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A THEORETICAL/EMPIRICAL VALENCE-BOND APPROACH TO UNAMBIGUOUS STRUCTURE DETERMINATION OF CHLORINATED DIBENZO-p-DIOXINS BY CARBON-13 NUCLEAR MAGNETIC RESONANCE AND FOURIER TRANSFORM INFRARED SPECTROSCOPY

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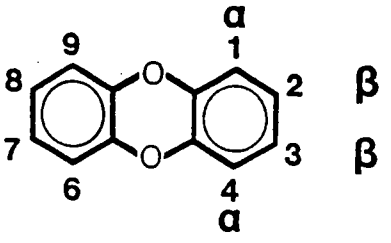
Carbon-13 Nuclear Magnetic Resonance (13C NMR) and Gas Chromatography/ Fourier transform infrared spectroscopy (GC/FTIR) were employed for structural identification of chlorinated dibenzo-p-dioxin (CDD) synthetic mixture components using qualitative steric and delocalization parameters derived from valence-bond theory. The relatively high symmetry of the dibenzo-p-dioxin (DPD) molecule allows for application of a model ring approach where model ring (DPD and CDDs chlorinated on a single ring) chemical shifts and ether linkage asymmetric stretching frequencies [Vcoc(asym)] can be treated as an unperturbed term and electronic and steric interactions from the second ring can be treated as a perturbation. The high degree of symmetry also results in chemical shifts for lateral (2,3,7 and 8), longitudinal (1,4,6 and 9) and heterocyclic carbons appearing in each of three distinctively separate regions of the spectrum. The infrared and 13C NMR spectrum of each isomer examined was unique. Infrared isomer identification was accomplished by examination of characteristic absorption patterns in the aromatic skeletal stretching frequency and ether linkage symmetric and asymmetric regions. Carbon atoms with chlorine substituents are characterized by downfield shifts within the defined region with intensities reduced by longer (relative to C-H) relaxation times. The valence-bond approach was incorporated to evaluate steric, inductive, and delocalization effects on infrared absorption bands and chemical shift, which resulted in a systematic method for FTIR and 13C NMR isomer differentiation of CDD synthetic mixture components.

INTRODUCTION

Chlorinated dibenzo-p-dioxin(CDD) isomer groups containing the toxic laterally (2,3,7 and 8) tetrachlorinated congeners have been the focus of isomer differentiation studies by a variety of analytical techniques employing chromatographically independent methods of structural identification [1-14]. X-ray crystallography[1-7], the definitive method for the determination of molecular structure, encounters difficulties in generation of pure crystals resulting in published structures for only eight of the 75 chlorinated dibenzodioxin congeners. Gas chromatography/Fourier transform infrared (GC/FTIR) spectroscopy[8-12] and proton nuclear magnetic resonance (1H NMR) spectroscopy [13,14] have both been utilized in development of systematic methods for CDD structural assignment. The GC/FTIR approach correlates aromatic skeletal stretching band [Vcc(arom)] absorptions and ether linkage asymmetric

stretching frequencies [Vcoc(asym)] with specific substitution patterns while the 1H NMR approach correlates proton chemical shifts and coupling constants[13] and proton relaxation times[14] with substitution pattern. Although the two techniques are in virtual agreement on isomer pair structural assignments, conflicting results were obtained for the 1,2,4,7-/1,2,4,8-tetrachlorodibenzodioxin isomer pair[9].



Dibenzodioxin numbering system

Carbon-13 Nuclear Magnetic Resonance(13C NMR) spectroscopy has also been used for characterization of CDD congeners[15,16], chlorinated dibenzofurans[17,18], and related aromatic compounds[19]. Kende, et. al.[15] reported 13C NMR spectra for CDD congeners and proposed molecular symmetry and regional chemical shifts as parameters for evaluating molecular structure. Although regional assignment of carbon resonances are of value in structure elucidation, a more definitive method involves correlation of individual aromatic ring carbon resonance patterns with chlorine substitution pattern. In this paper, the valence-bond and perturbation methods are presented as a mathematical foundation for a systematic approach for FTIR and 13C NMR isomer identification of chlorinated dibenzodioxins. The approach was developed using model ring [Vcoc(asym)] values (FTIR) and model ring chemical shifts (13C NMR) as a first approximation, then incorporating qualitative valence-bond parameters to evaluate electronic and steric effects on infrared absorption bands and chemical shift.

EXPERIMENTAL

Instrumentation. Carbon-13 NMR spectra were obtained by means of a Varian XL-300 spectrometer (Palo Alto, California) equipped with an XL data system and a 7.0T superconducting magnet. Samples (100-150 μg) of each CDD congener or isomer pair mixture were examined in ~.5 mL of acetone-d_6 at 30°C. Chemical shifts were calculated relative to TMS by referencing the residual acetone signal at 2.050 ppm.

FTIR spectra were obtained by means of a Nicolet (Madison, WI) model 170SX FTIR spectrometer equipped with an array processor for optimum speed and accuracy of FFT algorithms and with a broad band mercury cadmium telluride (MCT) detector, was used for all GC/FTIR measurements. Chromatographic separations were performed on a Hewlett-Packard (Palo Alto, CA) model 5880A gas chromatograph.

ISOMER PREPARATION. The chlorinated dibenzodioxin (CDD) congeners were prepared at the Centers for Disease Control and Prevention (CDC). The isomers were reaction products of dipotassium salts of chlorinated catechols with chlorinated benzenes or chlorinated nitrobenzenes in anhydrous DMSO. Structures of the synthesis products were verified by GC/FTIR, ^{1}H NMR, and GC/MS. Details of synthesis and purification are discussed by

Gelbaum, et. al.[20]. Dibenzodioxin (DPD), 1- and 2- monochlorodibenzodioxin (MCDD), 2,7- and 2,8-dichlorodibenzodioxin (DCDD), 1,2,3-, 1,2,4-, 2,3,7- and 1,7,8- trichlorodibenzodioxin (TrCDD) were obtained from commercial sources.

THEORY

Resonance theory derives from a qualitative treatment of the quantum mechanical description of a system by a wave equation. One approximate valence-bond method for solution of the wave equation is based on the assumption that the wave function can be expressed as a linear combination of known functions:

$$\Psi o = c_1\phi_1 + c_2\phi_2 + ... + c_n\phi_n = \Sigma c_i\phi_i \qquad (1)$$

where Ξo represents a wavefunction for a specific canonical form (delineated by a laterally stabilized electronic delocalization) and c represents a weighting coefficient. Each wave function corresponds to some definite value E for the energy of the system and can be correlated with delocalization parameters at a specific site. The number of wave mechanics problems with exact solutions is very small, and perturbation theory is one of several approximate methods successfully used to generate solutions. If the wavefunction for the nth state of a system can be expanded as:

$$\Psi_n = \Psi_n^{(o)} + \lambda\Psi_n^{(1)} + \lambda^2\Psi_n^{(2)} + ... \qquad (2)$$

where λ is a perturbation parameter ($0 \le \lambda \le 1$) chosen such that as $\lambda \to 0$, $\Psi_n \to \Psi_n^{(0)}$ and equation (2) can be used for the unperturbed state. The first and second order perturbation terms are represented by $\lambda\Psi_n^{(1)}$ and $\lambda^2\Psi_n^{(2)}$, respectively.

From infrared data, substitution patterns for dibenzo-p-dioxins can be determined from characteristic model ring aromatic skeletal stretching [Vcc(arom)] frequencies and the most intense aromatic skeletal stretching frequency band [Vcc(arom)MAX]. The degree of chlorine substitution can be determined from the [vcoc(asym)/Vcc(arom)] ratio. The identities of specific synthetic isomer pair components may be determined from:

$$Vcoc(asym) = V°coc(asym) + \Sigma nS + F + Q \qquad (3)$$

where V°coc(asym) is the value (cm^{-1}) of the dominant model ring, ΣnS represents the number (n) of laterally stabilized delocalizations (S) among chlorine substituents on the two aromatic rings in the system that affect the partial double bond character of the ether linkage, and F and Q are steric interaction terms that respectively describe nonbonded interaction effects among longitudinal substituents on different rings and the ether linkage(s) and nonbonded interaction effects among longitudinal substituents on the same ring and the ether linkages.

For NMR, the magnetic shielding constant [σ] derives from the influence of chemical effects on the precessional frequency of a nucleus. Contributions from three additive parameters[21-23] are represented by

$$\sigma = \sigma_{[dia]} + \sigma_{[para]} + \sigma_{[N]} \qquad (4)$$

where the shielding parameters represent diamagnetic, paramagnetic, and neigh-boring group anisotropy contributions, respectively. For 13C, the paramagnetic

shielding term ($\sigma_{[para]}$) dominates, and may be expressed as

$$\sigma_{[para]} = [C(\Delta E^{-1})(r^{-3})][Q_{aa} - \Sigma Q_{ax}] \qquad (5)$$

where C is a constant, ΔE is the average value for the electronic excitation energy, r is the distance between a 2p electron and the nucleus, and $[Q_{aa} - \Sigma Q_{ax}]$ represents the charge density/bond order matrix for the 2p electrons[23,24-27].

Karplus and Pople[23] derived an expression for carbon-13 chemical shifts in aromatic molecules by an LCAO-MO approach that predicts a significant difference on the π-electron density, which correlates with sign and magnitude with experimentally determined results. In conjugated systems, contributions from two-electron terms expressed in the same form as the mobile bond order were also found to be significant. A modification of this LCAO-MO approach was used to extend the concept of a variable effective nuclear charge to account for polarization effects in alternant aromatic hydrocarbons[27]. Bond polarization generated by steric repulsion interactions between γ substituents has been found to increase the effective nuclear charge parameter and decrease the value of the (r^{-3}) term [28,29] which results in upfield shifts for the impacted carbons. Extended HMO[30] calculations also predict small charge density increases at sterically perturbed nuclei. For delocalization effects on chemical shift, an upfield or a downfield shift can result from deviations from classical perfect pairing bond structures as a function of electron spin correlations associated with bond delocalization. Parallel spin pairing of two electrons centered on the same nucleus reduces the admixture of high energy orbitals which enhance the paramagnetic term and results in an upfield shift[29].

Jameson and Gutowsky [31] generated expressions for $\sigma_{[para]}$ in the LCAO-MO and valence-bond (VB) framework. In the VB approach, the shielding constant is defined in terms of the main valence structure (σ_{11}) and the electronic delocalization term ($\sigma_{[deloc]}$). The advantage of this method derives from the potential for treating deformations in valence structures with respect to the perfect pairing of electrons. For a system containing 2n electrons, the ground state wave function (Ψ_0) may be written as:

$$\Psi_0 = \Sigma c_n \phi_n \qquad (6)$$

where ϕ represents a set of canonical forms for the singlet wave functions where the independent valence-bond structures are

$$\phi_n = 2^{-n/2}\Sigma(-1)^R [(2n)!]^{-1/2} \Sigma(-1)^P Pa(1)\beta(1)w(2)\alpha(2) \qquad (7)$$

In this representation, α correlates with a positive spin, β correlates with a negative spin, P operates on the (2n!) permutations of the electrons among the orbitals and their associated spins, and R is associated with the 2^n interchanges of α and β for the pairs of orbitals which are bonded together in the structure. The orbitals a and b are considered to be on the nucleus under consideration, w and x are located at another site in the molecule.

Qualitative application of these concepts to CDD isomer differentiation can be accomplished by (1) using empirically determined chemical shift patterns of model rings as first approximation values for assigning chemical shifts for corresponding positions in CDD isomers, (2) factoring in small downfield shifts resulting from model ring interaction effects and (3) evaluating canonical forms involving laterally stabilized delocalizations to determine which positions in

specific isomers are expected to exhibit upfield shifts from electron density increases generated by delocalization and steric interactions.

RESULTS AND DISCUSSION

Carbon-13 NMR spectra and infrared spectra of chlorinated dibenzodioxin congeners show distinctive absorption and chemical shift patterns that vary in predictable ways for each compound examined. Infrared isomer identification was accomplished from Vcc(arom) patterns with Vcoc(asym) values determined from qualitative applications of valence-bond theory and perturbation theory. Carbon-13 NMR isomer identification of chlorinated dibenzodioxins based on molecular geometry and qualitative valence-bond approximations can be accomplished by evaluating electron density differences at corresponding carbon nuclei. Inductive effects, steric effects and laterally stabilized delocalizations were found to predict the chemical shift differences in CDD isomer pairs. Semi-preparative HPLC separations of five CDD isomer pairs established a relative intensity format for distinguishing individual model ring chemical shift patterns in isomer pair mixtures. The ^{13}C NMR determination of synthetic isomer pair component composition is totally consistent with determinations from GC/FTIR.

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