynuclear aromatic species detected. As in the Rappe et al. studies, however, no standards other than 2,3,6,7-TCBP were available for the PCBPs or PCPYs. Smith et al. (*4*) have also reported finding many PCBPs in soot produced during an electrical accident in a New York State office building, that involved the pyrolysis of PCBs.

In several recent studies, investigators have reported that certain chlorinated congeners of the dioxins and furans are present in human adipose tissue in a range of a few parts per trillion (pptr) up to hundreds of parts per trillion (*5-7*). A major source of these contaminants is probably municipal waste incinerators where organic waste containing chlorine is burned (*1*). It is of interest, therefore, to examine human adipose tissue for the chlorinated PCBPs and PCPYs as well as the PCDDs and PCDFs. Workers in several laboratories have synthesized many of the PCDD and PCDF congeners for analytical reference material (*8-14*). Some of these congeners are also commercially available, but are costly. A review of the literature of environmental chemistry and of suppliers indicated that these other two classes of potentially toxic and analytically interfering chlorinated aromatic compounds, PCBPs and PCPYs, were not available as either pure standards for reference or as mixtures for qualitative analyses. We therefore investigated a simple procedure for producing qualitative mixtures of chlorinated aromatics that would serve as reference compounds for analytical method development.

EXPERIMENTAL SECTION

Safety. Chlorinated aromatic hydrocarbons have been reported to be toxic to animals in minute quantities. Persons attempting laboratory manipulation of chlorinated aromatic compounds should be familiar with the appropriate techniques for their safe handling and disposal.

Reagents. Perchlorination kits (Analabs), sulfuryl chloride (Aldrich), and antimony pentachloride (Aldrich) were used as chlorinating reagents. The kit consisted of three different sulfuryl chloride mixtures supplied in sealed glass vials with directions for perchlorination of biphenyls, dibenzofurans, naphthalenes, and other aromatic compounds (*15, 16*). Reagent A is composed of equal volumes from vials A_1 (1% SO_2Cl_2) and A_2 (0.5% $AlCl_3$). Reagent B contains one part iodine in 200 parts of 10% $SbCl_5$ in SO_2Cl_2. Reagent C ($SbCl_5$ in SO_2Cl_2) is recommended for perchlorination of naphthalenes. The species chlorinated consisted of 3-aminobiphenyl, 4-fluorobiphenyl, pyrene, dibenzo-*p*-dioxin, dibenzofuran, [$^{13}C_{12}$]-2,3,7,8-TCDD, [$^{13}C_{12}$]-1,2,3,7,8-PnCDF, and biphenylene.

Liquid Chromatography. Nonpolar components of synthesis mixtures were eluted in hexane from a dry-packed silica gel column, 25 cm × 1 cm i.d. The silica gel (Kiesilgel-60, 70–230 mesh ASTM) was purchased from EM Science, West Germany. Resolution of components was effected with reverse-phase liquid chromatography on octadecylmethylsiloxane with acetonitrile and water (85–100%).

Gas Chromatography. Capillary columns were purchased from Supelco (SP 2330) and J&W (DB-5) and used with helium carrier gas. Flow rates and temperature programs were adjusted for specific separations and are given in the figure legends.

Mass Spectrometry. The gas chromatography/mass spectrometry (GC/MS) analyses were run on three different instruments. The Finnigan 4500 operated at ionizer temperature of 100 °C, with methane gas for negative-ion chemical ionization (NCI), electron energy of 70 eV. The VG instrument, ZAB-2F, operated with the source at 200 °C, 1500 resolution, 1 s/decade, EI mode, and mass range 50–700. The Finnigan 1020 manifold was set at 80 °C, the separator compartment at 300 °C, and EI ionization and the mass range at 50–600.

Nuclear Magnetic Resonance. The Varian XL 300 NMR spectrometer, with a 7.0-T superconducting magnet, was used in all analyses. The usual solvent was [$^2H_6$]acetone.

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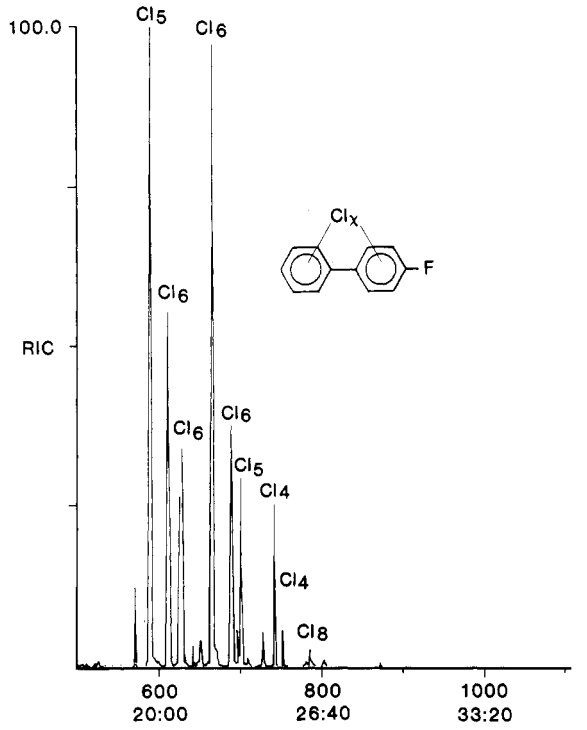


Figure 1. Reconstructed ion chromatogram of the chlorination products of 4-fluorobiphenyl. A 30-m, DB-5 capillary column was used in the Finnigan 4500, EI mode, 100 °C (1)–275 °C at 8 °C/min.

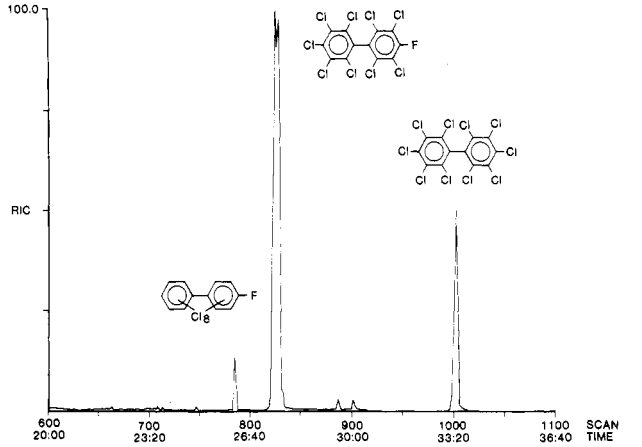


Figure 2. Reconstructed ion chromatogram of products of repeated chlorination reactions with 4-fluorobiphenyl, same conditions as in Figure 1.

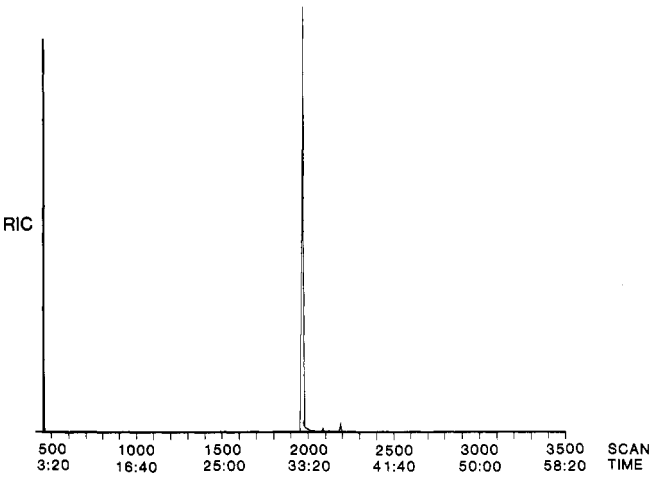


Figure 3. Reconstructed ion chromatogram of nonachloro-4-fluorobiphenyl following RPLC fractionation. The 30-m, DB-5 column was used in the Finnigan 1020 MAT, 100 °C (2)–275 °C at 10 °C/min and held.

Procedure. Typically, 20–100 mg of the biphenyls, dibenzo-*p*-dioxin, dibenzofuran, biphenylene, or pyrene were measured in a 5.0-mL reaction vial. Variable amounts of up to 3 mL of reagent were added. The kit reagents, A or C, were used either neat or in combination with SO_2Cl_2. Sulfuryl chloride (only), or in combination with $SbCl_5$, was also used for chlorination. The reaction mixture was heated to 70 °C and held at that temperature for a period of time (10 min to 2 h), cooled, and quenched with a few drops of 25% HCl (aqueous). The vial contents were rinsed with hexane onto a silica gel (2 cm, 1.0 × 20 cm, Merck Kieselgel 60) column. The chlorinated aromatic products were eluted in 100–200 mL of hexane, concentrated, and fractionated by reverse-phase liquid chromatography at 254-nm detection.

RESULTS AND DISCUSSION

Biphenyls. The chlorination products of fluorobiphenyl were obtained by following instructions for the kit procedure with reagent A. Many chlorinated species were present (Figure 1). Apparent molecular ion clusters indicate that there is a series of fluorobiphenyls with three to nine positions chlorinated on the molecules. The target molecule was the nonachloro derivative, so the initial product mixture was rechlorinated twice before the reaction mixture composition was satisfactory (Figure 2). Fractionation by reversed-phase liquid chromatography (RPLC) on ODS with 85% acetonitrile/water eluent resulted in a perchlorinated product of high purity by gas chromatography/flame ionization detection (GC/FID) and GC/MS. The material was suitable reference material for the gas chromatography/electron capture detector (GC/ECD) analysis (Figure 3).

From our initial experience with biphenyl chlorination (above), we recognized the advantages of that strategy in producing chlorinated aminobiphenyl congeners, requested as precursors for immunogens needed to produce antibodies to Aroclors (PCB congener mixtures, transformer oils). Kit reagent B, with aminobiphenyl, produced numerous compounds, most of which could not be characterized by MS patterns. Reaction of aminobiphenyl with SO_2Cl_2, however, produced congeners with two to six positions chlorinated.

Varying the pH of the reaction product before hexane or toluene extractions varied the composition of the mixture in terms of ratios of amounts of congeners to each other. The numerous products of a single synthesis procedure have the potential for variation in immunogenicity and perhaps for effective immunogenicity for rabbit antibody to Aroclors. These antibodies effect the detection system when samples are being screened for the presence of Aroclors.

Dibenzofuran. Reaction of dibenzofuran with reagent A for 2 h resulted in a mixture of chloro derivatives composed mainly (60%) of a trichlorodibenzofuran. Only monochloro isomers were detected as products, with SO_2Cl_2 as the lone reagent. All of the dibenzofuran congeners that have been detected in human tissue are chlorinated at the 2,3,7,8-positions (5–7), and therefore, the ^{13}C-labeled congeners are needed as internal standards in GC/MS analyses. The chlorination products of [$^{13}C_{12}$]-1,2,3,7,8-pentachlorodibenzofuran (obtained from Cambridge Isotopes, Inc., Woburn, MA) are characterized by GC/MS in Figure 4. The structural assignments of the hepta- and octachlorodibenzofurans are based on coelution with authentic ^{12}C congeners (Cambridge Isotopes, Inc.).

Dibenzo-*p*-dioxin. Several chlorination conditions were employed with both dibenzo-*p*-dioxin and [$^{13}C_{12}$]-2,3,7,8-TCDD. Overnight reaction of dibenzo-*p*-dioxin with SO_2Cl_2 at room temperature resulted in products composed mainly of a Cl_1 isomer (80–90%). Heating these SO_2Cl_2 products for 2 h at 75 °C resulted in dichloro- and trichlorodibenzo-*p*-dioxins by GC/MS analysis. The Cl_2 and Cl_3 product mixture was reacted with reagent A for 2 h at 75 °C to give a mixture

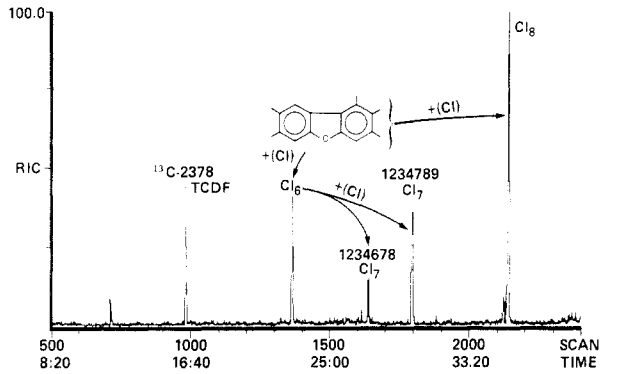


Figure 4. Reconstructed ion chromatogram of products of chlorination of [$^{13}C_{12}$]-1,2,3,7,8-pentachloridibenzofuran. The internal standard was [$^{13}C_{12}$]-2,3,7,8-TCDF in a Finnigan 4500, DB-5, 60-m, NCI 100 °C (2)–260 °C at 20 °C/min–300 °C at 1.0 °C/min and held.

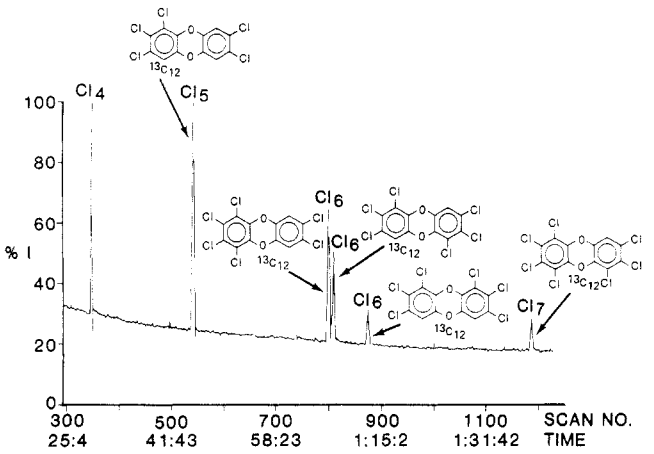


Figure 5. Chromatogram of the chlorination products of [$^{13}C_{12}$]-2,3,7,8-TCDD. Relative intensities, EI, were recorded on the ZAB-2F with isomer-specific analysis on the SP 2330, 60-m, programmed 170 °C (2)–220 °C at 20 °C/min and held.

that was >95% 2,3,7,8-TCDD, the highly toxic and most useful congener for method development and validation in environmental laboratories performing dioxin assays.

Because of the toxicities and presence in human tissues of the congeners chlorinated at the 2,3,7,8 positions, the $^{13}C_{12}$-labeled compounds were required for quantitation by isotope-dilution GC/MS analyses. The $^{13}C_{12}$ isomer of 2,3,7,8-TCDD was obtained (National Center for Toxicological Research, Jefferson, AR) and chlorinated to yield the more costly higher chlorinated species shown chromatographically (Figure 5). In this reaction 200 μL of reagent A and 200 μg of [$^{13}C_{12}$]-2,3,7,8-TCDD were held at 70 °C for 1 h before silica gel chromatography (hexane elution) and GC/MS analysis. The products were fractionated by reversed-phase liquid chromatography (RPLC) with acetonitrile/water (85–100%) eluent. Compounds in the fractions were quantified by GC/FID and GC/MS response factors, and fractions were combined to provide the reference standard of $^{13}C_{12}$-labeled compounds shown in Figure 6. The structural assignments are based on coelution of the ^{13}C compounds with the corresponding ^{12}C labeled congeners.

Biphenylenes. Chlorination of biphenylene has been reported (*16*). The chief chlorinated product was 2,3,6,7-tetrachlorobiphenylene. With reagents A, the products consisted predominantly of chlorinated species of molecular mass two units above that of polychlorinated biphenylenes (see Figures 7 and 8). Although the mass of the fragments and molecular ions are the same as those of a hexachlorobiphenyl, the NMR spectrum is different from those published for the hexachlorobiphenyls (*17*). Furthermore, previously we have reported the GC separation of several Cl_{10} isomers from this

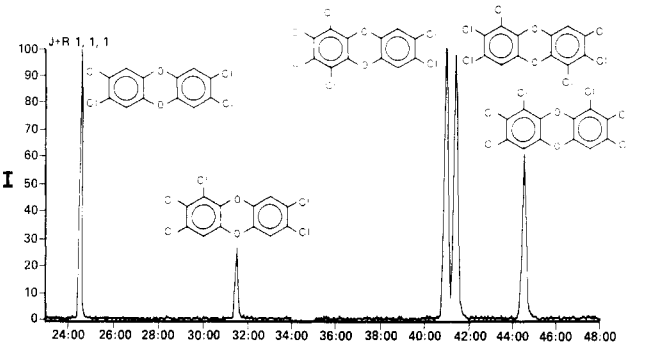


Figure 6. Chromatogram from VG 70/70E mass spectrometer of GC/MS reference standard. Prepared from chlorination of [$^{13}C_{12}$]-2,3,7,8-TCDD and RPLC fractionation. Conditions are the same as those in Figure 5.

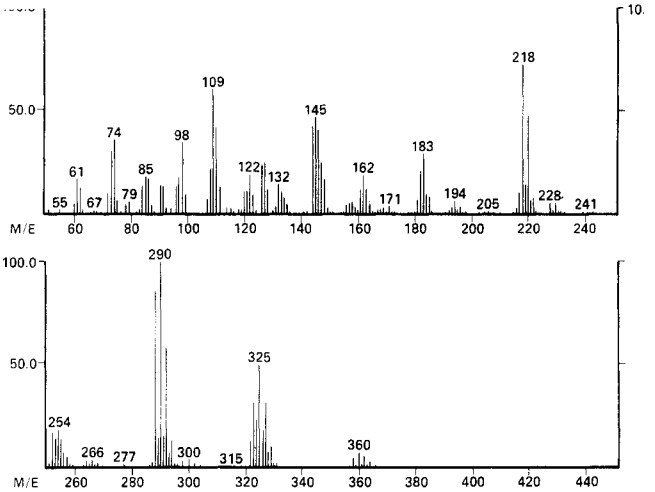


Figure 7. Mass spectrum of a hexachlorinated biphenylene derivative, Finnigan 4500.

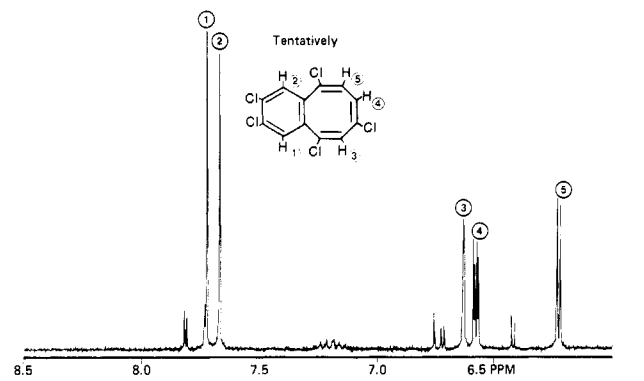


Figure 8. ^{1}H NMR spectrum of the hexachlorinated derivative of biphenylene in [$^2H_6$]acetone: δ 7.722 (s, 1 H), 7.670 (s, 1 H), 6.632 (dd, J = 0.85, 1.55 Hz, H_3), 6.579 (dd, J = 1.5, 4.3 Hz, H_4), 6.223 (dd, J = 0.8, 4.30 Hz H_5).

mixture with the molecular ion cluster of biphenyl, of which there is only one possible decachlorinated species (*16*).

The compounds in question are apparently due to chlorine addition at some positions and substitution at others on the biphenylene molecule. One set of chlorinating conditions produced PCDD-like compounds (by MS analysis), and preliminary infrared (IR) spectrometry data (*16*) indicate the presence of keto groups. These chloro derivatives of biphenylene are of interest as potential interferants in PCB/PCDD/PCDF analysis and perhaps as environmental contaminants. Production and purification of congeners for reference material and toxicity studies are under way.

Pyrene. The chlorination of pyrene with reagent C for 1 h resulted in both substitution and addition (*18*). The

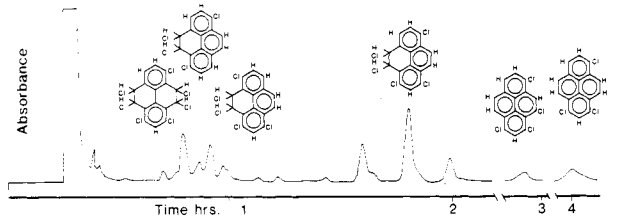


Figure 9. Chromatogram, RPLC, of pyrene chlorination products, 254 nm. The Dynamax ODS column, 25 cm × 10 mm, i.d., was eluted with 87–100% methanol, 9 mL/min.

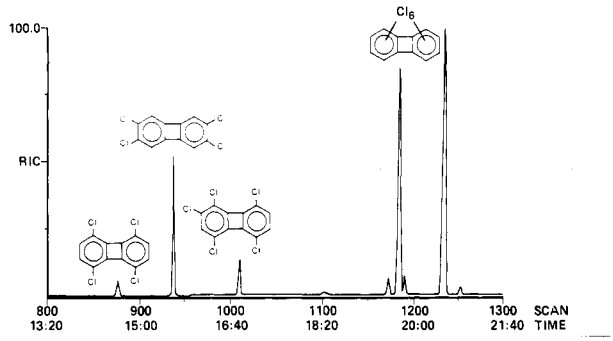


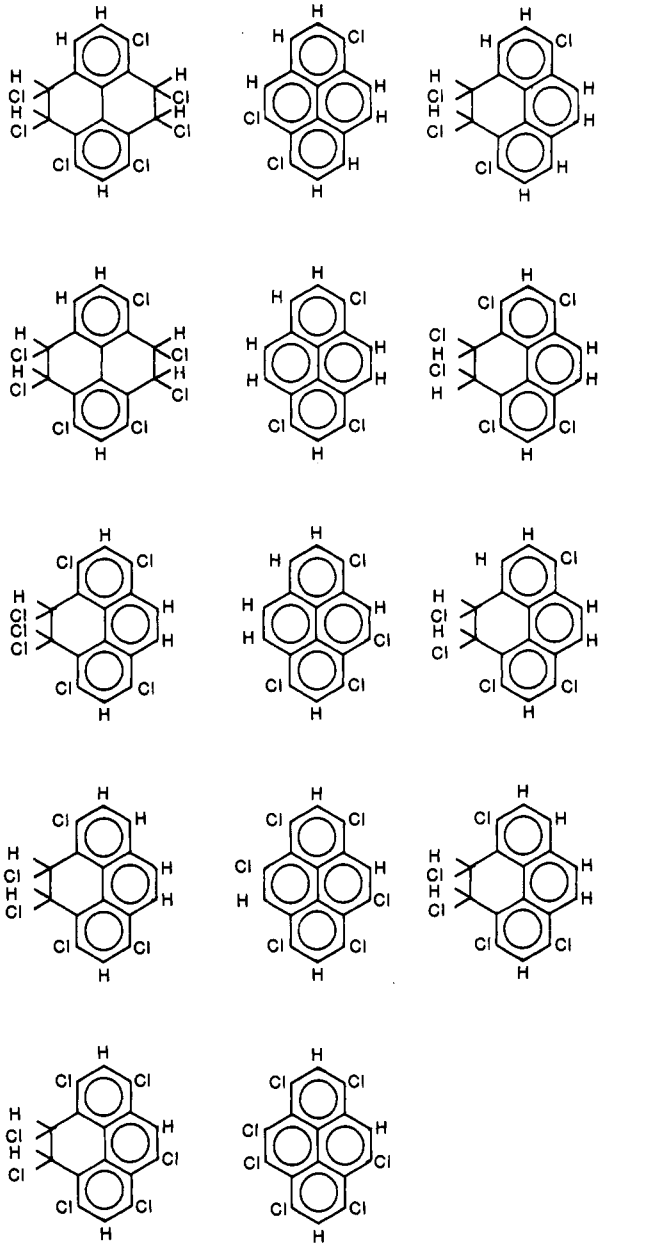
Figure 10. Chromatogram of photolysis products of OCBP with TCBP added. Conditions are given in Figure 4.

products were eluted from silica gel in four 50-mL hexane fractions. The residues were subjected to RPLC on an ODS column and eluted with 87% to 100% methanol in water, with the results from one of these fractions shown in Figure 9. The various HPLC peaks from the four fractions were collected and submitted to NMR analysis. The structures were assigned after we determined that we could recognize NMR proton signals influenced by proton environments in the same ring, as well as by influences from adjacent rings. The technique, which uses longitudinal relaxation rates, makes the unequivocal assignment of these compounds possible (*18*). The various chlorinated products of pyrene which have been isolated from the four fractions are given in Table I along with the structural assignments based on NMR (*18*).

On GC the chloro addition/substitution derivatives of pyrene apparently lose HCl in the injector and chromatograph poorly (*19*). Because GC/MS is the method of choice for analytical determinations in environmental incidents involving chlorinated aromatics, the less stable products like these may be overlooked, although they may pose a more serious health hazard than the stable products (*19*). Liquid chromatography/mass spectrometry might be a better procedure for analyzing for these unstable (hot GC injection ports) compounds.

Ultraviolet Photolysis. In general, the carbon atoms that are least favored to be chlorinated in the reactions described above remain chlorinated during the ultraviolet photolysis of the higher chlorinated analogues (*16*). In this way ultraviolet photolysis of the various perchlorinated compounds can lead to congeners which are not produced in the chlorination reaction of the unchlorinated precursors. Figure 10 shows the GC/MS analysis of a standard of biphenylenes which is a mixture of the photolysis products of octachlorobiphenylene and the major tetrachlorobiphenylene (2,3,6,7-TCBP) produced by chlorination of biphenylene. The structural assignment of the 2,3,6,7-TCBP isomer is based on the use of ^1H NMR longitudinal relaxation times (*20*). The NMR spectrum of this compound consists of a single proton resonance which indicates that the four protons in the molecule must be equivalent. There are two possible isomers of TCBP that fit this description, 1,4,5,8- and 2,3,6,7-TCBP. Because of the difference in substitution patterns, the protons in 1,4,5,8-TCBP have ortho-proton neighbors and the protons in 2,3,6,7-TCBP do not. If the unknown compound is

Table I. Structures of Chloro Derivatives of Pyrene



1,4,5,8-TCBP, the relative relaxation time should be in the range 0.56–0.99. If the compound is 2,3,6,7-TCBP, the relative relaxation should be within the range 1.54–1.96 (*20*). The relative relaxation time for the biphenylene compound was determined to be 1.74 which indicates that the biphenylene produced by chlorination is 2,3,6,7-TCBP. The structural assignment for the other tetra isomer and the pentachlorobiphenylene shown in Figure 10 must remain tentative since these congeners have not yet been isolated from the photolysis mixture and analyzed by NMR. The tentative structural assignments are based on the analogous photolysis of octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran. These two compounds preferentially lost the lateral chlorine atoms during UV photolysis (*16*).

CONCLUSION

In most cases, with this simple procedure, if precursors are at hand the required reference compounds can be synthesized and purified for analysis of a pertinent sample the same day it is received.

For laboratories engaged mainly in the analysis of environmentally persistent compounds of high toxicity, with

equipment generally available to analytical laboratories, simple chlorination and dechlorination reactions can produce a number of compounds, normally expensive or commercially unavailable, for reference material in GC/MS.

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LITERATURE CITED

(1) Rappe, C.; Marklund, S.; Berqvist, P.-A.; Hanson, M. *Chlorinated Dioxins and Dibenzofurans in the Total Environment*; Choudhary, G., Keith, L. H., Rappe, C., Eds.; Butterworth: Stoneham, MA, 1983; Chapter 7.
(2) Poland, A.; Glover, E.; Kende, A. S. *J. Biol. Chem.* **1976**, *251*, 4936-4940.
(3) Williams, C. H.; Prescott, C. L.; Stewart, P. B.; Choudhary, G. *Chlorinated Dioxins and Dibenzofurans in the Total Environment II*; Keith, L. H., Rappe, C., Choudhary, G., Eds.; Butterworth: Stoneham, MA, 1985; Chapter 32.
(4) Smith, L. W.; Stalling, D. L.; Johnson, L. *Anal. Chem.* **1984**, *56*, 1830-1834.
(5) Patterson, D. G.; Holler, J. S.; Smith, S. J.; Liddle, J. A.; Sampson, E. J.; Needham, L. L. *Chemosphere* **1986**, *15*, 2055-2060.
(6) Ryan, J. J.; Schecter, A. *Chlorinated Dioxins and Dibenzofurans in the Total Environment III*; Rappe, C., Choudhary, G., Keith, L. H., Eds.; Lewis: Chelsea, MI, 1986; Chapter 1.
(7) Schecter, A.; Tiernan, T. O.; Taylor, M. L.; VanNess, G. F.; Garrett, J. H.; Wagel, D. J.; Gitlitz, G.; Bogdasarian, M. *Chlorinated Dioxins and Dibenzofurans in the Total Environment II*; Keith, L. H., Rappe, C., Choudhary, G., Eds.; Butterworth: Stoneham, MA, 1985; Chapter 18.
(8) Gray, A. P.; Cepa, D. P.; Solomon, I. J.; Aniline, O. *J. Org. Chem.* **1976**, *41*, 2435-2437.
(9) Taylor, M. L.; Tiernan, T. O.; Ramalingam, B.; Wagel, D. J.; Garrett, J. H.; Salch, J. G.; Ferguson, G. L. *Chlorinated Dioxins and Dibenzofurans in the Total Environment II*; Keith, L. H., Rappe, C., Choudhary, G., Eds.; Butterworth: Stoneham, MA, 1984; Chapter 2.
(10) Nestrick, T. J.; Lamparski, L. L.; Stehl, R. H. *Anal. Chem.* **1979**, *51*, 2283-2276.
(11) Bell, R. A. *Chlorinated Dioxins and Dibenzofurans in the Total Environment*; Choudhary, G., Keith, L. H., Rappe, C., Eds.; Butterworth: Stoneham, MA, 1983; Chapter 4.
(12) Safe, S. H.; Safe, L. M. *J. Agric. Food Chem.* **1984**, *32*, 68-71.
(13) Bell, R. A.; Gara, A. *Chlorinated Dioxins and Dibenzofurans in the Total Environment II*; Keith, L. H., Rappe, C., Choudhary, G., Eds.; Butterworth: Stoneham, MA, 1983; Chapter 1.
(14) Gelbaum, L. T.; Patterson, D. G., Jr.; Groce, F. G. *Chlorinated Dioxins and Dibenzofurans in Perspective*; Rappe, C., Keith, L. H., Choudhary, G., Eds.; Lewis Publisher: Chelsea, MI, 1986; Chapter 31.
(15) Hutzinger, O.; Safe, S.; Zitko, V. *Int. J. Environ. Anal. Chem.* **1972**, *2*, 95-106.
(16) Barnhart, E. R.; Patterson, D. G., Jr.; MacBride, J. A. H.; Alexander, L. R.; Alley, C. A.; Turner, W. E. *Chlorinated Dioxins and Dibenzofurans in Perspective*; Rappe, C., Choudhary, G., Keith, L. H., Eds.; Lewis: Chelsea, MI, 1986; Chapter 33.
(17) Mullin, M. D.; Pochini, C. M.; McCrindle, S.; Romkes, M.; Safe, S. H.; Safe, L. M. *Environ. Sci. Technol.* **1984**, *18*, 468-476.
(18) Ashley, D. L.; Barnhart, E. R.; Patterson, D. G., Jr.; Hill, R. H. *Appl. Spectros.*, in press.
(19) Colmsjo, A.; Rannug, A.; Rannug, U. *Mutat. Res.* **1984**, *135*, 21-29.
(20) Ashley, D. L.; Barnhart, E. R.; Patterson, D. G., Jr.; Hill, R. H., submitted for publication in *Anal. Chem.*

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Single Potential-Alteration Surface Infrared Spectroscopy: Examination of Adsorbed Species Involved in Irreversible Electrode Reactions

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The feasibility of obtaining potential-difference infrared spectra for reactive electrochemical adsorbates with a Fourier transform infrared (FTIR) spectrometer using only a single potential alteration during the spectra data acquisition is illustrated and applied to examining the electrooxidation of carbon monoxide and formic acid via adsorbed CO at platinum–acidic aqueous interfaces. The form of such SPAIR (single potential-alteration infrared) spectra for such irreversible electrode reactions is compared with corresponding data obtained under more conventional conditions that utilize multiple potential steps between the base and sample potentials during the spectral acquisition. Several advantages of the former approach are apparent in these data, including information on the nature and extent of CO_2 product formation and the electrochemical reactivity of the adsorbed CO. The capability of obtaining SPAIR spectra for such adsorbed species within ca. 5 s or less is noted, and possible electrochemical kinetic applications are pointed out.

External reflectance infrared spectroscopy has recently emerged as a major technique for the in situ molecular characterization of electrode–solution interfaces (*1*, *2*). Besides the use of polarization modulation, the necessary subtraction of spectral interferences arising from the bulk solution is achieved by means of potential-difference methods, taking advantage of the sensitivity of the electrochemical surface structure to the electrode potential. Such potential-difference spectra are usually obtained in two ways (*1*, *2*). The first, termed "EMIRS" (electrochemically modulated infrared spectroscopy), involves relatively rapid (ca. 10 Hz) potential modulation between the chosen "base" and "sample" values while the infrared wavelength is scanned slowly with a grating spectrometer. The alternative approach, dubbed "SNIFTIRS" (subtractively normalized interfacial Fourier transform infrared spectroscopy), involves recording a series of interferometer scans sequentially at the base and sample potentials, data sets then being substracted from each other to yield the potential-difference infrared (PDIR) spectra (*1*, *2*).

While the former technique necessarily requires a large number of potential modulations in order to acquire a spectrum over a suitably wide frequency range, the latter method in principle only requires that a single potential alteration, from the base and sample potentials, be applied during the spectral data acquisition. Indeed, SNIFTIR spectra can often readily be obtained in this manner for electrogenerated so-

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