CHARACTERIZATION OF THE INTERNATIONAL HUMIC SUBSTANCES SOCIETY STANDARD AND REFERENCE FULVIC AND HUMIC ACIDS BY SOLUTION STATE CARBON-13 (^{13}C) AND HYDROGEN-1 (^{1}H) NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

U.S. GEOLOGICAL SURVEY

Water-Resources Investigations Report 89-4196

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U.S. GEOLOGICAL SURVEY

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CONVERSION FACTORS

Multiply	*By*	*To obtain*
millimeter (mm)		
milliliter (mL)	0.0002642	gallon
milligram (mg)	0.00003527	ounce, avoirdupois

The following terms and abbreviations also are used in this report:

megahertz (MHz)
hertz (Hz)
parts per million (ppm)

CHARACTERIZATION OF THE INTERNATIONAL HUMIC SUBSTANCES SOCIETY STANDARD AND REFERENCE FULVIC AND HUMIC ACIDS BY SOLUTION STATE CARBON-13 (^{13}C) AND HYDROGEN-1 (^{1}H) NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

By Kevin A. Thorn, Daniel W. Folan,
and Patrick MacCarthy[1]

ABSTRACT

Standard and reference samples of the International Humic Substances Society have been characterized by solution state carbon-13 and hydrogen-1 nuclear magnetic resonance (NMR) spectrometry. Samples included the Suwannee River, soil, and peat standard fulvic and humic acids, the Leonardite standard humic acid, the Nordic aquatic reference fulvic and humic acids, and the Summit Hill soil reference humic acid. Aqueous-solution carbon-13 NMR analyses included the measurement of spin-lattice relaxation times, measurement of nuclear Overhauser enhancement factors, measurement of quantitative carbon distributions, recording of attached proton test spectra, and recording of spectra under nonquantitative conditions. Distortionless enhancement by polarization transfer carbon-13 NMR spectra also were recorded on the Suwannee River fulvic acid in deuterated dimethyl sulfoxide. Hydrogen-1 NMR spectra were recorded on sodium salts of the samples in deuterium oxide. The carbon aromaticities of the samples ranged from 0.24 for the Suwannee River fulvic acid to 0.58 for the Leonardite humic acid.

INTRODUCTION

This report presents solution state carbon-13 (^{13}C) and hydrogen-1 (^{1}H) nuclear magnetic resonance (NMR) spectra that have been recorded on standard and reference samples of the International Humic Substances Society (IHSS). The objective is not to interpret the spectra in great detail, but to expeditiously make the data available to the IHSS in a convenient format. For detailed presentations of NMR theory, the reader is referred to several recent textbooks on NMR spectroscopy: Breitmaier and Voelter (1987), Farrar (1987), Sanders and Hunter (1987), Derome (1987), Wehrli, Marchand, and Wehrli (1988), and Brey (1988). The application of NMR spectroscopy to humic substances has been reviewed by Wershaw and Mikita (1987) and Wilson (1987). These latter two books also discuss the limitations in the quantitative reliability of solid state cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR when applied to humic substances. Further information on the IHSS Suwannee River samples is available in the review by Averett and others (1989).

To obtain solution state ^{13}C NMR spectra that are quantitative, that is, in which peak areas or intensities are in direct proportion to the number of ^{13}C nuclei resonating, two phenomena must be taken into consideration. First,

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differential saturation effects must be eliminated by recording the spectra using a pulse delay that is five times the longest spin-lattice relaxation time (T_1) present in the sample. T_1 is a time constant that is inversely proportional to the rate at which energy is transferred from the spins to the lattice, allowing the magnetization to return to its equilibrium value after a radio frequency pulse (Wehrli and Wirthlin, 1980). If too short a pulse delay is used, then ^{13}C nuclei that have shorter T_1's are exaggerated in intensity when compared to ^{13}C nuclei that have longer T_1's. Second, differential nuclear Overhauser enhancement (NOE) effects must be eliminated. This elimination is accomplished by employing inverse-gated decoupling, in which the decoupler is on during the acquisition time but off during the pulse delay. The NOE effect occurs because proton decoupling causes changes in the population distribution of the ^{13}C spins, resulting in an enhancement of the ^{13}C signals (Wehrli and Wirthlin, 1980). The signal increase resulting from NOE is equal to $1+\eta$, where η is the NOE factor. When ^{13}C spin-lattice relaxation occurs solely by ^1H-^{13}C dipole-dipole interactions, and within the extreme narrowing limit of fast molecular motion, η reaches its maximum value of 1.988. At its maximum, therefore, the NOE effect can result in a threefold increase in signal intensity ($1+1.988\approx3$).

Even if spectra are acquired under conditions that eliminate differential T_1 and differential NOE effects, problems may still arise with ^{13}C nuclei that are in close proximity to paramagnetic metal ions, stable organic free radicals, or quadrupolar nuclei. Such ^{13}C nuclei can have short spin-spin relaxation times (T_2's) and, therefore, linewidths too broad to be observed. No manipulation of the spectrometer can overcome this latter potential problem. In principle, however, spin-counting experiments (Vassallo, 1987) can be performed to determine whether the problem exists or not. This possibility may be an area of future investigation.

The quantitative distributions of ^{13}C nuclei in the fulvic and humic acids were determined by recording ^{13}C NMR spectra using inverse gated decoupling and pulse delays of sufficient duration to ensure quantitative results. The T_1's of the samples were measured using the progressive saturation method (Freeman and Hill, 1971) to determine the necessary pulse delays. The NOE factors can provide some insight into the relaxation mechanisms of the ^{13}C nuclei in the fulvic and humic acid molecules, and so it is convenient to measure these concurrently with the quantitative carbon distributions. This measurement is done by simply switching the decoupler between the continuous decoupling and the inverse gated decoupling modes when recording the quantitative spectra. The NOE factors are calculated by measuring the differences in peak areas between the two sets of spectra.

Sometimes qualitative information is suppressed in ^{13}C NMR spectra recorded under quantitative conditions. For this reason it also is useful to record spectra under nonquantitative conditions that maximize signal-to-noise. Under conditions of continuous decoupling and short pulse delays, the peak intensities of ^{13}C nuclei that have short T_1's and significant NOE factors are enhanced in comparison to spectra obtained under quantitative conditions. Additionally, because greater signal to noise is obtained in a nonquantitative ^{13}C NMR spectrum, a smaller line broadening can be applied to the free induction decay (FID) resulting in the resolution enhancement of discrete, narrow line resonances.

A number of spectral editing pulse sequences were introduced in the late 1970's and early 1980's. These pulse sequences are usually classified into spin-echo techniques, such as APT (Attached proton test; Patt and Shoolery (1982)), GASPE (Gated spin echo; Cookson and Smith (1983)), and PSCE (Part coupled spin echo; Snape (1983)), or polarization transfer techniques, such as INEPT (Insensitive nuclei enhancement by polarization transfer; Morris and Freeman (1979)) and DEPT (Distortionless enhancement by polarization transfer; Doddrell and others (1982)). APT spectra were recorded on the samples examined in this study. The APT experiment differentiates among methyl, methylene, methine, and quaternary carbons, and therefore allows more specific assignments of the ^{13}C NMR spectra. DEPTGL (GL version of DEPT sequence; Sorensen and others, 1983) spectra also were recorded on the Suwannee River fulvic acid.

EXPERIMENTAL CONDITIONS

NMR Spectrometry

The ^{13}C and ^{1}H spectra were recorded on a Varian* XL-300 NMR spectrometer at 75.4 and 299.9 megahertz (MHz), respectively. All ^{13}C NMR spectra were recorded using a Varian XL Series Generation III 10-millimeter (mm) broadband probe, which was modified to be free of any background carbon signal. For ^{13}C spectra, approximately 200 milligrams (mg) of sample was added to 1.5 milliliter (mL) H_2O and 0.5 ml D_2O, and adjusted to pH 7 with NaOD, in a 10 mm NMR tube. The ^{1}H spectra were recorded using a 5 mm broadband probe. Approximately 35 mg of sample was tirated to pH 8-9 in aqueous solution, and then dried in a rotary-evaporator. The sodium salts were redissolved in 100 percent D_2O, and then dried once again in a rotary-evaporator to exchange deuterium for hydrogen. The deuterium exchange step was repeated a second time, and the sodium salts were again dissolved in 100% D_2O for NMR analysis. In most cases, the residual HOD peaks in the ^{1}H-NMR spectra were narrow enough so homonuclear decoupling of the HOD peaks was unnecessary.

Acquisition Parameters

Progressive saturation experiments were recorded using a 30,000 hertz (Hz) spectral window (SW), 90° pulse width (PW), 0.2 second acquisition time (AT), and continuous decoupling in the WALTZ Modulation mode (CWD). Pulse delays (PD) were arrayed at 0.2, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 seconds. The samples were not degassed to remove dissolved oxygen, which may contribute to ^{13}C relaxation through the electron dipolar relaxation mechanism (Martin and others, 1980).

NOE measurements were recorded using a 50,000 Hz SW, 90° PW, 0.2 second AT, and 10.0-12.0 second PD. The decoupler was switched between the continuous and inverse-gated decoupling modes for each block size of 100 transients. Spectra recorded using inverse gated decoupling represented the quantitative spectra.

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*Use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

Nonquantitative spectra were recorded using a 30,000 Hz SW, 45° PW, 0.2 second AT, 1.0 second PD, and CWD decoupling. Dioxane, assumed to be 67.4 parts per million (ppm), was used as an internal reference standard.

Attached Proton Test spectra were recorded using the pulse sequence described by Patt and Shoolery (1982):

$$(\mathrm{PD} - \mathrm{PW} - \tau(\text{decoupler off}) - 180^\circ - (\tau+\Delta) - 180^\circ - \Delta - \text{acquire})_n$$

The acquisition parameters included a 30,000 Hz SW, and 0.2 second AT. PD was 0.0 seconds, PW was 45°, τ was 7.0 milliseconds, and Δ was 1.0 millisecond. The value of 7.0 milliseconds for τ corresponds to $\tau=1/J$, where J equals 142.5 Hz, the average of the aromatic C-H ($^1J_{CH}$=160 Hz) and aliphatic C-H ($^1J_{CH}$=125 Hz) one bond coupling constants. When τ is set equal to 1/J, methylene and quaternary carbons are positive, and methyl and methine carbons negative.

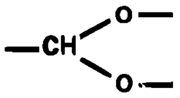
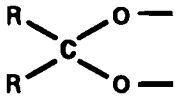
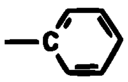
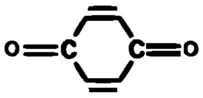
Proton spectra were recorded using an 8,000 Hz SW, 25° PW, 1.9 second AT, and 5.0 second pulse delay. Dioxane, assumed to be 3.6 ppm, was used as an internal reference standard. The line broadenings applied to the FIDS are listed with the individual ^{13}C and ^1H NMR spectra.

DEPTGL spectra of the Suwannee River fulvic acid were recorded using the pulse sequence of Sorensen and others (1983). The GL version of the DEPT sequence was introduced to minimize J-cross talk in edited subspectra where there is significant variation in the values of $^1J_{CH}$. The acquisition parameters included a 30,000 Hz SW, 0.2 second AT, and 2.0 second delay for proton relaxation. Maximum and minimum values of $^1J_{CH}$ were assumed to be 200 Hz and 125 Hz, respectively. Spectra were recorded on 200 mg of the H-saturated sample dissolved in 2.0 g of dimethylsulfoxide-d_6, 99.9 atom percent ^{12}C. The spectra were referenced on the residual dimethylsulfoxide peak at 39.5 ppm.

RESULTS AND DISCUSSION

The ^{13}C NMR spectra of the fulvic and humic acids all exhibit the five major bands characteristic of humic substances. These bands are the aliphatic carbons from about 0 to 60 ppm, the hetero-aliphatic carbons from about 60 to 90 ppm (primarily sp^3 hybridized carbons bonded to oxygens), the aromatic carbons from about 90 to 165 ppm, the carboxyl carbons from about 165 to 190 ppm, and the ketone and quinone carbons from about 190 to 220 ppm. (In aqueous solution, the chemical shift positions of carboxyl and phenolic carbons vary with the degree of dissociation of these functional groups, as a function of pH (Preston and Schnitzer, 1988).) These are general assignments; overlap of many functional groups occurs within these regions. For example, methoxyl carbons occur in the region from about 50 to 62 ppm. Acetal and hemiacetal carbons may overlap with aromatic carbons in the region from 90 to 110 ppm; olefinic carbons also may overlap with aromatic carbons in the region from 110 to 150 ppm. Lactone, ester, and amide carbons may overlap with the carboxyl carbons in the region from 165 to 190 ppm. Chemical structures that occur in these regions are summarized in table 1. Progressive saturation experiments are shown in figures 1 through 10, and the T_1 values estimated from these experiments are listed in table 2.

Table 1.--*Approximate chemical shift ranges for carbon-13 nuclei in parts per million relative to tetramethylsilane (Adapted from Breitmaier and Voelter)*

H_3C-C<	primary carbon	0-30
H_3C-O-		40-60
H_3C-N<		20-45
H_3C-S-		10-30
-CH_2-C<	secondary carbon	25-45
-CH_2-O-		40-70
-CH_2-N<		40-60
-CH_2-S-		25-45
>CH-C<	tertiary carbon	30-60
>CH-O-		60-75
>CH-N<		50-70
>CH-S-		40-55
>C-C	quaternary carbon	35-70
>C-O-		70-85
>C-N		65-75
>C-S		55-70
	acetal carbon	90-100
	ketal carbon	95-105
>C=C<	olefinic carbon	110-150
	aromatic carbon	90-165
-COOR	ester	155-175
-CONHR	amide	160-180
-COOH	carboxylic acid	160-175
-CHO	aldehyde	175-205
	quinone	170-190
-RCOR'	ketone	175-225

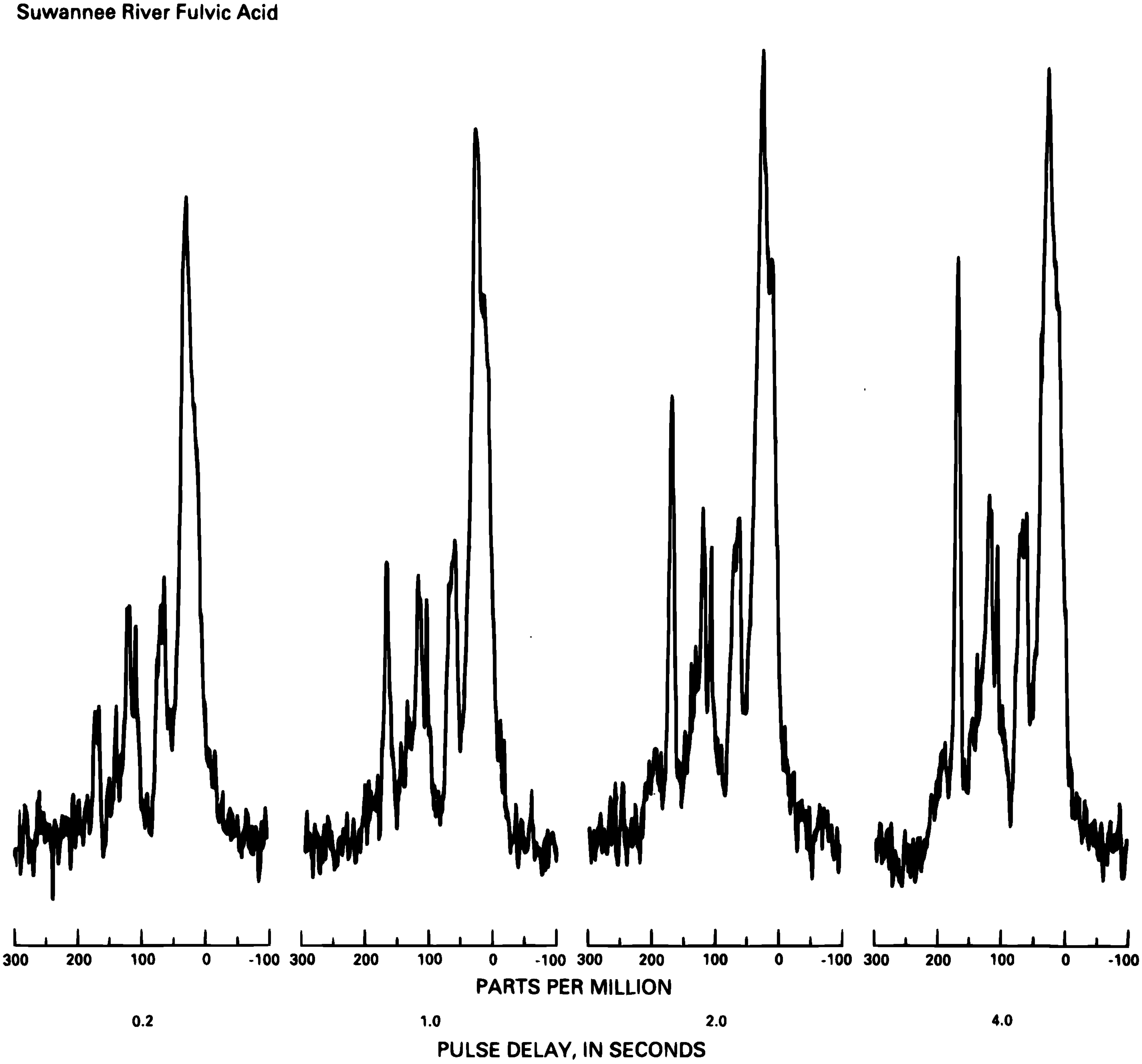


Figure 1.--Progressive saturation experiment for Suwannee River fulvic acid. Line broadening = 100.0 Hertz.

Suwannee River Fulvic Acid

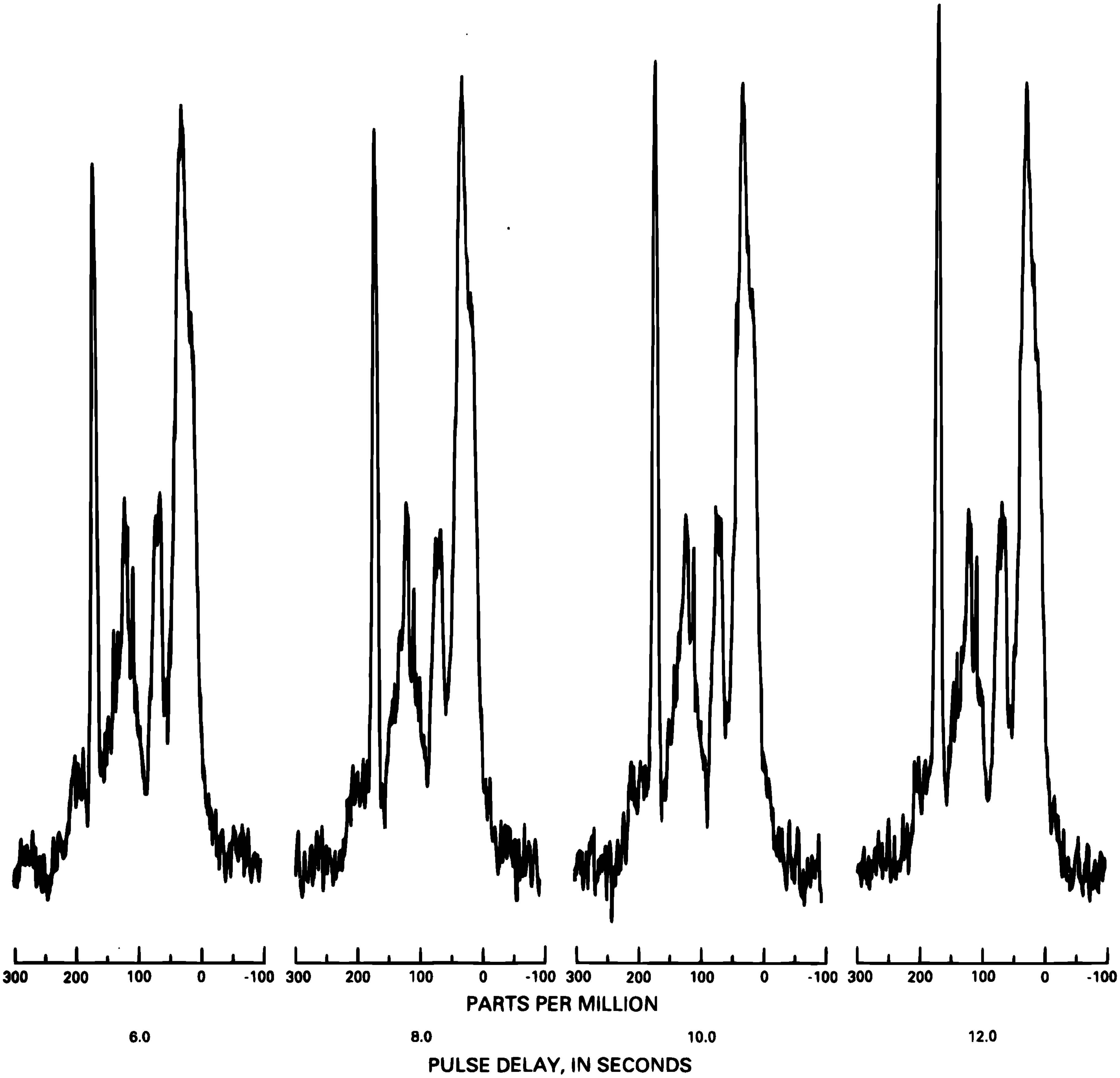


Figure 1.--Progressive saturation experiment for Suwannee River fulvic acid. Line broadening = 100.0 Hertz--Continued.

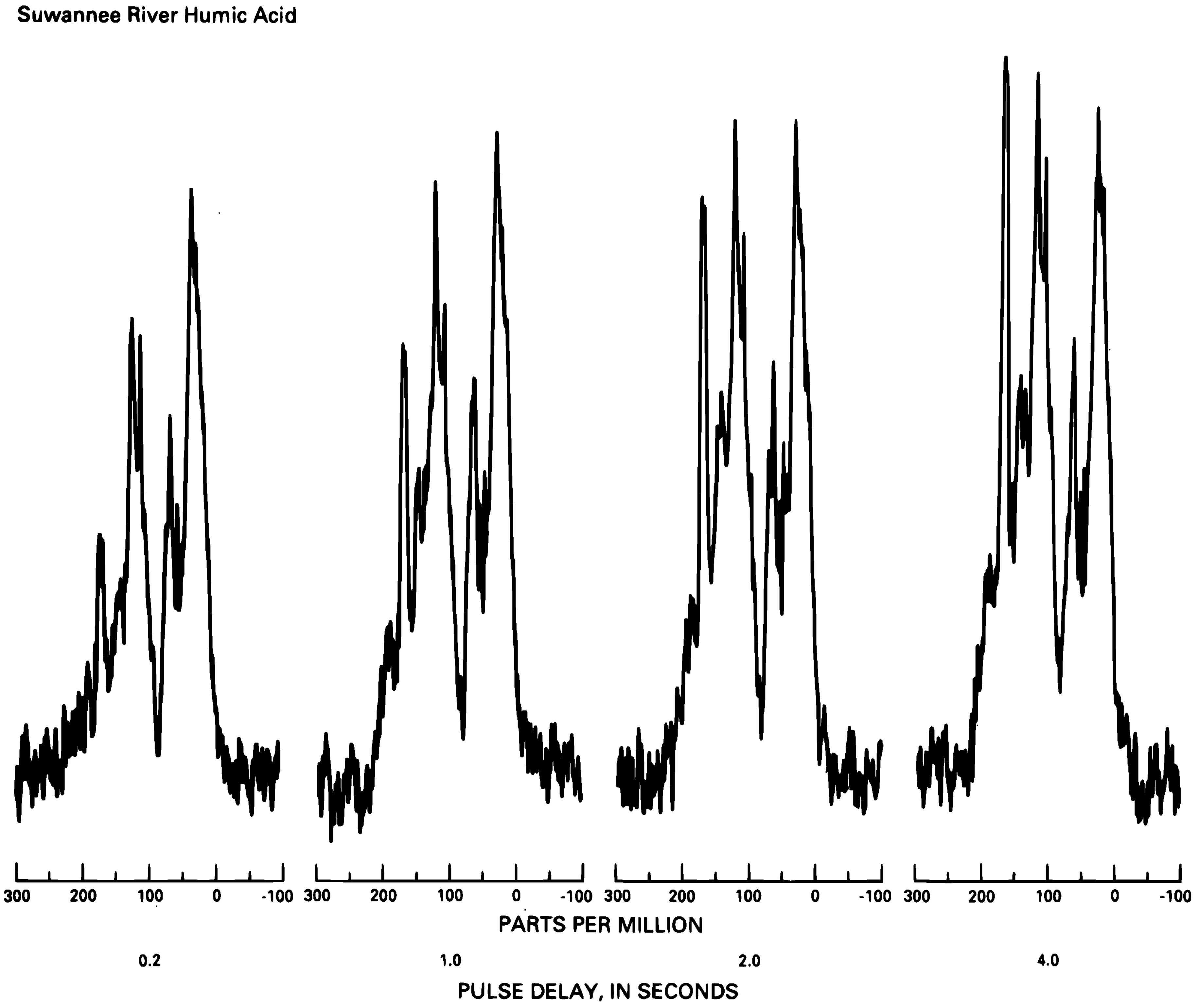


Figure 2.--Progressive saturation experiment for Suwannee River humic acid. Line broadening = 100.0 Hertz.

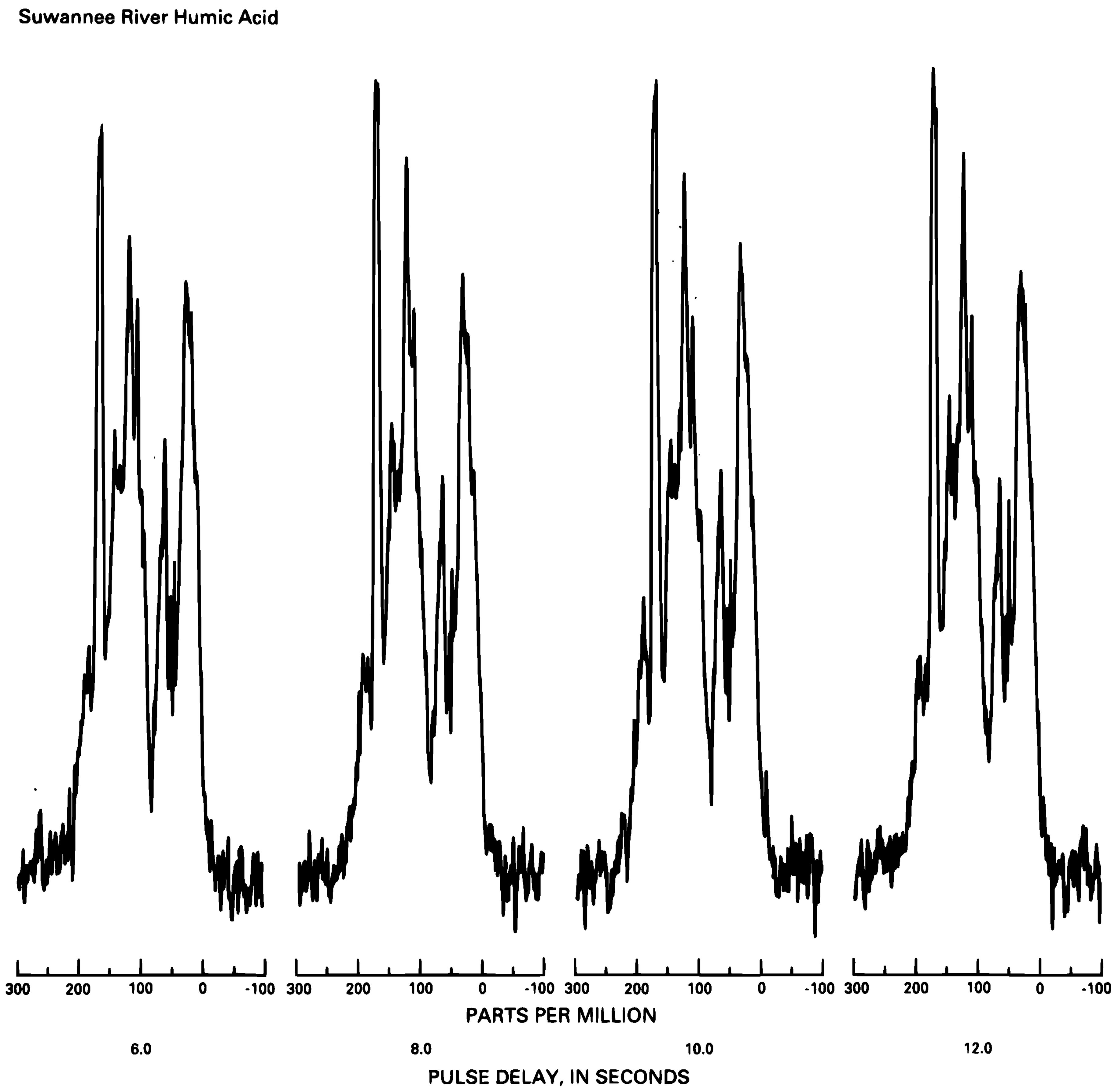


Figure 2.--Progressive saturation experiment for Suwannee River humic acid. Line broadening = 100.0 Hertz--Continued.

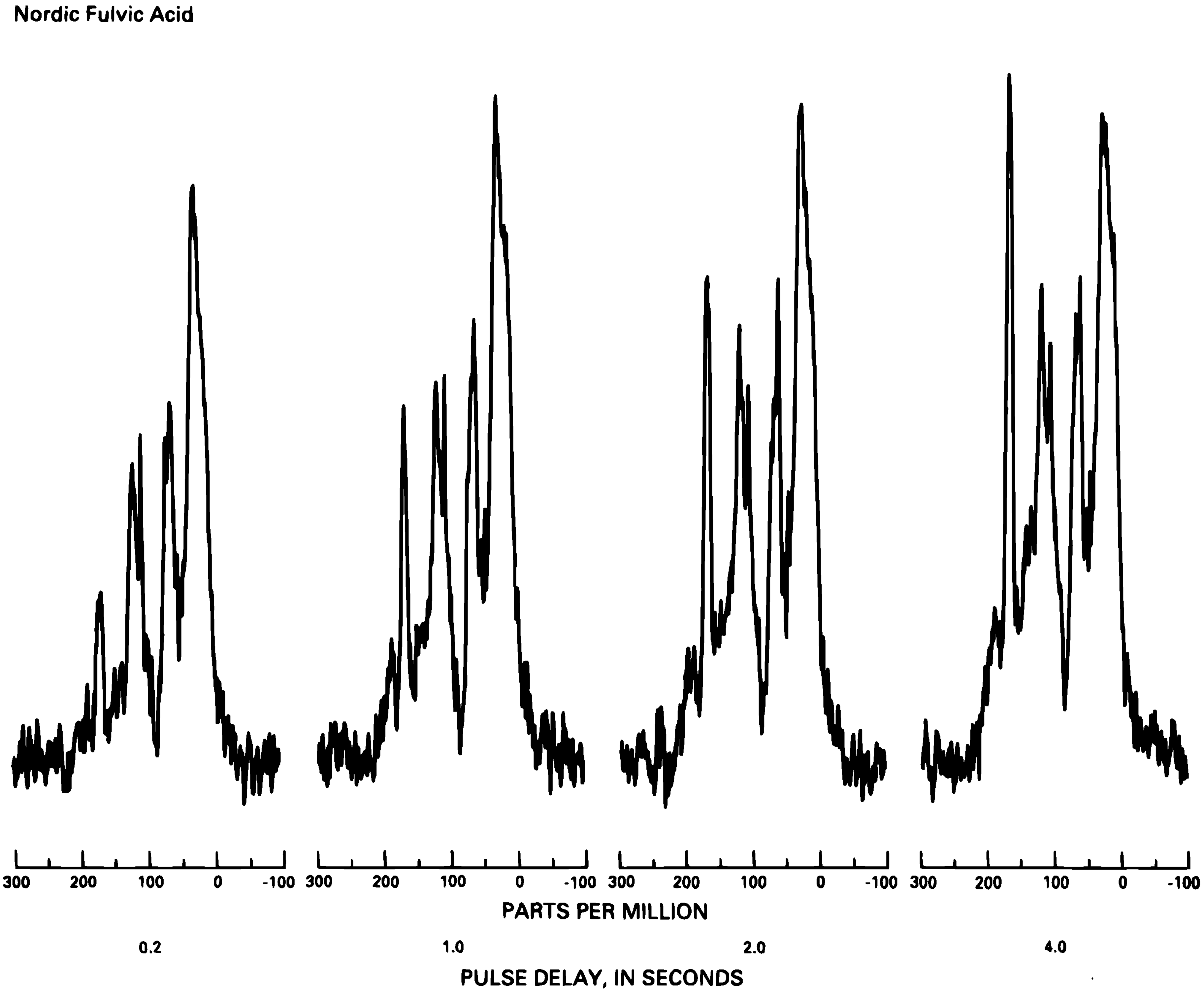


Figure 3.--Progressive saturation experiment for Nordic fulvic acid. Line broadening = 100.0 Hertz.

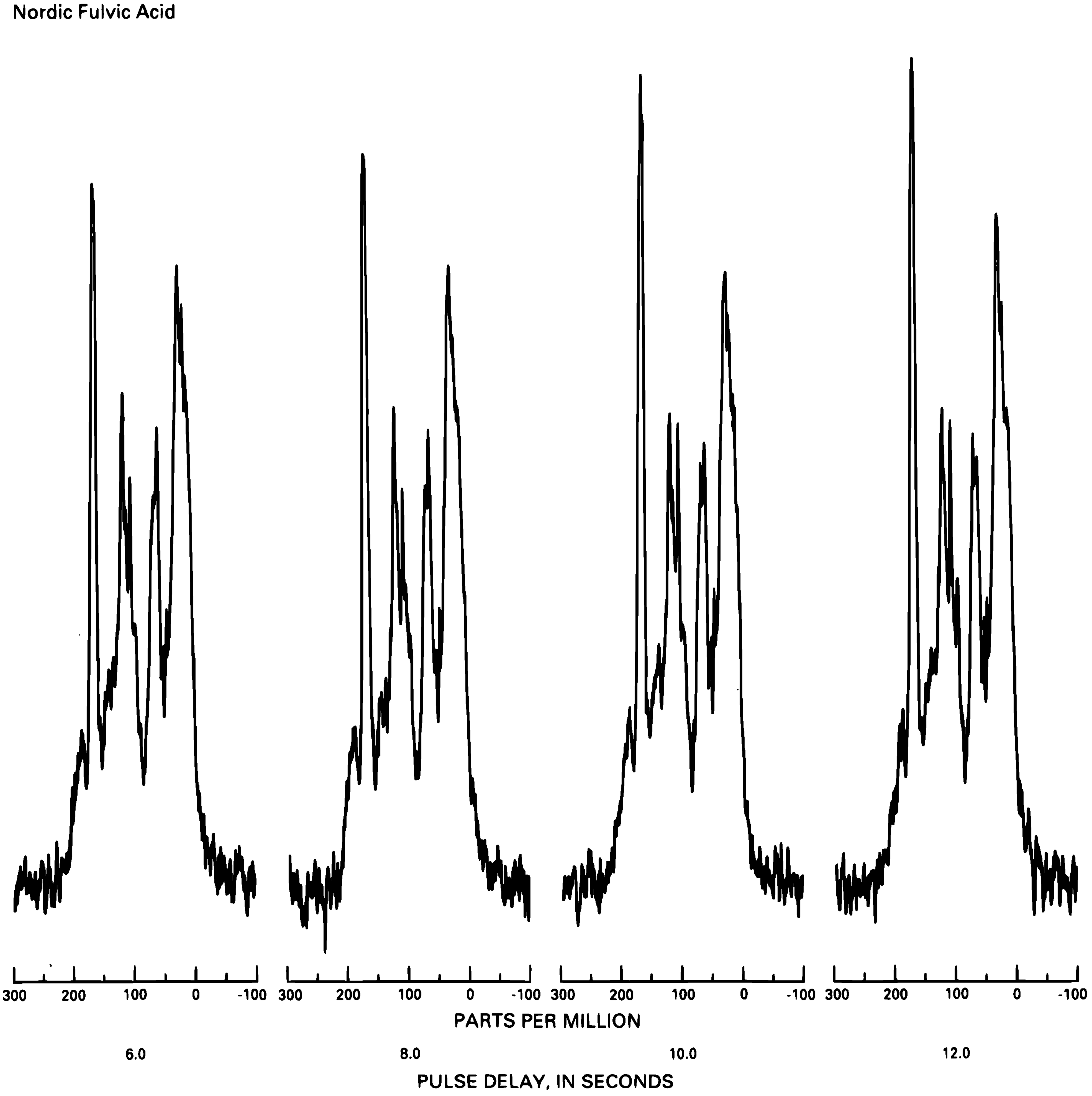


Figure 3.--Progressive saturation experiment for Nordic fulvic acid. Line broadening = 100.0 Hertz--Continued.

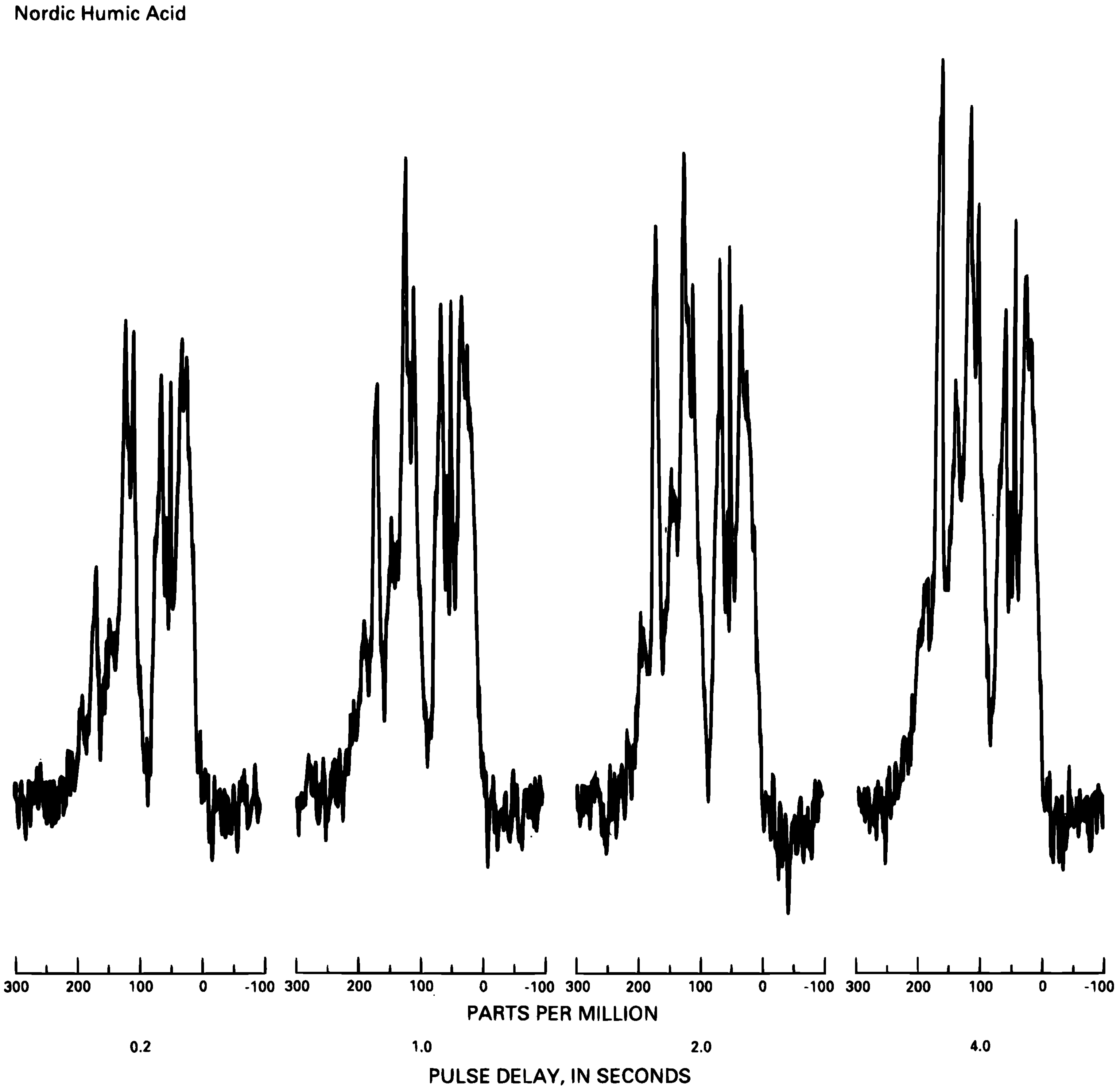


Figure 4.--Progressive saturation experiment for Nordic humic acid. Line broadening = 100.0 Hertz.

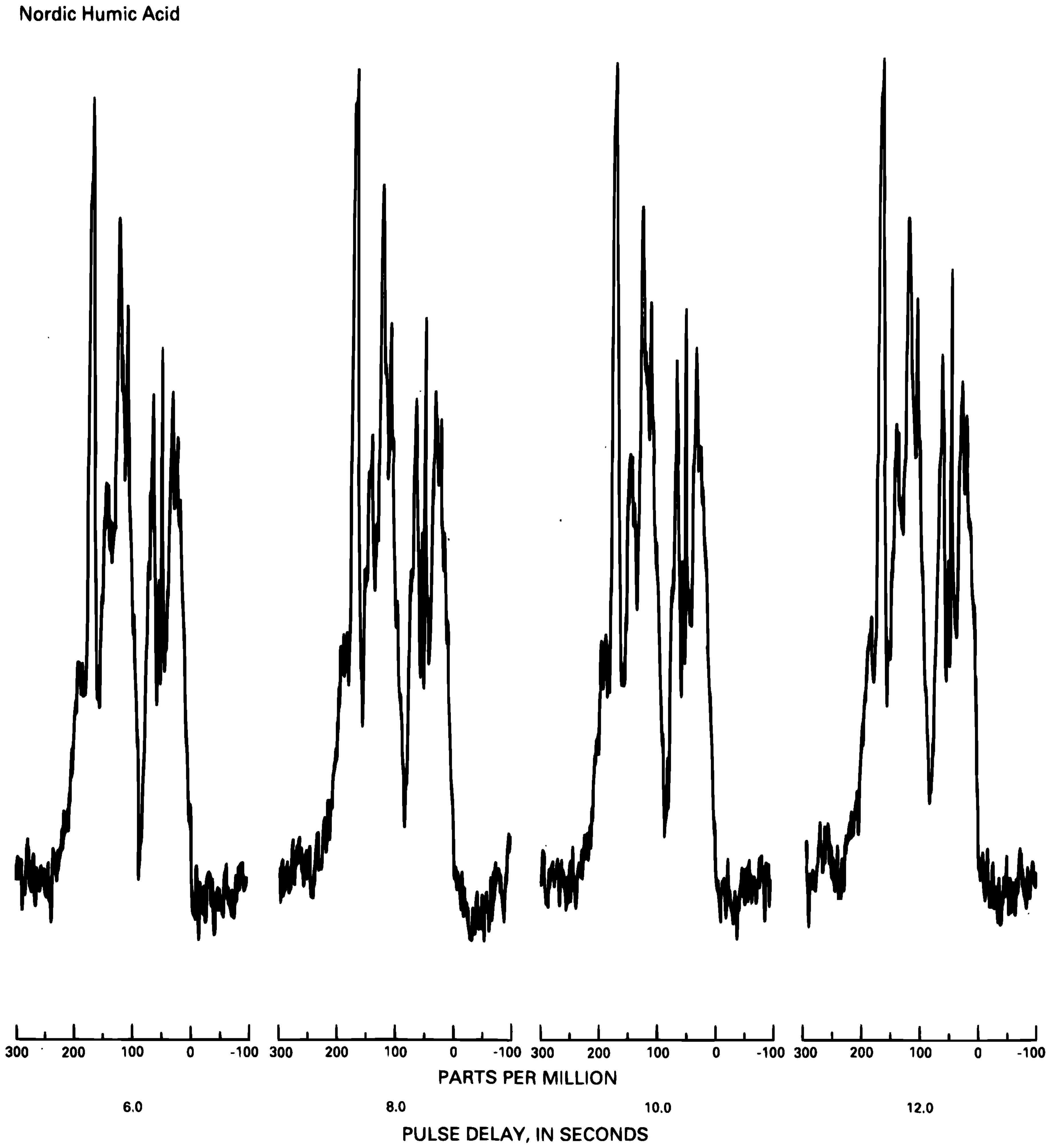


Figure 4.--Progressive saturation experiment for Nordic humic acid. Line broadening = 100.0 Hertz--Continued.

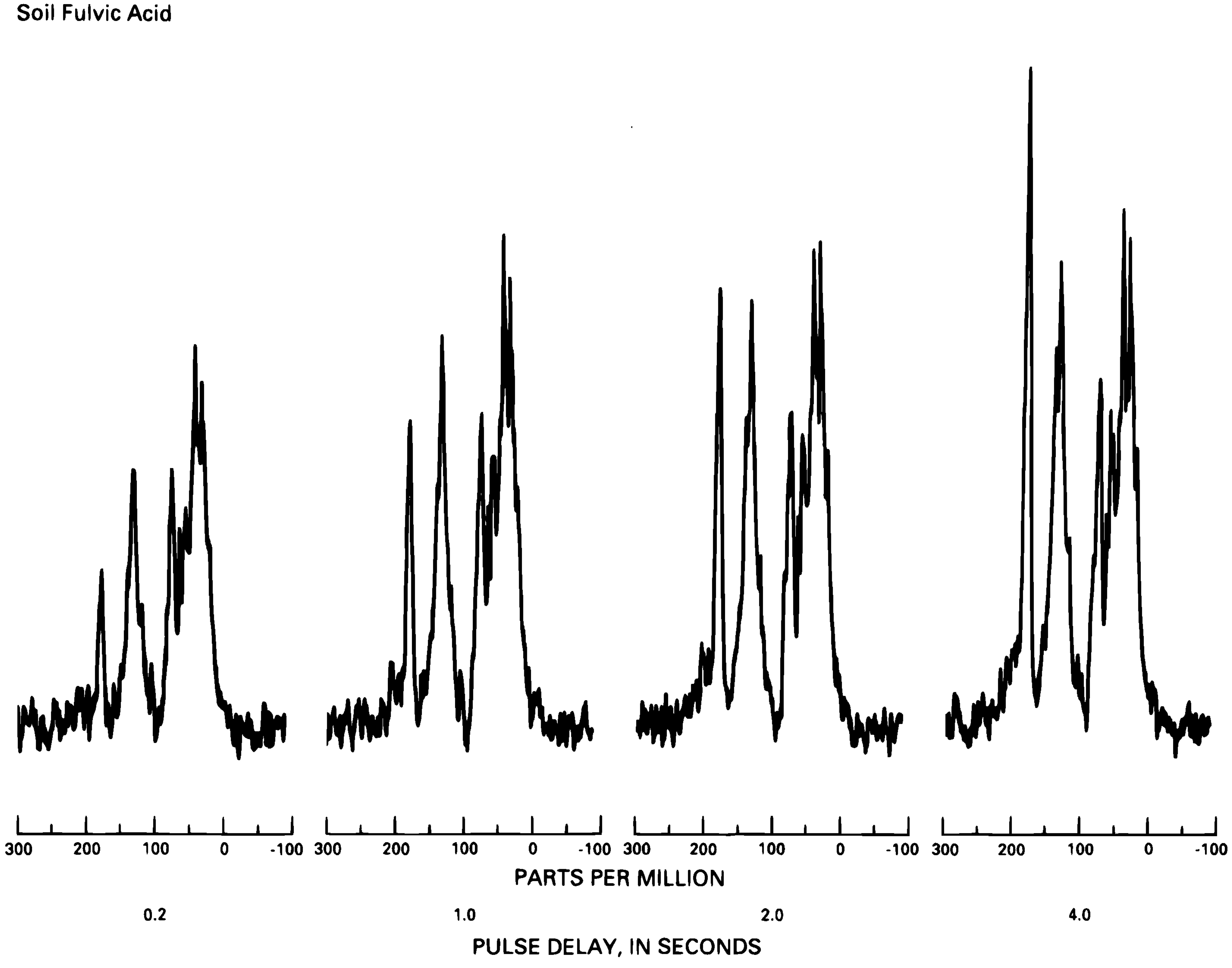


Figure 5.--Progressive saturation experiment for Soil fulvic acid. Line broadening = 100.0 Hertz.

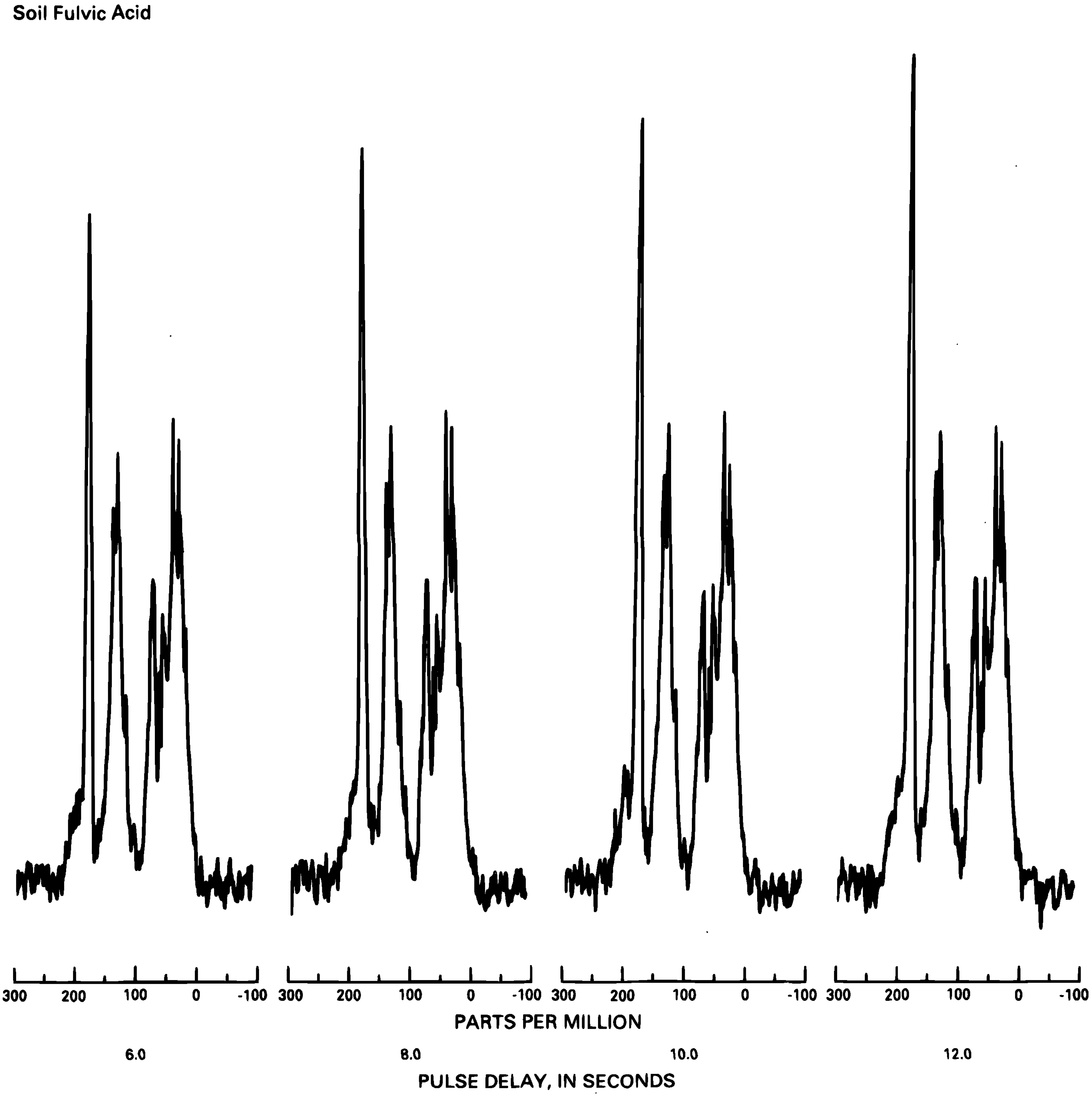


Figure 5.--Progressive saturation experiment for Soil fulvic acid.
Line broadening = 100.0 Hertz--Continued.

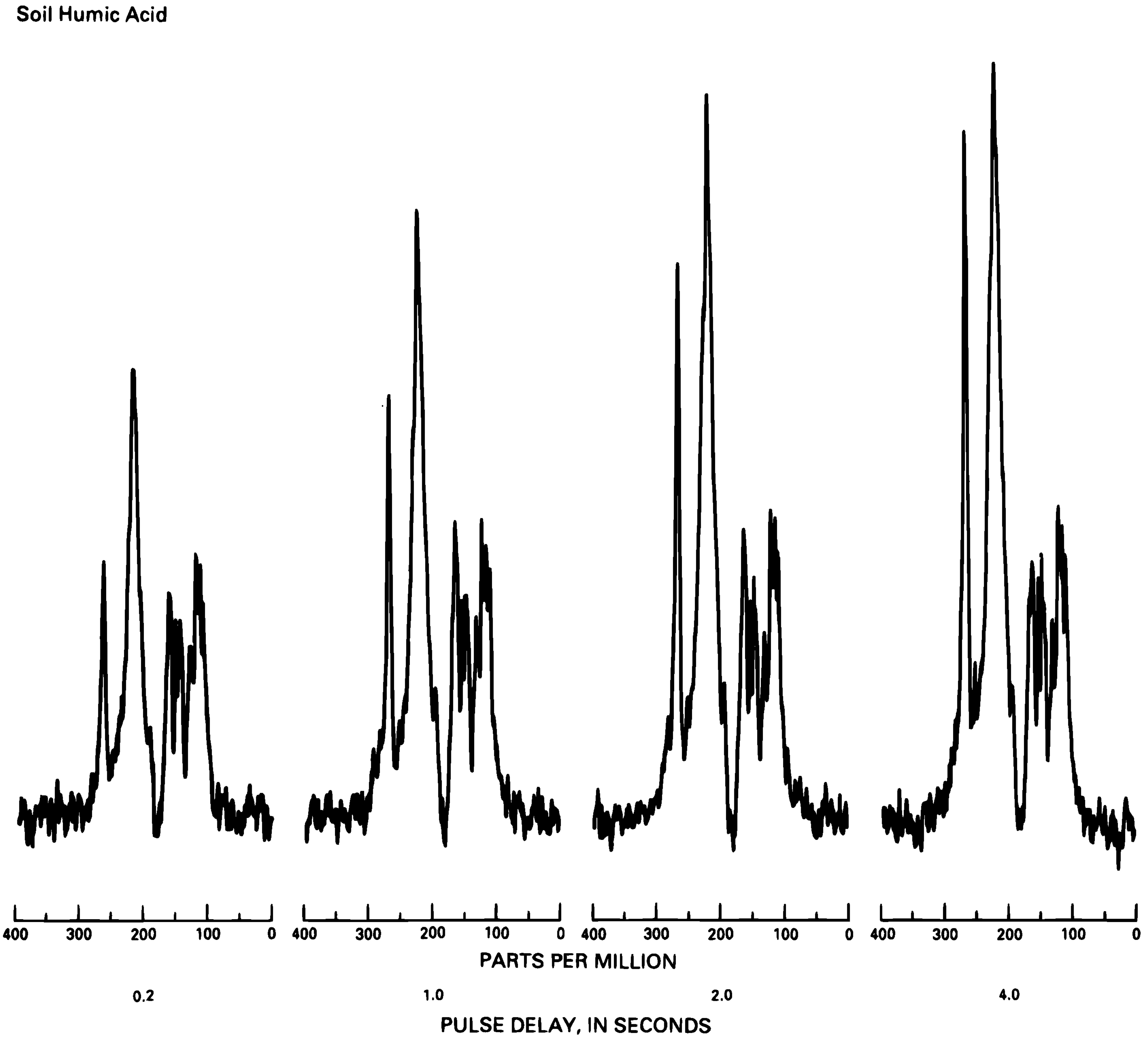


Figure 6.--Progressive saturation experiment for Soil humic acid.
Line broadening = 100.0 Hertz.

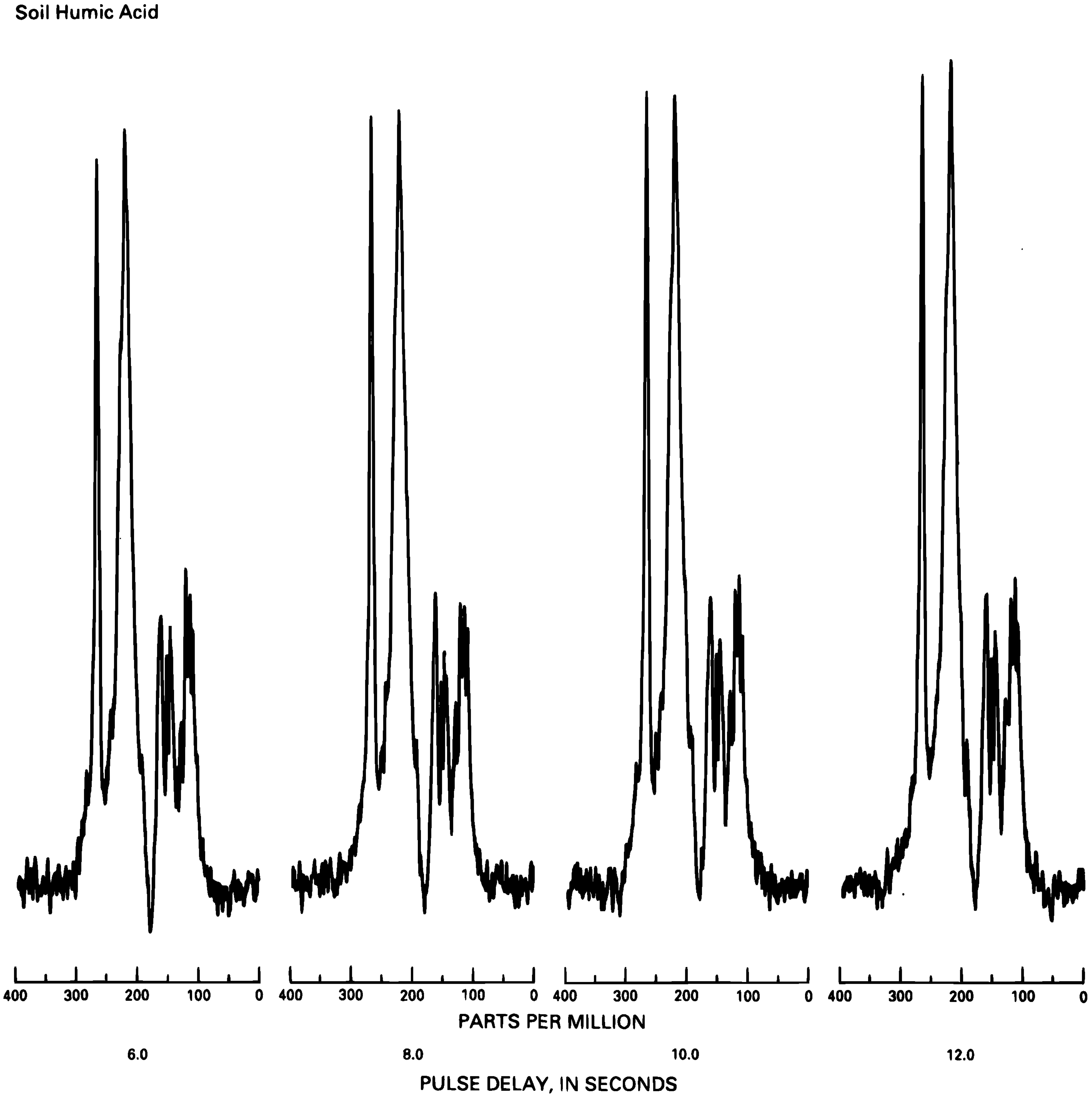


Figure 6.--Progressive saturation experiment for Soil humic acid. Line broadening = 100.0 Hertz--Continued.

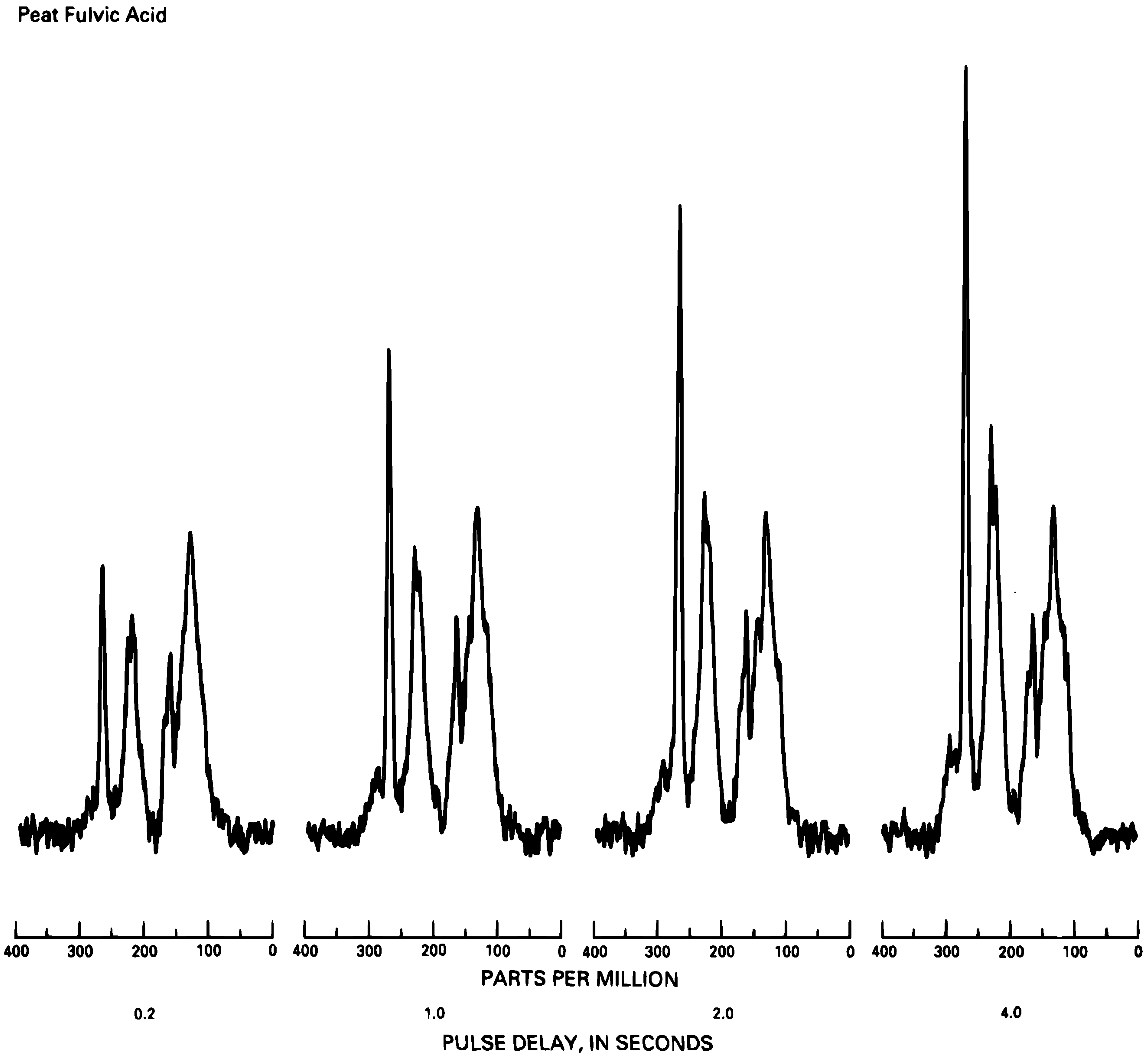


Figure 7.--Progressive saturation experiment for Peat fulvic acid. Line broadening = 100.0 Hertz.

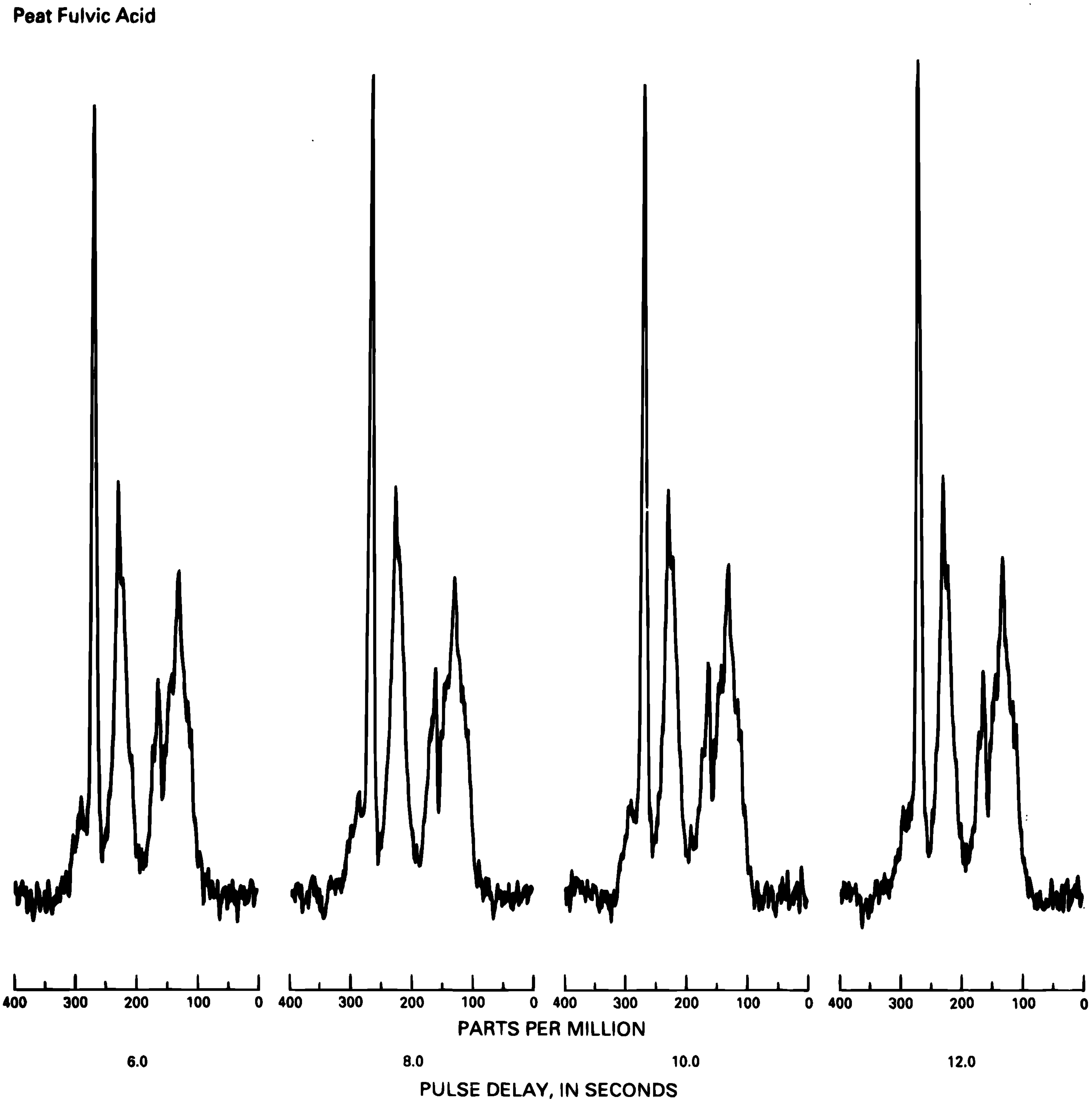


Figure 7.--Progressive saturation experiment for Peat fulvic acid. Line broadening = 100.0 Hertz--Continued.

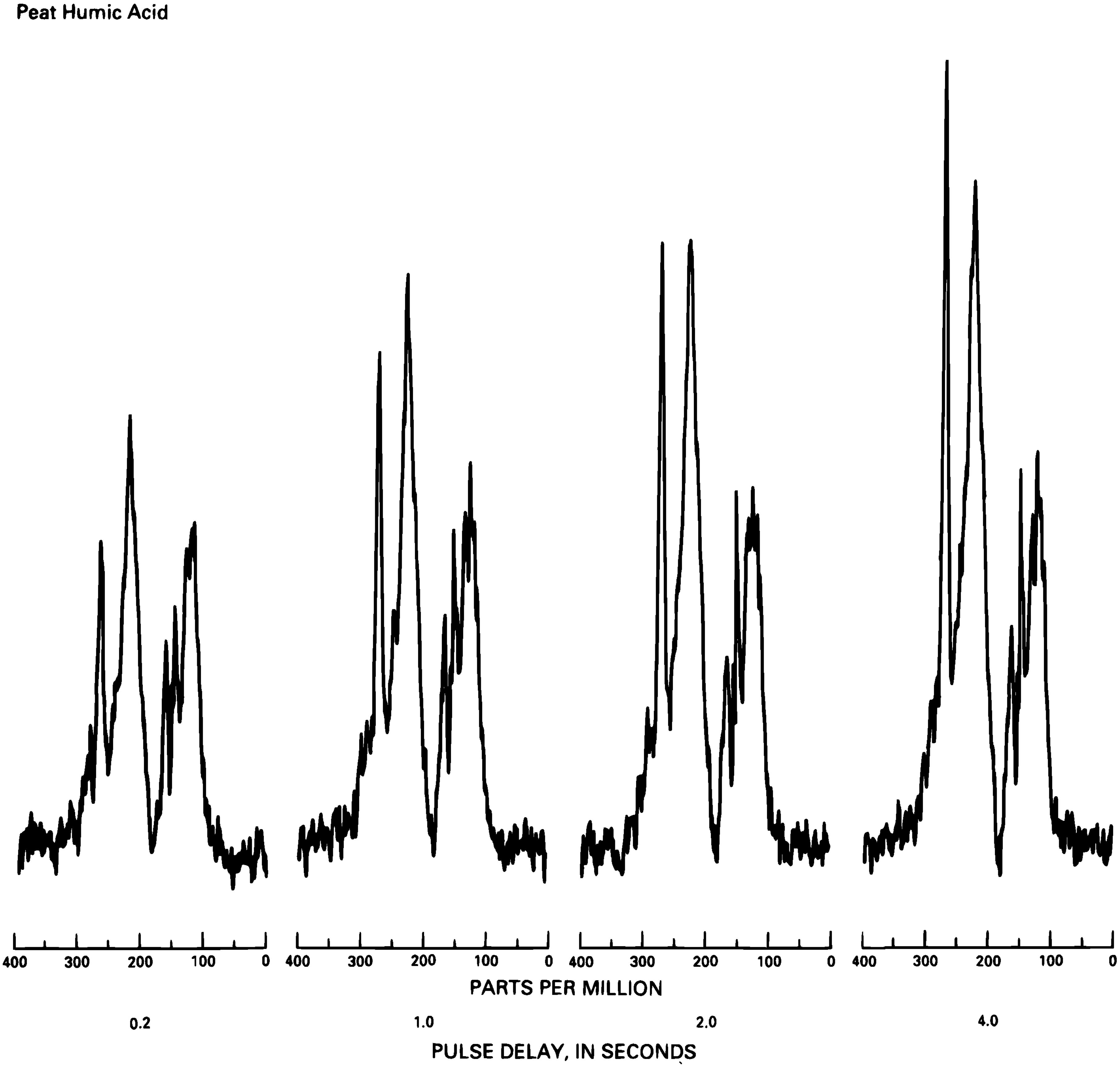


Figure 8.--Progressive saturation experiment for Peat humic acid. Line broadening = 100.0 Hertz.

Peat Humic Acid

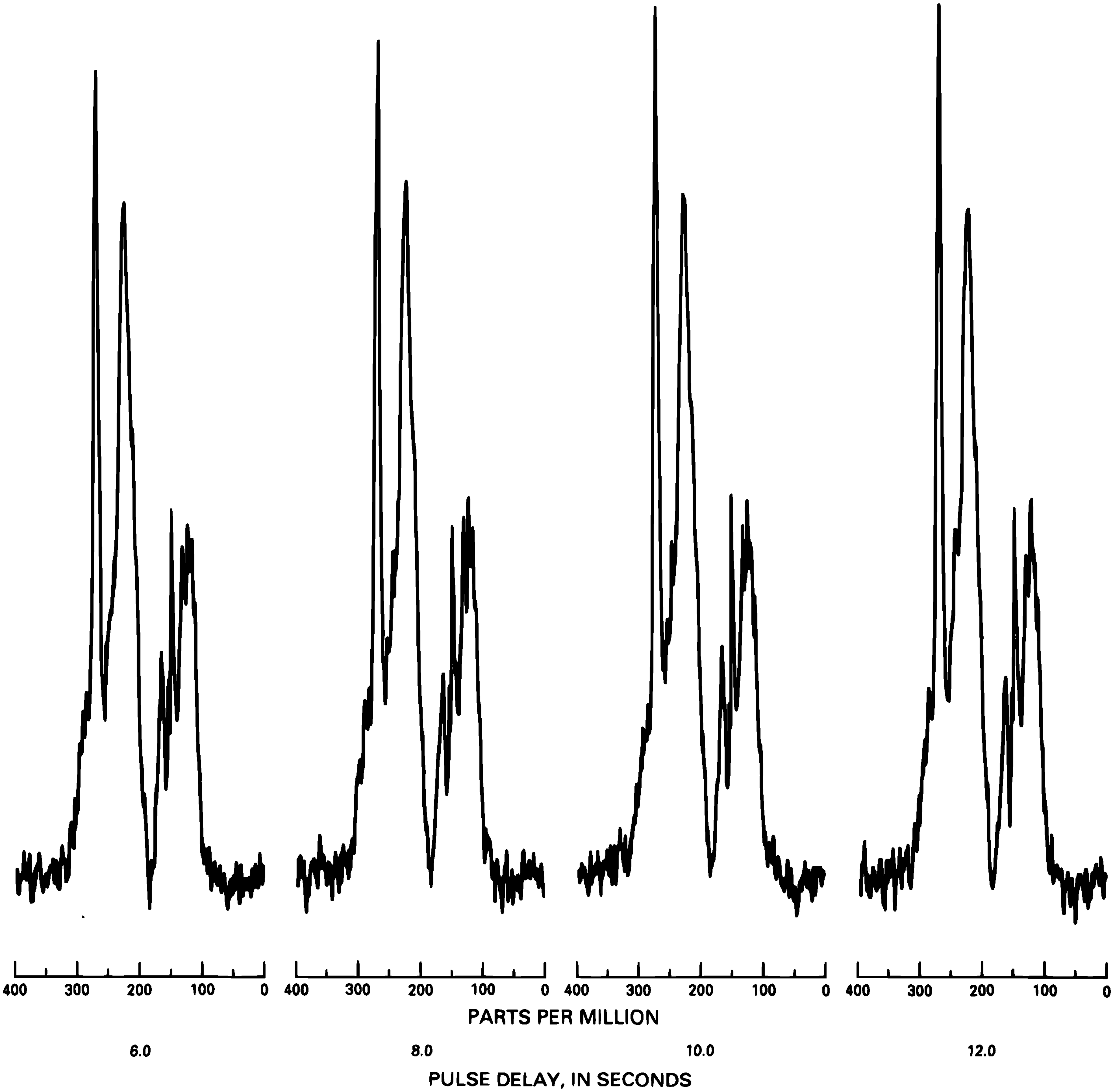


Figure 8.--Progressive saturation experiment for Peat humic acid. Line broadening = 100.0 Hertz--Continued.

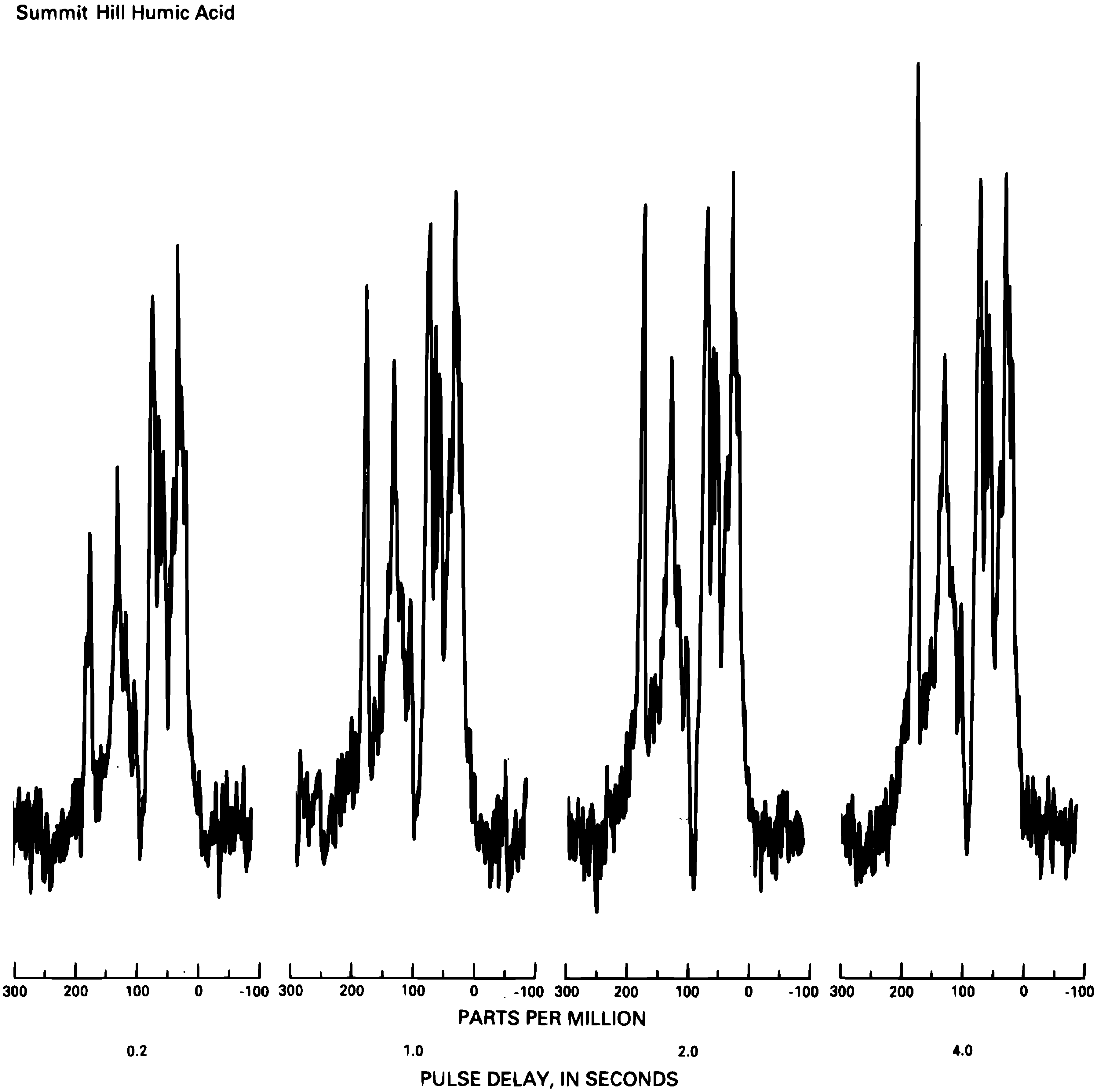


Figure 9.--Progressive saturation experiment for Summit Hill humic acid. Line broadening = 100.0 Hertz.

Summit Hill Humic Acid

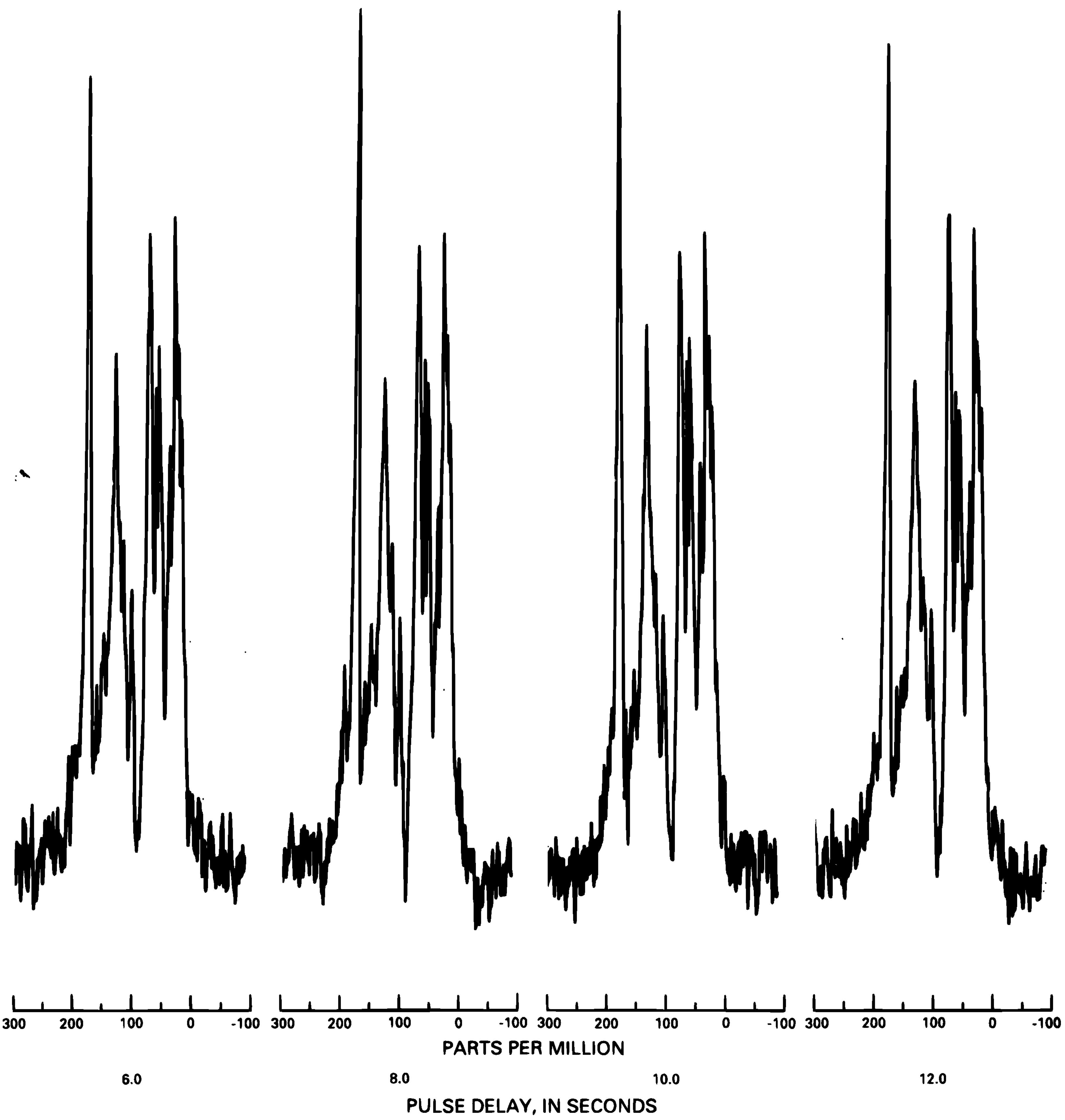


Figure 9.--Progressive saturation experiment for Summit Hill humic acid. Line broadening = 100.0 Hertz--Continued.

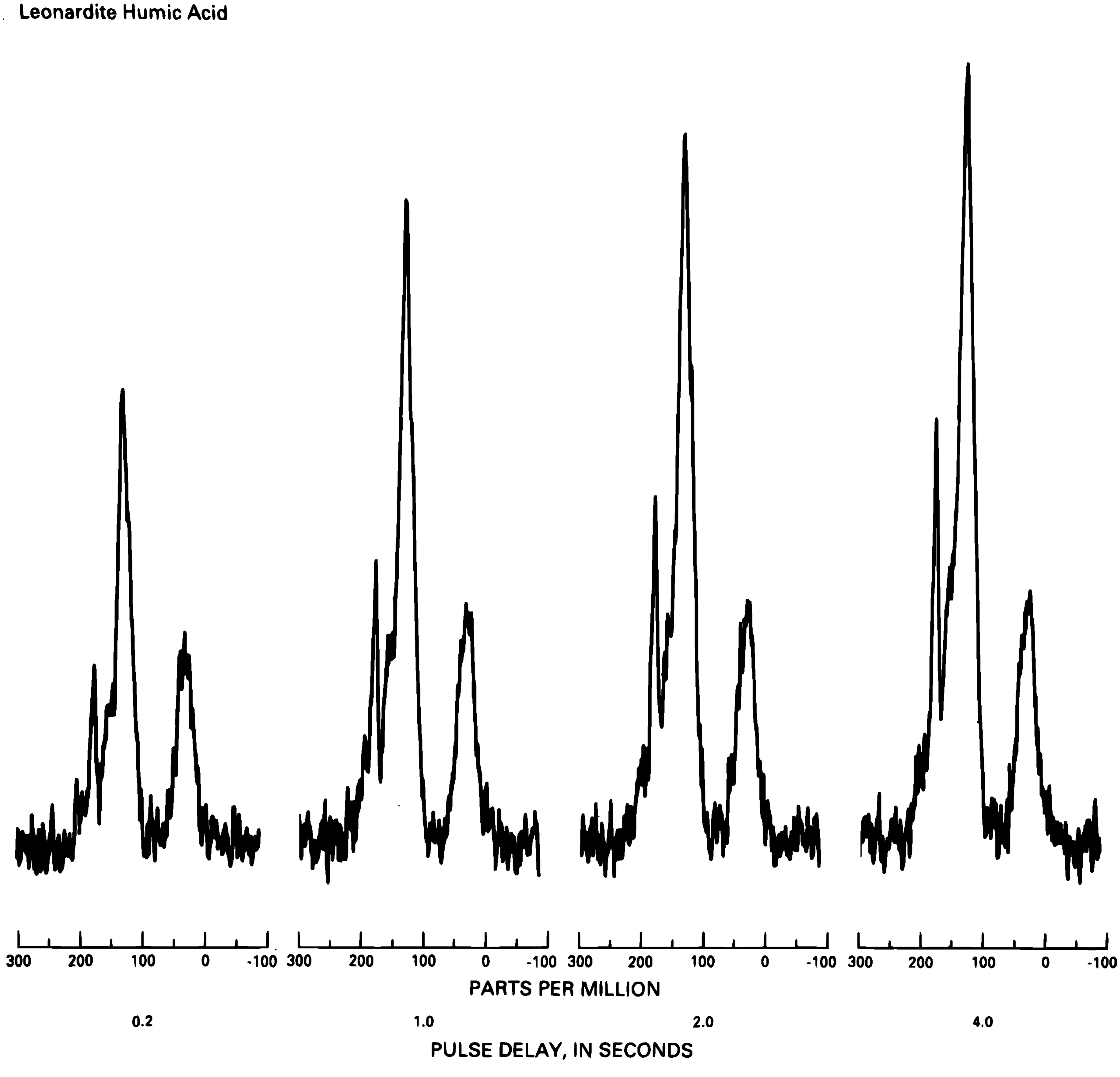


Figure 10.--Progressive saturation experiment for Leonardite humic acid. Line broadening = 100.0 Hertz.

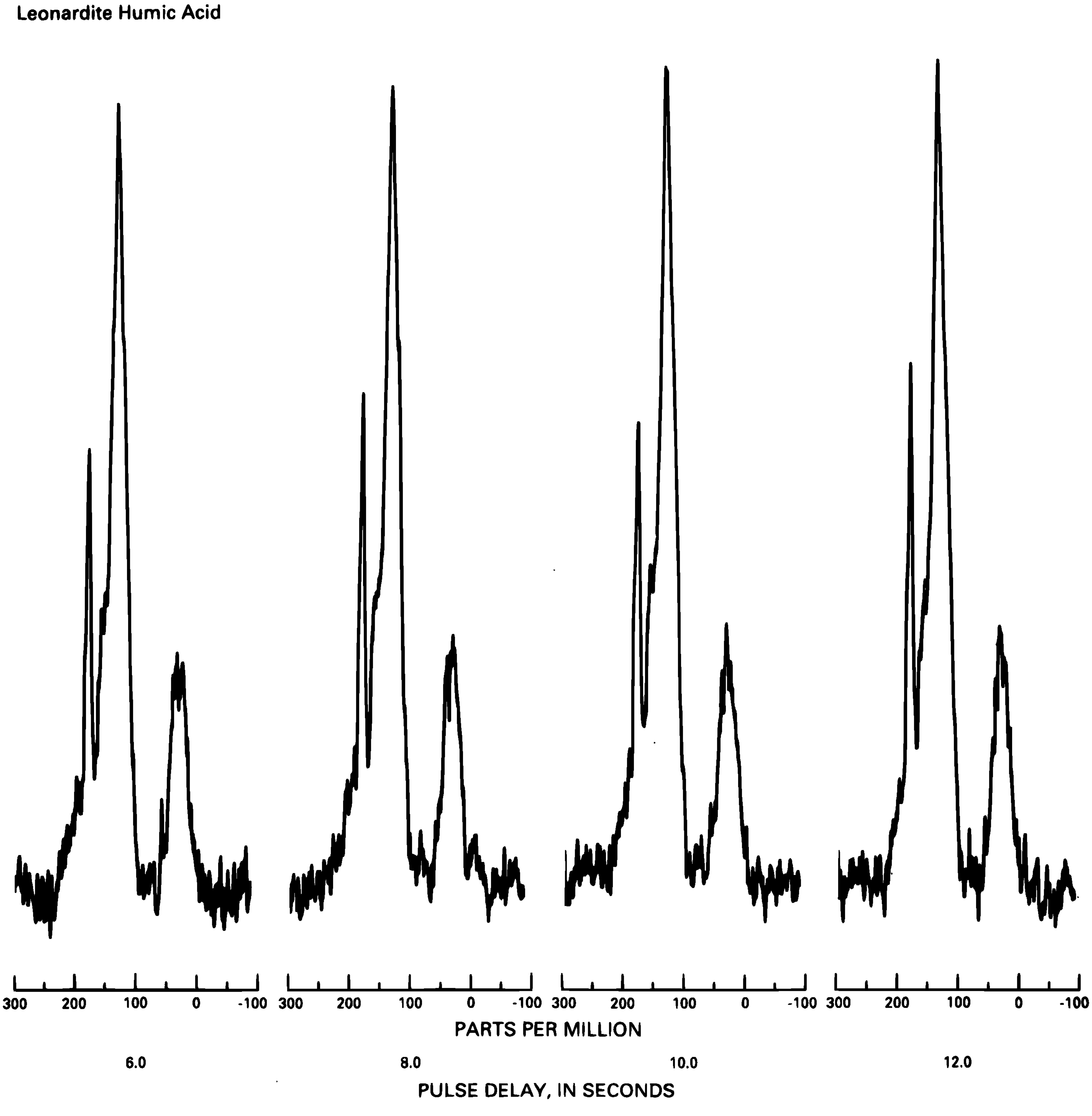


Figure 10.--Progressive saturation experiment for Leonardite humic acid. Line broadening = 100.0 Hertz--Continued.

Table 2.--*Spin-lattice relaxation times* (T_1*'s), in seconds, for major regions of carbon-13 nuclear magnetic resonance spectra of humic and fulvic acids estimated from progressive saturation experiments*

[ppm, parts per million; ≧, equal to or greater than]

Sample	Ketone/ quinone 220-190 ppm	Carboxyl 190-165 ppm	Aromatic 165-90 ppm	Hetero-aliphatic 90-60 ppm	Aliphatic 60-0 ppm
Suwannee fulvic		≧2.4	1.2	0.8	0.4
Suwannee humic		1.6	1.6	0.8	0.2
Nordic fulvic		2.4	1.2	0.4	0.2
Nordic humic		1.6	0.8	0.4	0.4
Soil fulvic		2.4	1.6	0.8	0.8
Soil humic		2.4	2.4	0.4	0.4
Peat fulvic		1.6	1.2	0.4	0.2
Peat humic		2.0	1.2	0.4	0.4
Summit Hill humic		1.6	1.2	0.4	0.4
Leonardite humic		2.4	2.0		0.2

The carboxyl and ketone/quinone carbons are the most slowly relaxing carbons in the samples, and these carbons have completely relaxed by 2.0 seconds in almost all the samples. Therefore, pulse delays of 10.0 to 12.0 seconds in conjunction with 90° pulses and inverse gated decoupling should be adequate for quantitative spectra.

Quantitative Carbon-13 Nuclear Magnetic Resonance Spectra and Measurement of Nuclear Overhauser Enhancement Factors

The quantitative spectra are shown in figures 11 through 20, and the NOE measurements in figures 21 through 30. The distributions of carbons in the major regions of the quantitative spectra are listed in table 3, and the NOE factors for these carbons are listed in table 4. In general, there is good agreement between the numbers generated from electronic integration and from cutting and weighing of the spectra as listed in table 3. The two exceptions are in the aliphatic peak of the Nordic fulvic acid and in the ketone/quinone peak in the soil fulvic acid. Aromaticities, f_a's, measured from electronic integration of the spectra, also are listed in table 3. The f_a's were calculated by dividing the area of the spectrum from 110 to 165 ppm by the total area of the spectrum. The f_a's range from a low of 0.24 for the Suwannee River fulvic acid to a high of 0.58 for the Leonardite humic acid. In addition to its high aromaticity, the Leonardite also is distinguished from the other samples in that it contains a low concentration of hetero-aliphatic carbons (60-90 ppm).

The NOE factors (η's) listed in table 4 follow the expected pattern. For most samples, the NOE factors are greatest for the aliphatic carbons (0 to 60 ppm), and subsequently decrease downfield with the hetero-aliphatic carbons (60 to 90 ppm), aromatic carbons (90 to 165 ppm), and carbonyl carbons (165 to 220 ppm), as the degree of protonation decreases. The largest NOE factor measured is about 1.4 for the aliphatic region of the Suwannee River fulvic acid, and the smallest is 0.002 for the ketone/quinone region of the peat humic acid. The negative values of η for the ketone/quinone region of the soil humic, soil fulvic, and peat fulvic acids are unrealistic, as negative NOE factors are possible only for nuclei with negative magnetogyric ratios, of which ^{13}C is not one. The NOE factors for the ketone/quinone peaks have the greatest error associated with them, because these peaks are broad, of low intensity and poor signal-to-noise, and in most cases poorly resolved from the carboxyl peaks. Therefore, these peak areas are difficult to measure accurately. The NOE factors listed in table 4 are all less than the theoretical maximum value of 2. The reduction of NOE values below the theoretical maximum of 2 may result from both slow molecular motion in solution (outside the extreme narrowing limit) and the presence of relaxation mechanisms other than the ^{13}C-^{1}H dipole-dipole interaction.

Nonquantitative Carbon-13 Nuclear Magnetic Resonance Spectra

Nonquantitative ^{13}C NMR spectra of the fulvic and humic acids are shown in figures 31-45. These spectra exhibit superior signal to noise and resolution compared to the quantitative spectra and afford a more detailed view of the structural features of the samples. There are a number of discrete, sharp line resonances in the aliphatic region of the soil and peat fulvic and humic acids. Horizontal expansions of the aliphatic region of the spectra of these four samples also are shown.

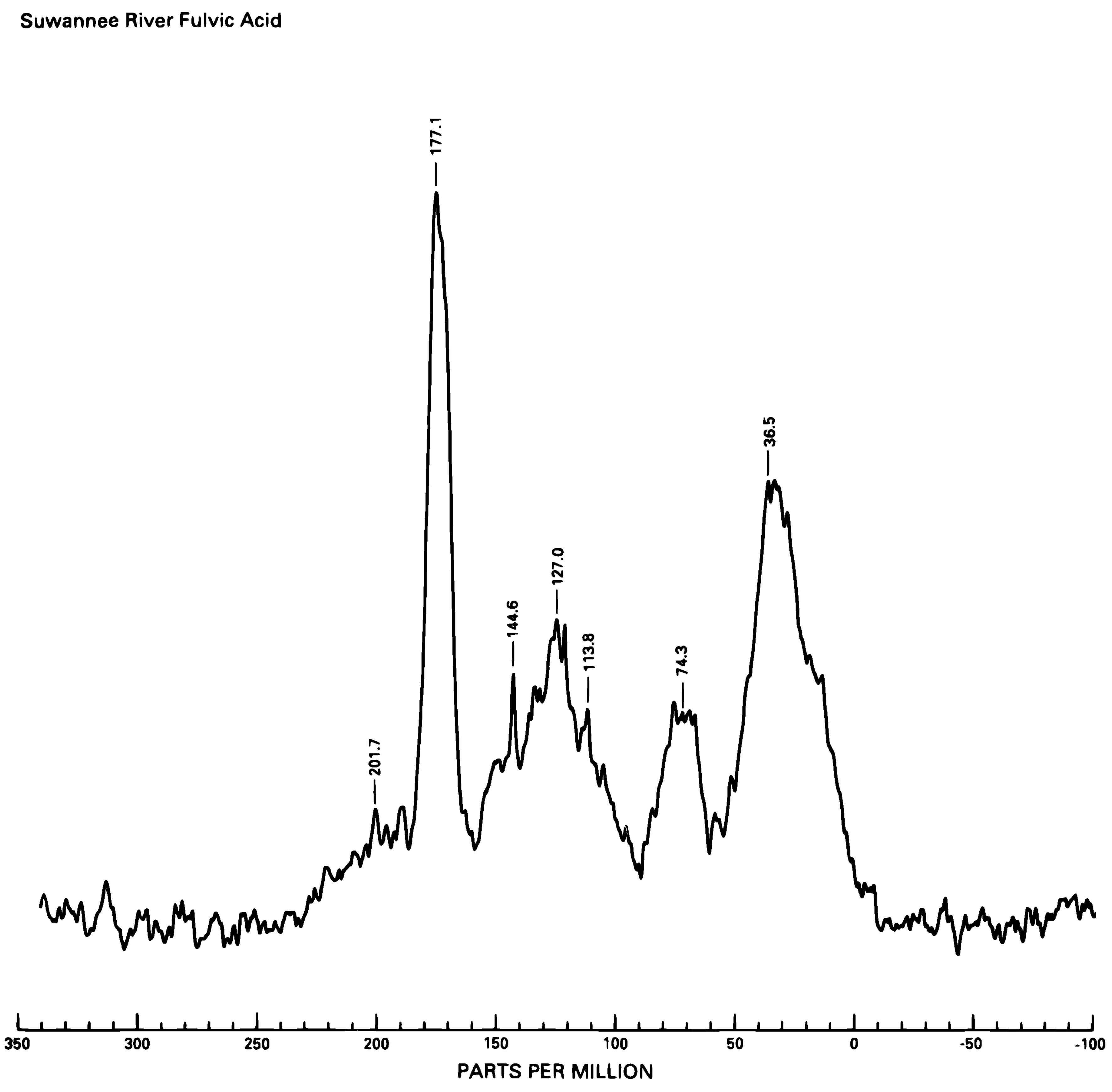


Figure 11.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Suwannee River fulvic acid. Line broadening = 100.0 Hertz.

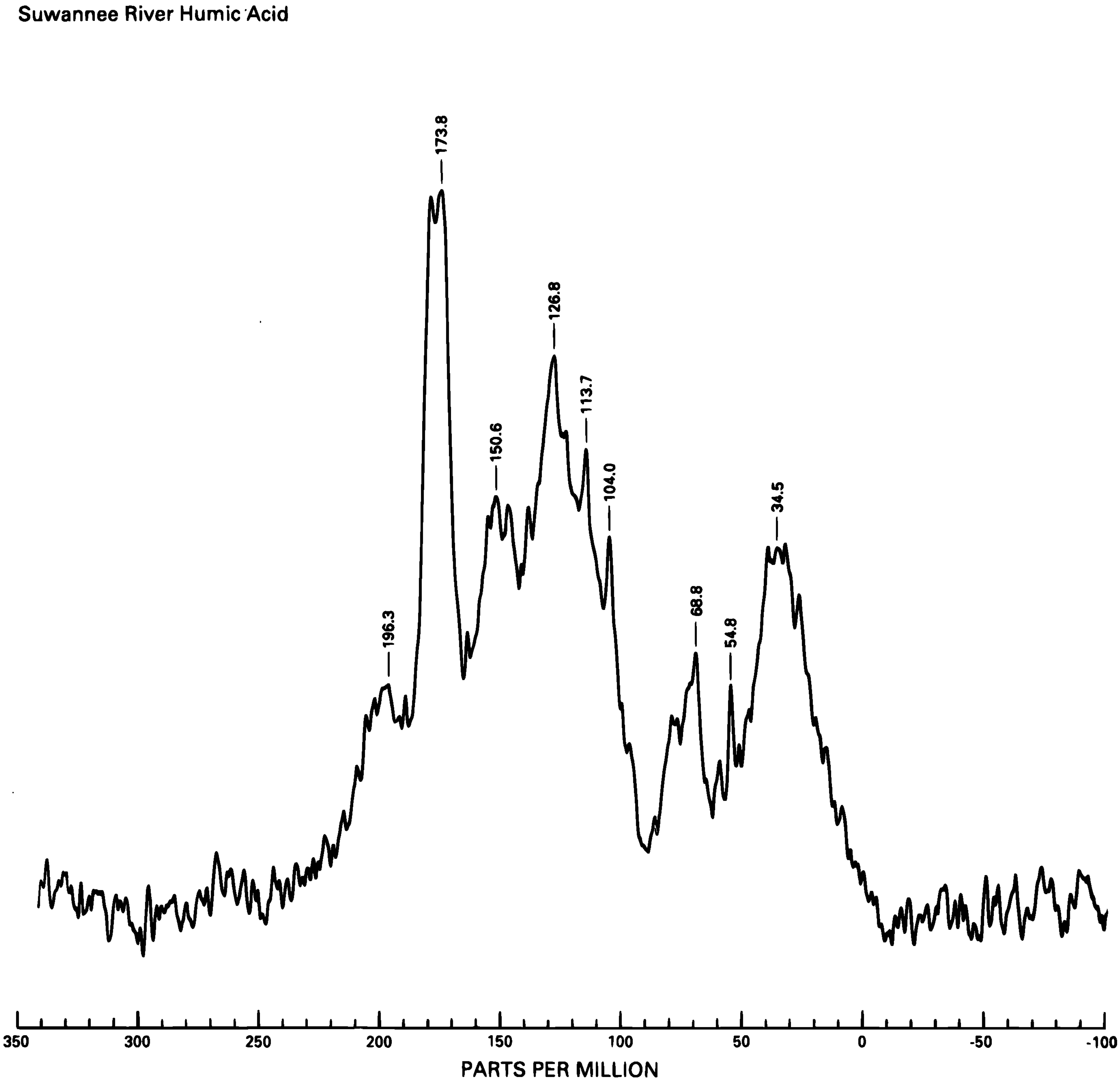


Figure 12.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Suwannee River humic acid. Line broadening = 100.0 Hertz.

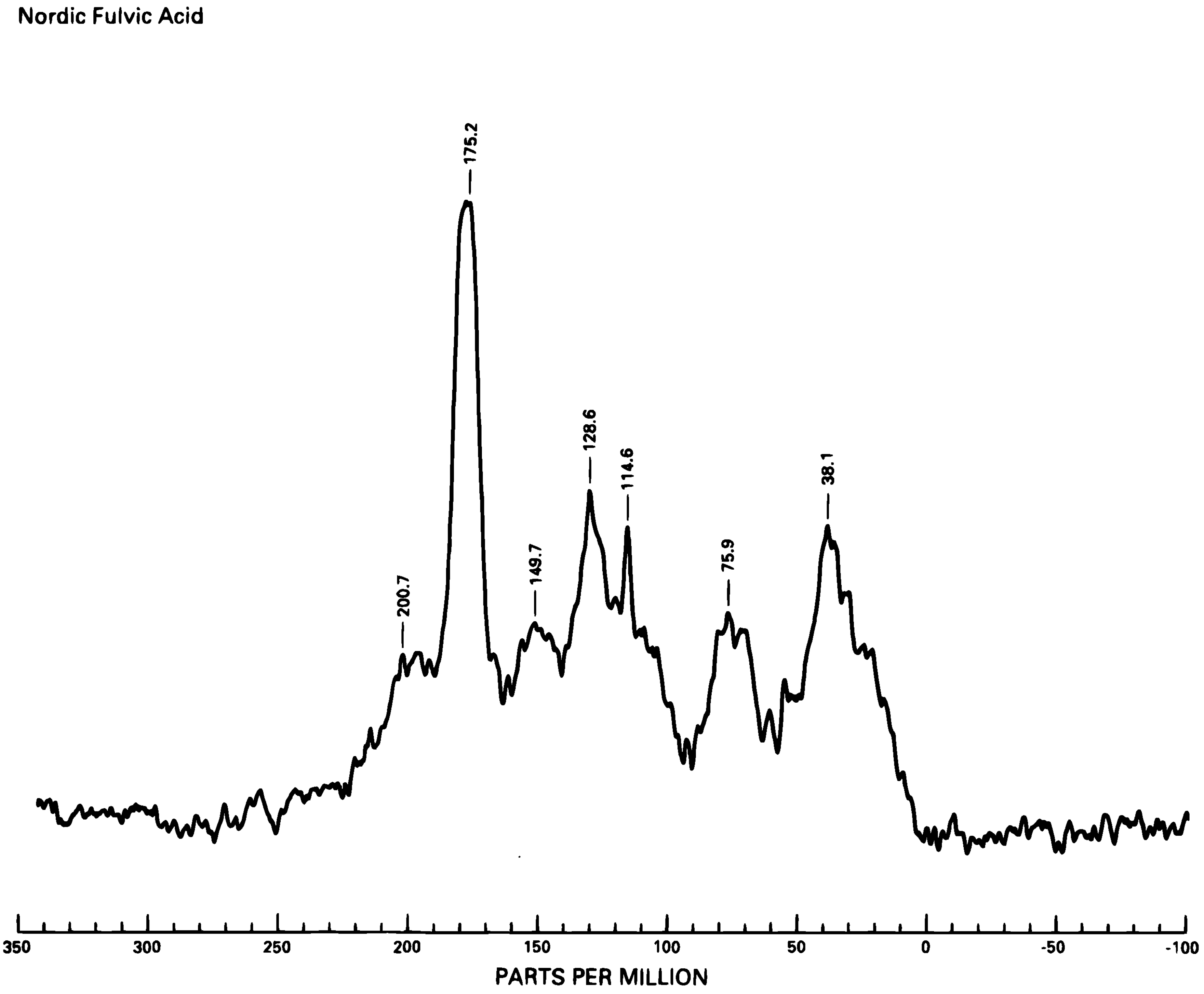


Figure 13.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Nordic fulvic acid. Line broadening = 100.0 Hertz.

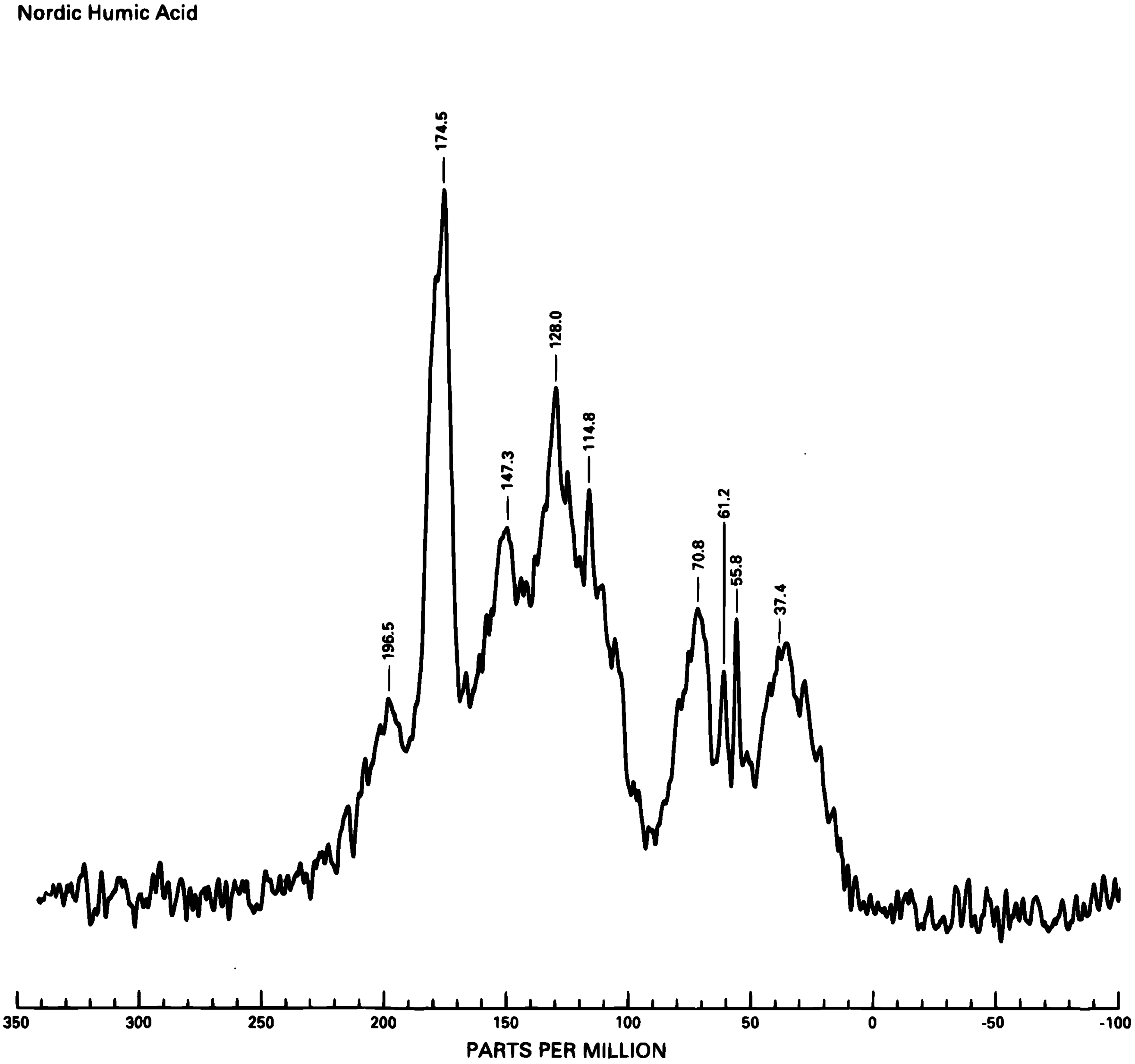


Figure 14.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Nordic humic acid. Line broadening = 100.0 Hertz.

Soil Fulvic Acid

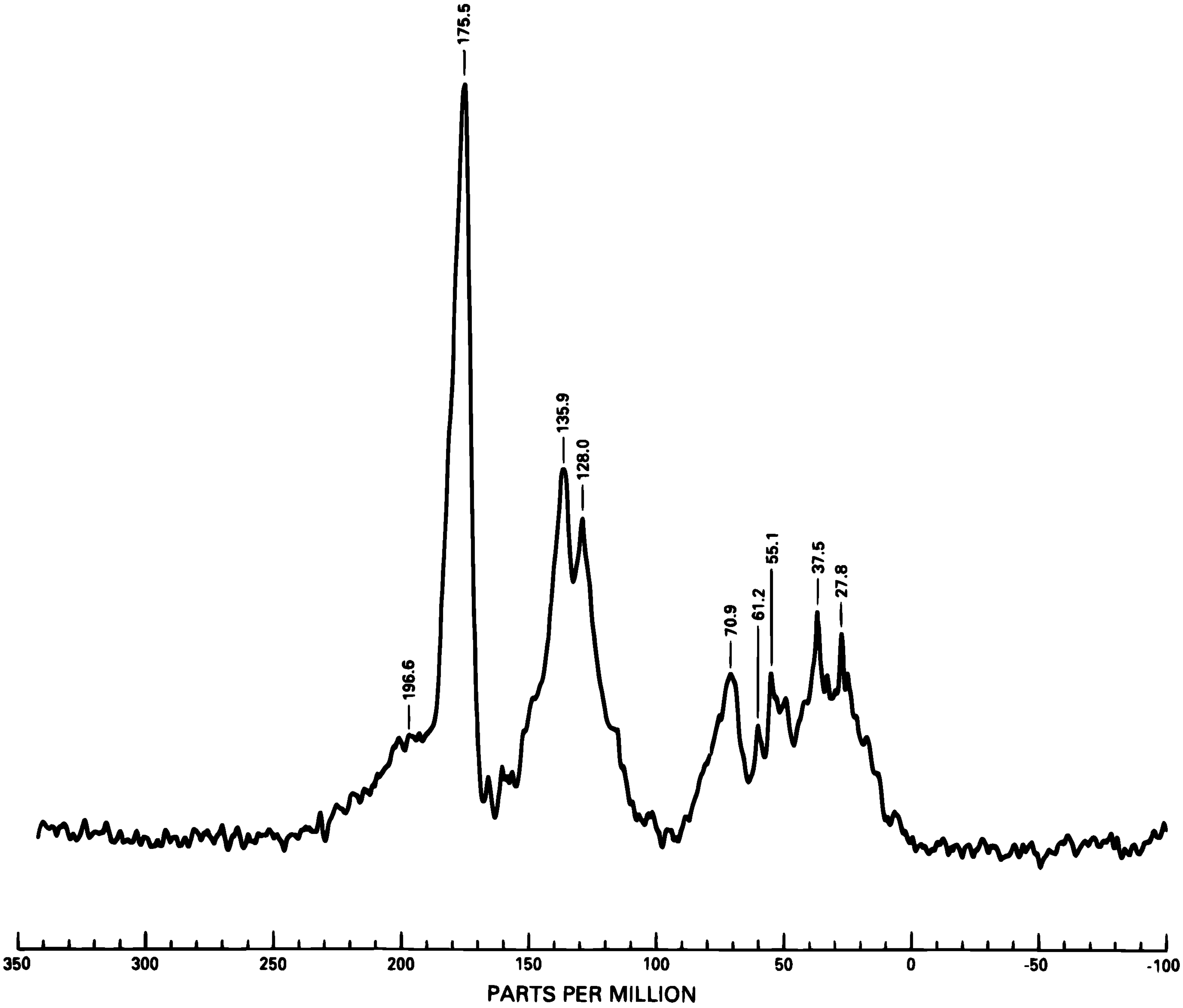


Figure 15.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Soil fulvic acid. Line broadening = 100.0 Hertz.

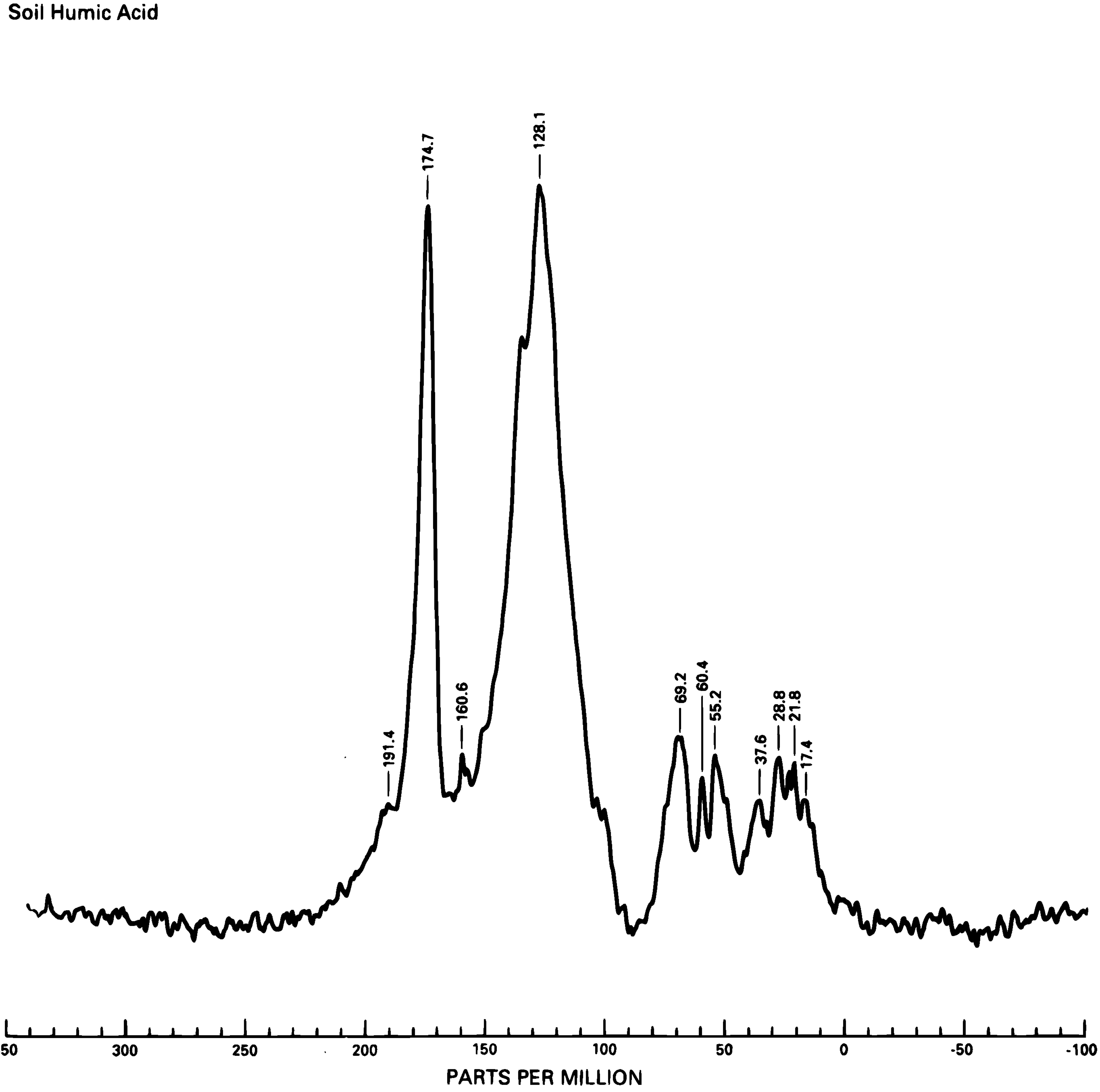


Figure 16.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Soil humic acid. Line broadening = 100.0 Hertz.

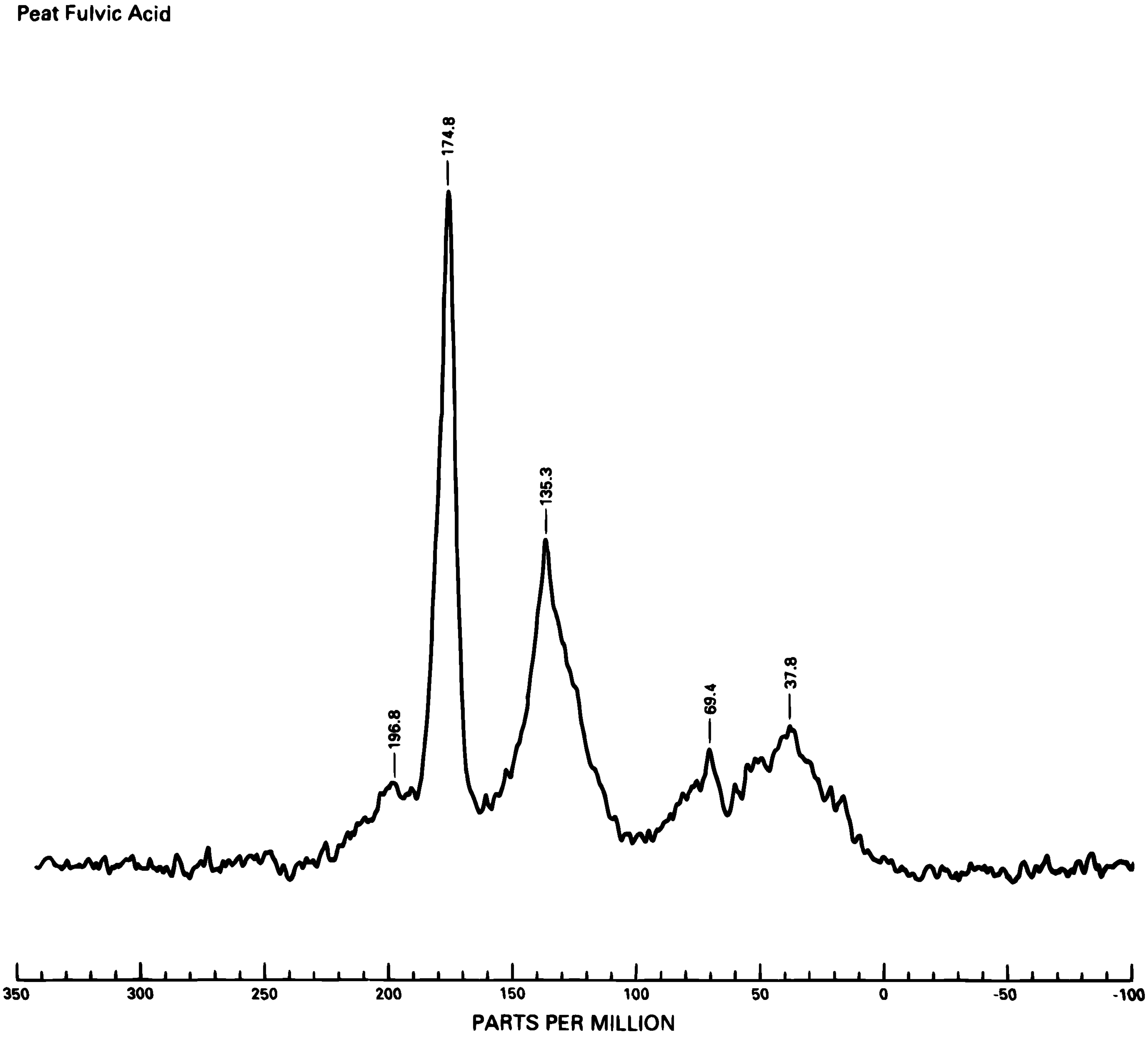


Figure 17.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Peat fulvic acid. Line broadening = 100.0 Hertz.

Peat Humic Acid

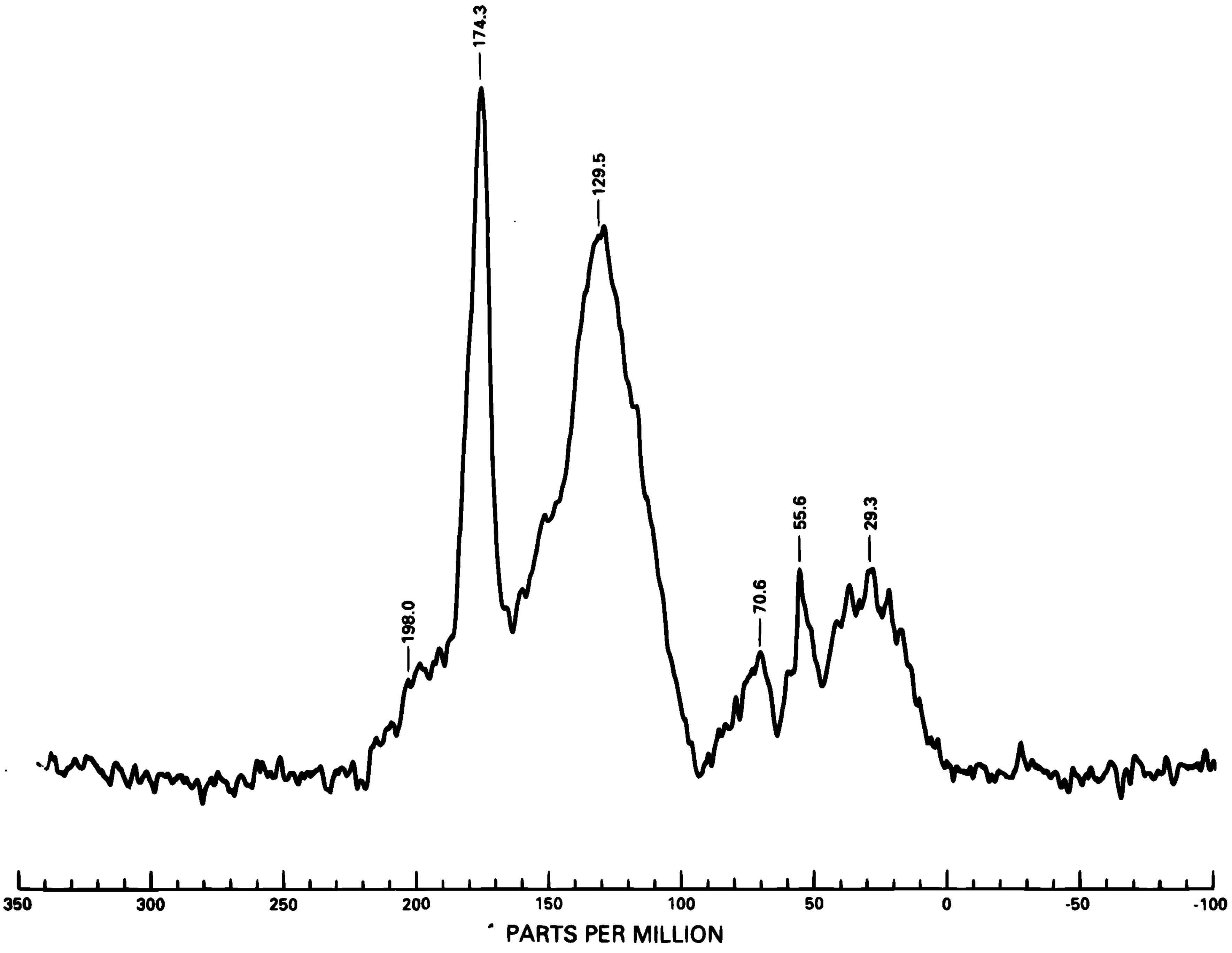


Figure 18.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Peat humic acid. Line broadening = 100.0 Hertz.

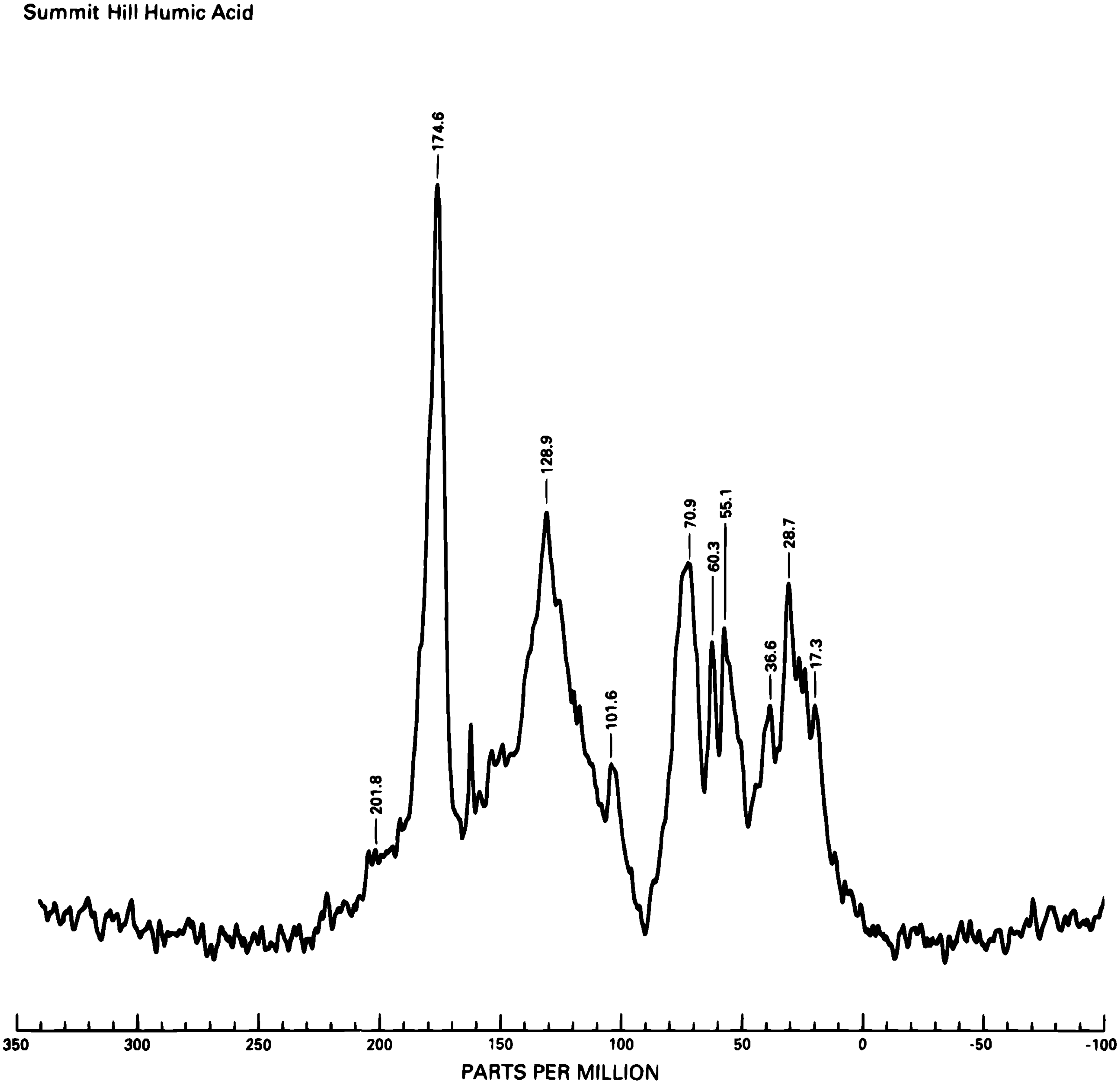


Figure 19.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Summit Hill humic acid. Line broadening = 100.0 Hertz.

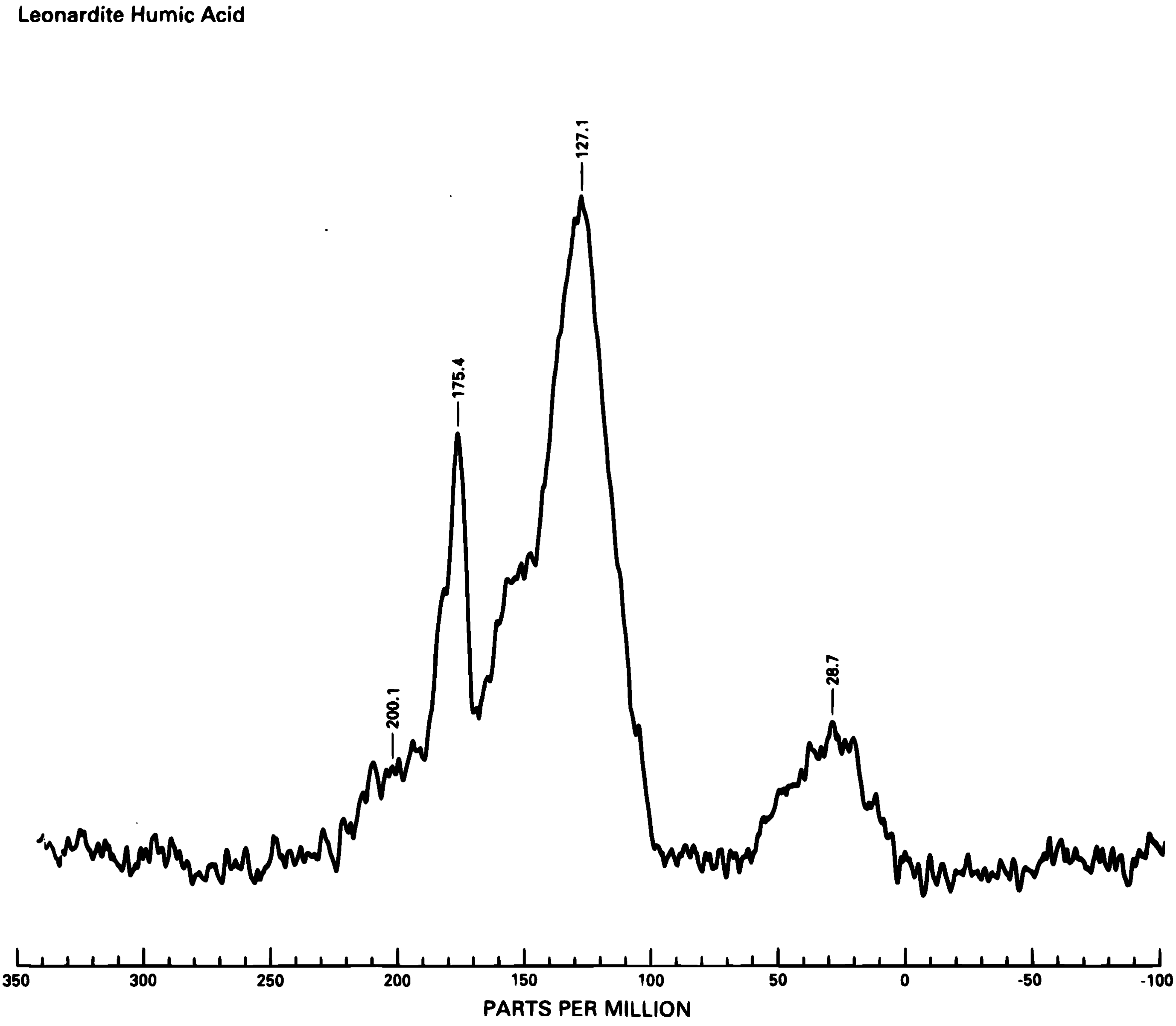


Figure 20.--Quantitative carbon-13 nuclear magnetic resonance spectrum of Leonardite humic acid. Line broadening = 100.0 Hertz.

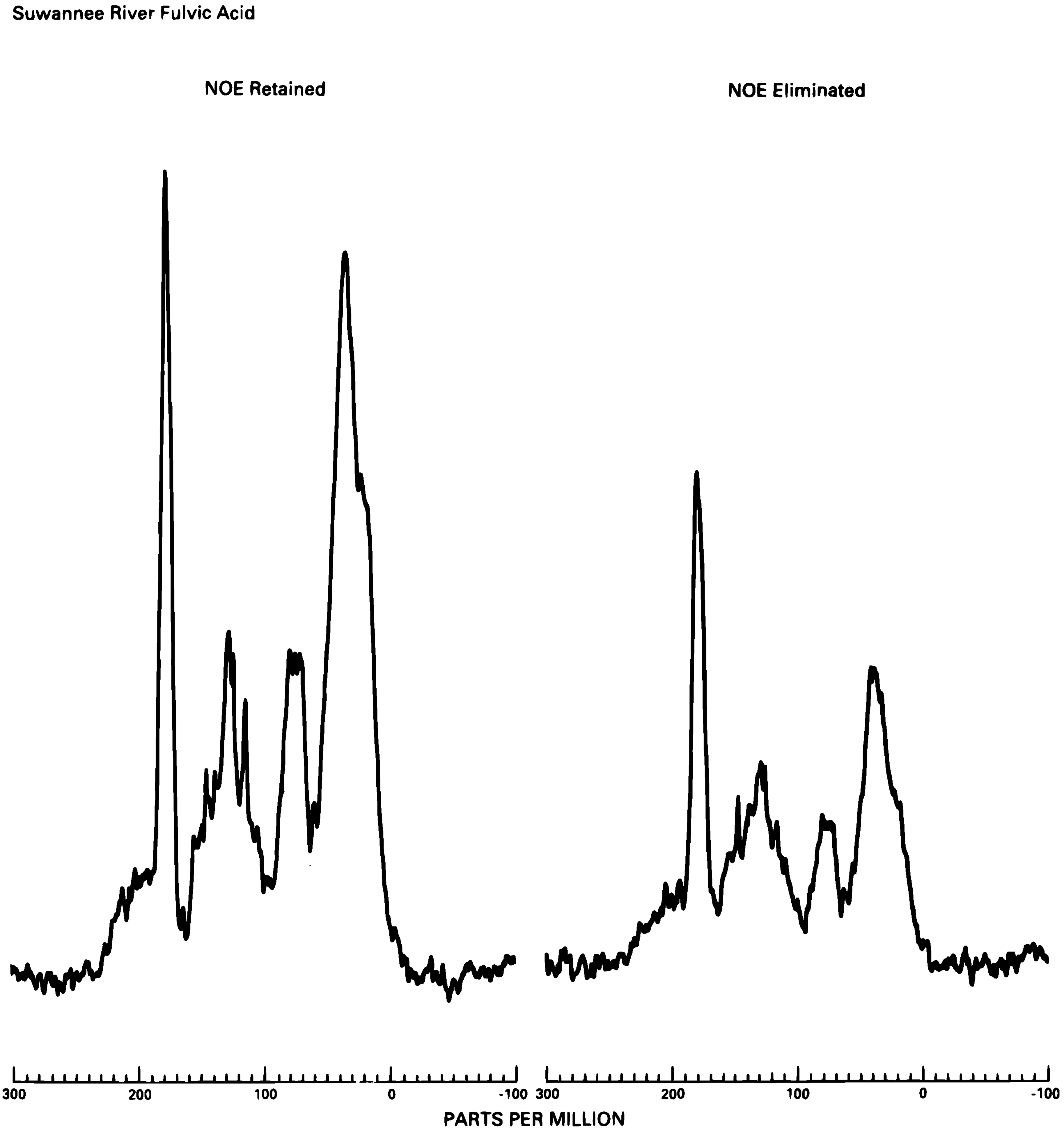


Figure 21.--Nuclear Overhauser enhancement measurement of Suwannee River fulvic acid. Line broadening = 100.0 Hertz.

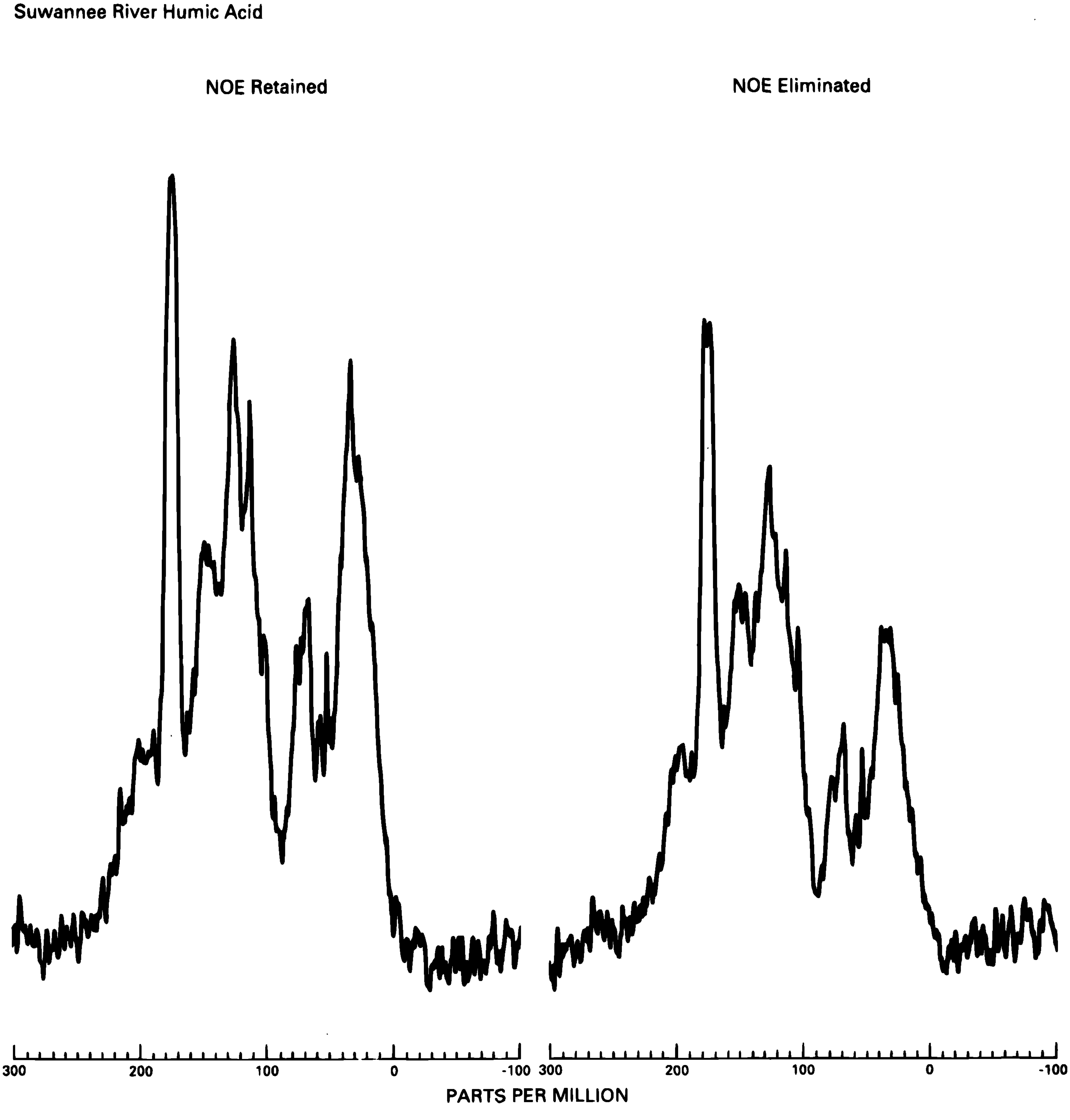


Figure 22.--Nuclear Overhauser enhancement measurement of Suwannee River humic acid. Line broadening = 100.0 Hertz.

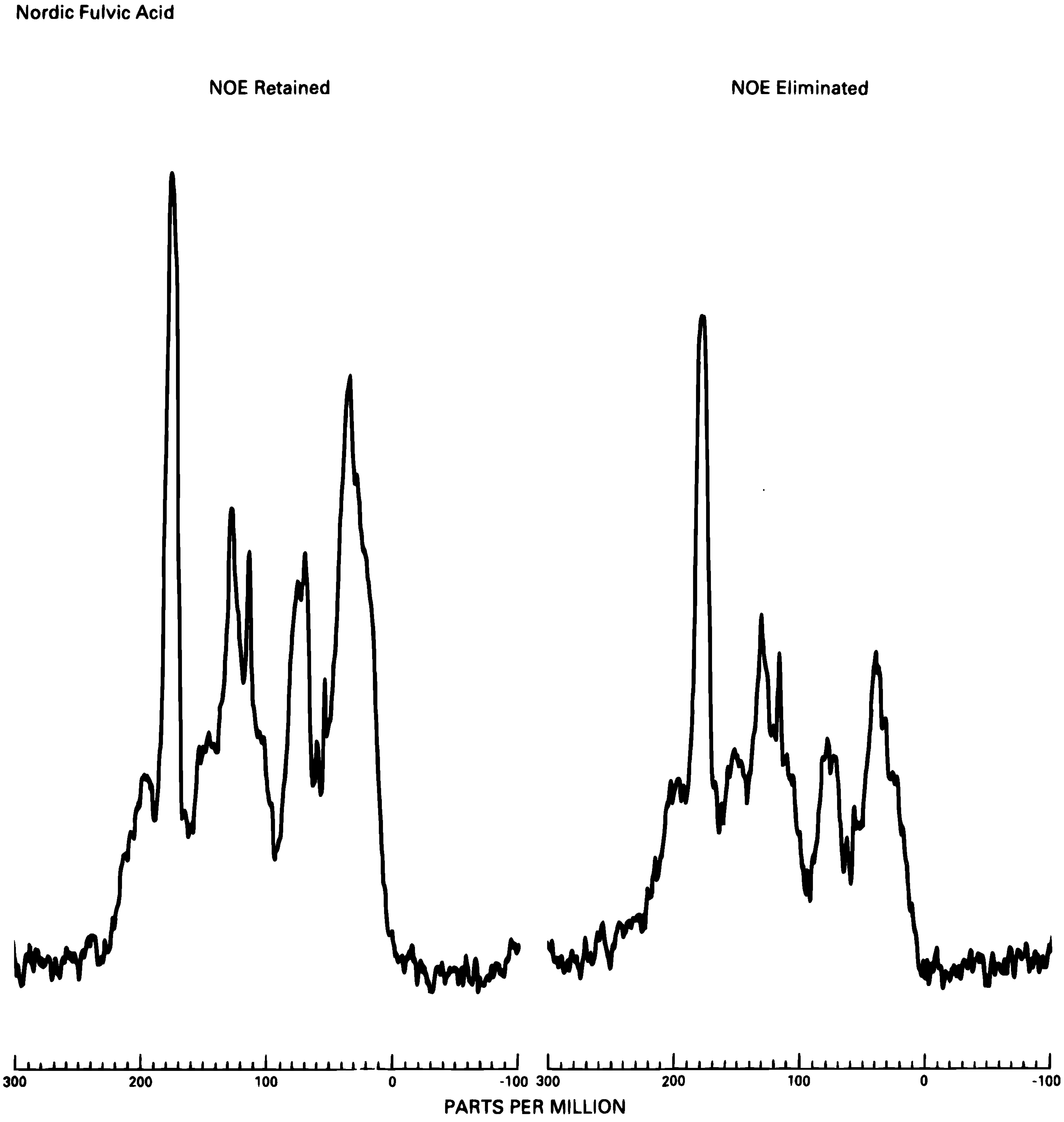


Figure 23.--Nuclear Overhauser enhancement measurement of Nordic fulvic acid. Line broadening = 100.0 Hertz.

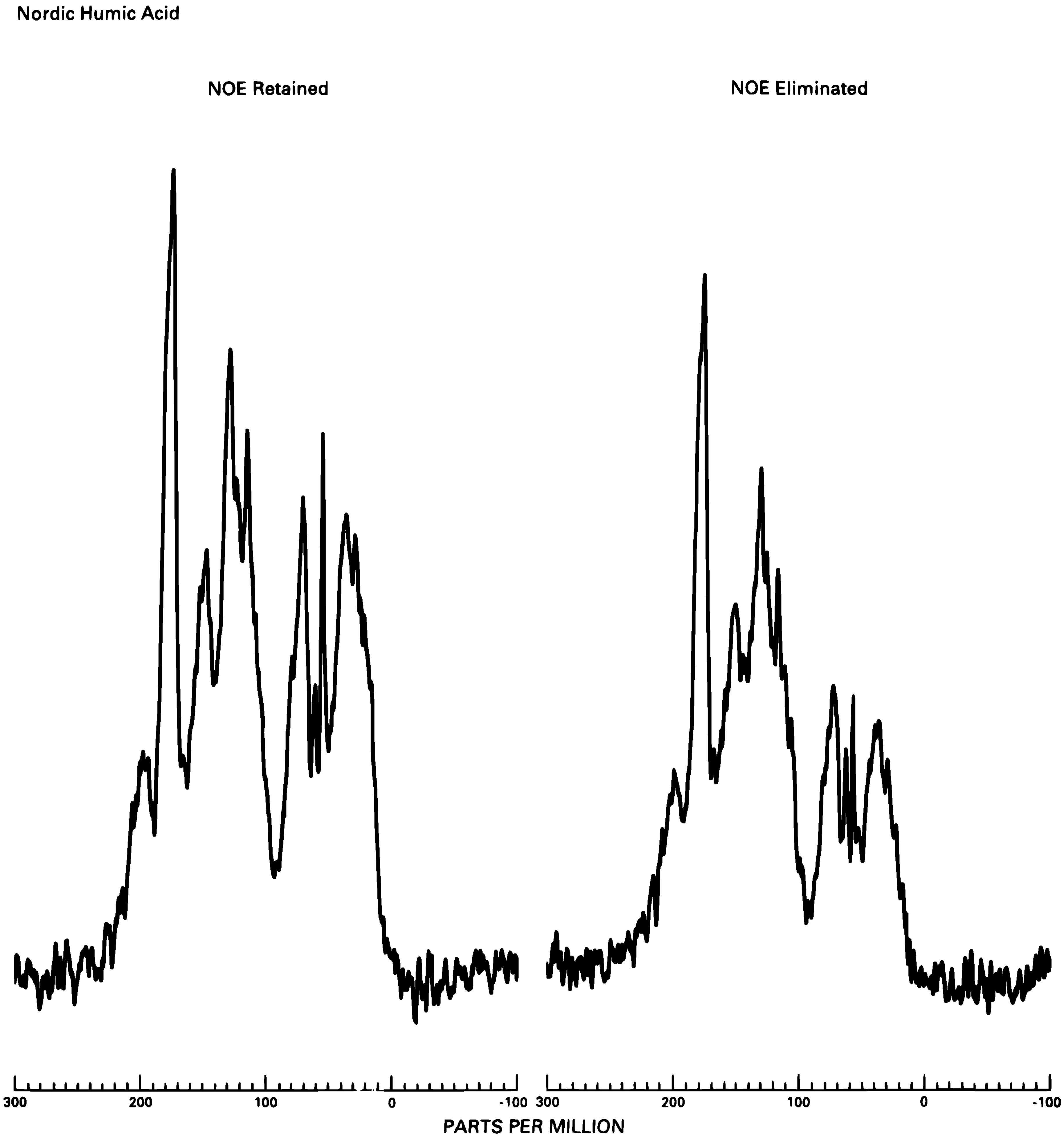


Figure 24.--Nuclear Overhauser enhancement measurement of Nordic humic acid. Line broadening = 100.0 Hertz.

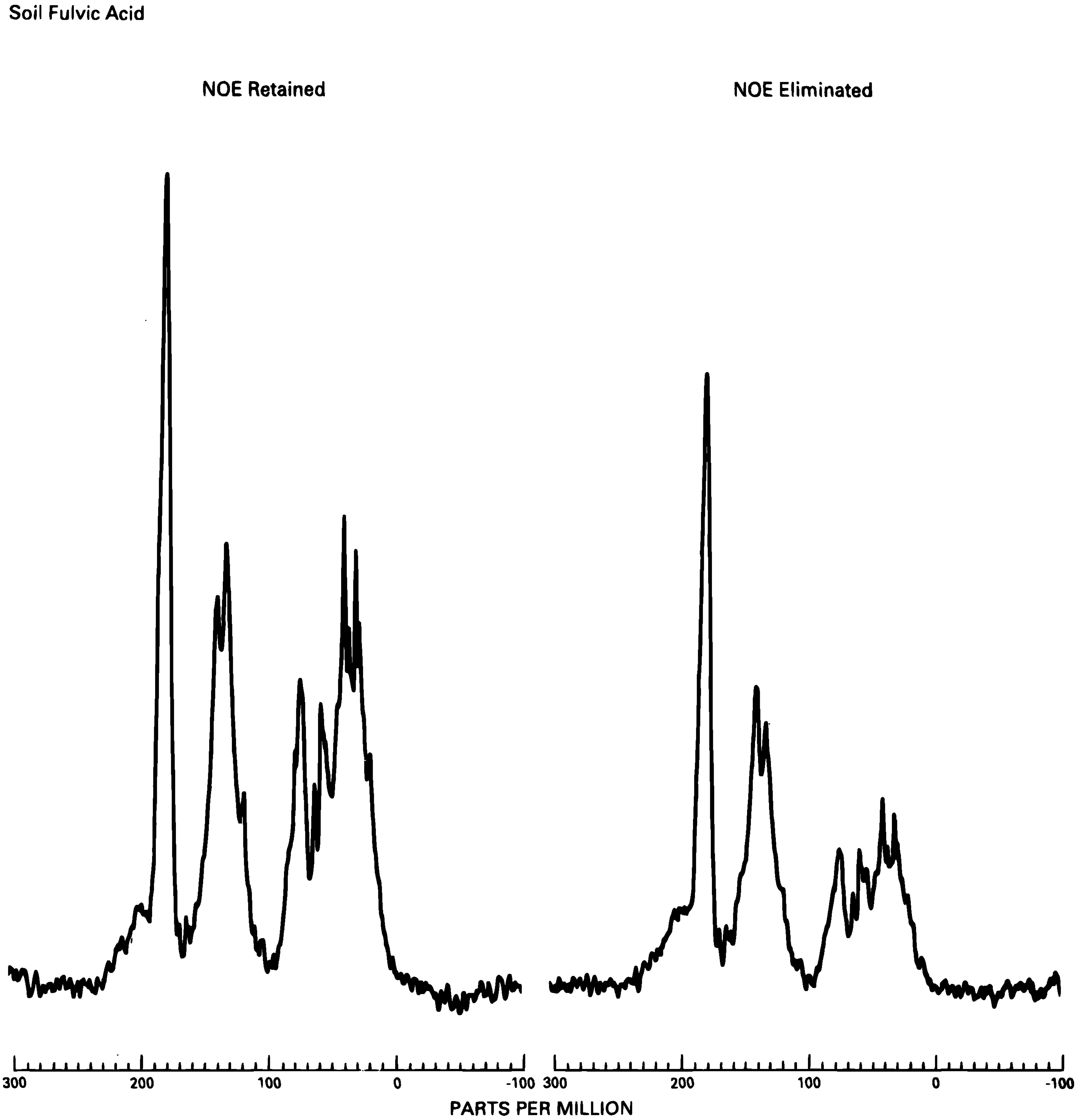


Figure 25.--Nuclear Overhauser enhancement measurement of Soil fulvic acid. Line broadening = 100.0 Hertz.

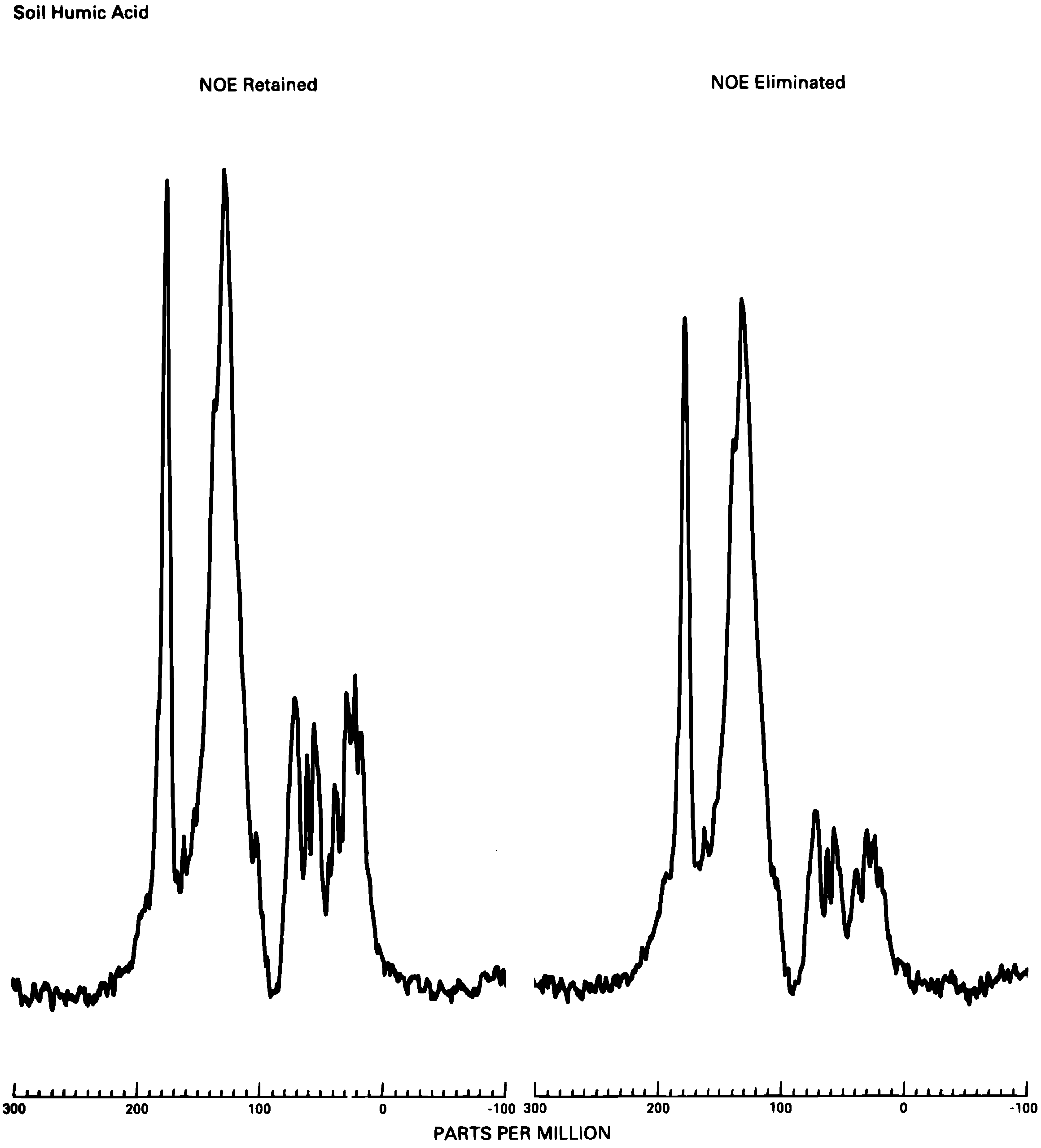


Figure 26.--Nuclear Overhauser enhancement measurement of Soil humic acid. Line broadening = 100.0 Hertz.

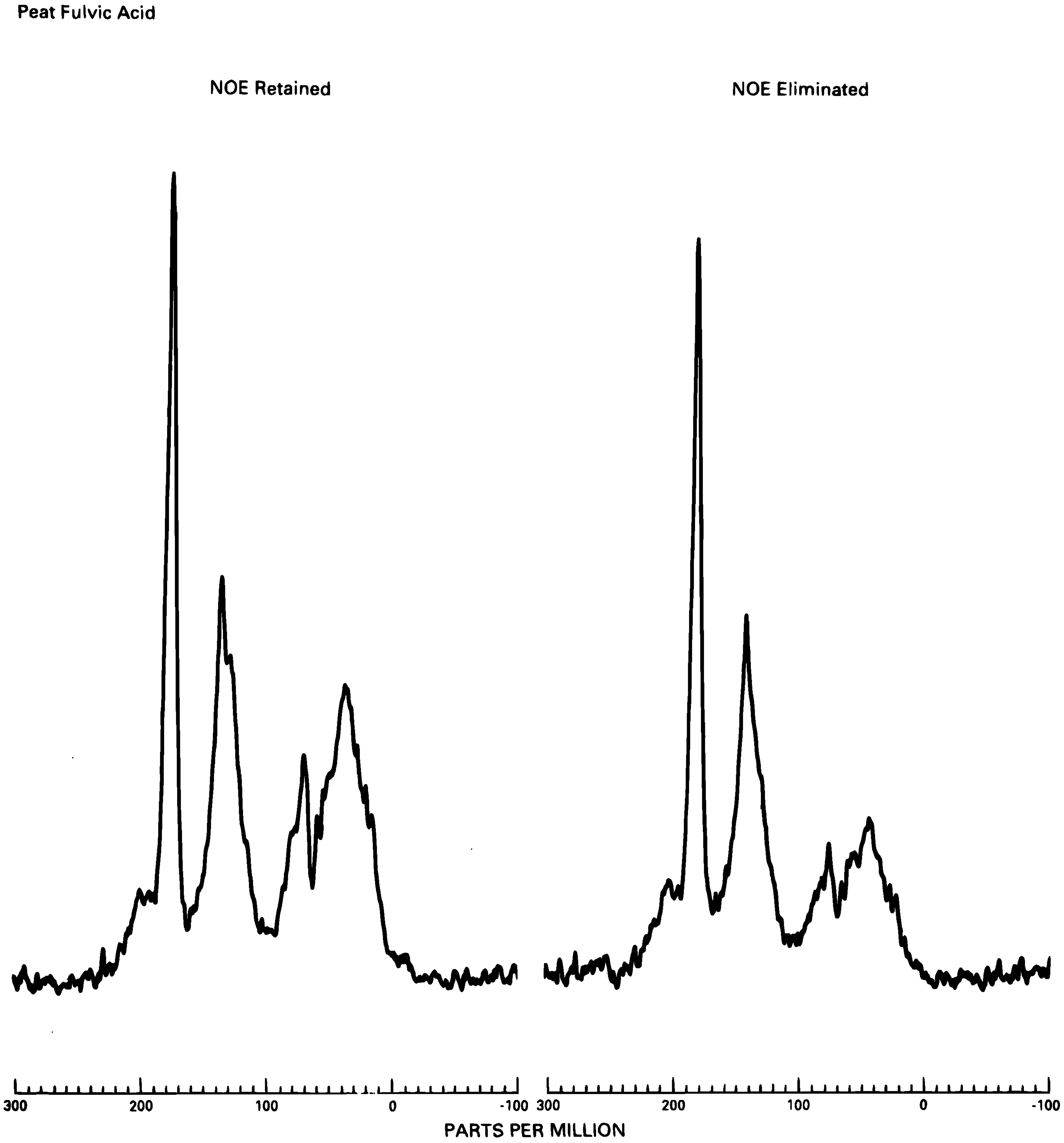


Figure 27.--Nuclear Overhauser enhancement measurement of Peat fulvic acid. Line broadening = 100.0 Hertz.

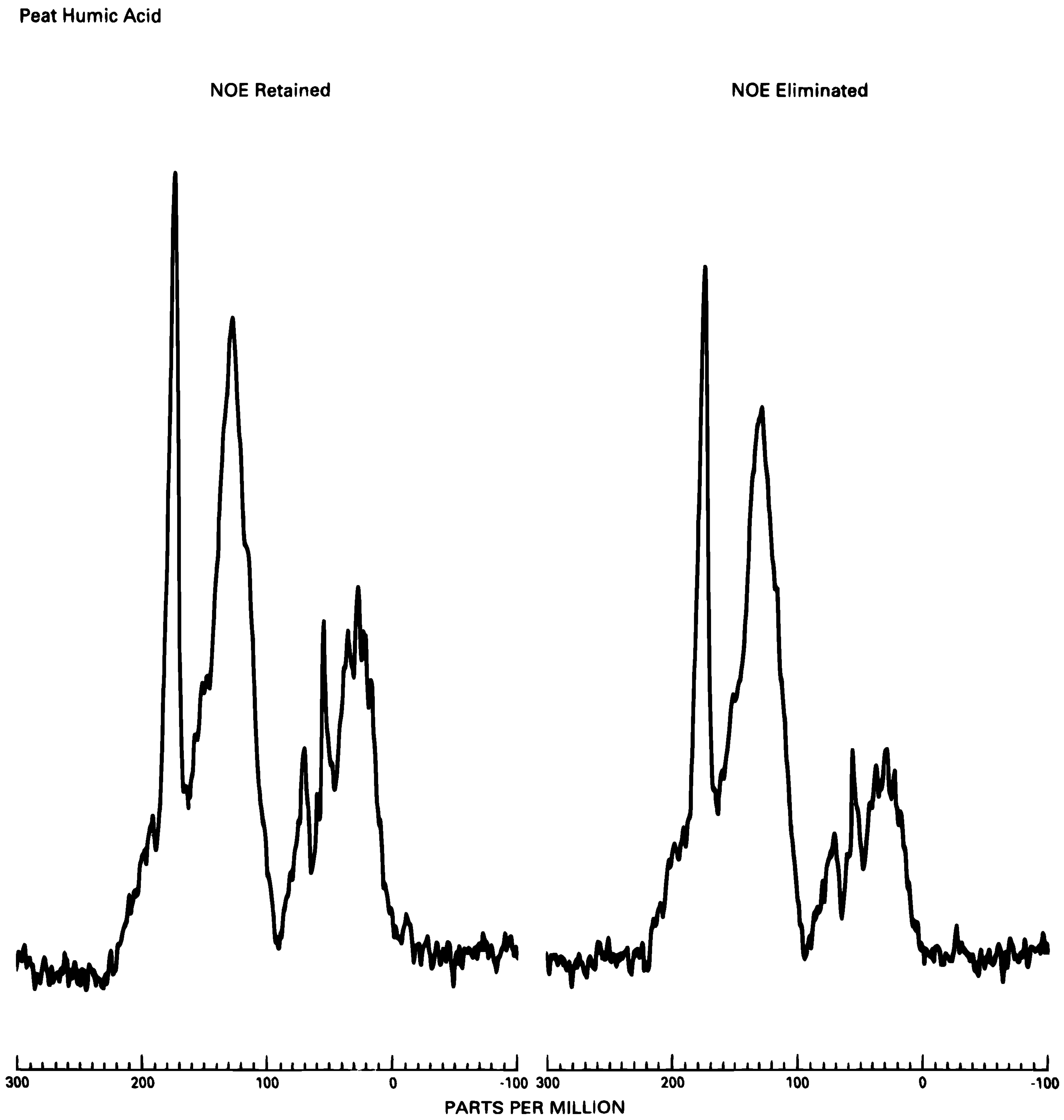


Figure 28.--Nuclear Overhauser enhancement measurement of Peat humic acid.
Line broadening = 100.0 Hertz.

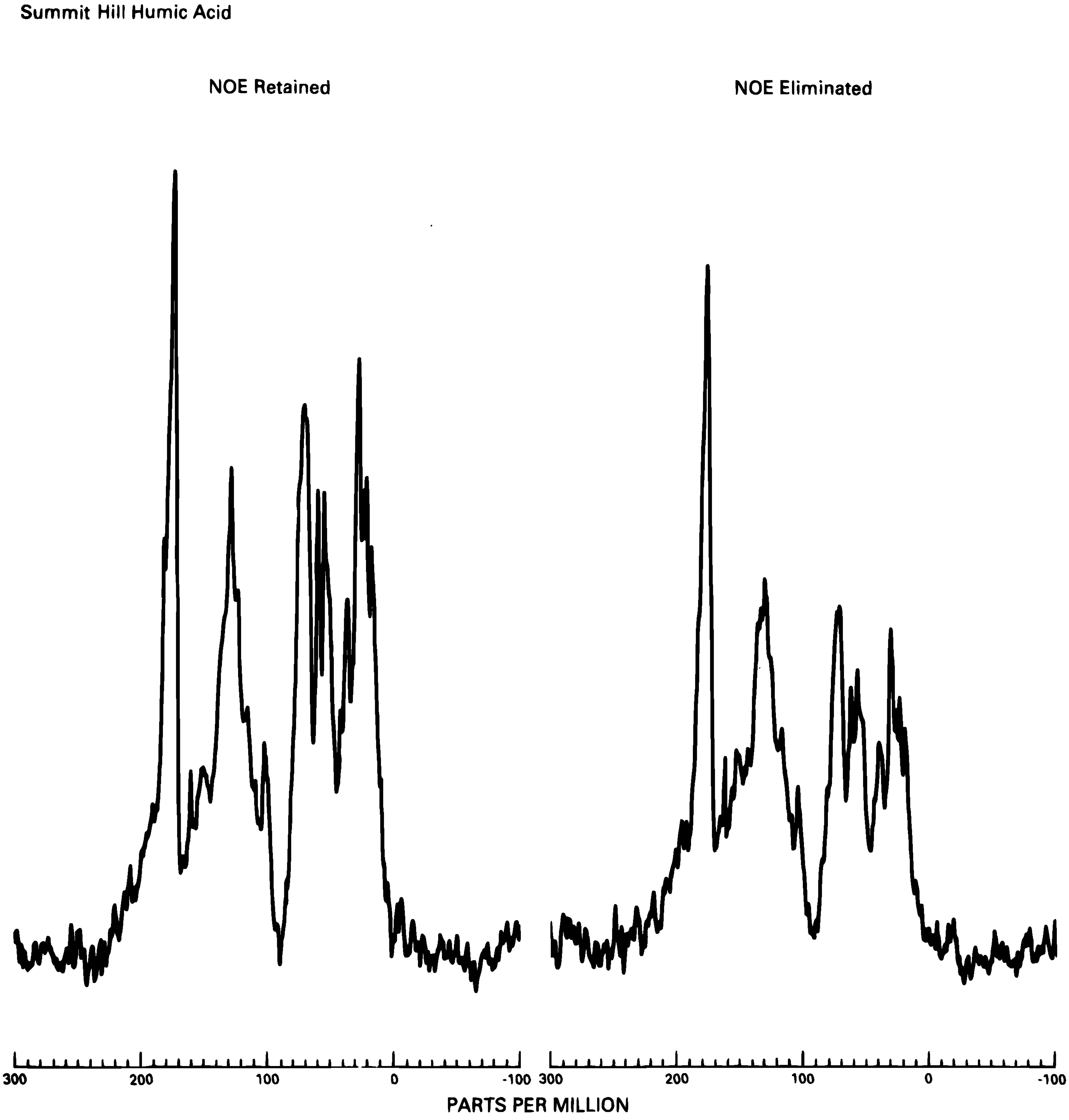


Figure 29.--Nuclear Overhauser enhancement measurement of Summit Hill humic acid. Line broadening = 100.0 Hertz.

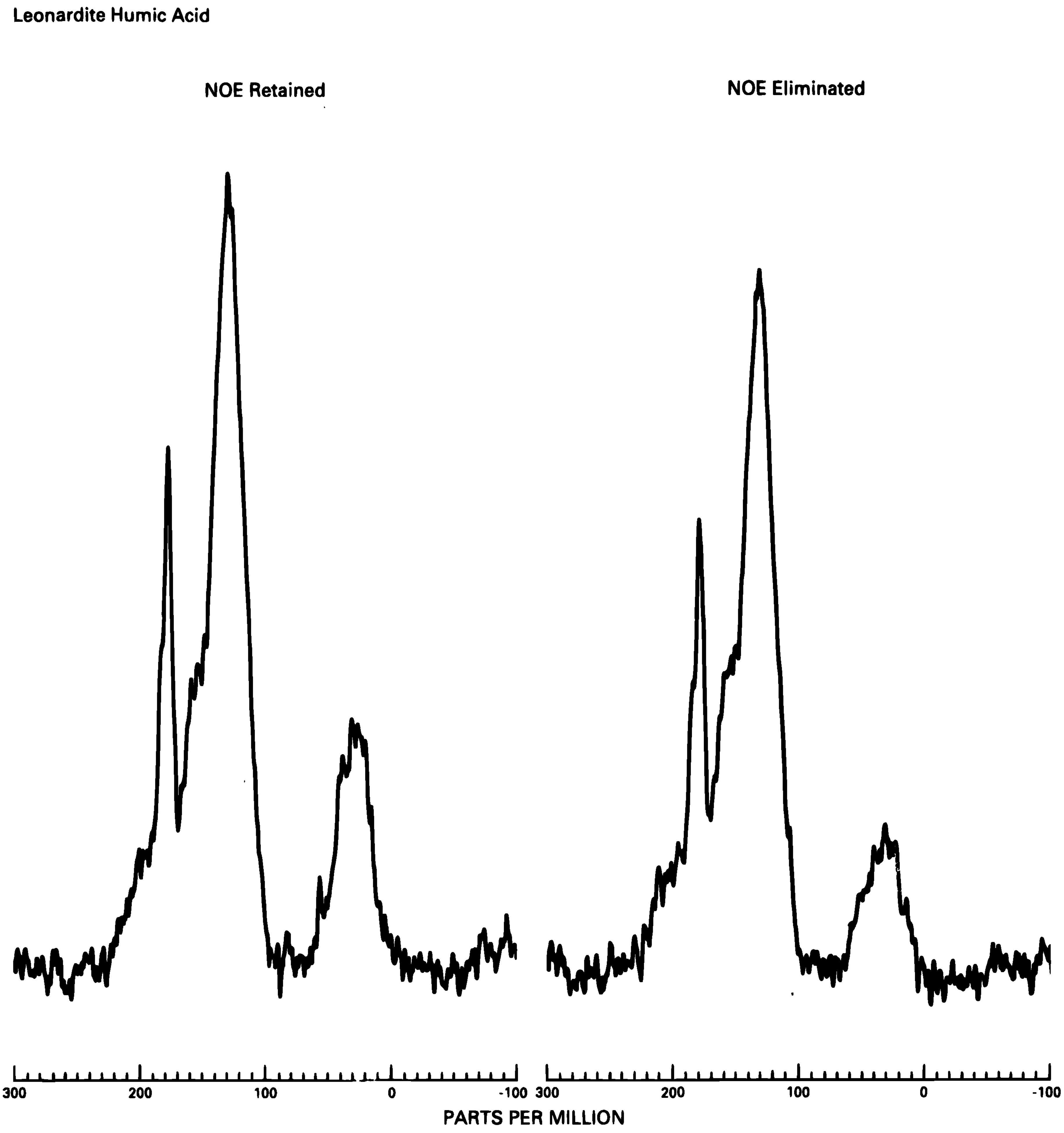


Figure 30.--Nuclear Overhauser enhancement measurement of Leonardite humic acid. Line broadening = 100.0 Hertz.

Table 3.--*Carbon distributions of humic and fulvic acids determined from quantitative carbon-13 nuclear magnetic resonance spectra*

[Numbers represent percent of total spectrum area; Top number determined from electronic integration, bottom number from cutting weighing of spectra; ppm, parts per million; f_a, spectrum area from 110 to 165 ppm divided by total spectrum area]

Sample	Ketone/ Quinone 220-190 ppm	Carboxyl 190-165 ppm	Aromatic 165-90 ppm	Aromatic 165-110 ppm	Acetal/ aromatic 110-90 ppm	Hetero-aliphatic 90-60 ppm	Aliphatic 60-0 ppm	Aromaticity f_a
Suwannee fulvic	7	20	28	24	5	11	33	0.24
	8	21	21			11	32	
Suwannee humic	8	19	45	37	9	7	21	.37
	9	18	43			8	22	
Nordic fulvic	10	24	37	31	7	12	18	.31
	9	19	34			11	23	
Nordic humic	10	19	45	38	7	11	15	.38
	9	19	43			12	18	
Soil fulvic	12	25	31	30	1	9	22	.30
	9	27	31			8	25	
Soil humic	6	18	54	50	4	6	16	.50
	5	20	52			6	17	
Peat fulvic	7	28	36	34	3	9	20	.34
	8	27	36			8	20	
Peat humic	5	20	51	47	4	5	19	.47
	6	20	48			5	19	
Summit Hill humic	7	19	35	30	4	14	25	.30
	6	19	35			12	28	
Leonardite humic	8	15	61	58	4	1	14	.58
	7	16	62			0	15	

Table 4.--*Nuclear Overhauser enhancement (NOE) factors* (η) *for major spectral regions of carbon-13 nuclear magnetic resonance spectra of humic and fulvic acids*

[Values measured from cutting and weighing of spectra; ppm, parts per million]

Sample	Ketone/ Quinone 220-190 ppm	Carboxyl 190-165 ppm	Aromatic 165-90 ppm	Hetero-aliphatic 90-60 ppm	Aliphatic 60-0 ppm
Suwannee fulvic	0.25	0.45	0.42	1.23	1.37
Suwannee humic	.04	.16	.10	.48	.53
Nordic fulvic	.11	.13	.23	.73	1.00
Nordic humic	.06	.19	.17	.35	1.15
Soil fulvic	-.05	.36	.47	1.11	1.32
Soil humic	-.14	.13	.11	.65	.60
Peat fulvic	-.03	.13	.10	.58	.79
Peat humic	.02	.08	.08	.29	.67
Summit Hill humic	.30	.14	.18	.86	.79
Leonardite humic	.12	.18	.11		.61

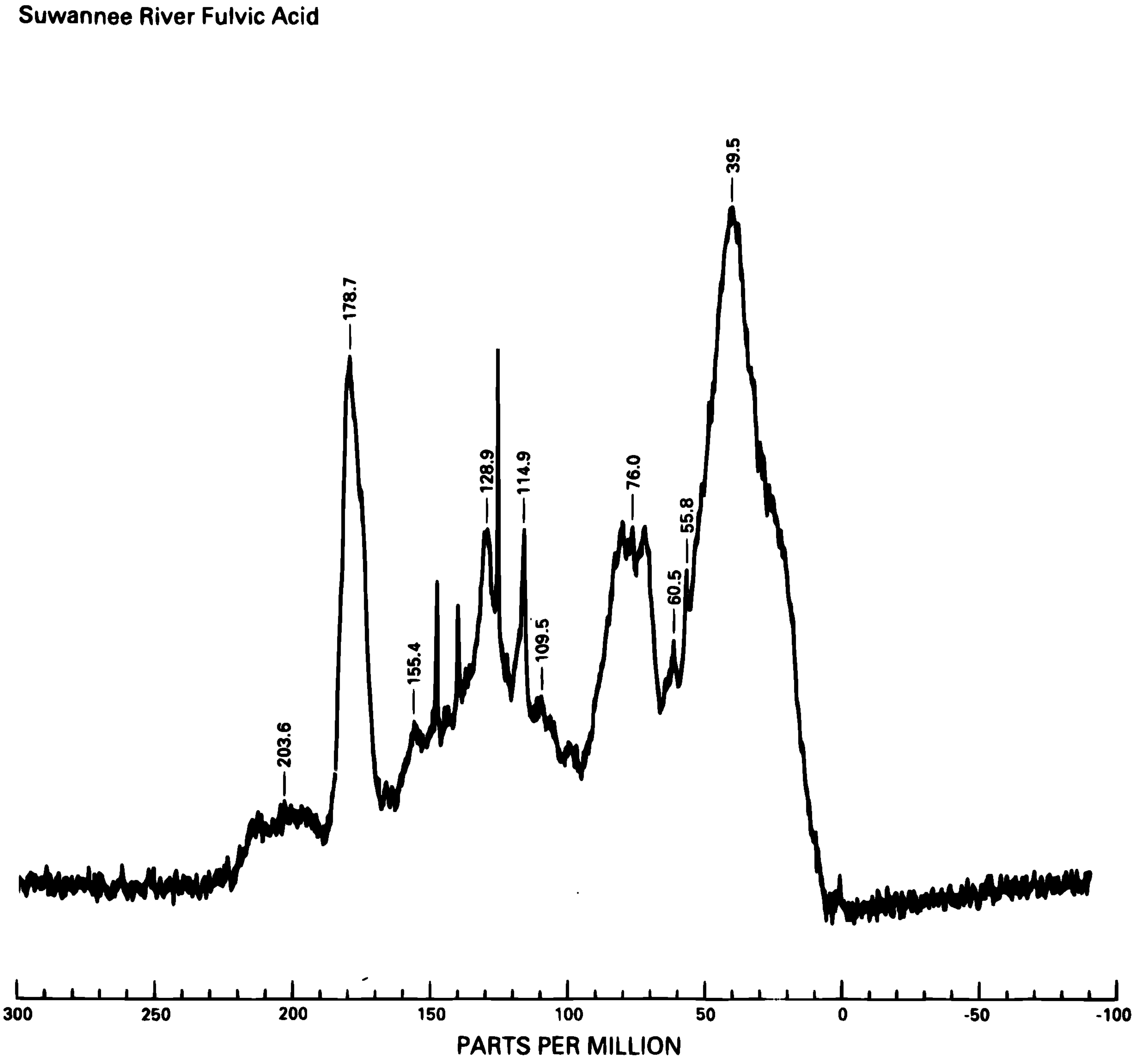


Figure 31.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Suwannee River fulvic acid. Line broadening = 15.0 Hertz.

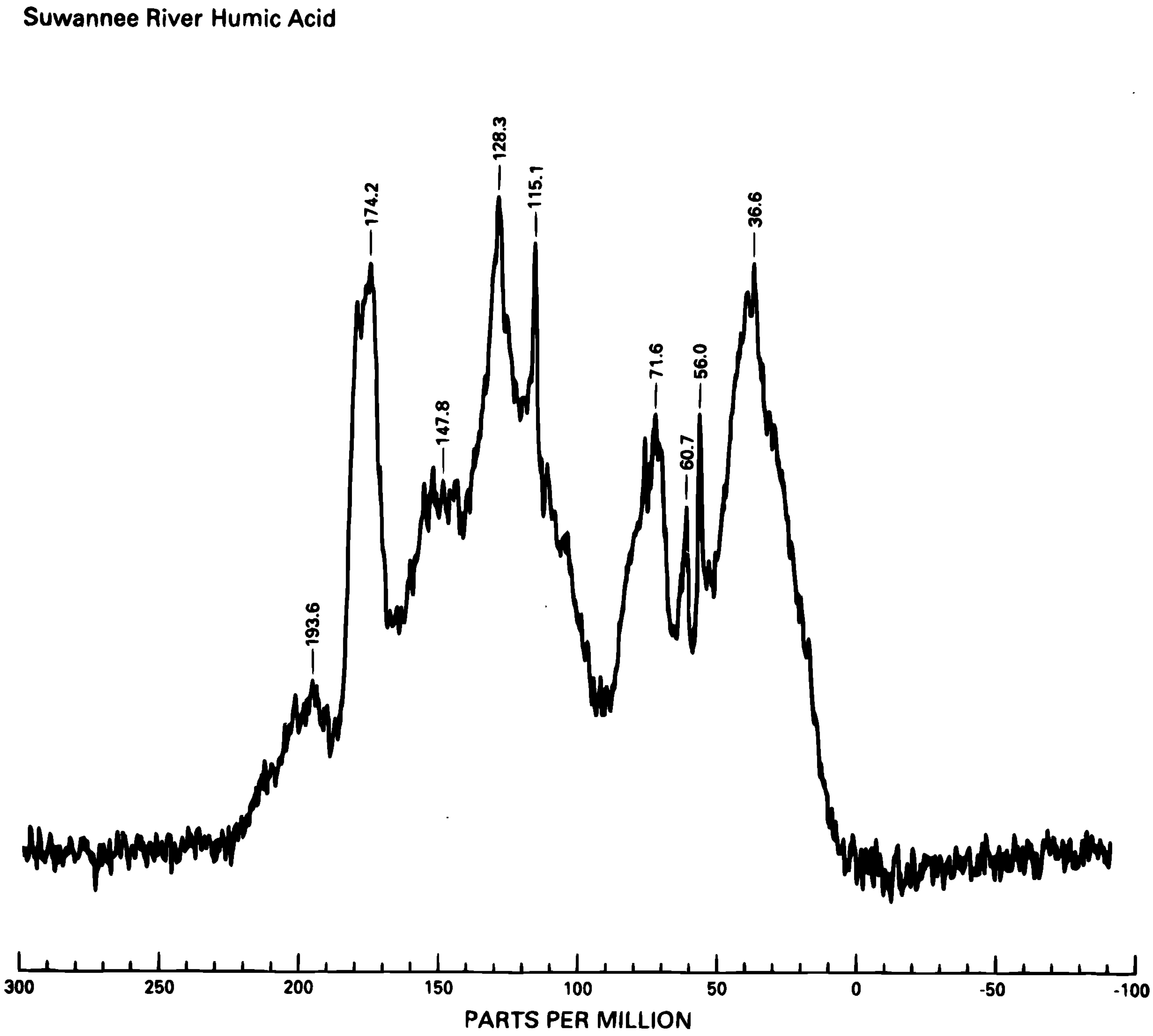


Figure 32.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Suwannee River humic acid. Line broadening = 30.0 Hertz.

Nordic Fulvic Acid

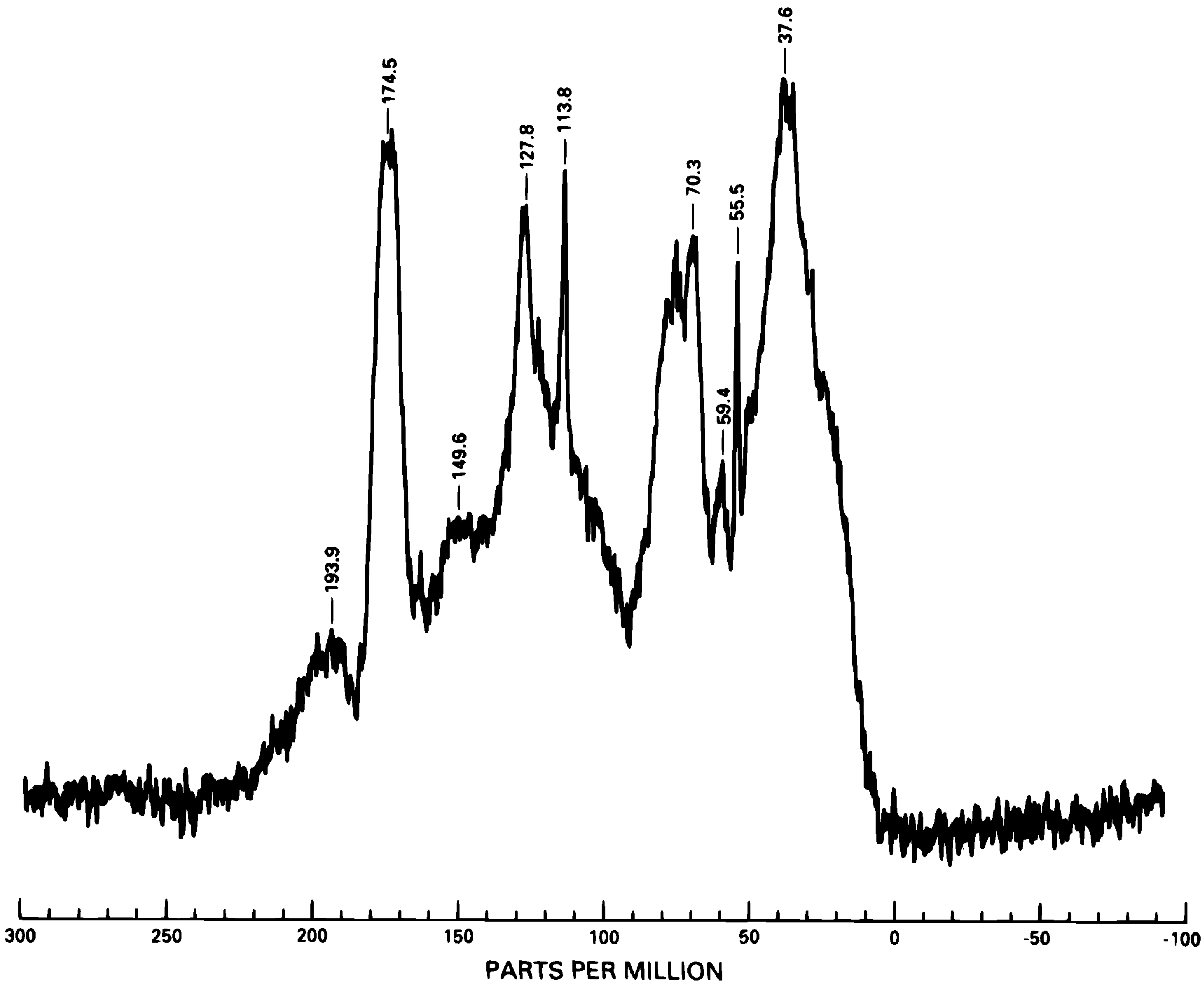


Figure 33.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Nordic fulvic acid. Line broadening = 20.0 Hertz.

Nordic Humic Acid

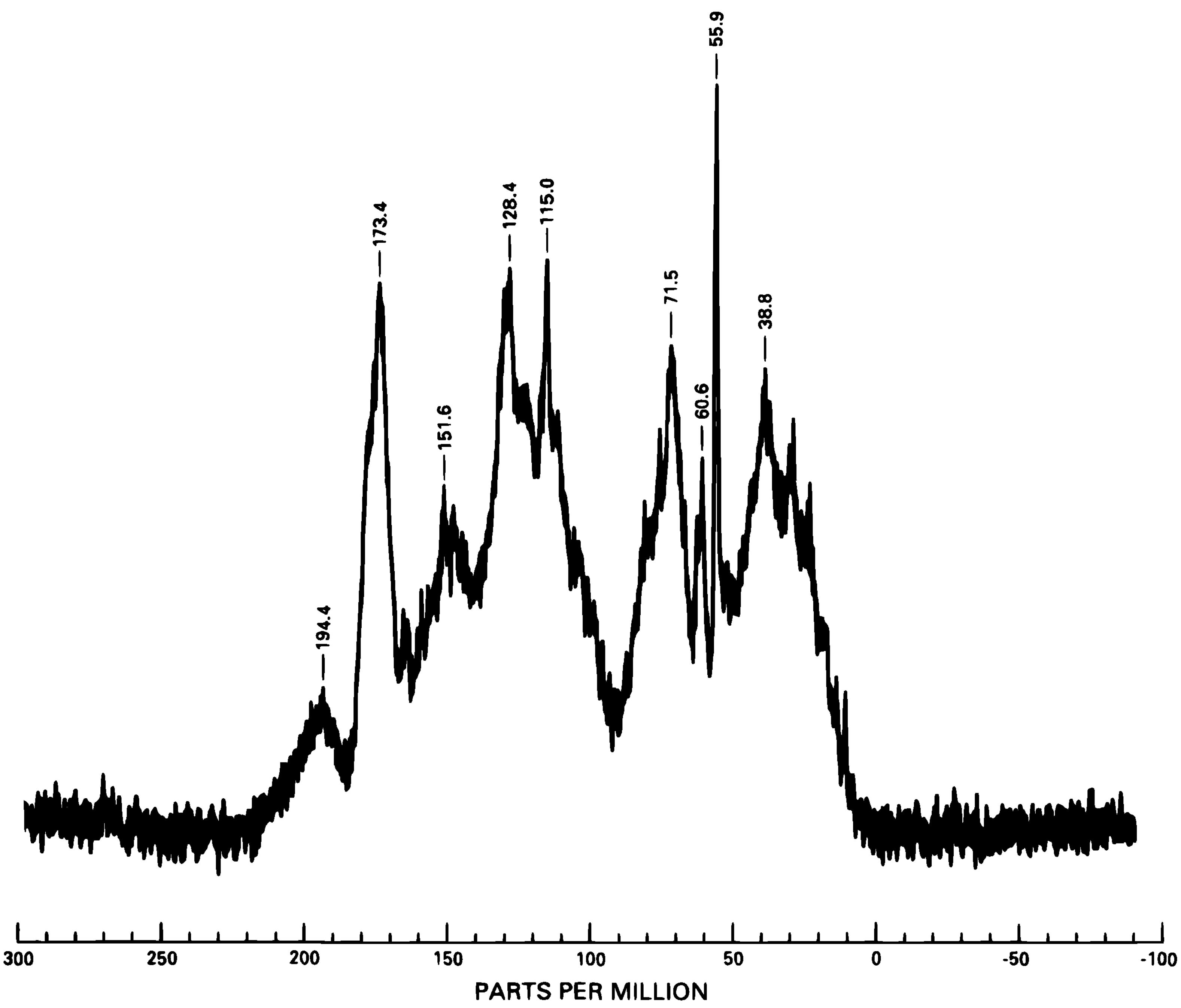


Figure 34.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Nordic humic acid. Line broadening = 20.0 Hertz.

Soil Fulvic Acid

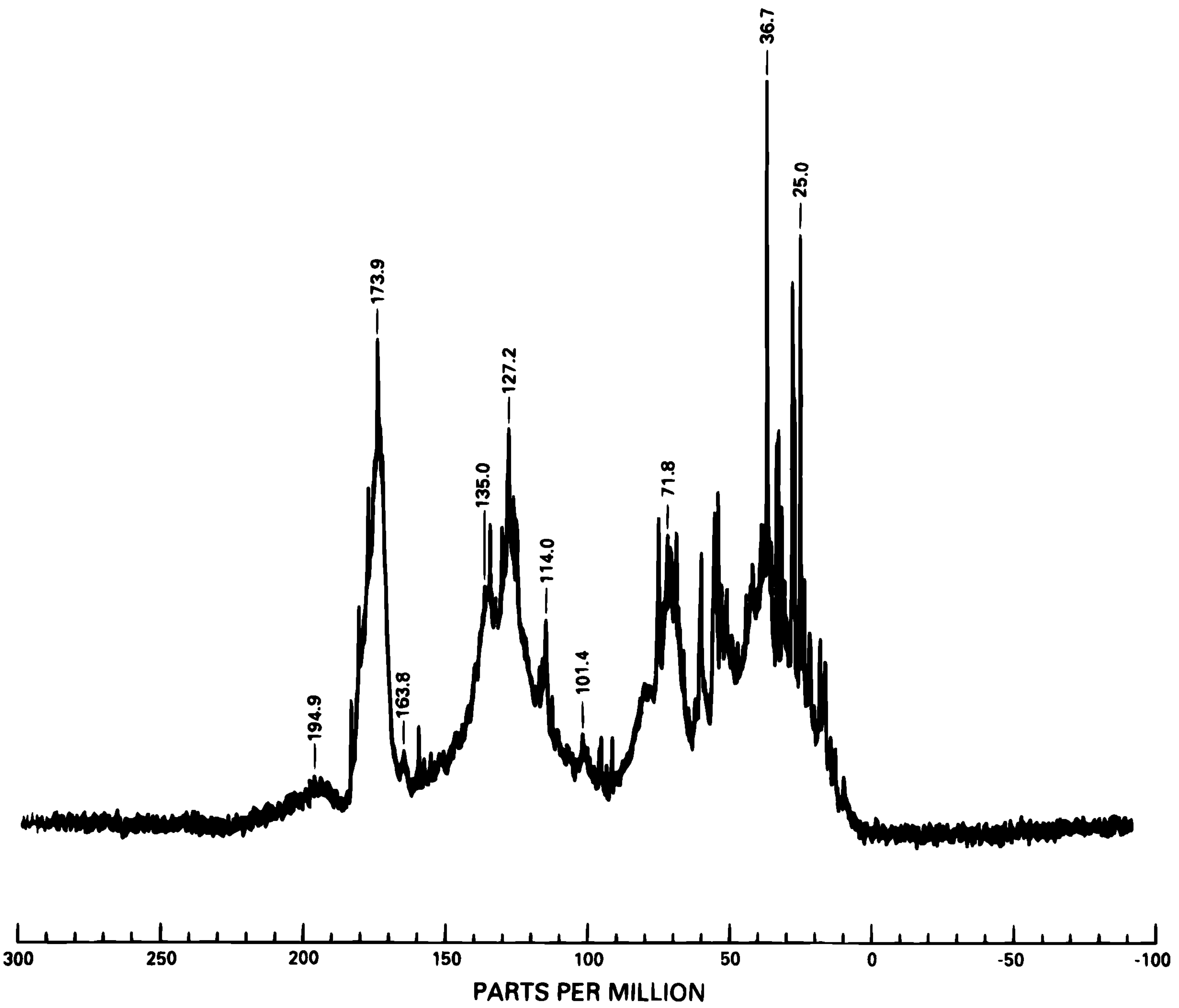


Figure 35.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Soil fulvic acid. Line broadening = 5.0 Hertz.

Soil Fulvic Acid

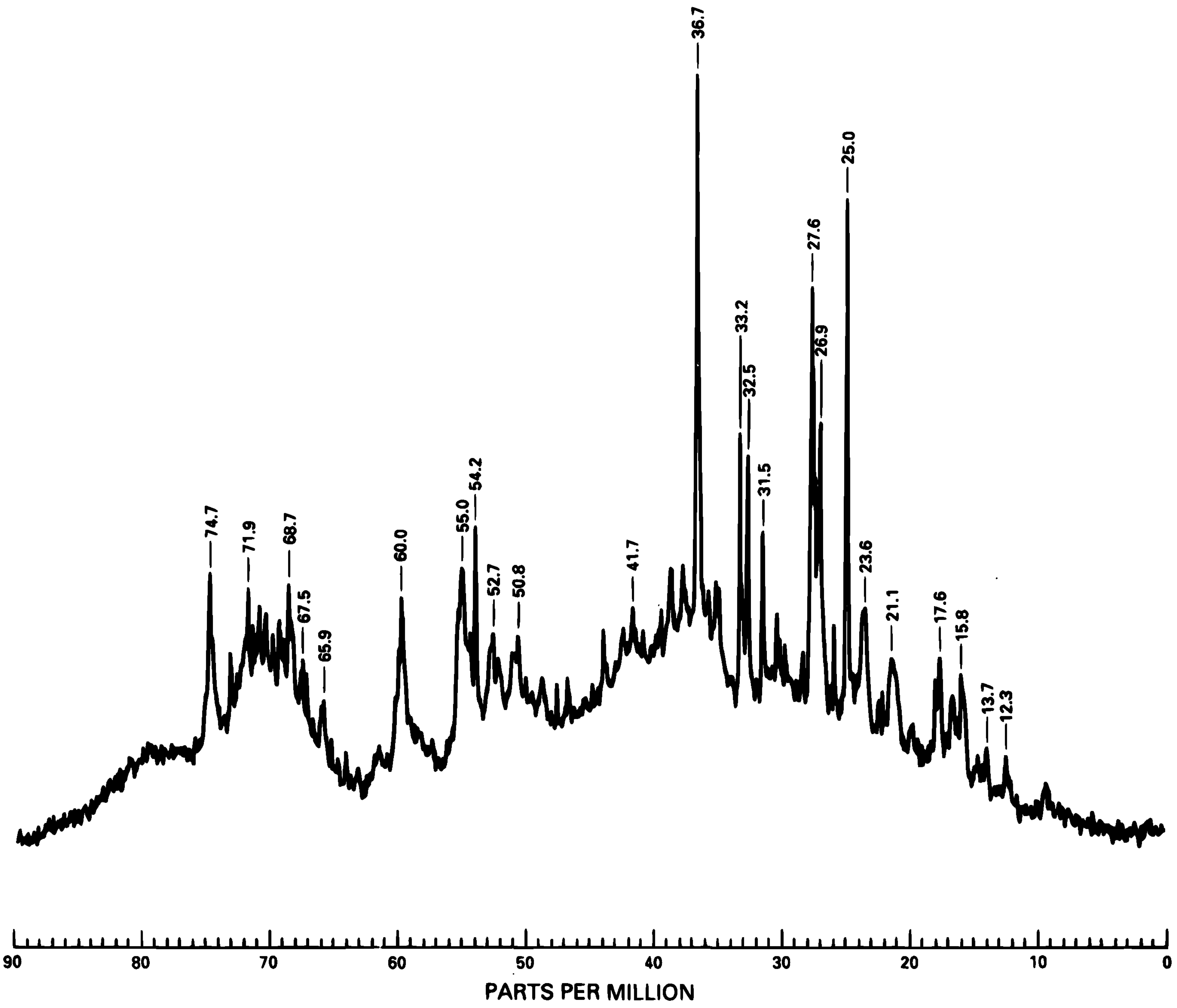


Figure 36.--Horizontal expansion of aliphatic region of nonquantitative carbon-13 nuclear magnetic resonance spectrum of Soil fulvic acid. Line broadening = 3.0 Hertz.

Soil Humic Acid

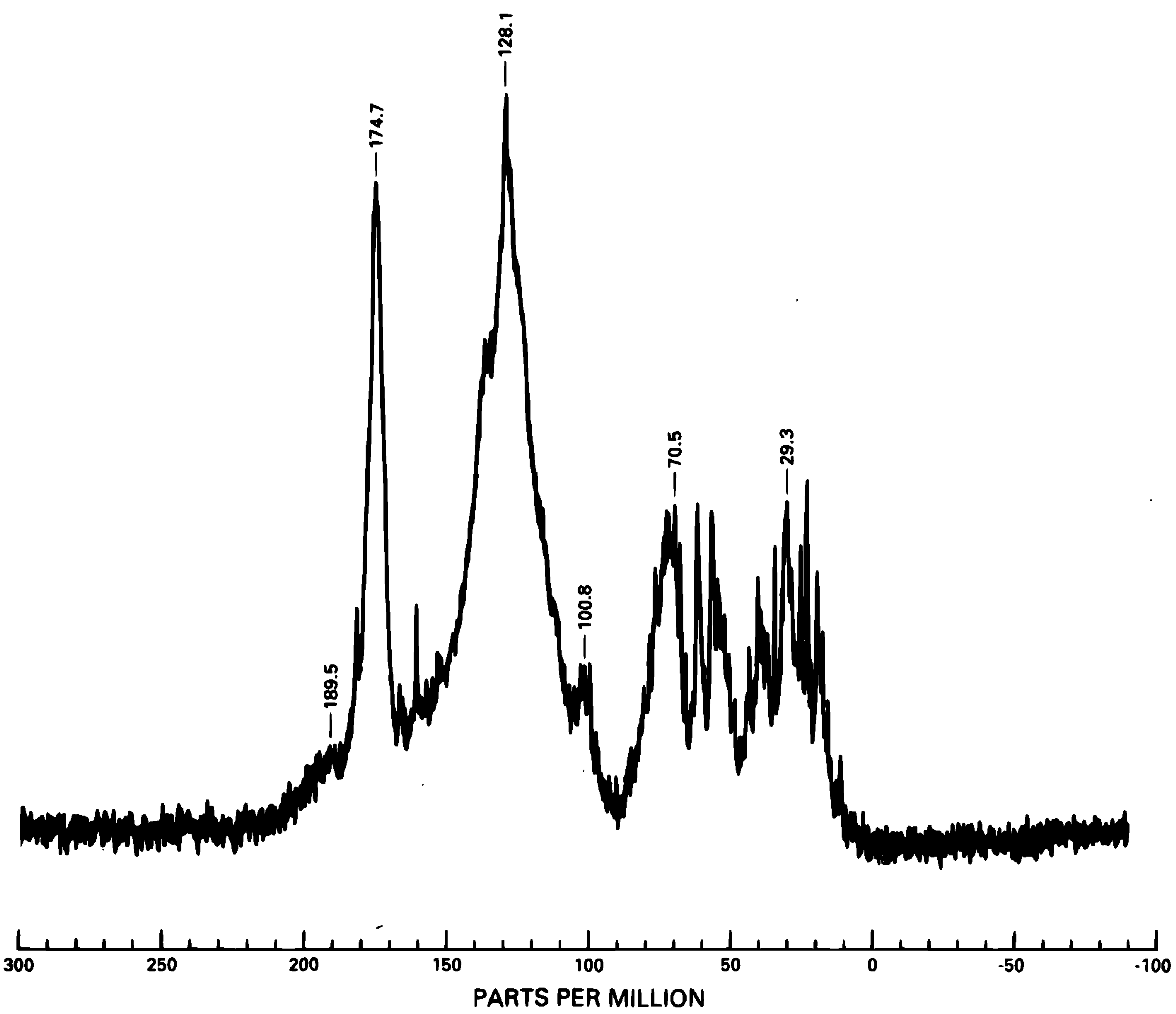


Figure 37.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Soil humic acid. Line broadening = 10.0 Hertz.

Soil Humic Acid

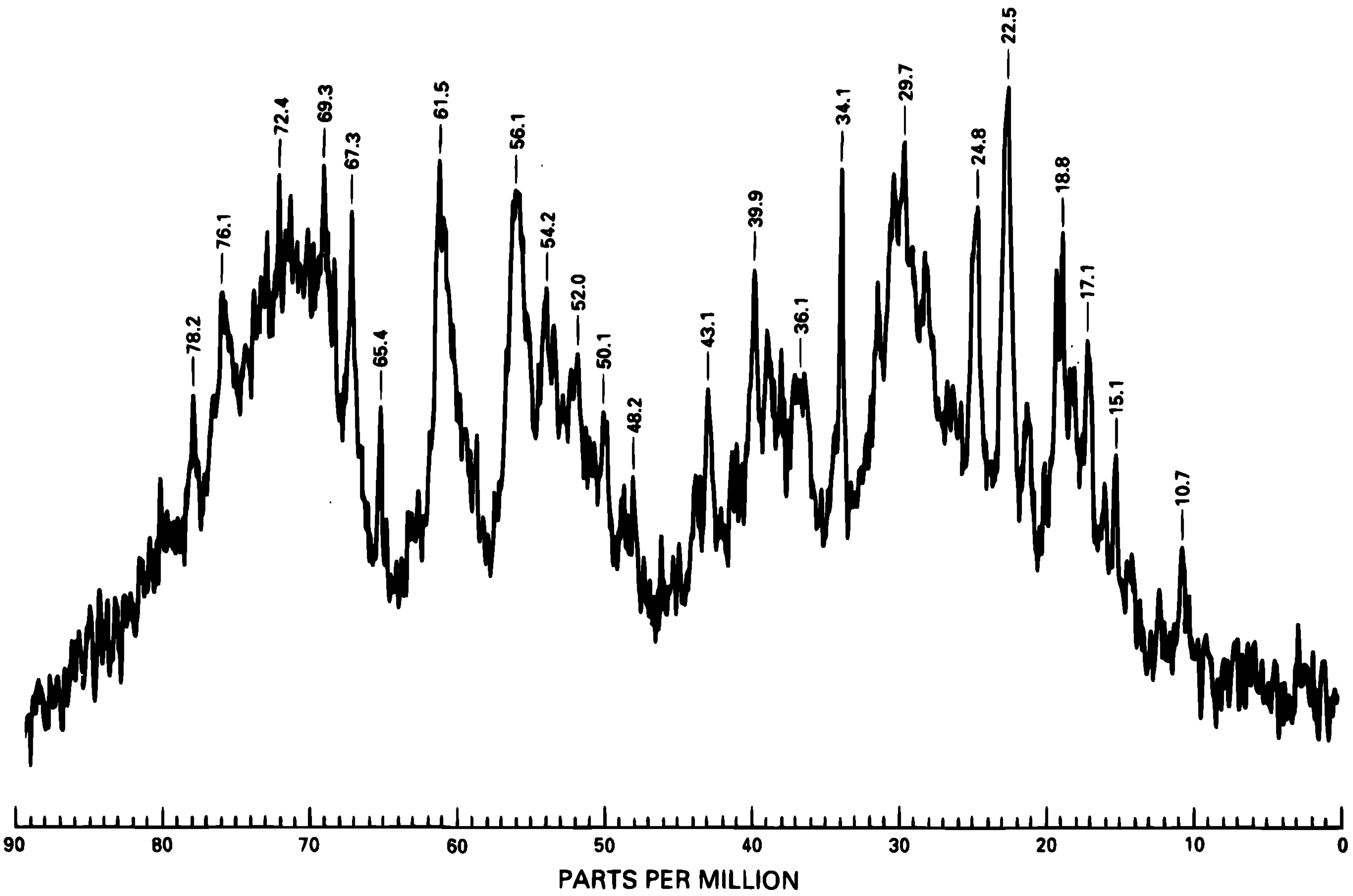


Figure 38.--Horizontal expansion of aliphatic region of nonquantitative carbon-13 nuclear magnetic resonance spectrum of Soil humic acid. Line broadening = 5.0 Hertz.

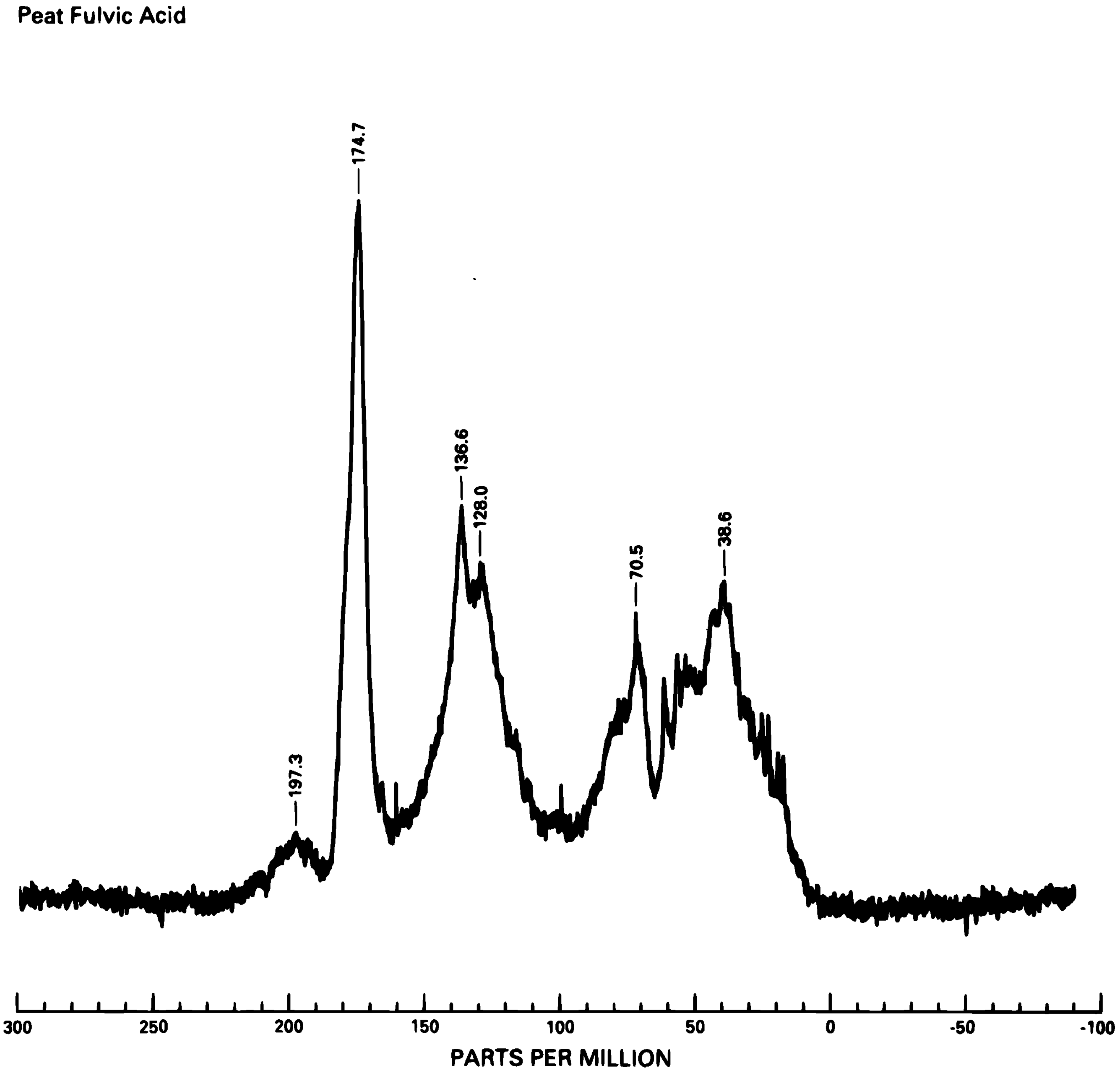


Figure 39.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Peat fulvic acid. Line broadening = 10.0 Hertz.

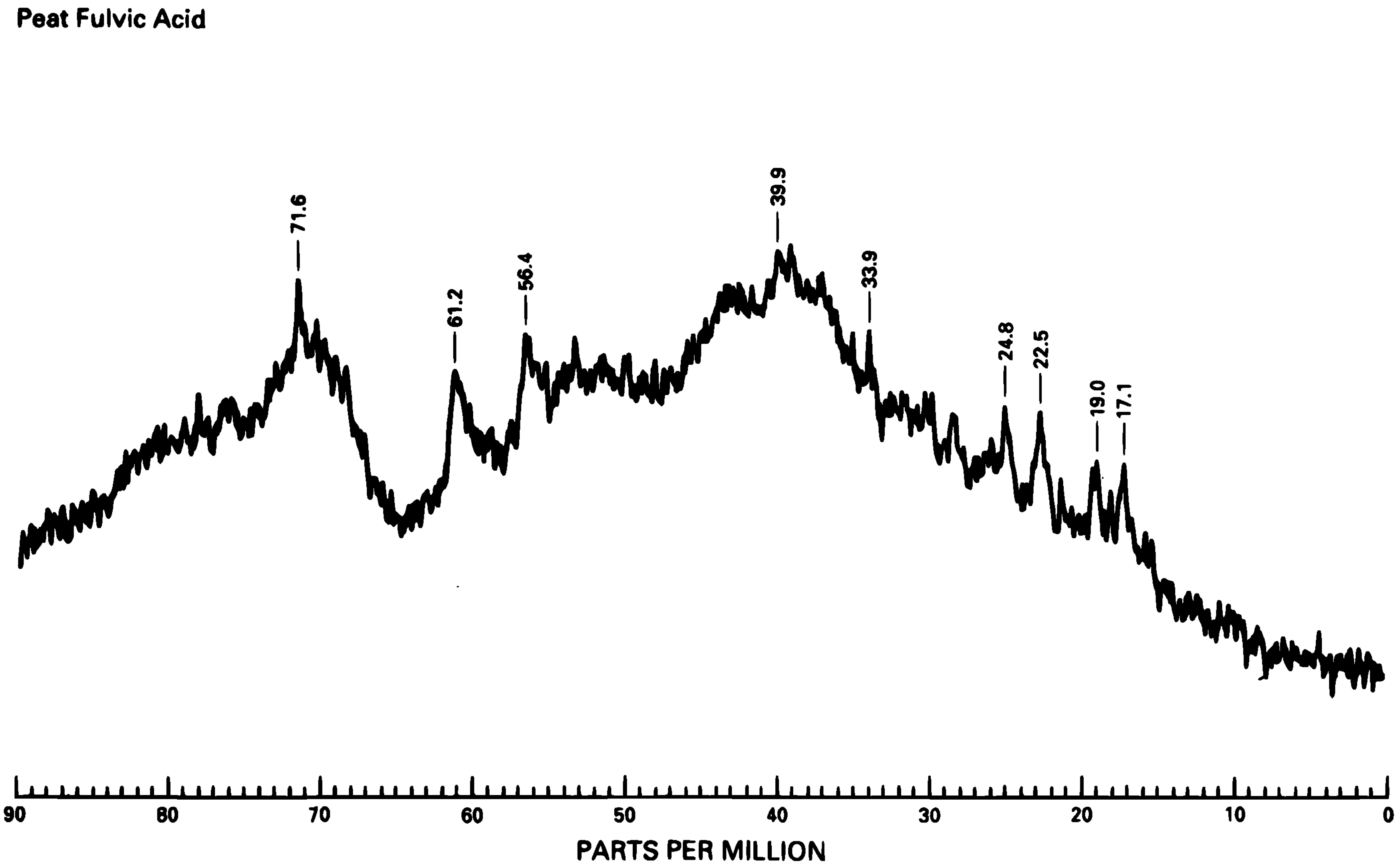


Figure 40.--Horizontal expansion of aliphatic region of nonquantitative carbon-13 nuclear magnetic resonance spectrum of Peat fulvic acid. Line broadening = 5.0 Hertz.

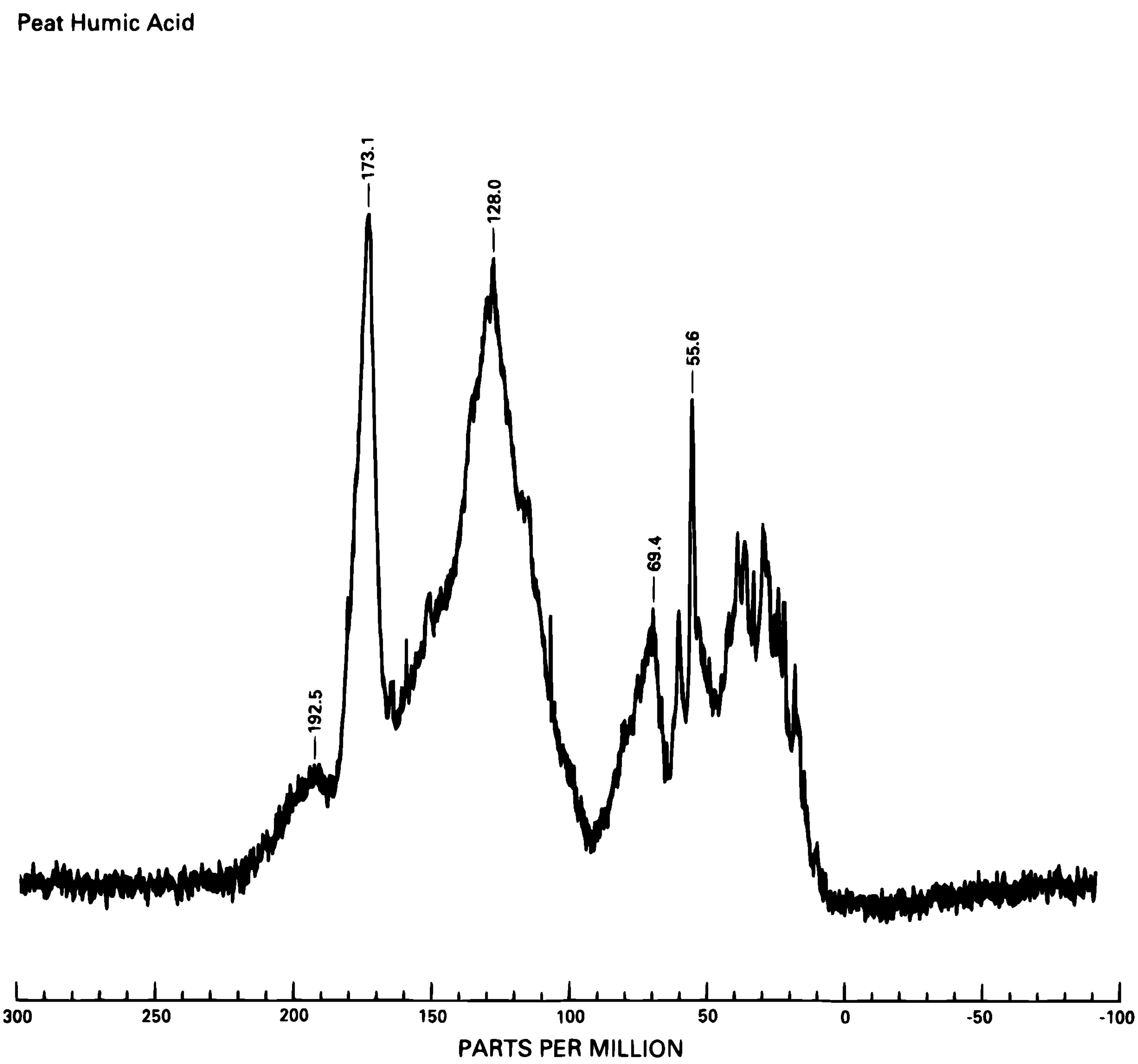


Figure 41.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Peat humic acid. Line broadening = 10.0 Hertz.

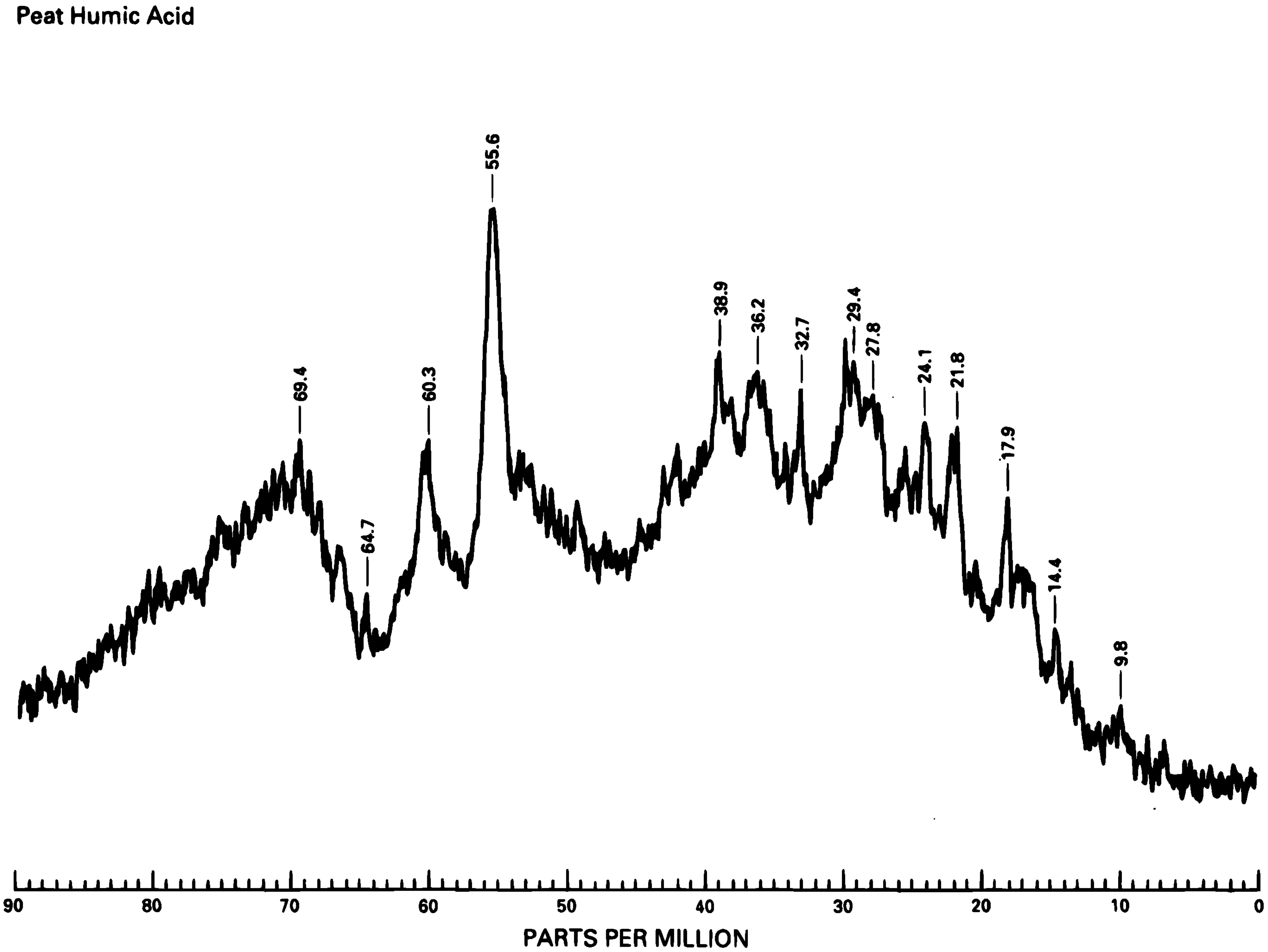


Figure 42.--Horizontal expansion of aliphatic region of nonquantitative carbon-13 nuclear magnetic resonance spectrum of Peat humic acid. Line broadening = 5.0 Hertz.

Summit Hill Humic Acid

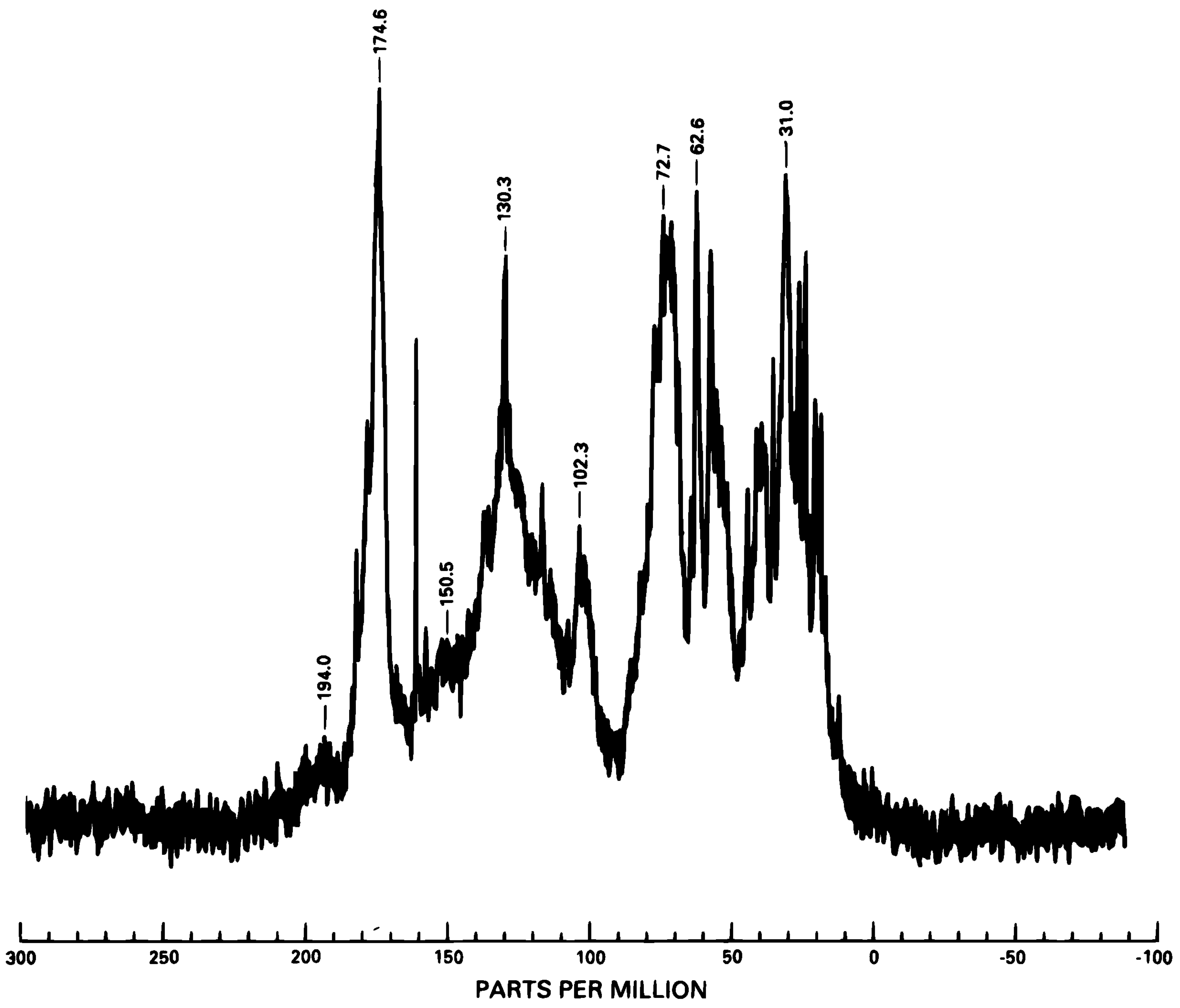


Figure 43.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Summit Hill humic acid. Line broadening = 10.0 Hertz.

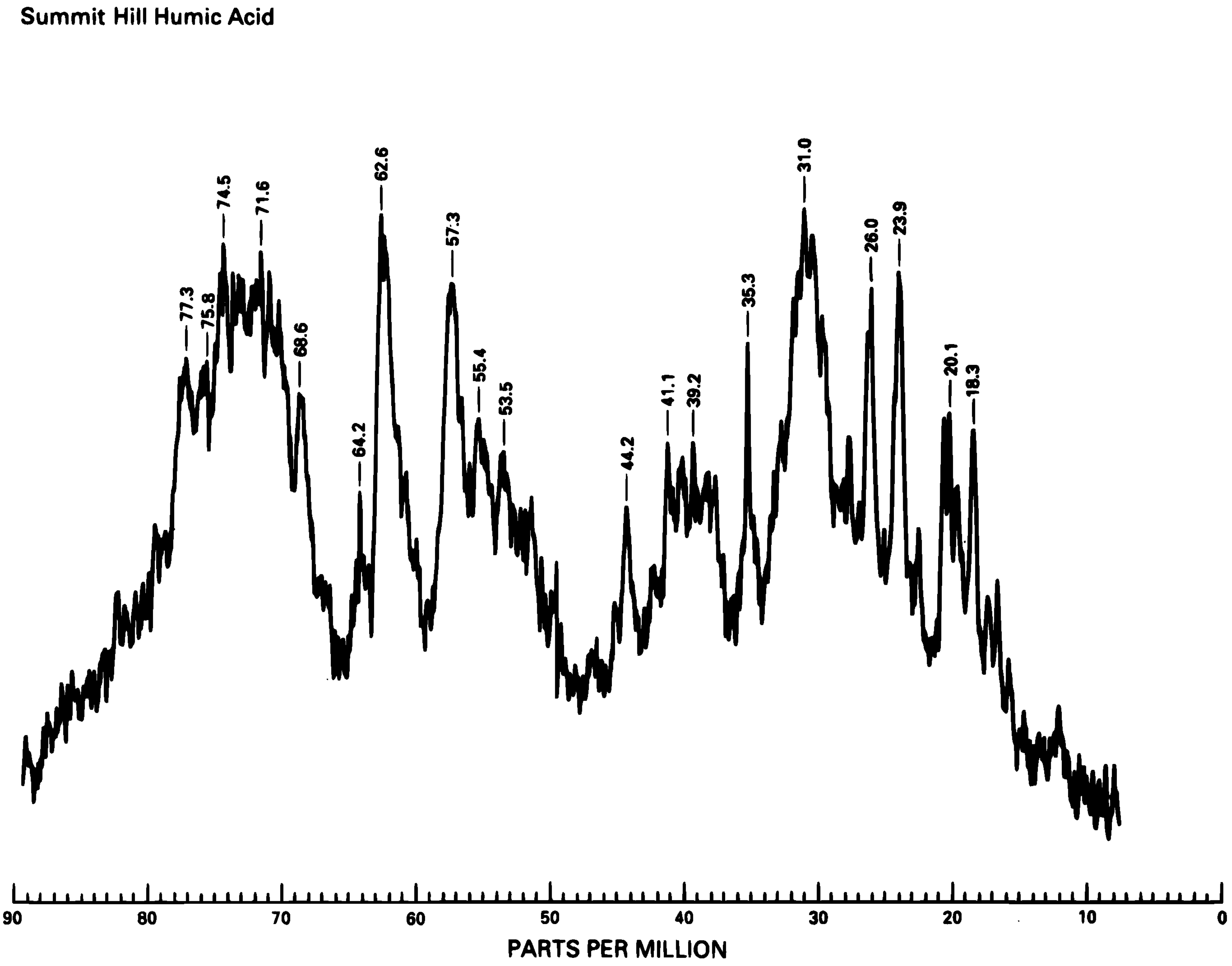


Figure 44.--Horizontal expansion of aliphatic region of nonquantitative carbon-13 nuclear magnetic resonance spectrum of Summit Hill humic acid. Line broadening = 5.0 Hertz.

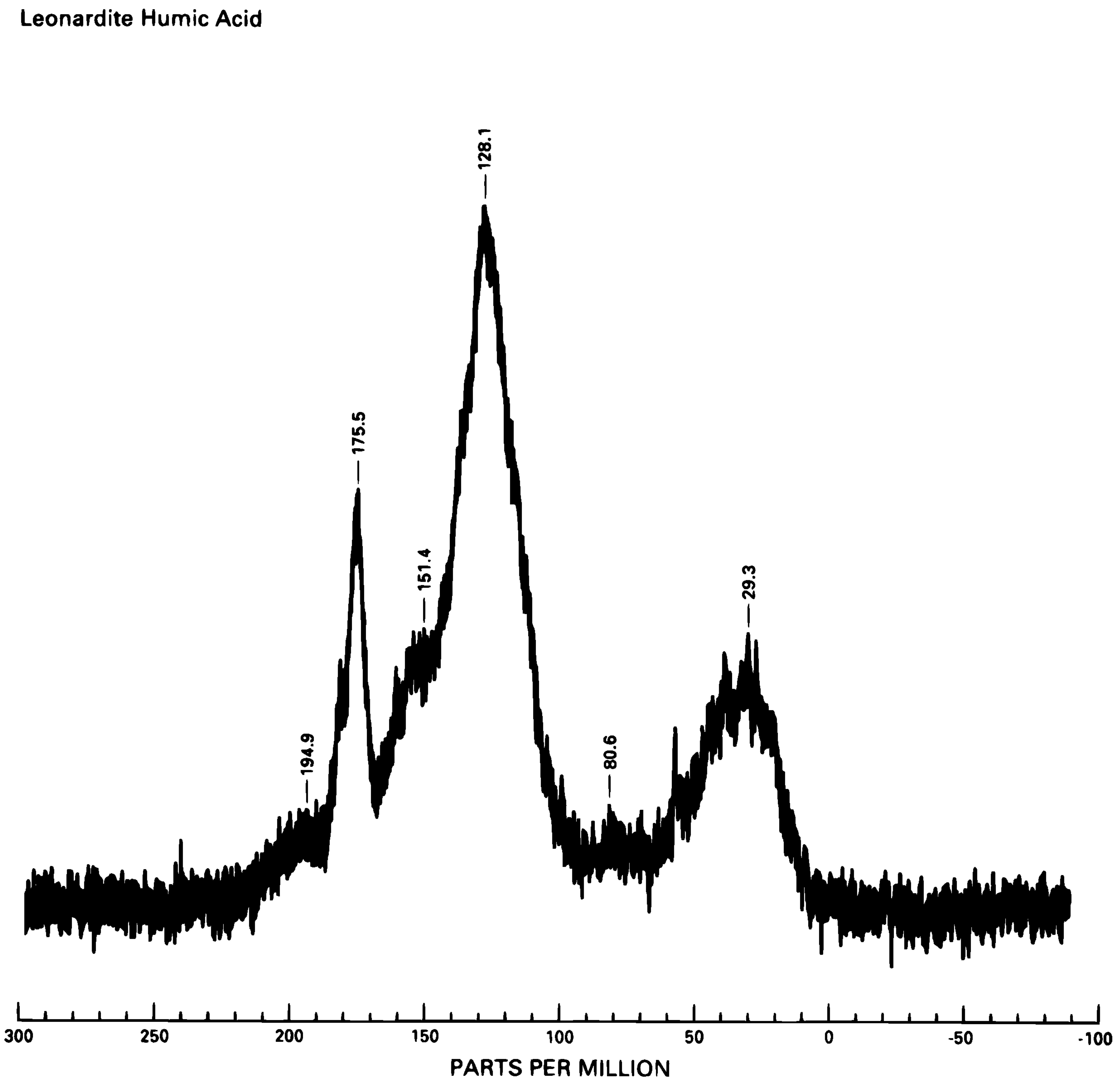


Figure 45.--Nonquantitative carbon-13 nuclear magnetic resonance spectrum of Leonardite humic acid. Line broadening = 5.0 Hertz.

Attached Proton Test Carbon-13 Nuclear Magnetic Resonance Spectra

In the APT spectra shown in figures 46-55, methyl and methine carbons are positive, and methylene and quaternary carbons are negative. The spectra all exhibit a similar pattern. Based upon chemical shift considerations and previous work on the Suwannee River samples in which APT spectra were recorded with different tau delays to further differentiate between methylene and quaternary carbons and between methyl and methine carbons (Thorn, 1989), the following general assignments can be made. The aliphatic carbons (0 to 60 ppm) are resolved into methyls (0 to 26 ppm), methylenes (26 to 43 ppm), and methines (43 to 60 ppm). The hetero-aliphatic carbons (60 to 90 ppm) are methines, and therefore are predominated by secondary alcohols and secondary ethers. The region from 90 to 165 ppm is resolved into peaks from 90 to 110 ppm (acetal, hemiacetal, or protonated aromatic carbons), from 110 to 133 ppm (protonated aromatic carbons), and from 133 to 165 ppm (nonprotonated aromatic carbons). The carboxyl (165 to 190 ppm) and ketone/quinone (190 to 220 ppm) carbons are positive in the APT spectra.

Hydrogen-1 Nuclear Magnetic Resonance Spectra

^{1}H-NMR spectra of the fulvic and humic acids are shown in figures 56-65. These spectra were recorded on sodium salts of the samples in D_2O, and show only the non-exchangeable protons. General assignments for the four major regions of the proton spectra are as follows: (1) 0 to 1.6 ppm, protons on methyl and methylene carbons directly bonded to other carbons; (2) 1.6 to 3.0 ppm, protons on methylene and methine carbons alpha to aromatic rings, carboxyl, and carbonyl groups; (3) 3.3 to 5.5 ppm, protons on methyl, methylene, or methine carbons directly bonded to oxygen or nitrogen, including carbohydrate and amino acid protons; and (4) 5.5 to 9.0 ppm, protons attached to unsaturated carbons and aromatic protons. Chemical shift ranges for protons are summarized in table 5.

DEPTGL Carbon-13 Nuclear Magnetic Resonance Spectra

DEPTGL ^{13}C NMR spectra of the Suwannee River fulvic acid are shown in figures 66-70. An advantage of the DEPT sequence compared to the APT sequence is that four subspectra can be generated from the DEPT experiment showing all protonated carbons, methine carbons only, methylene carbons only, and methyl carbons only. The DEPTGL spectra show results similar to the APT spectra. The peak maximum for the methyl carbons occurs at 33.3 ppm. The peak maximum for the methylene carbons occurs at 33.3 ppm. Methylene carbons also occur from about 57 to 78 ppm, with peak maxima at 59.7 and 68.3 ppm. These represent primary alcohol and ether carbons which are not discernible from the APT spectra. Peak maxima for the methine carbons occur at 44.9, 75.3, 95.5, 114.1, and 127.4 ppm. The absence of any resonances in the region from about 190 to 200 ppm in the spectra showing all protonated carbons and methine carbons only indicates that the concentration of aldehyde carbons in the fulvic acid is insignificant.

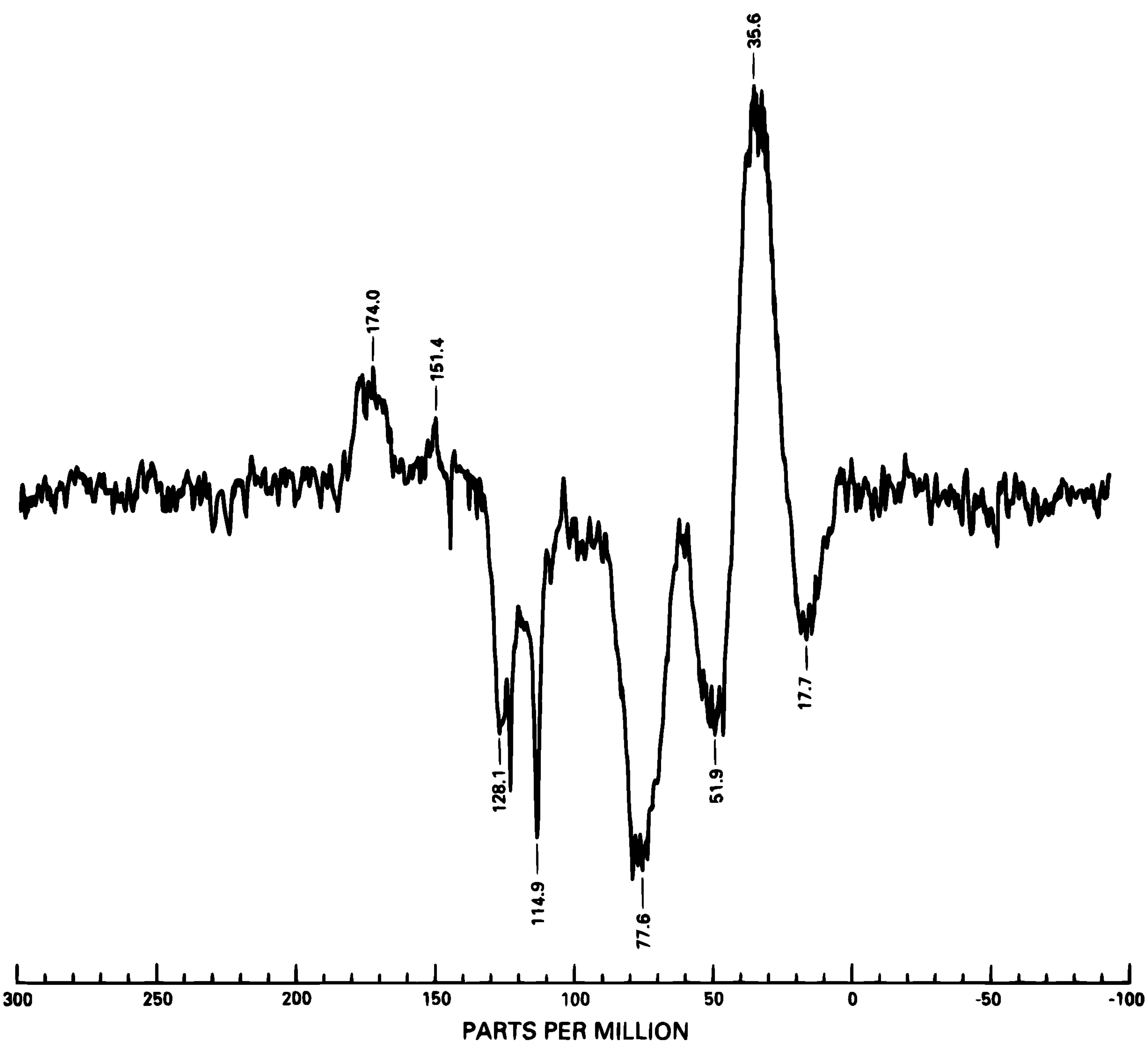


Figure 46.--^{13}C attached proton test spectrum of Suwannee River fulvic acid. Line broadening = 50.0 Hertz.

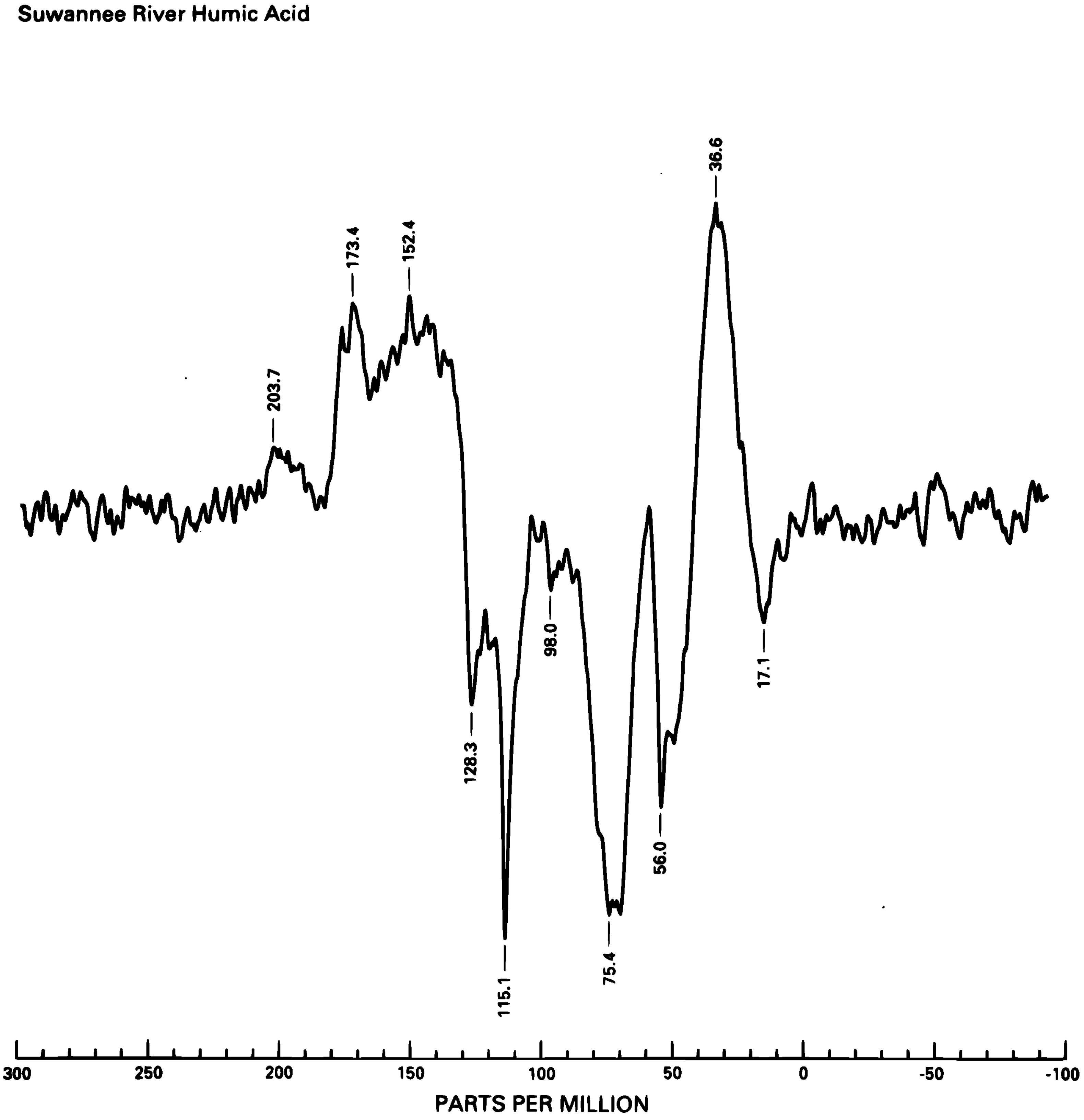


Figure 47.--^{13}C attached proton test spectrum of Suwannee River humic acid. Line broadening = 100.0 Hertz.

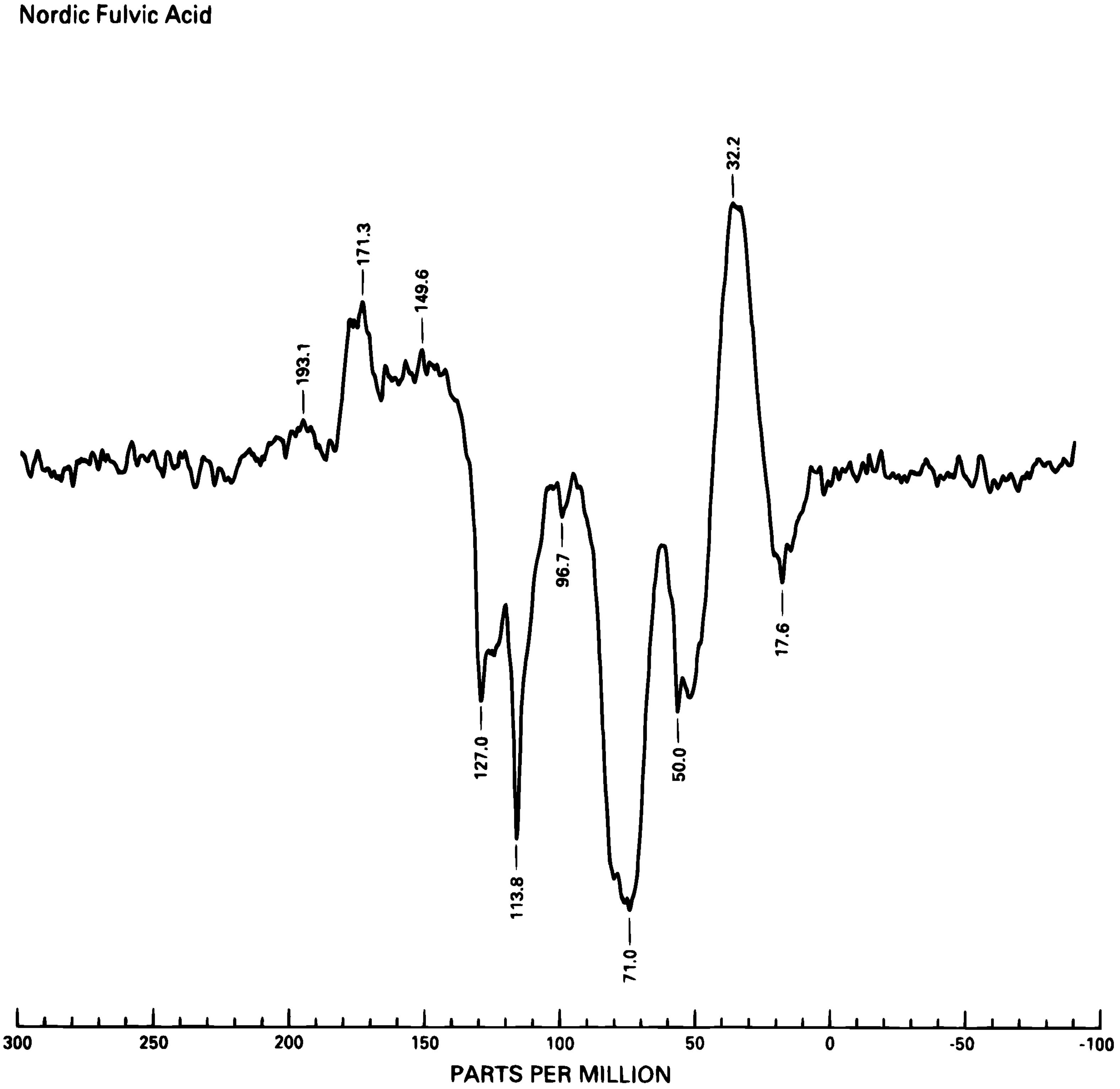


Figure 48.--^{13}C attached proton test spectrum of Nordic fulvic acid. Line broadening = 100.0 Hertz.

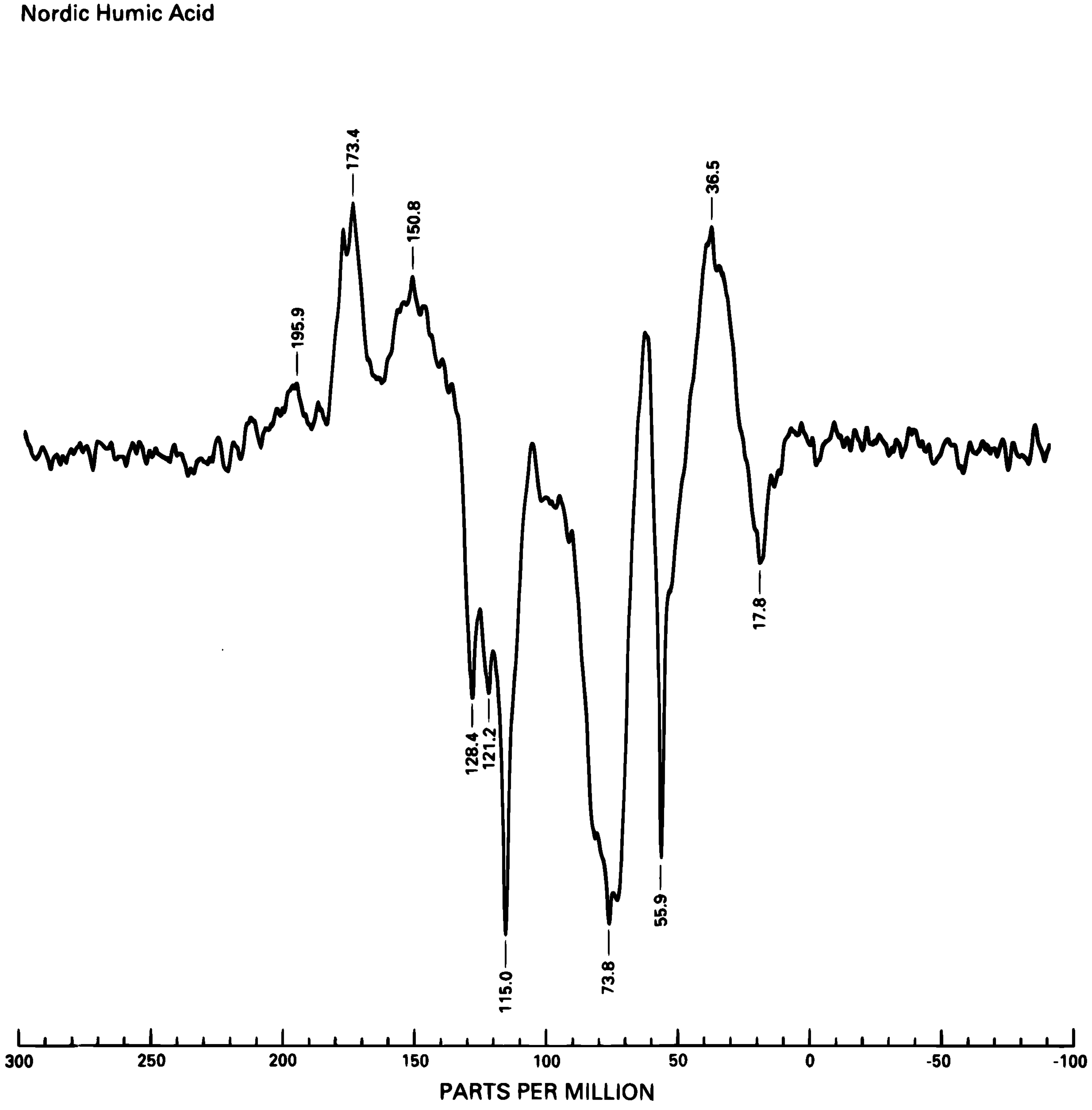


Figure 49.--^{13}C attached proton test spectrum of Nordic humic acid. Line broadening = 100.0 Hertz.

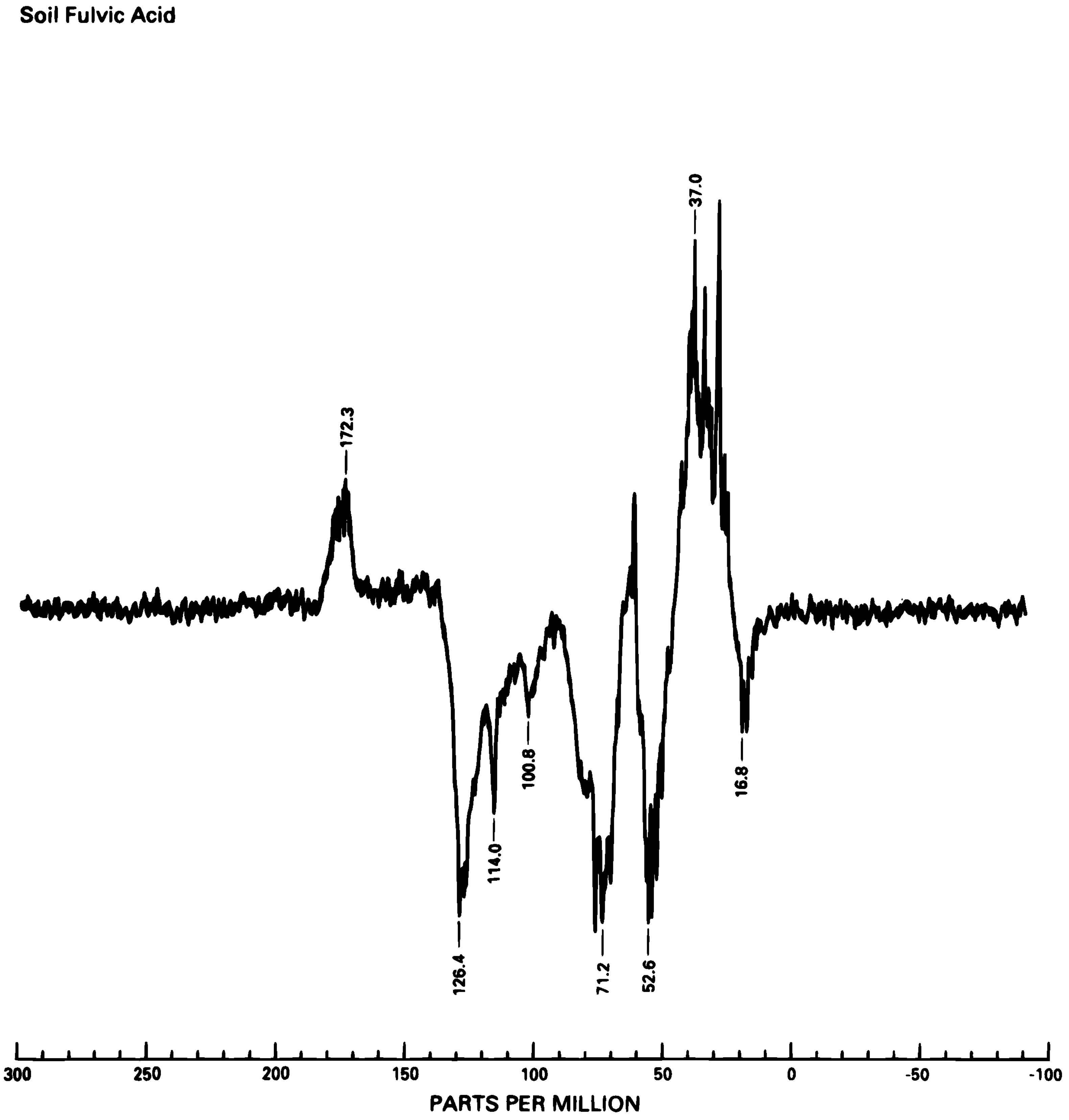


Figure 50.--^{13}C attached proton test spectrum of Soil fulvic acid. Line broadening = 20.0 Hertz.

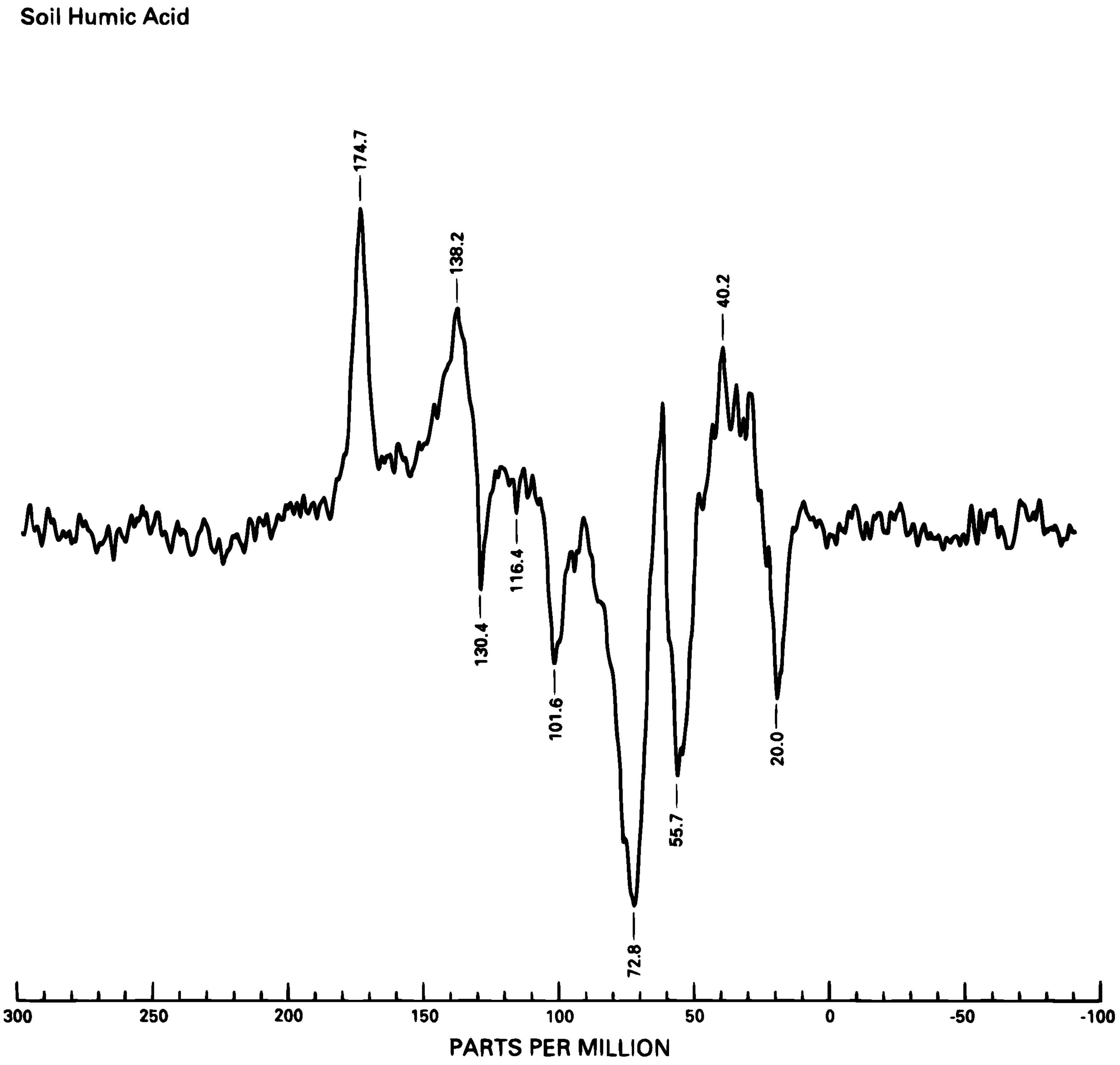


Figure 51.--^{13}C attached proton test spectrum of Soil humic acid. Line broadening = 100.0 Hertz.

Peat Fulvic Acid

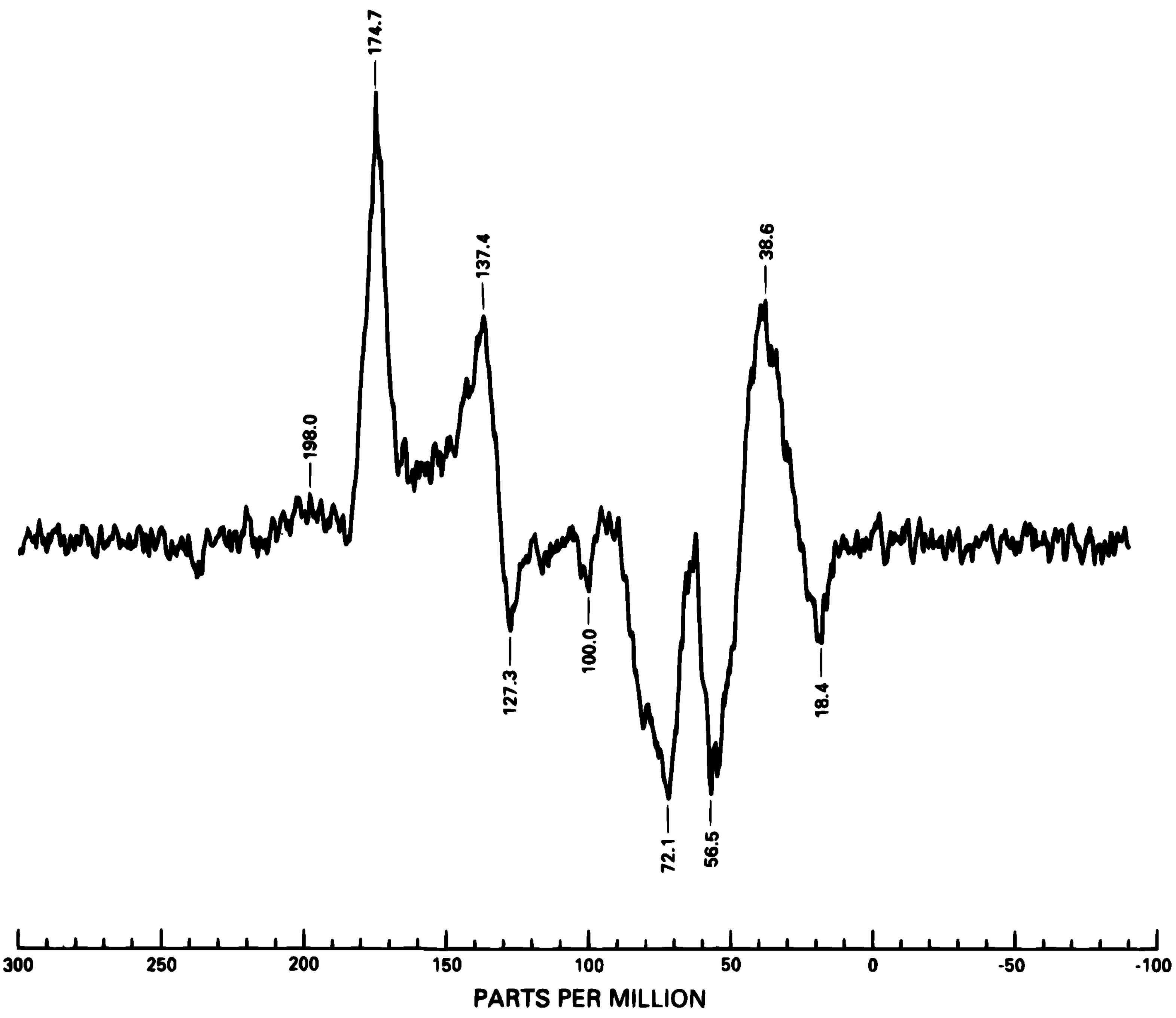


Figure 52.--^{13}C attached proton test spectrum of Peat fulvic acid. Line broadening = 50.0 Hertz.

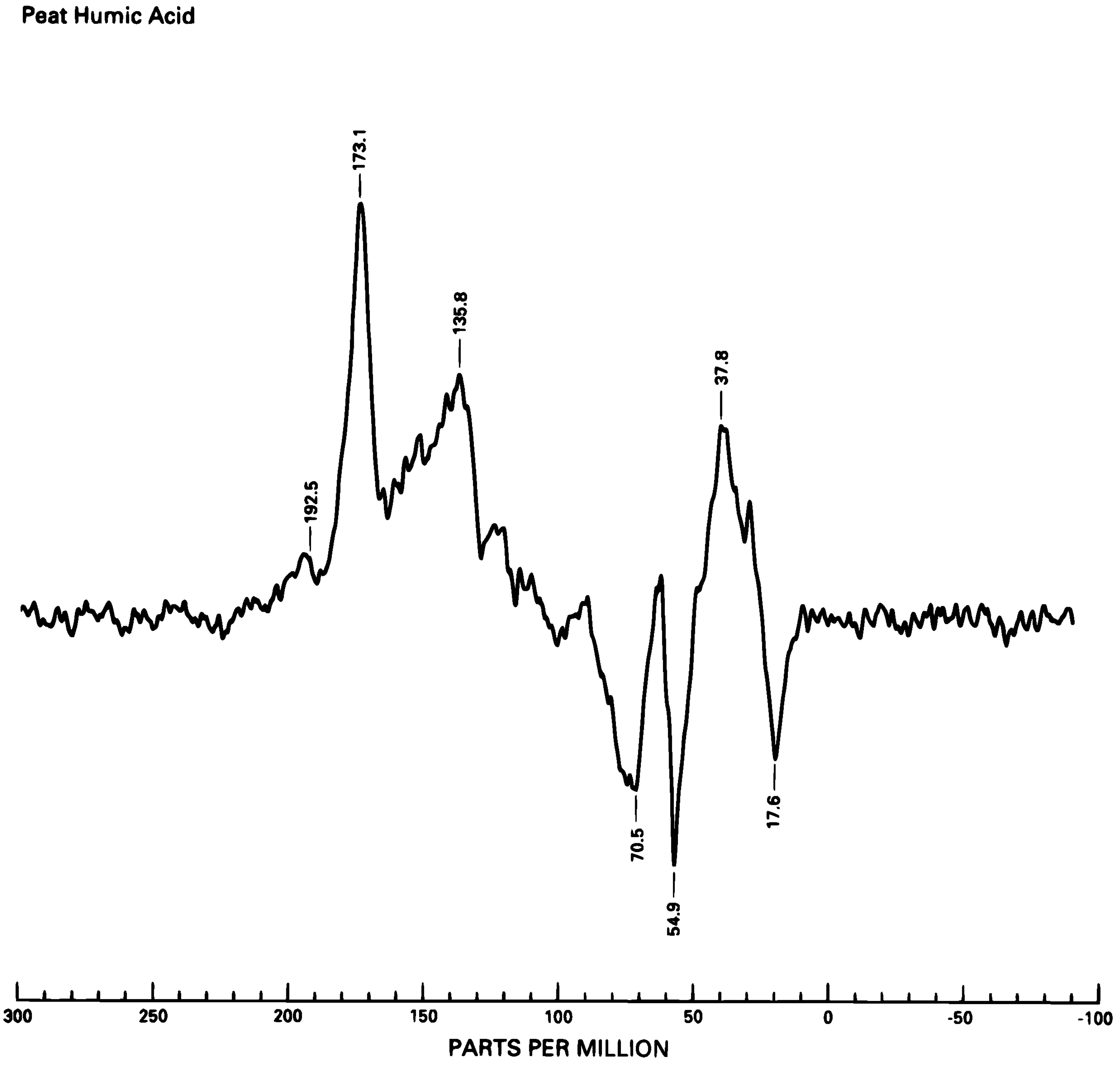


Figure 53.--^{13}C attached proton test spectrum of Peat humic acid. Line broadening = 100.0 Hertz.

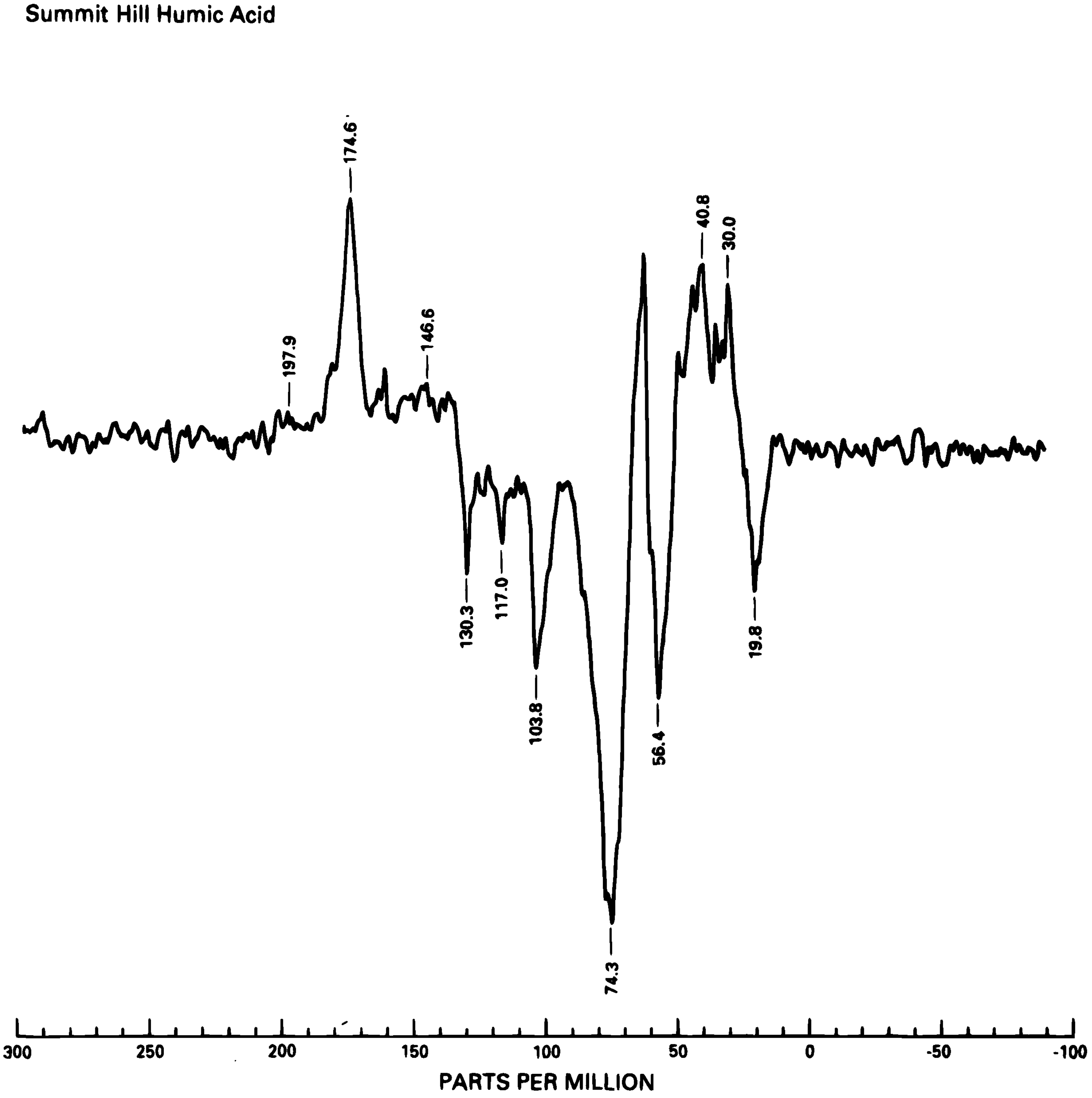
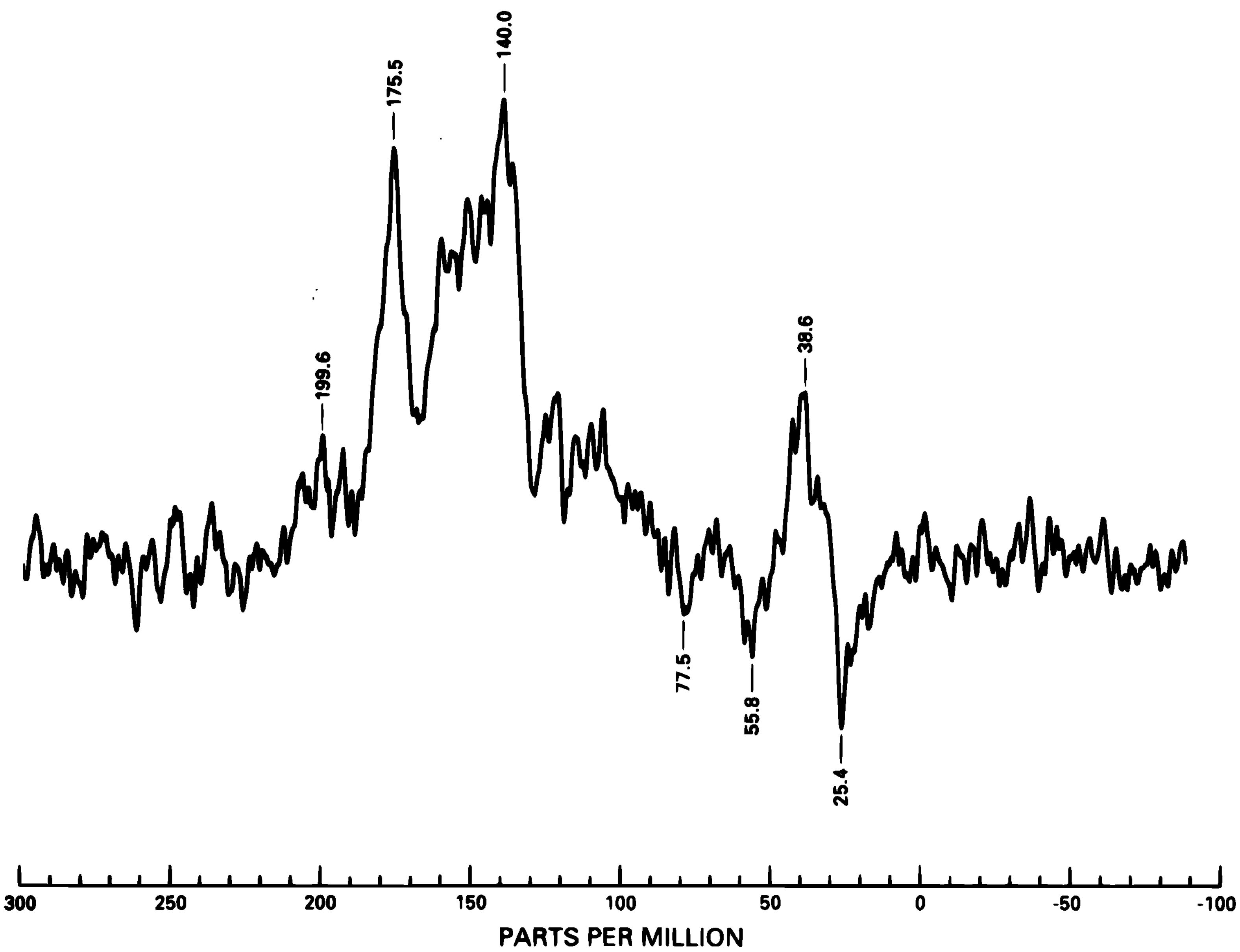


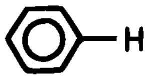
Figure 54.--^{13}C attached proton test spectrum of Summit Hill humic acid. Line broadening = 100.0 Hertz.



Leonardite Humic Acid

Figure 55.--^{13}C attached proton test spectrum of Leonardite humic acid. Line broadening = 100.0 Hertz.

Table 5.--*Approximate chemical shift ranges for protons in parts per million relative to tetramethylsilane. R = alkyl, X = halogen, -OR, -NHCOR, -OCOR*

$CH_3\text{-}R$	methyl proton	1.9-0.75
$R\text{-}CH_2\text{-}R'$	methylene proton	2.25-1.25
$>C{=}C\text{-}CH_2\text{-}R$	methylene proton	2.75-1.75
$>C{=}C\text{-}CHR_2$	methine proton	3.75-1.75
$CH_3\text{-}NR_2$	methyl proton	2.5-2.0
$RCH_2\text{-}NR_2$	methylene proton	2.75-2.25
$R_2CH\text{-}NR_2$	methine proton	3.25-2.75
$CH_3\text{-}X$	methyl proton	4.25-2.25
$R\text{-}CH_2\text{-}X$	methylene proton	4.5-3.2
$R_2CH\text{-}X$	methine proton	5-3.5
$>C{=}CH$	olefinic proton	8.0-3.8
	aromatic proton	9.0-6.5
RCHO	aldehydic proton	11.0-9.0
RCOOH	carboxylic acid proton	12.0-9.75

Suwannee River Fulvic Acid

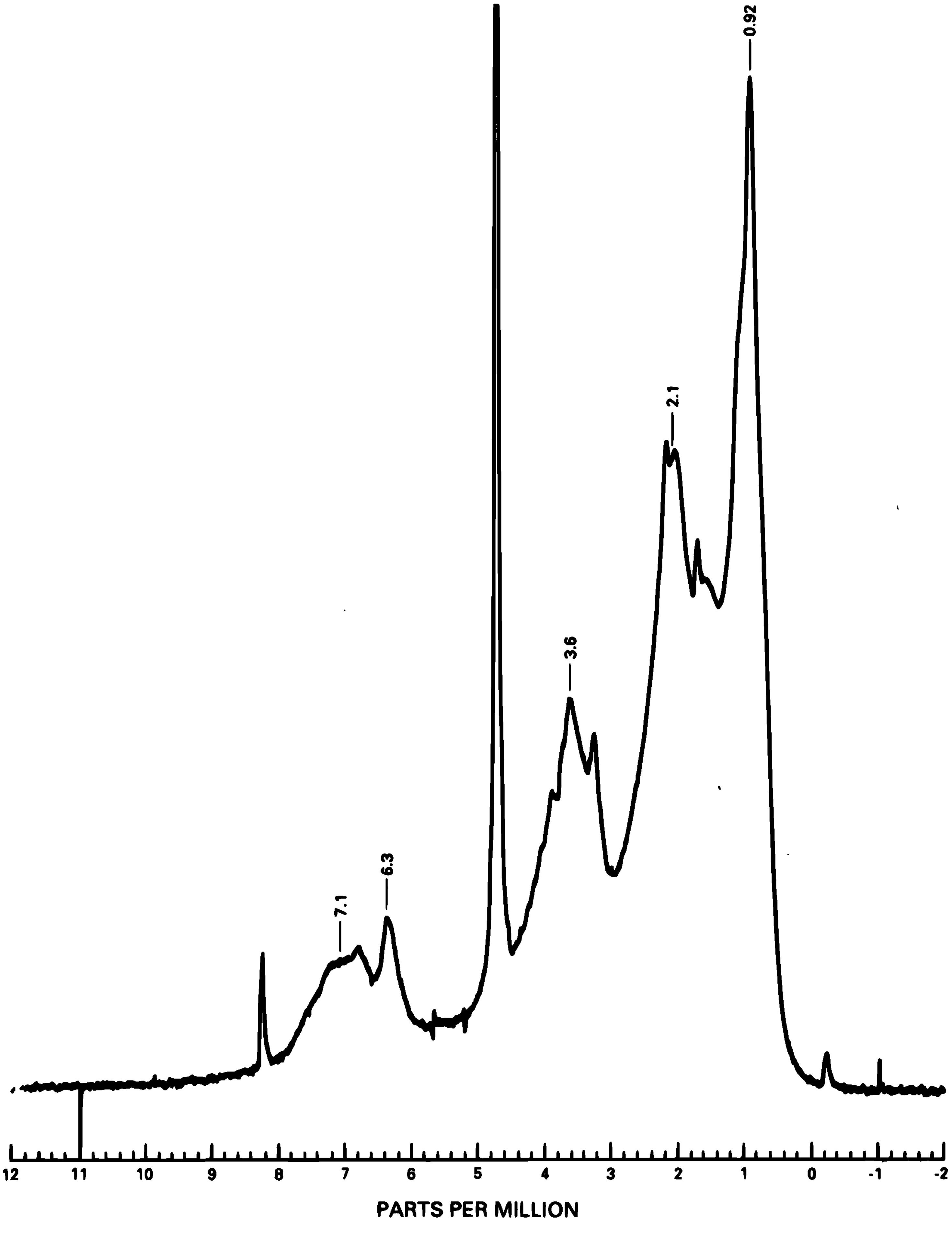


Figure 56.--^{1}H nuclear magnetic resonance spectrum of Suwannee River fulvic acid.

Suwannee River Humic Acid

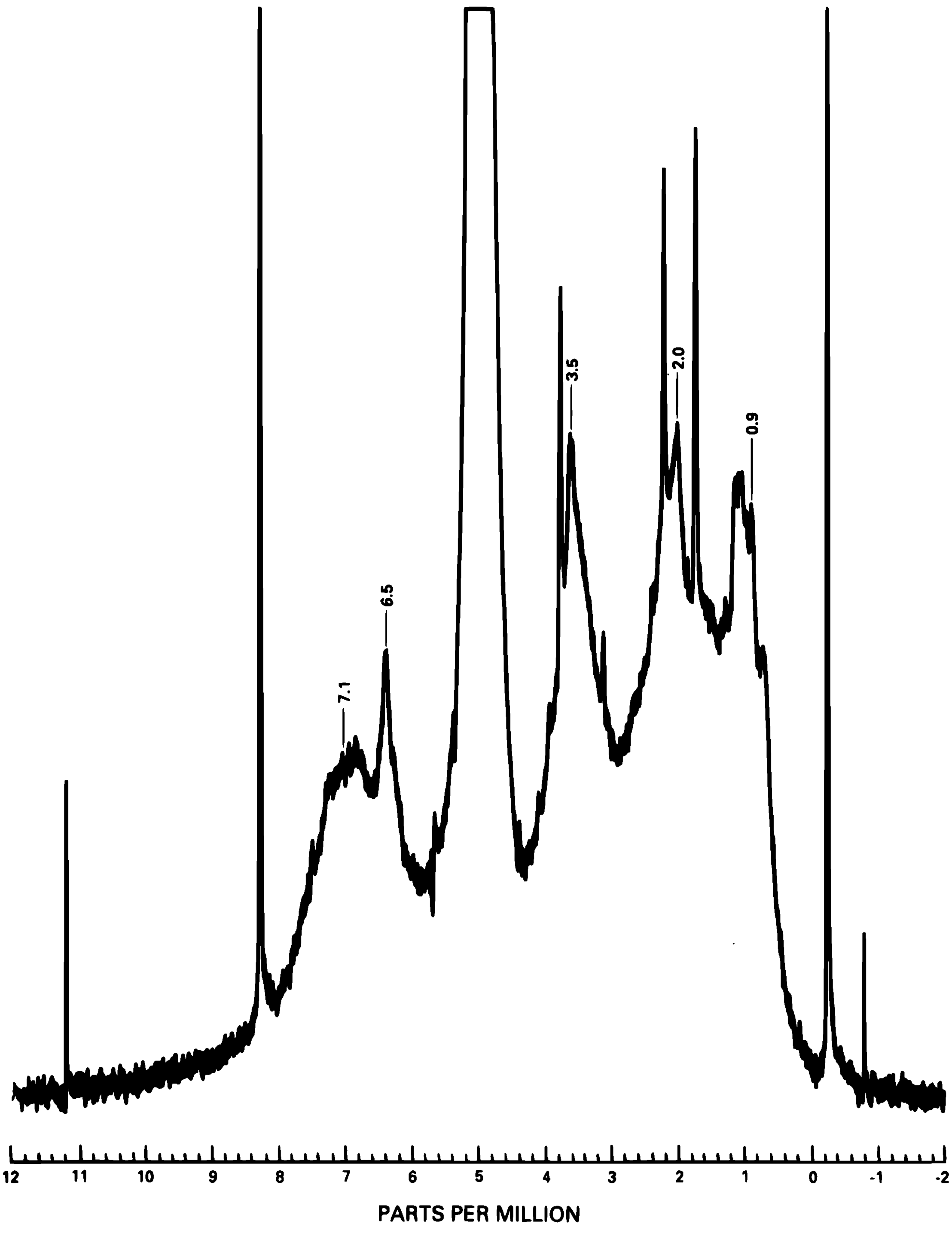


Figure 57.--^{1}H nuclear magnetic resonance spectrum of Suwannee River humic acid.

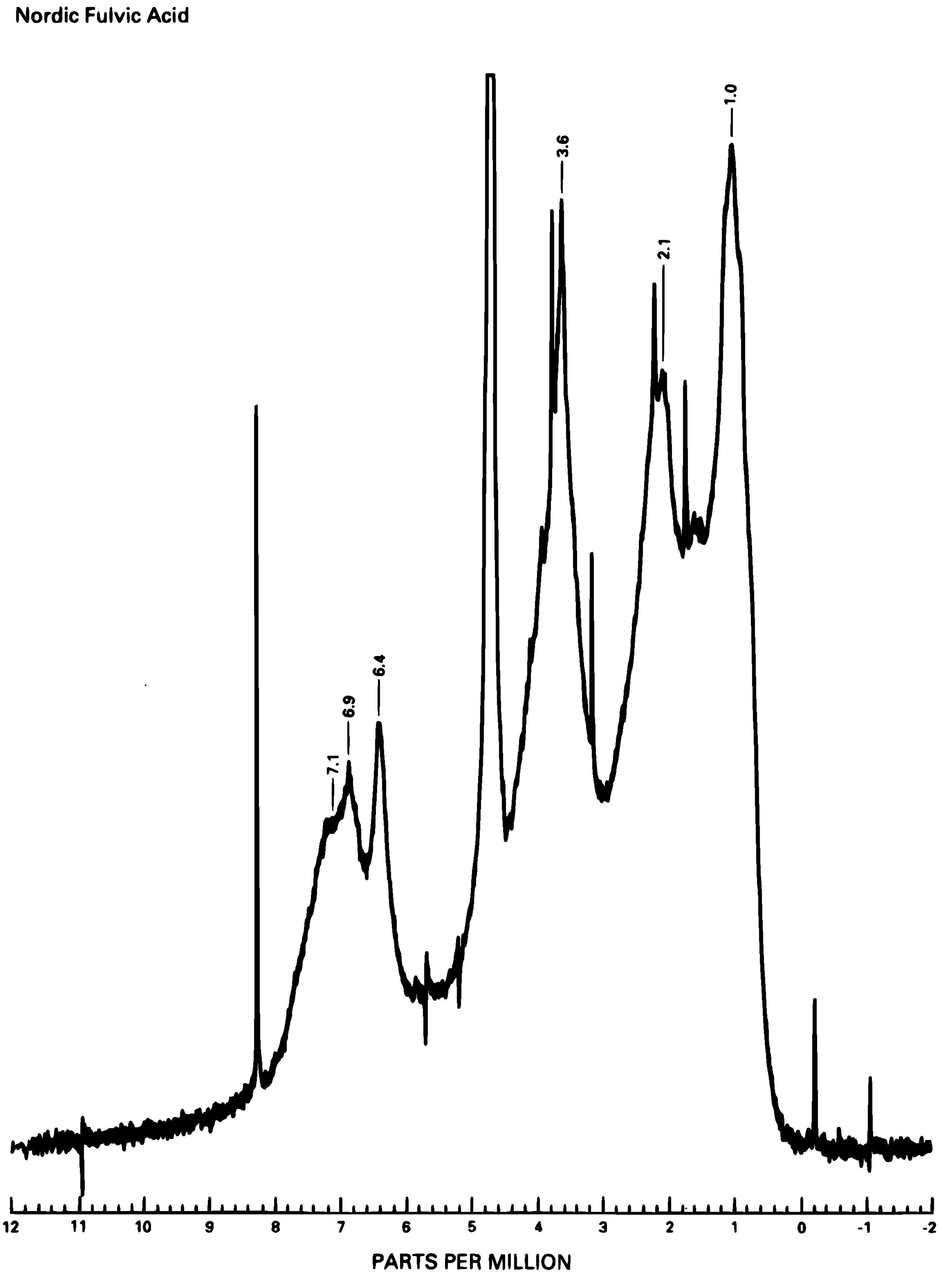


Figure 58.--^1H nuclear magnetic resonance spectrum of Nordic fulvic acid.

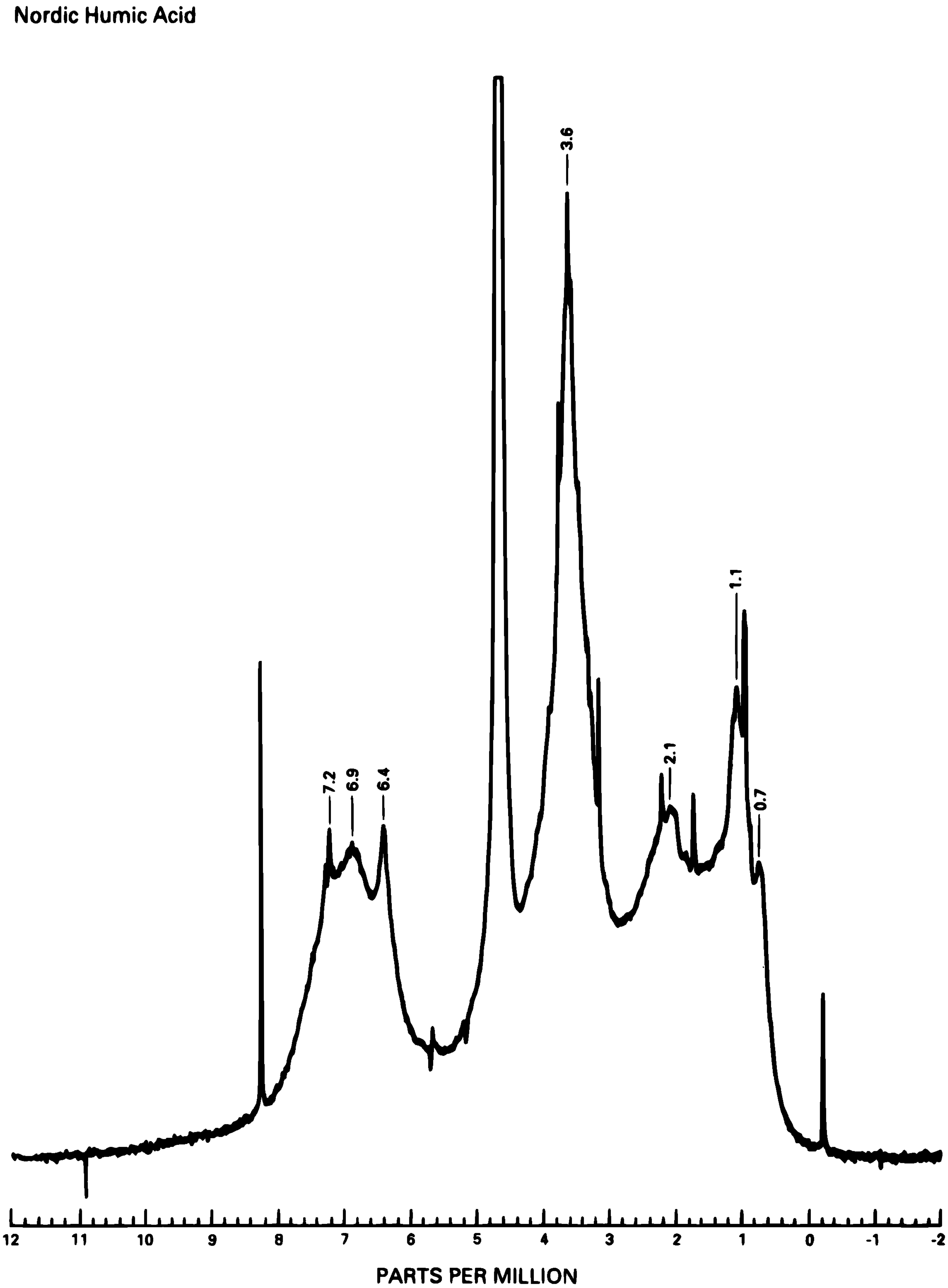


Figure 59.--^{1}H nuclear magnetic resonance spectrum of Nordic humic acid.

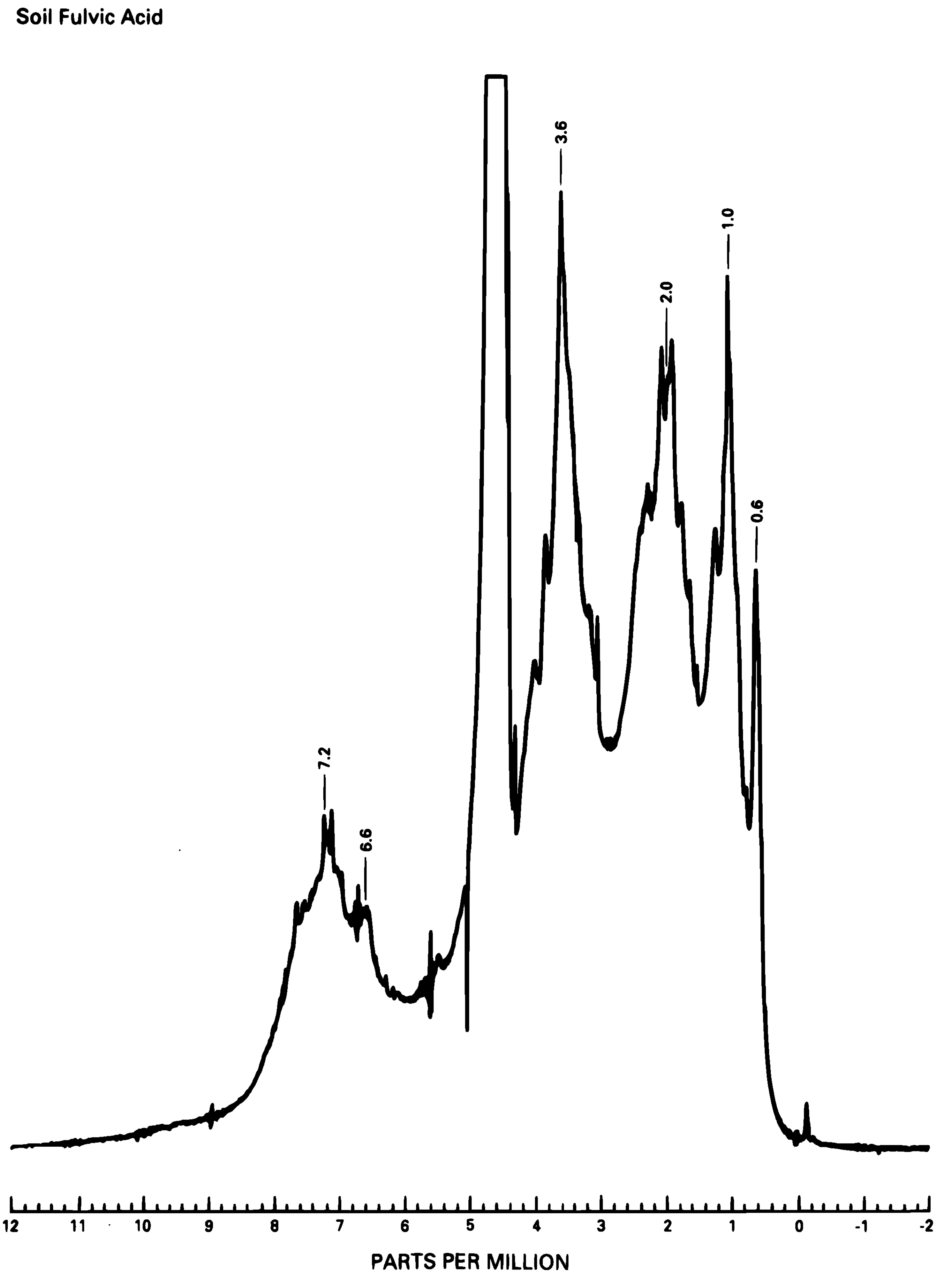


Figure 60.--^{1}H nuclear magnetic resonance spectrum of Soil fulvic acid.

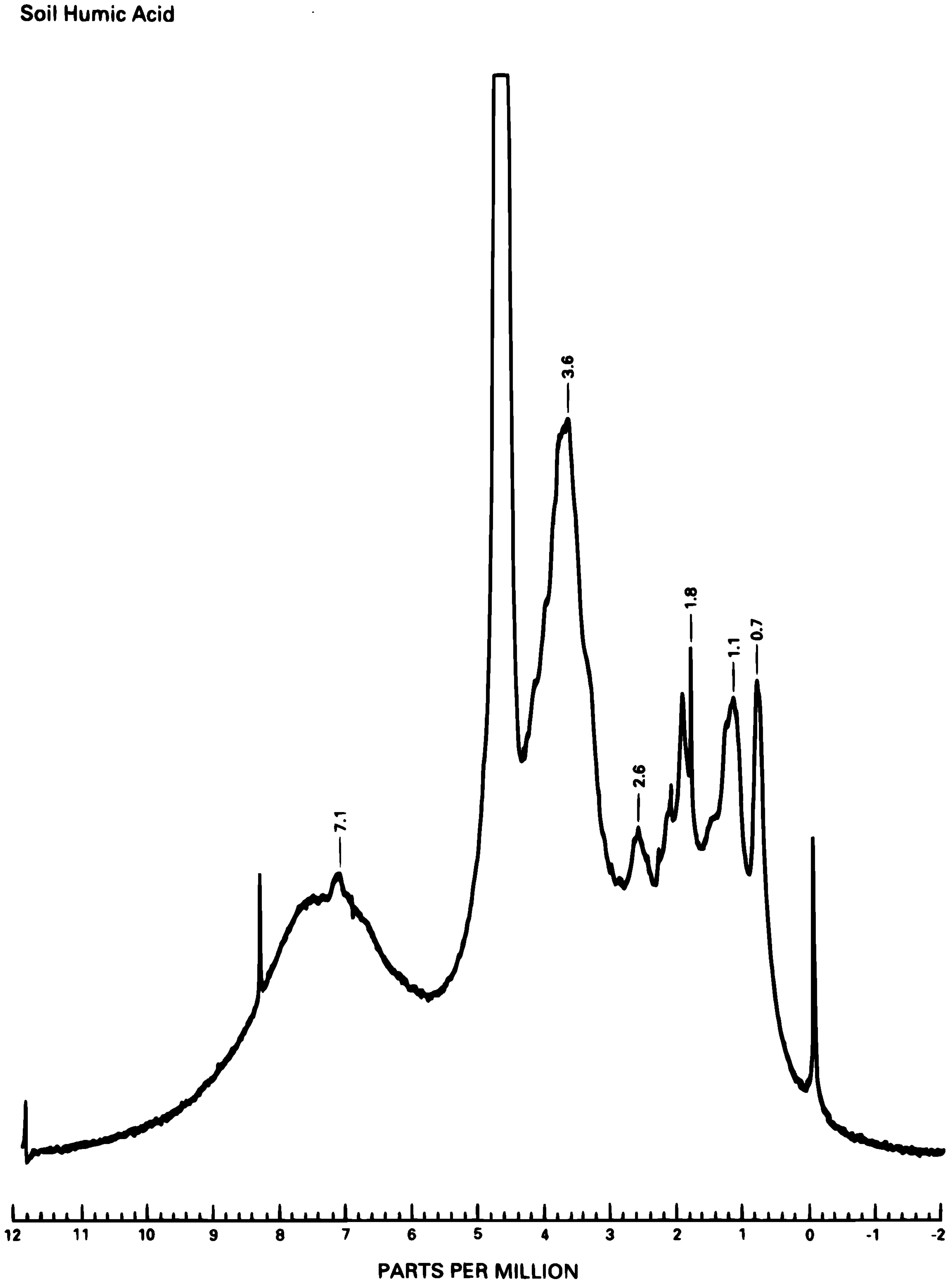


Figure 61.--^{1}H nuclear magnetic resonance spectrum of Soil humic acid.

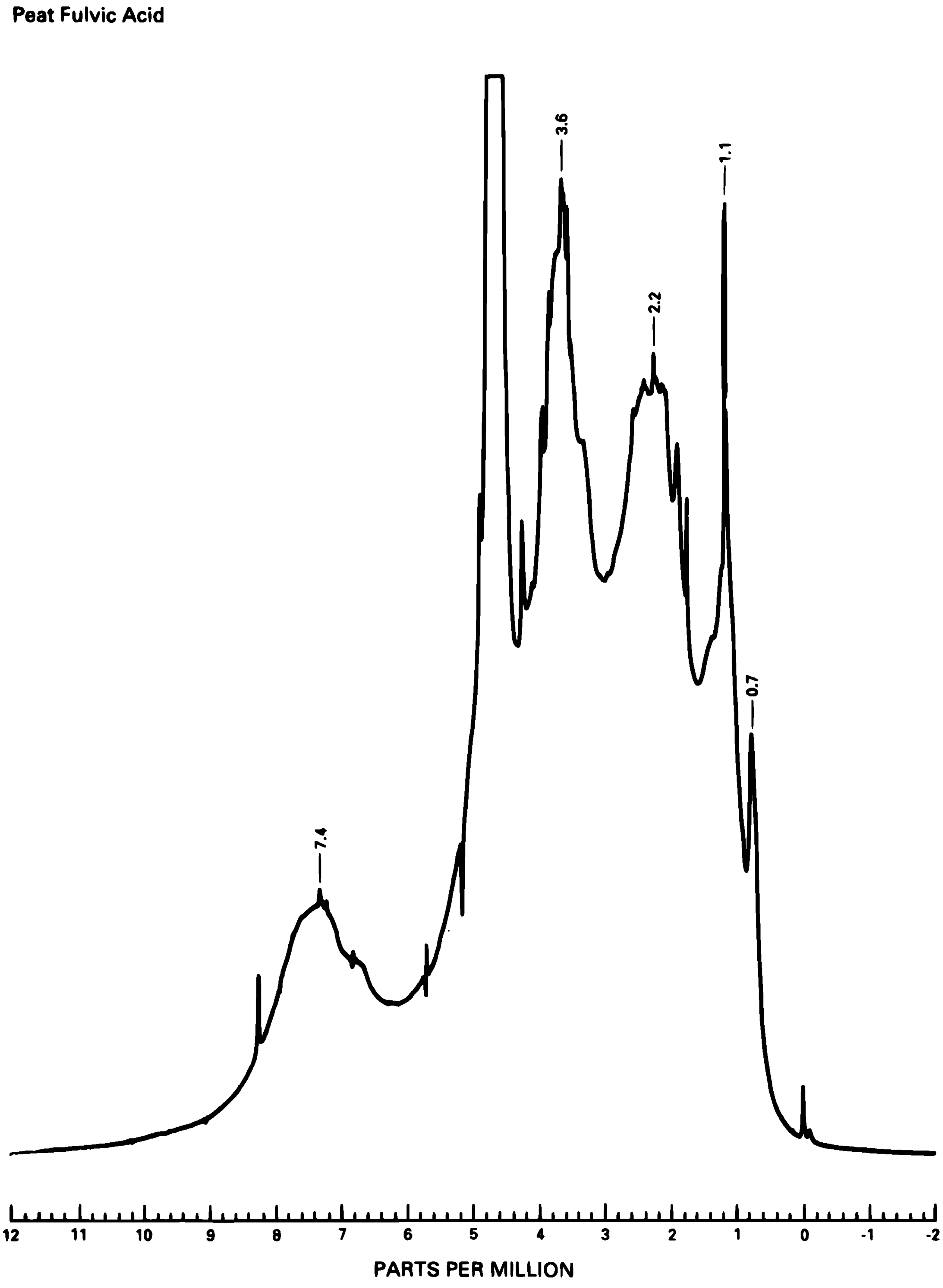


Figure 62.--^1H nuclear magnetic resonance spectrum of Peat fulvic acid.

Peat Humic Acid

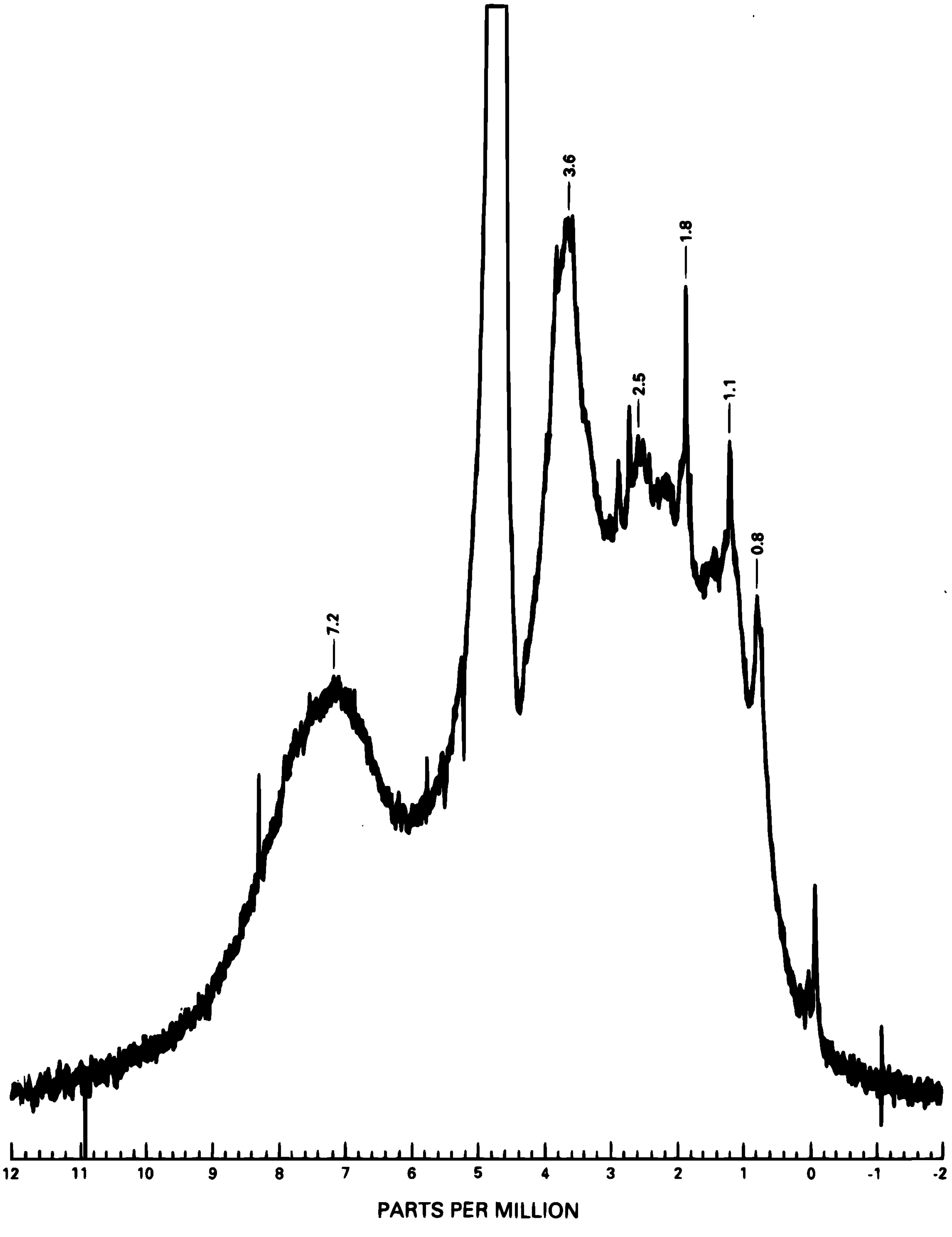


Figure 63.--^{1}H nuclear magnetic resonance spectrum of Peat humic acid.

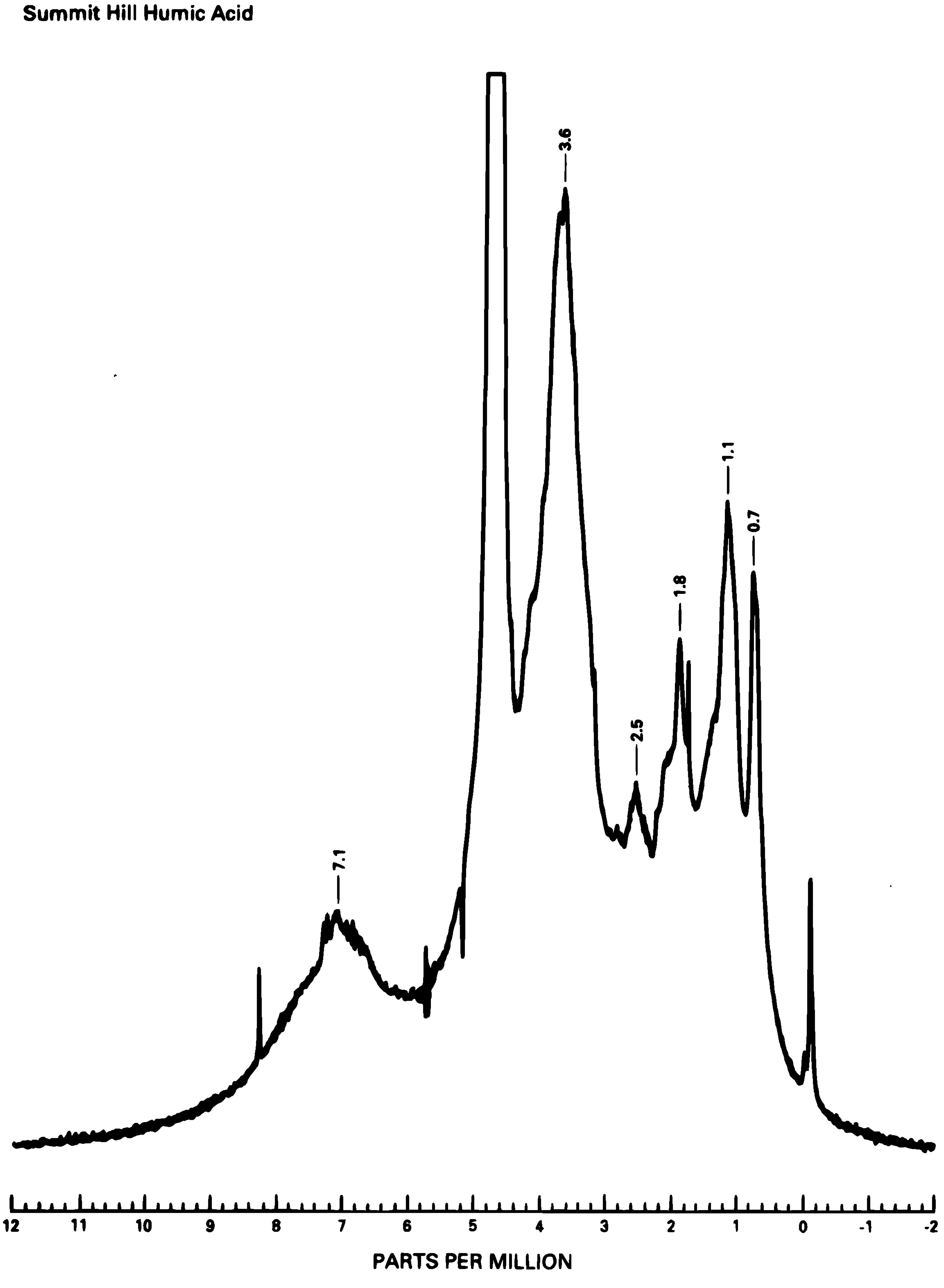


Figure 64.--^{1}H nuclear magnetic resonance spectrum of Summit Hill humic acid.

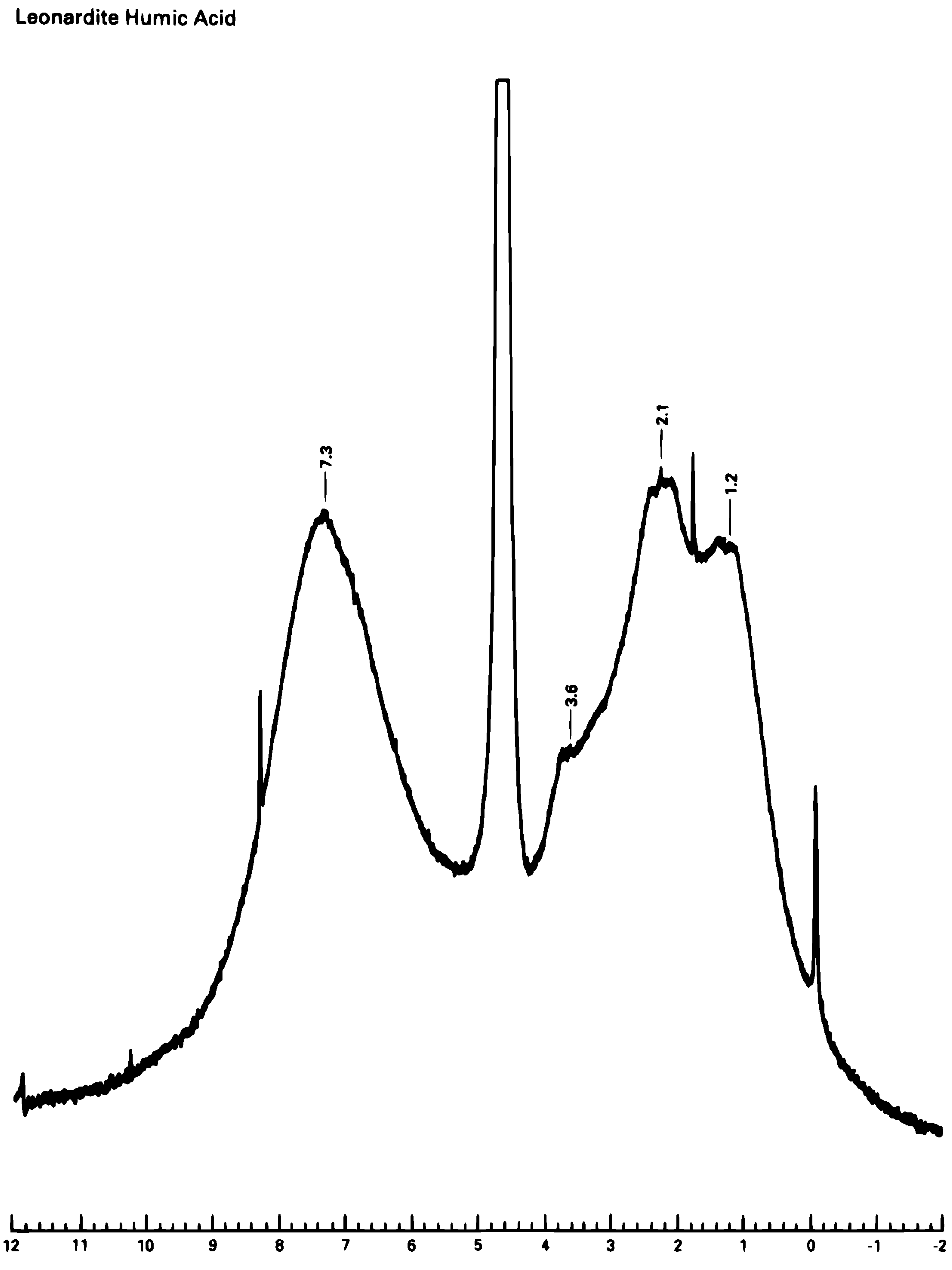


Figure 65.--^{1}H nuclear magnetic resonance spectrum of Leonardite humic acid.

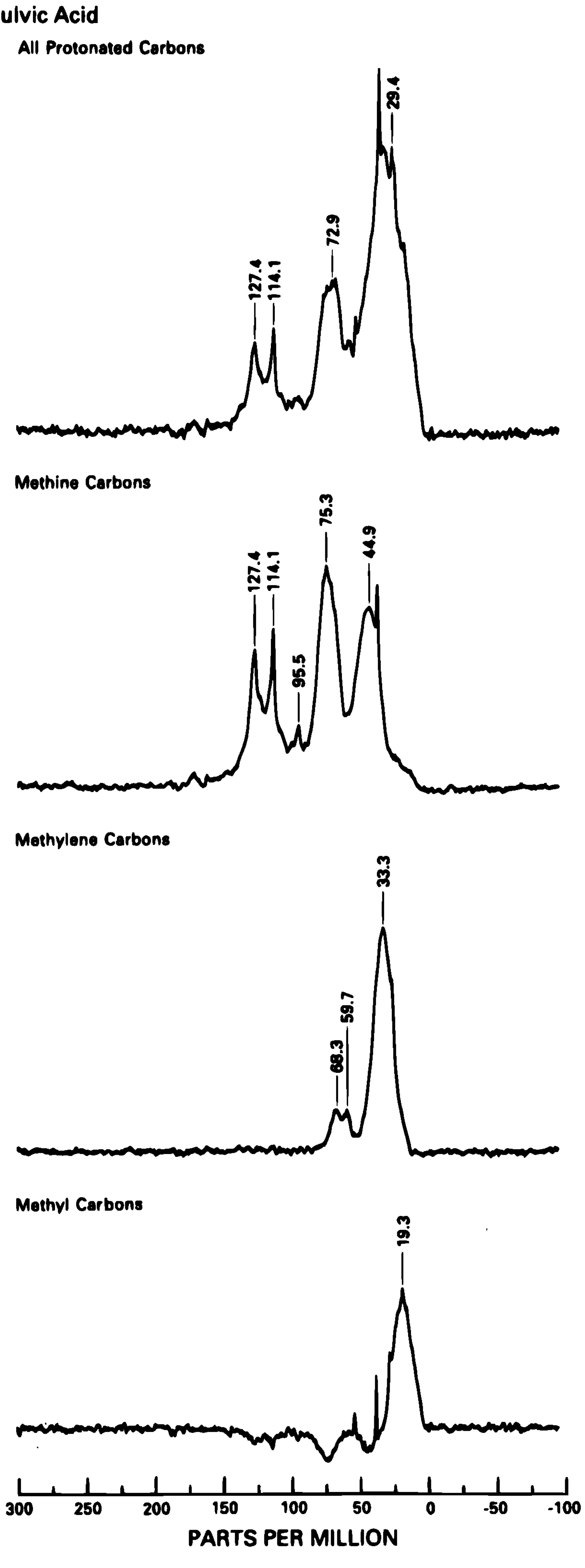


Figure 66.--Distortionless enhancement by polarization transfer (GL version) carbon-13 nuclear magnetic resonance spectra of Suwannee River fulvic acid in dimethylsulfoxide-d_6. Line broadening = 50.0 Hertz. *A*, spectrum showing all protonated carbons; *B*, spectrum showing only methine carbons; *C*, spectrum showing only methylene carbons; *D*, spectrum showing only methyl carbons.

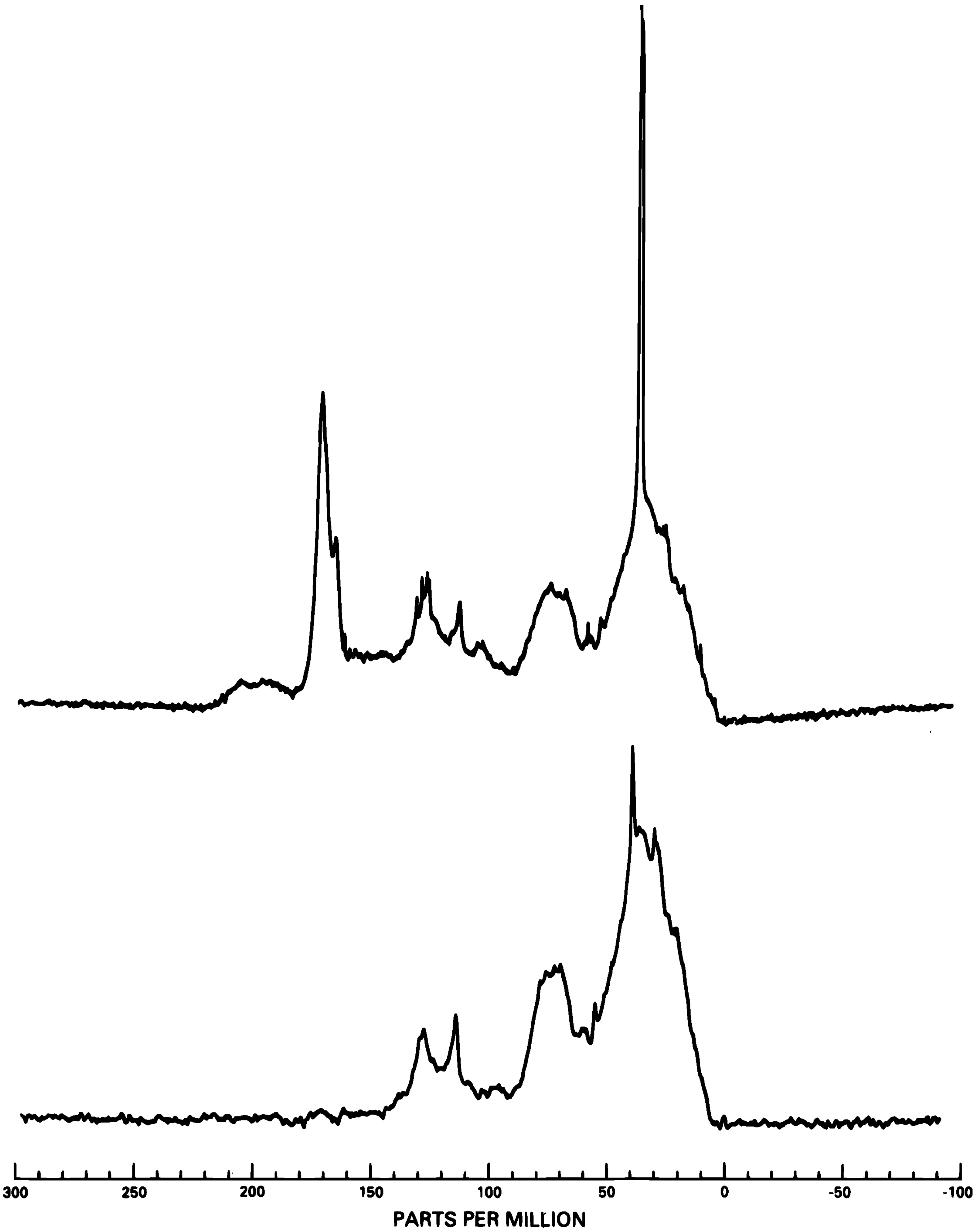


Figure 67.--Carbon-13 nuclear magnetic resonance spectra of Suwannee River fulvic acid in dimethylsulfoxide-d_6. Top = continuous decoupled spectrum; Bottom = DEPTGL spectrum showing all protonated carbons.

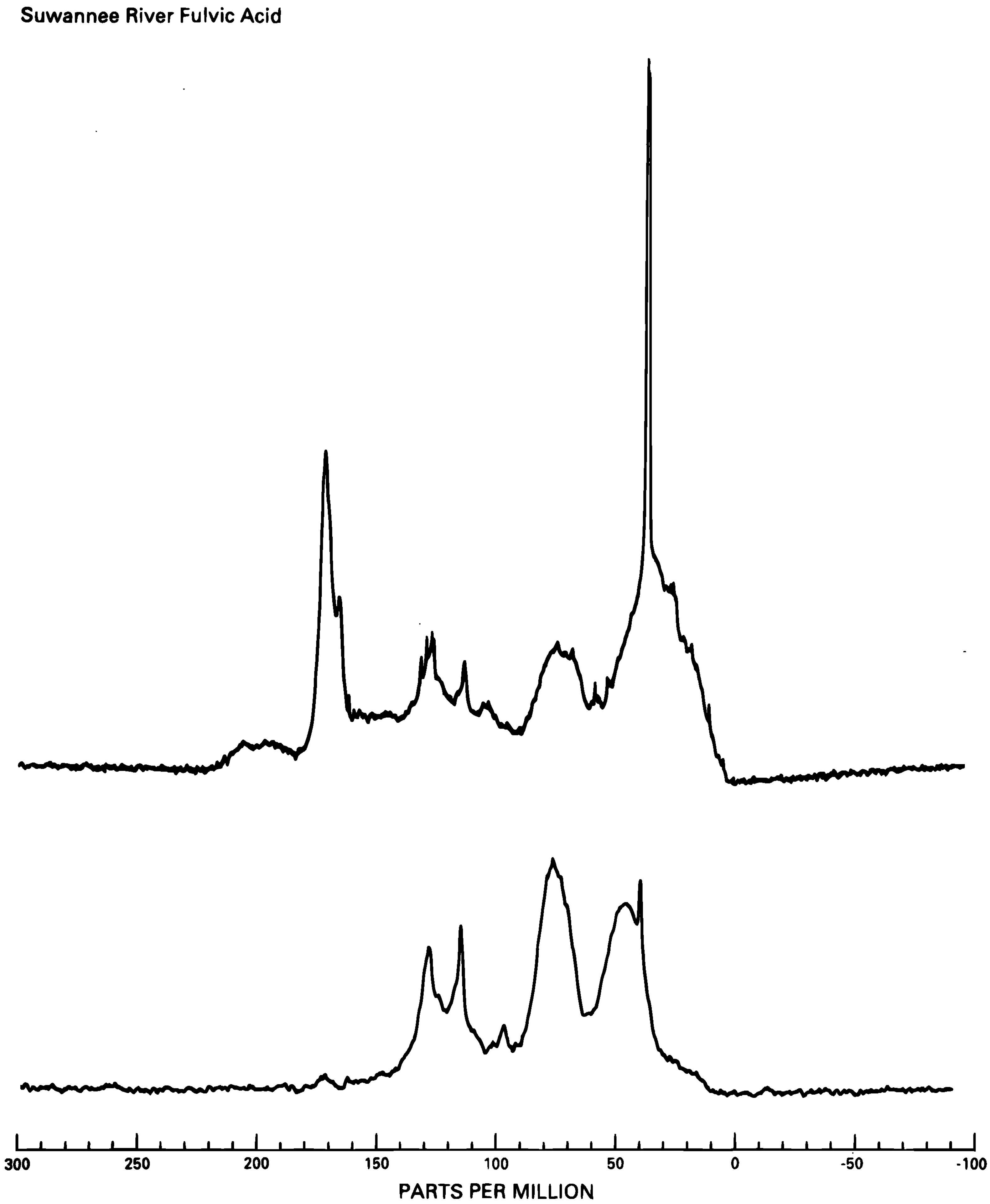


Figure 68.--Carbon-13 nuclear magnetic resonance spectra of Suwannee River fulvic acid in dimethylsulfoxide-d_6. Top = continuous decoupled spectrum; Bottom = DEPTGL spectrum showing only methine carbons.

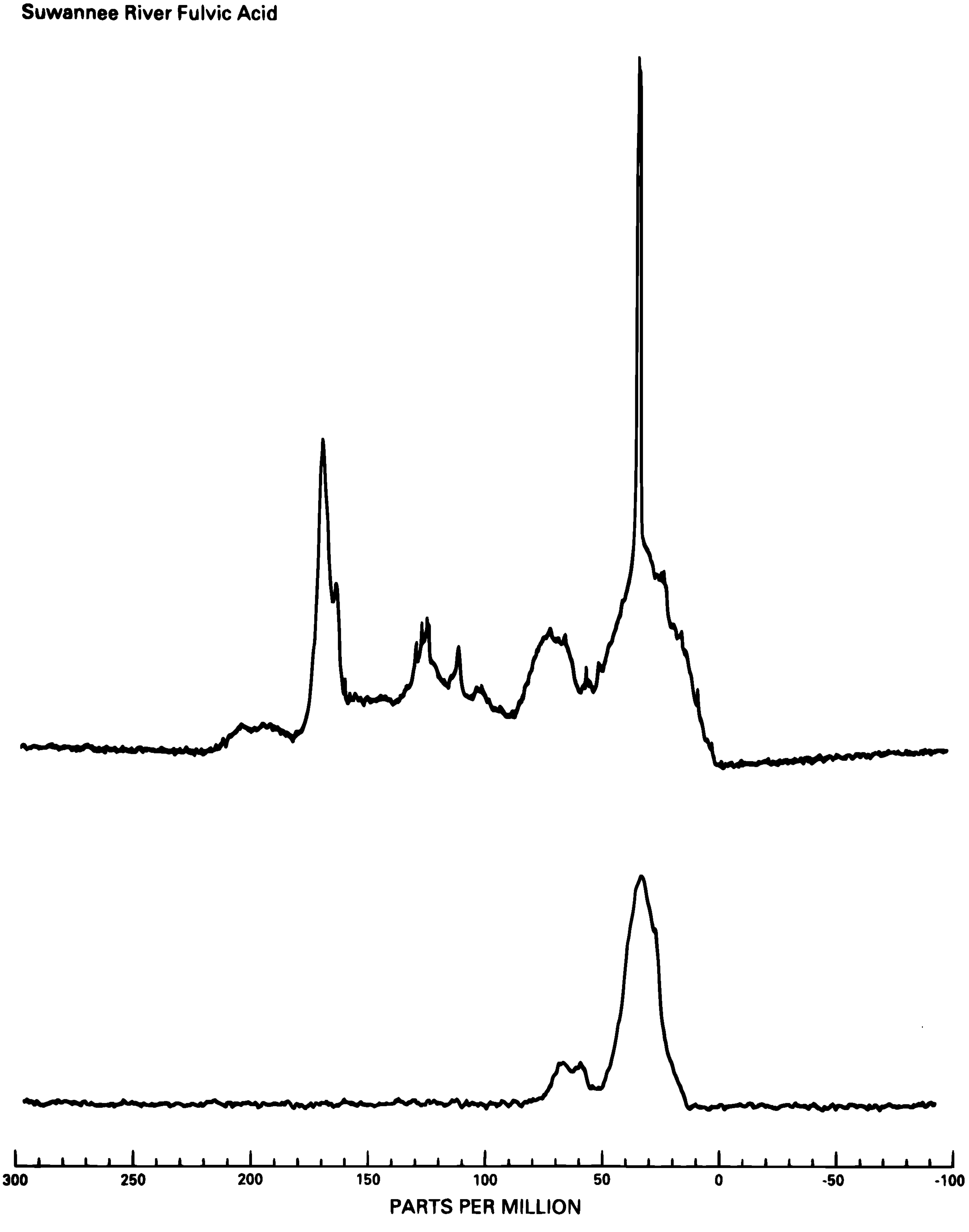


Figure 69.--Carbon-13 nuclear magnetic resonance spectra of Suwannee River fulvic acid in dimethylsulfoxide-d_6. Top = continuous decoupled spectrum; Bottom = DEPTGL spectrum showing only methylene carbons.

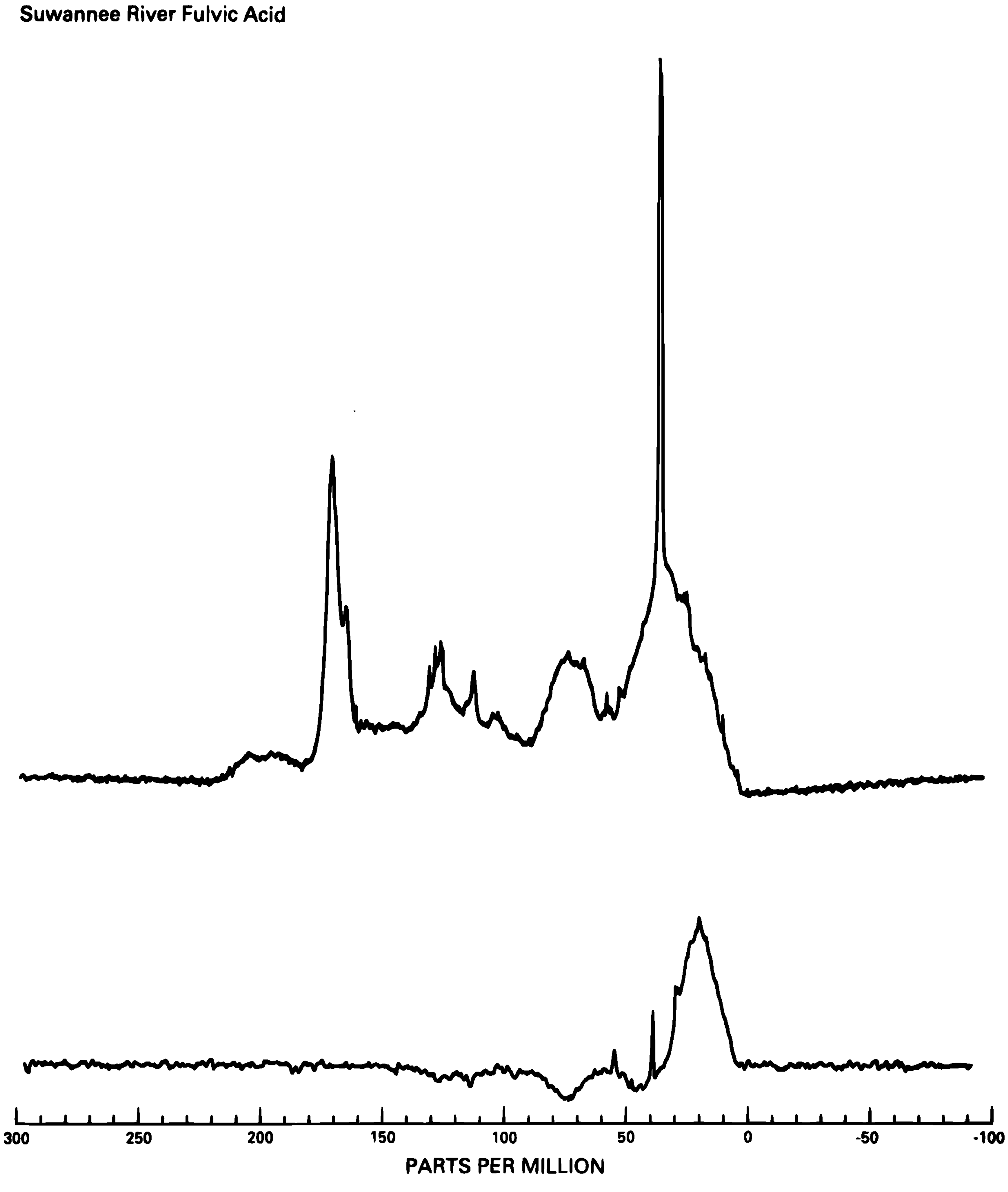


Figure 70.--Carbon-13 nuclear magnetic resonance spectra of Suwannee River fulvic acid in dimethylsulfoxide-d_6. Top = continuous decoupled spectrum; Bottom = DEPTGL spectrum showing only methyl carbons.

SUMMARY AND CONCLUSIONS

The NMR spectra presented in this report have provided useful information on the structural characteristics of the IHSS fulvic and humic acid standard and reference samples. There is still a need, however, for further NMR analyses of these samples. Continued application of the DEPTGL sequence, quaternary carbon only spectra, and spectra of samples for which functional groups have been derivatized with spin labelled reagents, may all provide additional structural information. Some of these other analyses are currently being undertaken in our laboratory.

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