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Strong-Acid, Carboxyl-Group Structures in Fulvic Acid from the Suwannee River, Georgia. 1. Minor Structures

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An investigation of the strong-acid characteristics (pK_a 3.0 or less) of fulvic acid from the Suwannee River, Georgia, was conducted. Quantitative determinations were made for amino acid and sulfur-containing acid structures, oxalate half-ester structures, malonic acid structures, keto acid structures, and aromatic carboxyl-group structures. These determinations were made by using a variety of spectrometric (^{13}C-nuclear magnetic resonance, infrared, and ultraviolet spectrometry) and titrimetric characterizations on fulvic acid or fulvic acid samples that were chemically derivatized to indicate certain functional groups. Only keto acid and aromatic carboxyl-group structures contributed significantly to the strong-acid characteristics of the fulvic acid; these structures accounted for 43% of the strong-acid acidity. The remaining 57% of the strong acids are aliphatic carboxyl groups in unusual and/or complex configurations for which limited model compound data are available.

Introduction

An understanding of the structural characteristics of humic substances is important for many geochemical studies of the properties and early diagenesis of organic matter in the environment. Current models and mixtures of model compounds derived from degradative studies generally describe the aromatic carbon moieties and associated functional groups in humic substances (*1–4*). Little is known about aliphatic structures and associated functional groups in humic substances because these structures are destroyed or modified in degradative studies used in structure elucidation (*5*). The proximity of carboxyl groups to each other and to other functional groups in humic substances is particularly important for studies of acidity and trace metal complexation characteristics (*6*).

In a recent study (*7*) of acid-group heterogeniety in a number of fulvic acids from soil and aqueous environments, pK_{a1} values of 2.0 ± 0.3 were calulated for four- and five-site models of carboxyl groups. A survey (*8*) of pK_a values for all organic acids containing only carbon, hydrogen, and oxygen (567 pK_a values; *9*, *10*) found only 58 carboxylic acids listed with pK_a values less than 3.0 and only 29 acids with pK_a values less than 2.5. These strong acids are mainly polycarboxylic acids on aromatic rings or in aliphatic structures with electron-withdrawing groups (carboxylic acid, ketone, ester, ether, hydroxyl, π-electrons in double and triple bonds) in proximity to the strongly acidic carboxyl group. The small number of possible carboxylic acid structures imposed by the strong carboxylic acid acidity of fulvic acid was an impetus to conduct a systematic study whose objective was to determine molecular configurations of carboxyl groups that produced the observed acidity of fulvic acid. Fulvic acid isolated from the Suwannee River, Georgia, was selected for this study because of its availability and extensive previous characterization (*11*, *12*).

The molecular heterogeniety of humic substances requires that structural investigations of strong acid functionality be rigorous in addressing all possible structural combinations that can be hypothesized to account for carboxyl-group acidity. The objective of this first of a series of two papers is to set limits on carboxyl-group structures listed in published data that contribute to the acidity of a pK_a of 3.0 or less that are present in fulvic acid from the Suwannee River, Georgia.

Experimental Section

Determination of Oxalate Half-Esters. A total of 2 g of fulvic acid from the Suwannee River, isolated by column chromatography (*12*), was dissolved in 1.0 M NaOH (100 mL) and was refluxed for 4 h. After being cooled, the mixture was acidified to pH 2 with HCl and was passed through a 800-mL bed-volume column of Amberlite XAD-8 resin to adsorb and remove the hydrolyzed fulvic acid. The column effluent was adjusted to pH 8 with NaOH, and saturated $CaCl_2$ solution (10 mL) was added to precipitate oxalate. The precipitate was washed once with distilled water, dried under vacuum, and weighed. An infrared spectrum of the precipitate was obtained to confirm its identity.

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Determination of Malonic Acids. Malonic acids can be determined as barbituates by their condensation with urea using acetic anhydride as the condensation reagent (*13*). The procedure first was tested and optimized by reacting urea with malonic, methylmalonic, dimethylmalonic, butylmalonic, phenylmalonic, and tartronic acids purchased from Aldrich Chemical Co.

After the purity of the barbituate derivatives was determined by infrared and ^{13}C-NMR spectrometry, the yields were determined by ultraviolet (UV) spectrometry. The UV spectrum was determined in water in a 1-cm quartz cell at pH 7 by a Perkin-Elmer Lambda-4B spectrophotometer. The various barbituate derivatives of the model malonic acids all gave a peak near 257 nm of comparable molar absorbtivities. After the spectra of the derivatives were obtained, yields were determined in the reaction mixture by measuring absorbance at 257 nm in the sample cell and by using the reaction mixture minus the urea in the reference cell to subtract the reagent background.

Based upon results for the malonic acid standards, malonic acid groups in fulvic acid were derivatized to barbituate groups by the following procedure:

(1) Fulvic acid (200 mg, 1.8 mequiv of carboxyl groups) was dissolved in acetic anhydride (4 mL).

(2) 1.2 mmol of urea was dissolved in acetic acid (4 mL) and added to the solution from step 1.

(3) The reaction mixture was heated to 60 °C for 3 h.

(4) The mixture was vacuum evaporated to dryness.

(5) The mixture was dissolved in water (20 mL), and the pH was maintained near 7.0 for 24 h.

(6) The pH was adjusted to 2.0 with HCl, and the solution was passed through a column (100-mL bed volume) of Amberlite XAD-8 followed by 250 mL of 0.01 M HCl rinse to remove urea.

(7) The fulvic acid derivative was eluted from the column with 50 mL of 75% acetonitrile/25% water, and the eluent was evaporated to dryness.

(8) Yield of the barbituate derivative dissolved in water at pH 7 was assayed by UV spectrometry.

Determination of Keto Acids. Fulvic acid, isolated from the Suwannee River by column chromatography (*12*), was titrated before and after reduction of ketones to estimate the effect of keto acids on acidity. Reduction of ketone groups in fulvic acid was performed by adding sodium borohydride (0.3 g) to fulvic acid (0.3 g) in water (10 mL) and heating at 40 °C for 4 h. Acetic acid (1 mL) was added to destroy excess sodium borohydride, and the solution was evaporated under vacuum to dryness. Boric acid was removed as the volatile trimethyl borate ester by evaporation of three separate 50-mL portions of methanol added to the sample. Sodium ions then were exchanged with hydrogen ions by passing the sample, dissolved in 50 mL of distilled water, through a 20-mL bed-volume column of MSC ^1H cation-exchange resin. Water was removed by evaporation under vacuum.

Titrimetric Procedures. The titrations were performed using 200–400 mg of fulvic acid suspended in 4 mL of 0.5 M NaCl and titrating with 0.5 M NaOH. The large concentration of fulvic acid was necessary to determine the pK_a values of the strong-acid groups. The effect of the phase change (solid to dissolved) of fulvic acid with increasing pH was minimized by titrating in 0.5 M NaCl to diminish electrostatic effects. An Orion Model 501 pH meter with an Orion 91-05 glass electrode was used. The pH meter was standardized at pH 1 with 0.1 M HCl in 0.5 M NaCl and at pH 4 and 7 with buffer solutions. The slope of the pH response was linear from pH 1 to pH 7. The inflection end point of the carboxyl-group portion of the titration curve varied between 8.0 and 8.5, depending on the nature of the sample and the titration conditions.

Determination of pK_a Values for Fulvic Acid. A polyprotic acid model was assumed for the determination of intrinsic pK_a values for fulvic acid for given ionic strengths. Determinations of intrinsic pK_a values for polyprotic acids are usually made by assuming that each ionization step occurs independently of the next so that the titration data can be treated as a series of successive titrations (*14*). This assumption is true if the pK_a values differ by a factor of 2 or greater; separate inflections for each pK_a value are observed only if pK_a values differ by a factor of 4 or greater. If the stoichiometry of the acid is known, but separate inflections of the titrition curve are not observed, each pK_a can be determined by dividing the titration curve into equal segments corresponding to the number of the acid groups titrated, and the pH at the midpoint of each segment corresponds to the pK_a of the acid group titrated in that segment if the acid concentration is sufficiently great to suppress ionization. If the two pK_a values of a diprotic acid differ by less than 2, the successive titration assumption is no longer strictly valid. The exact solution for each pK_a is a cubic equation that is difficult to solve, and the pK_a values are determined by a method that uses a series of successive approximations for each pK_a value (*14*). However, even if the two pK_a values of a diprotic acid only differ by 1, the second ionization constant only introduces an error of 0.0414 in the pK_a determination of the first ionization constant.

We have determined, from the measured average molecular weight of the molecules in fulvic acid and the measured number of milliequivalents per gram of fulvic acid, that an average molecule in fulvic acid from the Suwannee River possesses four carboxylic acid groups. Therefore, a tetraprotic model was assumed for fulvic acid from the Suwannee River, although the authors recognize that a wide variety of polycarboxylic carboxyl groups exist with various numbers of carboxyl groups per molecule. When titration data of fulvic acid is graphed as the pK_a value (y-axis) versus the percent of ionized carboxyl groups (x-axis), both the slope and the y-intercept of this curve are mainly independent of the assumption of the number of acid groups per molecule. Therefore, this tetraprotic model and graph was used to assess the carboxylic acidity characteristics of fulvic acid.

The observed pK_a value at any given degree of dissociation, α, may be represented by eq 1 (*6*):

$$pK_{apparent} = pH - \log \frac{\alpha}{1-\alpha} \qquad (1)$$

If there is an average of four carboxyl groups per molecule of fulvic acid, the titration curve is divided into four equivalent segments corresponding to 25, 50, 75, and 100% of the carboxylic acid groups. Values of 37.5, 62.5, and 87.5% of the carboxyl-group content then correspond to pH values that equal pK_{a2}, pK_{a3}, and pK_{a4}, respectively, because the log term in eq 1 is zero when each acid group is 50% dissociated. The value for pK_{a1} at 12.5% of the carboxyl-group content is calculated by substituting in $\alpha = (0.5[COOH_t] + 4[H^+])/[COOH_t]$ in eq 1 where $[COOH_t]$ is the molar concentration of total carboxyl group content

TABLE 1

Reported Carboxyl-Group Contents of Fulvic Acid from the Suwannee River, Georgia

source of fulvic acid	method of determination	content (mmol/g)	ref
International Humic Substances Society (IHSS) reference fulvic acid	aqueous potentiometric titration	6.1	15
IHSS reference fulvic acid	aqueous potentiometric titration	5.65	7
IHSS reference fulvic acid	nonaqueous potentiometric titration	4.15	16
fulvic acid isolated by Thurman and Malcolm (*17*)	aqueous potentiometric titration	6.0	18
fulvic acid isolated by Thurman and Malcolm (*17*)	solid-state ^{13}C-NMR spectrometry	6.2	18
fulvic acid isolated by Thurman and Malcolm (*17*)	methylation with ^{13}C-labeled reagents, ^{13}C-NMR spectrometry	6.0	18
fulvic acid isolated by Leenheer (*12*)	methylation, ^{1}H-NMR spectrometry	6.8	19

at the titration volume measured at 12.5% of the carboxyl group content. The reason for the difference in the method of calculating pK_{a1} is because greater than 50% of the first carboxyl group in a polyprotic acid is dissociated at 12.5% of the total carboxyl-group content in a forward titration of acid with base.

^{13}C-Nuclear Magnetic Resonance Spectrometry. The ^{13}C-NMR spectra were measured with a Varian XL-300 spectrometer at 75.429 MHz; solutions at a concentration of 100 mg/mL in 10-mm tubes were used. Aqueous solutions of the fulvic acid were dissolved as the sodium salt form (pH 7) in H_2O/D_2O (3:1); the acid form and methylated fulvic acid samples were dissolved in ^{13}C-depleted dimethyl sulfoxide. Quantitative spectra were measured using inverse-gated decoupling in which the proton decoupler was on only during the acquisition of the free-induction decay curve. The transmitter was set for a 45° tip angle, and an 8-s delay was used. The acquisition time was 0.2 s, and the sweep width was 30 000 Hz.

Results and Discussion

Variations in Total Carboxyl-Group Content. Reported values for total carboxyl-group content of fulvic acid from the Suwannee River are listed in Table 1. Differences in the method of determination causes greater variations in total carboxyl group content as listed in Table 1 than does the source of the fulvic acid. Base-hydrolysis studies (*15, 20*) have shown that fulvic acid from the Suwannee River contains labile groups (likely esters) that hydrolyze during the course of aqueous titrations. One possible explanation of the variability between different determinations is the differing proportions of carboxylic acids and esters in the sample depending on the method of sample isolation, sample drying that creates ester groups, and titration (including time of titration and selection of end point). Another significant variable is the change in intramolecular hydrogen-bonding between acid groups between aqueous and nonaqueous solvents that affect acidity determinations. The most specific determinations for carboxyl-group content are the methylation procedures (using diazomethane; *18, 19*). Methylation minimizes hydrogen-bonding interactions; methylation followed by ^{13}C-NMR measurements distinguish between carboxyl groups and phenol and enol groups; and methylation with diazomethane is not as susceptable to disrupting ester linkages as are aqueous titrimetric methods. The ^{13}C-methylation values in Table 1 agree well with the aqueous titrimetry values, and authors of this study will use the value of 6.0 mmol/g for the total carboxyl-group content of fulvic acid from the Suwannee River.

Amino Acid and Sulfur Acid Structures. Although the low nitrogen content (0.7%, *21*) of fulvic acid from the Suwannee River severely limits the quantity of carboxyl groups associated with amino groups, they were considered because the pK_{a1} of amino acids ranges from 1.7 to 2.4 (*22*). If all the nitrogen were free amino acid nitrogen, and half of this nitrogen (typical of amino acid mixtures) were amino groups on the carbon α to the carboxyl group, 0.25 mmol/g of carboxyl groups with pK_a near 2.0 could be accounted. However, Thurman and Malcolm (*23*) found that only 8.7% of the nitrogen could be accounted as amino acids, and they estimated that greater than 90% of these amino acids are combined as amides with proteins and fulvic acid structures. These two factors reduce the strong-acid carboxyl groups associated with amino acids to about 0.002 mmol/g.

The organic sulfur content of fulvic acid from the Suwannee River is 0.56% (*21*). If all this sulfur contributed to strong-acid acidity as sulfonic acids and organic sulfate esters, it would account for 0.18 mmol/g of strong-acid acidity. Therefore, nitrogen and sulfur structures together could only account for a maximum of 0.182 mmol/g of acidity (3.0% of the total carboxyl acidity).

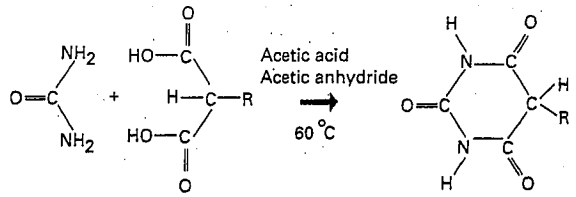
Oxalate Half-Ester Structures. Oxalic acid has a pK_{a1} near 1.2 (depending on variations in temperature and ionic strength, *24*), and its half esters might be similarly acidic, although no literature data was found for oxalate half esters. Oxalate half esters were suspected to be present because a base hydrolysis study of fulvic acid from the Suwannee River coupled with ^{1}H-NMR detection (*25*) revealed that formic, acetic, and succinic acids were present. These acids were postulated to be bound by ester linkages to various hydroxyl groups in fulvic acid. Oxalic acid is not observable by ^{1}H-NMR spectrometry. A gas chromatography–mass spectrometric study of base-hydrolysis products released from fulvic acids isolated from Lake Drummond in southeastern Virginia and from Black Lake on the North Carolina coastal plain found 0.013 mmol/g oxalic acid for fulvic acid from Black Lake and 0.017 mmol/g oxalic acid for fulvic acid from Lake Drummond (*26*).

The limit of detection for the gravimetric method used in this study to determine oxalic acid released by base hydrolysis of fulvic acid from the Suwannee River was 0.02 mmol/g based on the solubility product of calcium oxalate. No calcium oxalate precipitate was detected by infrared analysis of the trace amount of precipitate produced. The precipitate that resulted from CO_2 absorption during the base hydrolysis procedure was calcium carbonate.

Malonic Acid Structures. Substituted malonic acids, attached to fulvic acid at the center carbon α to the two carboxyl groups, were suspected to occur for two reasons: (1) the strong acidity of fulvic acid; (2) and carbon methylation was detected by ^{13}C-NMR after methylation of

the sample with methyl iodide catalyzed by sodium hydride in *N,N*-dimethylformamide. Values of pK_{a1} for various substituted malonic acids range from 2.4 to 2.8 (*10*). The carbon-methylation reaction might indicate other possible structures as well as substituted malonic acids (*27*).

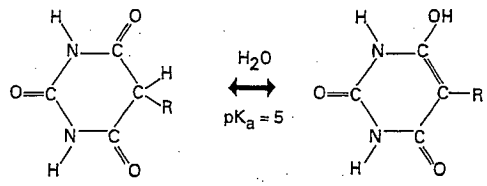
The synthesis of barbituric acid derivatives from monosubstituted malonic acids is shown in eq 2.



R = remainder of fulvic-acid structure (2)

Acid chloride and ethyl ester derivatives of fulvic acid also were tested as intermediates in the condensation reaction with urea to form barbituate derivatives, but condensation catalyzed by acetic anhydride (*13*) gave the best yields (48–104%) with the least side reactions for malonic, methylmalonic, butylmalonic, and phenylmalonic acids. The alkyl-substituted malonic acids gave nearly 100% yields; phenylmalonic acid had a tendency to decarboxylate during synthesis and yields were lower (48%). Disubstituted malonic acids failed to react as well as oxygen-substituted malonic acids such as tartronic acid. Disubstituted malonic acids are not likely as a significant constituent because quaternary carbons present in these structures were not detected in the attached proton test ^{13}C-NMR spectra (*28*). Oxygen-substituted malonic acids are a hypothetical possibility, but aryloxy malonic acids slowly decarboxylate at room temperature to aryloxyacetic acid (*29*).

Barbituric acids tautomerize in aqueous solution as shown in eq 3.



(3)

The enol form that predominates at pH 7 strongly absorbs UV radiation near 257 nm, and the strength and position of this absorption maximum are nearly independent of the alkyl or aryl substituent on the barbiturate derivative. The UV spectra of the barbituate derivatives of butylmalonic acid and fulvic acid from the Suwannee River are shown in Figure 1. No barbiturate derivative peak was detected in the fulvic acid spectrum. The limit of detection for carboxylic groups in the substituted malonic acid configuration is 0.024 mmol/g.

Barbiturate derivatives using ^{13}C-labeled urea of butylmalonic acid and fulvic acid from the Suwannee River also were synthesized and the ^{13}C-NMR spectra determined. The ^{13}C-NMR spectrum of the fulvic acid derivative did not exhibit any barbiturate formation. Urea derivatives of fulvic acid formed ureides that gave chemical shifts for the ^{13}C-labeled urea that differed from the barbituate derivative.

The barbituate derivative study did not give any definitive evidence for malonic acid structures in fulvic acid. However, it is possible that minor malonic acid structure might still exist and that they were not detected because of steric

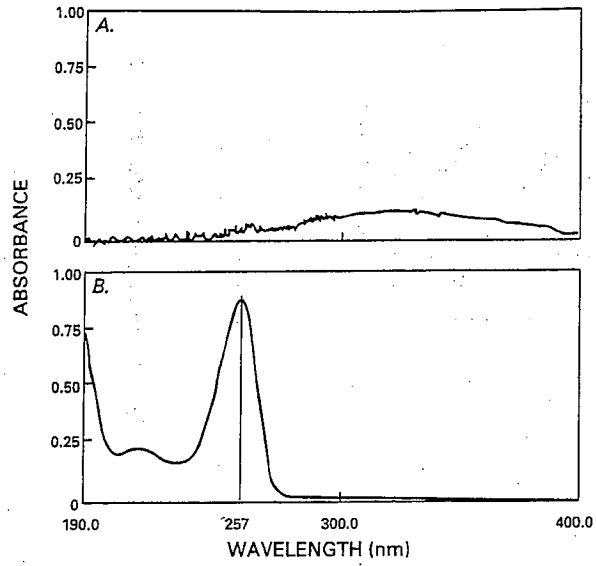


FIGURE 1. Ultraviolet spectra of 108 mg/L of the barbiturate derivative of fulvic acid from the Suwannee River, pH 7, in water (A) and of 6.3 mg/L of butyl barbituric acid, pH 7, in water (B). Acetylated fulvic acid titrated to pH 7 in water was in the reference cell for spectrum A, and water was in the reference cell for spectrum B.

hinderance limitations to the formation of the barbiturate derivative in fulvic acid.

Keto Acid Structures. Fulvic acid from the Suwannee River has a ketone-group content of 2.6 mmol/g (*11*). A survey (*10*) of ionization constants of keto acids showed that β-keto monocarboxylic acids had pK_a values from 3.5 to 3.8; α-keto monocarboxylic acids had pK_a values from 2.2 to 2.6; and α-keto, short-chain dicarboxylic acids had pK_{a1} values of 1.8–2.0.

To determine if ketone groups had an effect on the acidity of fulvic acid, sodium borohydride in aqueous solution was used to reduce the ketone groups in fulvic acids to alcohols; hydroxy acids are substantially weaker than keto acids. The extent of ketone-group reduction to alcohols by sodium borohydride was measured by the ^{13}C-NMR spectrometry. The ketone content decreased from 2.6 to 0.9 mmol/g as measured by the decrease in ketone band in the region from 185 to 220 ppm or by the increase in alcohol from 60 to 90 ppm. Aqueous sodium borohydride efficiently reduced α-keto acids (*30*); however, certain aromatic ketones and quinones are readily reoxidized during the processing and isolation of the reduced sample. The chemical shift of the residual ketones in the reduced sample is between 185 and 200 ppm; this spectral region is consistent with the presence of reoxidized aromatic ketones and quinones. These residual aromatic ketones and quinones should not significantly affect the acidity of the carboxyl groups, and quantitative reduction of keto acids will be assumed.

Four pK_a values were calculated for the fulvic acid titration curves before and after sodium borohydride reduction. A plot of these average pK_a values (connected by straight-line segments) versus the percentage of total carboxyl groups ionized in fulvic acid (Figure 2) indicated a 3.4% decrease of the percentage of carboxyl groups at pK_a 3.0 after sodium borohydride reduction. This decrease in acidity is equivalent to 0.2 mmol/g as keto acids or to 7.7% of the total ketone content as keto acids with pK_a values of 3.0 or less.

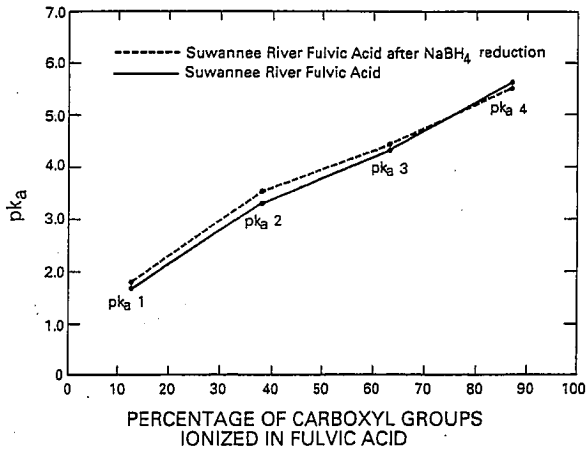


FIGURE 2. Plot of pK_a versus percentage of ionized carboxyl groups in fulvic acid from the Suwannee River before and after reduction with sodium borohydride.

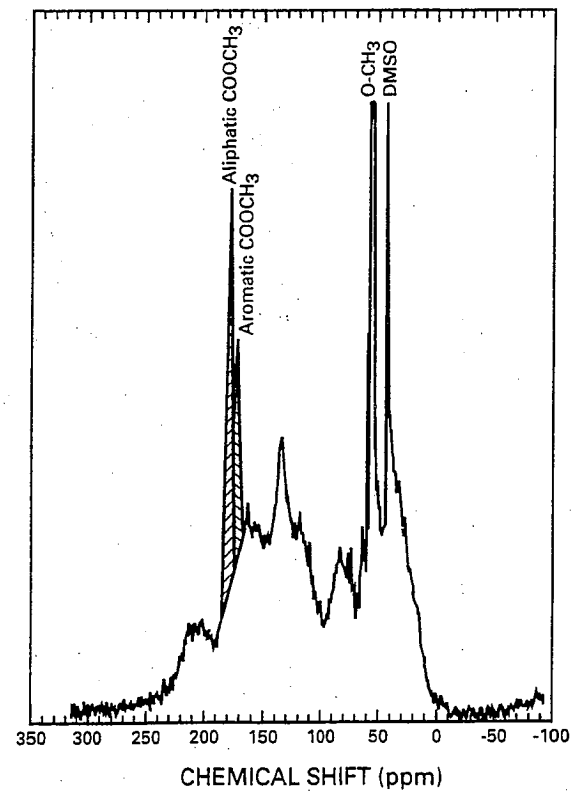


FIGURE 3. ^{13}C-NMR spectrum of methyl ester derivative of fulvic acid from the Suwannee River dissolved in dimethyl sulfoxide-d_6.

Aromatic Carboxyl-Group Structures. The best method for distinguishing aliphatic carboxyl groups from aromatic carboxyl groups is by liquid-state, ^{13}C-NMR spectrometry. Thorn (*28*) showed that the carboxyl-group peak in the ^{13}C-NMR spectra of fulvic acid from the Suwannee River was partially resolved into two peaks (174 ppm for aliphatic carboxyl groups and 168 ppm for aromatic carboxyl groups) when the free-acid form sample was dissolved in dimethyl sulfoxide-d_6. α,β-Unsaturated carboxyl groups are also included in the peak at 168 ppm. The carboxyl-group peak also includes quinone carbonyl groups (178–188 ppm) and ester carbonyl groups (165–172 ppm). Ester carbonyl groups are potentially a major interference in the quantitation of carboxyl groups by ^{13}C-NMR spectrometry because of their abundance (2.0–2.7 mmol/g, *11*) relative to carboxyl groups (6.0 mmol/g) and because the position

TABLE 2

Summary of Carboxyl-Group Structures (pK_a of 3.0 or Less) Determined in Fulvic Acid from the Suwannee River

acid-group structure	content (mmol/g)	% of total carboxyl groups
carboxyl groups of pK_a 3.0 or less[a]	2.00	33.4
sulfur and nitrogen acids	0.18	3.0
oxalate half-esters[b]	0.02	0.3
substituted malonic acids[b]	0.02	0.3
keto acids[a]	0.20	3.0
aromatic and olefinic acids	0.46	7.7
unaccounted carboxyl groups of pK_a 3.0 or less	1.12	19.1

[a] Data determined from Figure 3. [b] Data listed are limits of detection.

of the ester carbonyl carbon peaks are shifted about 3 ppm lower than the free-acid form carboxyl peak, which tends to weight the aromatic plus α,β-unsaturated carboxyl peak with error due to the ester.

The ester error was minimized by methylating the fulvic acid with diazomethane to convert carboxyl groups to methyl esters (*19*), and the quantitative ^{13}C-NMR spectrum is shown in Figure 3. Aliphatic methyl esters give a peak at 171 ppm, and aromatic plus α,β-unsaturated methyl esters give a peak at 165 ppm. Integration of these peaks was done by dividing the peaks at 167 ppm down to a baseline that was drawn between the minima at 162 and 185 ppm (see Figure 3). This subjective integration procedure was selected to minimize errors due to esters and quinones. Aliphatic carboxyl groups were determined to be 4.7 mmol/g (78% of total carboxyl groups), and aromatic carboxyl groups were 1.3 mmol/g (22% of total carboxyl groups).

With the exception of maleic acid (pK_{a1} of 1.9, *24*), α,β-unsaturated acids are not sufficiently acidic to account for fulvic acid acidity near pK_a 3.0. As an estimate of the distribution of carboxyl groups on aromatic rings in fulvic acid that have pK_a values less than 3.0, the aromatic acids that were released by base hydrolysis of fulvic acid from Black Lake and Lake Drummond were considered (*26*). The base-hydrolysis data were considered to be more valid for estimating carboxyl-group distributions on aromatic structures than aromatic acids released by permanganate oxidation of fulvic acid because permanganate oxidation produces additional aromatic acids by side-chain oxidation. These fulvic acids were isolated by the same method as fulvic acid from the Suwannee River (*17*), and these black waters occur in environments similar to the Okefenokee Swamp where the Suwannee River was sampled. Fulvic acid from Black Lake had 34% and fulvic acid from Lake Drummond had 36% of the carboxyl groups occurring on aromatic ring compounds whose pK_a values were less than 3.0, according to a compilation of pK_a values of aromatic acids (*24*). By using an intermediate value of 35% of aromatic carboxyl groups, fulvic acid from the Suwannee River was calculated to contain 0.46 mmol/g (7.7% of total carboxyl acidity) of aromatic carboxyl groups whose pK_a is 3.0 or less.

Summary and Conclusions

A summary of the carboxyl-group (pK_a of 3.0 or less) content and percentages determined in various structural arrange-

ments for fulvic acid from the Suwannee River is given in Table 2. An upper limit of 43% of the carboxyl groups of pK_a 3.0 or less could be attributed to the structures investigated in this paper. Certain of these structures, such as malonic acid, phthalic acid, and salicylic acid structures, are commonly hypothesized to account for the strong-acid and metal-binding characteristics of dissolved humic substances; however, the data in Table 2 indicate these structures are not major acid constituents of fulvic acid from the Suwannee River. The remaining strong-acid carboxyl groups must be aliphatic in nature and must be in unusual and/or complex configurations for which limited model compound data are available.

Acknowledgments

Any use of trade names in this paper is for descriptive purposes only and does not constitute endorsement by the U.S. Geological Survey.

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