ISOMER IDENTIFICATION OF CHLORINATED DIBENZO-p-DIOXINS BY ORTHOGONAL SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT

Isomer differentiation of chlorinated dibenzo-p-dioxin isomer pair components was examined by three orthogonal (gas chromatography, high performance liquid chromatography, and micellar electrokinetic chromatography) chromatographic and three orthogonal (Fourier transform infrared, carbon-13 and proton nuclear magnetic resonance) spectroscopic techniques. These six independent methods used in analysis of the same samples produced a general agreement in isomer pair assignments.

INTRODUCTION

Chlorinated dibenzo-p-dioxin (CDD) congeners and isomer groups have been separated by a variety of chromatographic techniques[1-6], and characterized by several spectroscopic methods[7-13] with isomer structures assigned by each method. X-ray crystallography, the definitive technique for assigning isomer structure, has been restricted by difficulties in growing usable crystals to the extent that crystal structures have been reported for only eight of the 75 possible dibenzo-p-dioxin congeners. Although isomer assignments have been designated in some publications with no indication of a systematic method for arriving at isomer structure, several studies employing systematic methods of isomer structural assignment have generated contradictory or inconclusive results[6,7,10,13] when determinations of isomer pair percentages were compared.

Early systematic gas chromatographic (GC) assignments were based on mass

spectral (MS) analysis and chromatographic retention times for pyrolysis and photolysis products[1,2]. Subsequent GC studies have resulted in CDD retention indices[5] and structural assignments based on Fourier transform infrared (FTIR) or mass spectral detection, with chromatography parameters employed as the primary factors for structure determination. Recently, a systematic reversed-phase high performance liquid chromatography (HPLC) method was used for analysis of all 75 CDD congeners and for identification of isomer pair components based on elecron donor and acceptor properties of each of four stationary phases[6].

Systematic spectroscopic methods for identification of CDD isomers have been developed using gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR)[7-11], proton nuclear magnetic resonance (^{1}H NMR)[12], and carbon-13 nuclear magnetic resonance (^{13}C NMR)[13]. Each of these methods evaluates isomer structure by orthogonal molecular parameters. In this work,isomer pair component percentages obtained by several independent techniques (GC/MS, HPLC, GC/FTIR, ^{1}H NMR and ^{13}C NMR) on the same samples are compared with each other and with gamma-cyclodextrin/micellar electrokinetic chromatography (γ-CD/MEKC) results.

EXPERIMENTAL

Isomer preparation. The CDD isomer pairs were prepared at the Centers for Disease Control. The isomers were the reaction products of dipotassium salts of chlorinated catechols with chlorinated nitrobenzenes in anhydrous dimethylsulfoxide[12].

GC/FTIR Instrumentation. A Nicolet(Madison, WI) Model 170SX Fourier transform infrared spectrometer equipped with an array processor and a mercury-cadmium-telluride (MCT) detector was used for all GC/FTIR measurements.Chromatographic separations were performed by a Hewlett-Packard (Palo Alto, CA) Model 5880A containing a J&W Scientific (Rancho Cordova, CA) fused silica capillary column with a 1μ DB5 film.

NMR Instrumentation. Carbon-13 NMR spectra were obtained with a Varian XL-300 (Palo Alto, CA) spectrometer equipped with an XL data system and a 7.0 T superconducting magnet. Samples (100-150 μg) of each isomer pair mixture were examined in approximately 0.5 mL of acetone-d_6 at 30.0 °C. Chemical shifts were calculated relative to TMS by referencing the residual acetone signal at 2.050 ppm.

MEKC Instrumentation. Separation of CDD isomer groups was accomplished by means of a Spectra-Physics (Palo Alto ,CA) Phoresis 1000 equipped with a variable temperature oven, a UV detector and a 44cm x 50μ fused silica capillary column. The buffer consisted of 100mM borate, 100 mM SDS, 5M urea, and 40 mM γ-CD at pH 9. Separations were generally accomplished at 15 kV and 15.0°C.

RESULTS AND DISCUSSION

Percentages of representative isomer pairs obtained by the six orthogonal methods are presented in Table 1. The GC/MS assignments were based on projected structure/retention characteristics of the individual isomer pair components as a function of dipole moment. The 3-(p-nitro-phenoxy)propylsilyl

TABLE 1. Isomer Pair Component Percentages Determined By Orthogonal Chromatographic and Spectroscopic Techniques

Isomer Pair	Isomer pair Percentages GC/MS	HPLC[6] (NPO)	CD/MEKC	GC/FTIR	^{13}C NMR[13]	^{1}H NMR[12]
12	16	16**	16	31*	21	
14	84	84	84	69	79	
16		75	74		77	
19		25	26		23	
17		69	72	71*	70	
18		31	28	29	30	
126		78	77	75	77	
129		22	23	25	23	
127		71	74	72	61	
128		29	26	28	39	
136	58	58	56	60		
139	42	42	44	40		
1236	67	81	81	83	69*	76
1239	33	19	19	17	31	24
1237		65	61	62	63	61
1238		35	39	38	37	39
1267	81	81	78	86	74	81
1289	19	19	22	14	26	19
1247		55	55	57[6]	55	44
1248		45	45	43	45	56
12368	57	57	58	57		
12379	43	43	44	43		
123679		52		55		
123689		48		45		

(NPO) data[6] was selected for reversed-phase HPLC isomer assignments since the NPO column (the most polar of the HPLC columns used) separated all the CDD isomer pairs. GC/FTIR assignments for DB-5 separated isomer pairs were based on aromatic skeletal stretching frequencies [$Vcc_{(arom)}$] and ether linkage asymmetric stretching frequencies [$Vcoc_{(asym)}$] that have been quantitatively correlated with chlorine substitution patterns[7-11]. Proton NMR isomer differentiation was based on chemical shift and coupling parameters.[12] Carbon-13 NMR CDD isomer pair assignments were established from empirically derived steric, inductive and delocalization perturbations of model ring chemical shift patterns[13].

Isomer pair structural assignments (Table 1.) determined for the same samples by the orthogonal techniques employed show an excellent correspondence in terms of isomer pair percentages. Isomer pairs resulting from different samples are designated by an asterisk. The NPO data for the 1,2-/1,4-dibenzo-p-dioxin pair percentages in Table 1. show a reversal of the percentages originally determined by HPLC[6] because a different sample was used. Results from the samples analyzed show that isomer assignments by the various orthogonal chromatographic and spectroscopic methods are systematically consistent and show an excellent agreement in terms of isomer structural assignments and percentages.

CONCLUSIONS

Orthogonal spectroscopic (Fourier transform infrared, proton and carbon-13 nuclear magnetic resonance) and chromatographic (gas chromatography, liquid chromatography and micellar electrokinetic chromatography) techniques were found to generate consistent results for isomer identification and percentage composition of the chlorinated dibenzo-p-dioxin isomer pairs examined. The excellent correspondence of the independently determined assignments using a variety of different parameters and approaches substantially increases the confidence level in isomer identification and systematic internal consistency of the individual techniques.

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