A Theoretical/Empirical Model Ring Approach to Approximating Molecular Structure and Structure-Activity Relationships for Chlorinated Dibenzo-p-dioxins by Cyclodextrin-modified Micellar Electrokinetic Chromatography and Orthogonal Spectroscopic Techniques.

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INTRODUCTION

The high degree of symmetry of the dibenzo-p-dioxin molecule allows for application of a model ring approach to determine systematic spectroscopic identification of isomer structure [1-5] and to determine approximations of migration order in CDD isomer groups [6]. Carbon-13 chemical shift values and ether linkage asymmetric stretching frequency [Vcoc(asym)] values for CDD isomer pairs have been determined by treating the model ring values as an unperturbed function ($\lambda = 0$) as defined by equation 1, which are subsequently affected by electronic and steric interactions of substituents on the second ring ($\lambda \neq 1$). The effects of these electronic and steric interactions on the carbon

$$\Psi_n = (1 + \lambda)\, \varphi_n^{(0)} + \lambda^2 \varphi_n + \ldots \qquad (1)$$

-13 chemical shifts or ether linkage asymmetric stretching frequencies of the individual components of isomer pairs are determined from the contributions of relevant canonical

$$\psi_0 = \Sigma c_n \phi_n \qquad (2)$$

forms on the specific carbon model ring chemical shift or [Vcoc(asym)] value. In this work, the model ring approach was examined to determine whether model ring values correlate with average capacity factor values to define migration order within an isomer group, and whether partitioning parameters and spectroscopic parameters correlate with dibenzo-p-dioxin toxicity.

EXPERIMENTAL

Cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) was conducted on a Spectraphysics (Palo Alto, CA) Phoresis 1000 system with a variable temperature oven and a variable wavelength detector. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ) used for electrophoretic separations were 50u ID X 44 cm. The 100 mM borate buffer (pH 9) contained 100 mM SDS, 5 M urea and 40 mM gamma cyclodextrin.

Carbon-13 NMR spectra were obtained by means of a Varian XL-300 spectrometer (Palo Alto, California) with an XL data system and a 7.0T superconducting magnet. Samples of each isomer pair mixture were examined in approximately 0.5 mL acetone-d_6 at 30°C. Chemical shifts were calculated relative to TMS by referencing the residual acetone signal at 2.050 ppm.

GC/FTIR spectra were obtained with a Nicolet (Madison, WI) model 170SX FTIR spectrometer equipped with an array processor and a broad band mercury-cadmium-telluride (MCT) detector. Chromatographic separations were performed with a Hewlett-Packard (Palo Alto, CA) model 5880A gas chromatograph.

RESULTS AND DISCUSSION

The 75 chlorinated dibenzo-p-dioxin (CDD) isomers were separated into five isomer/congener groups (the DPD, MCDD and DCDD group, the TrCDD group, the TCDD group, the PnCDD group and the HxCDD, HpCDD, and OCDD group) for cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) separations. The separations of the hydrophobic CDD isomers are the result of differential partitioning between the cyclodextrin phase and the micellar phase with selectivity determined by the relative tendency of the isomer to form a CD cavity inclusion complex [7]. Capacity factors (k') for CD-MEKC are defined by Equation 3. where n_{mc} and n_{CD} are the quantities of the analyte in the micellar and cyclodextrin phases, respectively, where t_R and t_0 are the respective migration times for the analyte and for the bulk solution.

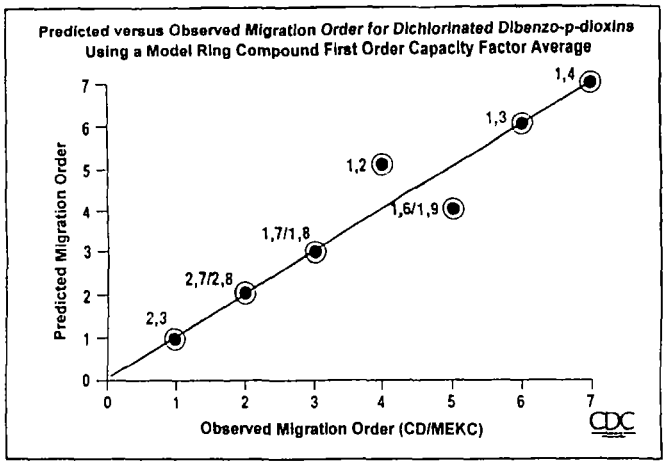
$$k' = \frac{n_{mc}}{n_{CD}} = \frac{t_R - t_0}{t_0} \qquad (3)$$

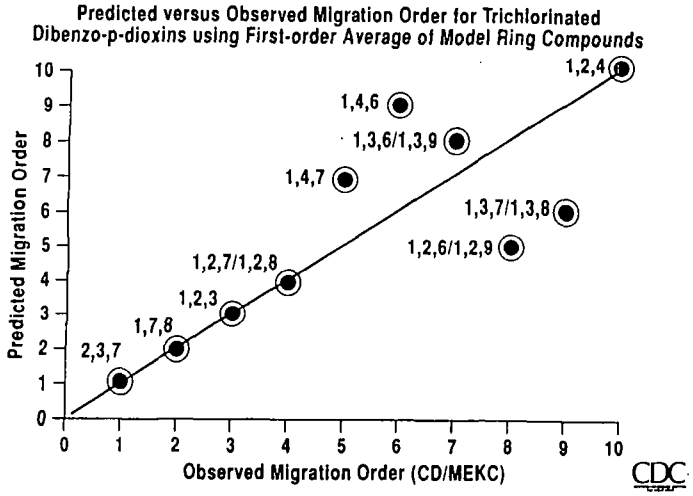
Experimentally determined model ring capacity factors from isomer group mixtures, individual components and from a model ring mixture are presented in Table 1. Migration orders calculated from averaged model ring capacity factor values for CDD isomer pairs are presented in Table 2. The observed migration order for the model ring mixture and for individual model ring compounds is 23>2>DPD>1>123>12>13 >14 >1234>124. The higher mobilities of the laterally substituted (2,3,7 or 8) model ring compounds indicate the promotion of CD complex formation by lateral chlorine substitution while longitudinal (1,4,6, or 9) substitution appears to inhibit complex formation. Calculated capacity factors determined for model ring compounds were

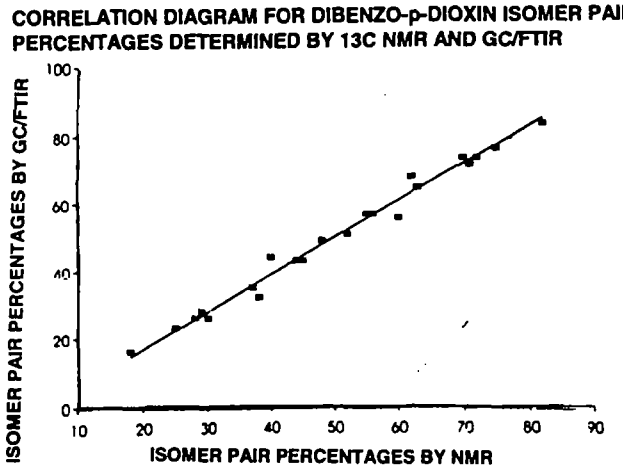
averaged to project first order approximations of migration order for CDD isomer group components. The projected migration orders are generally consistent with observed migration orders for DCDD and TrCDD isomer groups, with deviations attributed to steric interactions between model ring substituents not accounted for in first order approximations. Additional chlorine substitution results in increased steric interactions in TCDD isomers. Inclusion of a steric interaction term is expected to generate a more descriptive model for migration order and for biological structure activity relationships.

Average Capacity Factor Values For Di- and Trichlorinated Dibenzodioxin Isomer Pairs From Model Ring Compound Capacity Factors.

	Capacity Factor $[(k')^{-1}]$ Values			
Isomer	Isomer Group Mixture (Calculated)	Isomer Group Mixture (Observed)	Model Ring Mixture (Calculated)	Weighted Average(Calculated)
23	1.389 (1)	2.564 (1)	.952 (1)	1.430 (1)
27/28	1.020 (2)	.680 (2)	.667 (2)	.684 (2)
17/18	.820 (3)	.520 (3)	.592 (3)	.552 (3)
12	.654 (5)	.485 (4)	.417 (5)	.301 (5)
16/19	.690 (4)	.410 (5)	.535 (4)	.421 (4)
13	.535 (6)	.370 (6)	.350 (6)	.238 (6)
14	.535 (7)	.370 (7)	.342 (7)	.226 (7)
237	1.449 (1)	.704 (1)	.971 (1)	1.470 (1)
178	1.086 (2)	.671 (2)	.826 (2)	1.339 (2)
123	.637 (4)	.454 (3)	.488 (3)	.386 (3)
127/8	.658 (3)	.258 (4)	.422 (4)	.323 (4)
147	.593 (7)	.244 (5)	.345 (7)	.249 (6)
146	.481 (9)	.234 (6)	.325 (9)	.114 (10)
136/9	.483 (8)	.233 (7)	.331 (8)	.235 (7)
126/9	.568 (5)	.232 (8)	.392 (5)	.192 (8)
137/8	.546 (6)	.227 (9)	.353 (6)	.254 (5)
124	.365 (10)	.214 (10)	.267 (10)	.169 (9)







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