

Isomer Differentiation of Chlorinated Dibenzo-p-dioxins by Carbon-13 Nuclear Magnetic Resonance

J. Grainger, Z. Liu, S. Sirlmanne, V.L. Francis, & D.G. Patterson, Jr.

Centers for Disease Control & Prevention, National Center for Environmental Health, Division of Environmental Health Laboratory Sciences, Atlanta, Georgia, U.S.A.

A systematic method for structural identification of specific compounds within the chlorinated dibenzo-p-dioxin (CDD) series is essential for unambiguous isomer assignment. X-ray crystallography, the definitive method for assigning structure, encounters difficulties in generating pure crystals which have resulted in published results for only eight of the 75 CDD congeners. Gas chromatography/Fourier transform infrared [GC/FTIR] spectroscopy and proton nuclear magnetic resonance (^1H NMR) spectroscopy have both been utilized in development of systematic methods of dibenzodioxin structural assignment. The GC/FTIR approach correlates aromatic skeletal stretching band [$\nu_{\text{cc}}(\text{arom})$] absorptions and ether linkage asymmetric stretching frequencies [$\nu_{\text{oc}}(\text{asym})$] with specific substitution patterns and the ^1H NMR approach correlates proton chemical shifts and coupling constants with substitution pattern. Although the two techniques are in virtual agreement on isomer pair structural assignments, several assignments are still in question.

Carbon-13 nuclear-magnetic resonance (^{13}C NMR) spectroscopy has also been used for characterization of CDD congeners. The relatively high symmetry of the dibenzo-p-dioxin molecule results in the lateral (2, 3, 7, and 8) carbons, longitudinal (1, 4, 6 and 9) carbons, and heterocyclic (11, 12, 13, and 14) carbons appearing in each of three distinctively separate regions of the spectrum. Carbon atoms with chlorine substituents are characterized by downfield shifts within the defined region with reduced intensities generated by longer (relative to C-H) spin-lattice relaxation times. Chemical shifts and relative intensities for dibenzo-p-dioxin (DPD) and 17 chlorinated congeners are presented in the following tables.

Although regional assignment of carbon resonances are of some value in structural assignment, a more systematic method involves correlation of chemical shifts with the substitution pattern. In this approach inductive, steric, and resonance contributions to the chemical shift are quantified. Synthetic

ANA

isomer pairs of chlorinated dibenzo-p-dioxins were separated by prep HPLC and the carbon resonances of the individual isomers were established. A systematic method for ^{13}C NMR isomer differentiation was formulated using the chemical shifts on single rings and incorporating empirically determined modifications introduced by electronic and steric effects. Isomer assignments were compared with FTIR and ^1H NMR results.

Table 1: Fourier Transform ^{13}C NMR for DPD, Mono-, DI, and Trichlorinated Dibenzodioxins in Acetone- d_6 , Chemical Shift (ppm) and Relative Peak Heights

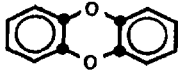
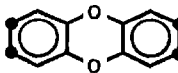
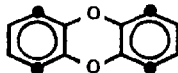
Substitution Pattern			
DPD	141.8 (.18)	123.8 (1.00)	116.0 (.72)
1	142.9 (.21) 141.3 (.23) 141.1 (.21) 138.4 (.18)	124.5 (1.00) 124.2 (.69) 123.7 (.71)	120.5 (.21) 116.3 (.83) 116.1 (.71) 114.8 (.75)
2	142.3 (.21) 141.3 (.18) 140.9 (.09) 140.7 (.10)	127.7 (.16) 124.3 (.38) 124.2 (.35) 123.6 (.37)	117.2 (.35) 116.1 (1.00) 113.1 (.21)
1,3	143.2 (.29) 140.8 (.27) 140.7 (.35)	127.5 (.29) 124.8 (.98) 124.6 (.86) 123.9 (.66)	121.2 (.27) 116.3 (1.00) 116.2 (.95) 115.1 (.75)
2,3	141.2 (.24) 140.8 (.23)	125.8 (.28) 124.5 (1.00)	117.6 (.70) 116.2 (.98)
2,7	142.0 (.41) 140.1 (.54)	128.0 (.29) 123.9 (.88)	117.3 (.98) 116.3 (1.00)
2,8	141.7 (.20) 140.3 (.22)	128.0 (.32) 124.0 (1.00)	117.3 (.80) 116.2 (.97)


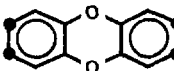
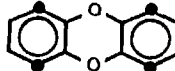
Table 1. Continued

123 ^a	141.5 (.23)	126.5 (.27)	116.4 (.94)
	140.5 (.34)	126.3 (.54)	116.2 (.83)
	139.6 (.70)	125.0 (1.00)	115.8 (.81)
		124.8 (.82)	
178	142.0 (.25)	26.5 (.19)	120.7 (.15)
	140.8 (.30)	126.2 (.28)	117.8 (.76)
	140.6 (.25)	125.1 (1.00)	117.6 (.86)
	139.6 (.28)	124.3 (.96)	115.0 (.92)
237	141.4 (.15)	128.3 (.16)	117.6 (1.00)
	140.8 (.13)	126.3 (.14)	117.4 (.46)
	140.6 (.13)	126.1 (.14)	116.4 (.49)
	139.8 (.13)	124.2 (.43)	
146	141.9 (.21)	125.5 (.88)	120.9 (.31)
	139.6 (.18)	124.8 (.94)	119.6 (.27)
	139.1 (.20)	124.7 (1.00)	119.2 (.25)
	137.5 (2.0)	124.5 (.74)	115.0 (.83)
147	141.2 (.09)	128.7 (.14)	119.3 (.15)
	139.6 (.08)	124.6 (1.00)	119.3 (.11)
	139.1 (.09)	124.4 (.98)	117.5 (.44)
	138.9 (.05)		116.4 (.45)

^aLow intensity peaks not determined or unresolved.

Table 2: Fourier Transform ^{13}C NMR Data For Several Tetrachlorinated Dibenzodioxin Isomers In Acetone- d_6

Chemical Shift (ppm) and Relative Peak Heights

Substitution Pattern			
1278 ^a	140.6 (.42) 140.3 (.47)	127.4 (.44) 126.9 (.33) 126.3 (.40) 124.5 (.90)	119.7 (.37) 117.9 (.86) 117.6 (.86) 115.3 (1.00)
1378 ^a	142.3 (.43) 140.3 (.51) 139.6 (.62)	128.1 (.40) 126.8 (.40) 126.6 (.42) 124.5 (.90)	121.5 (.48) 117.9 (.85) 117.7 (.86) 115.3 (1.00)
1478	140.1 (.36) 138.6 (.23)	126.9 (.32) 124.8 (.81)	119.4 (.38) 117.8 (1.00)
2378	139.6 (1.00)	126.9 (.41)	117.6 (.42)
1234 ^a	140.2 (.34) 139.2 (.17)	126.3 (.12) 125.3 (.86)	119.4 (.12) 116.3 (1.00)
1469	139.6 (1.00)	125.1 (.73)	119.5 (.49)

^aLow intensity peaks not determined or unresolved.