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# Strong-Acid, Carboxyl-Group Structures in Fulvic Acid from the Suwannee River, Georgia. 2. Major Structures

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Polycarboxylic acid structures that account for the strong-acid characteristics ($pK_{a1}$ near 2.0) were examined for fulvic acid from the Suwannee River. Studies of model compounds demonstrated that $pK_a$ values near 2.0 occur only if the α-ether or α-ester groups were in cyclic structures with two to three additional electronegative functional groups (carboxyl, ester, ketone, aromatic groups) at adjacent positions on the ring. Ester linkage removal by alkaline hydrolysis and destruction of ether linkages through cleavage and reduction with hydriodic acid confirmed that the strong carboxyl acidity in fulvic acid was associated with polycarboxylic α-ether and α-ester structures. Studies of hypothetical structural models of fulvic acid indicated possible relation of these polycarboxylic structures with the amphiphilic and metal-binding properties of fulvic acid.

## Introduction and Approach

In the preceding paper (*1*), a quantitative study of acid-group structures in fulvic acid from the Suwannee River concluded that only 43% of the strong-acid acidity ($pK_3$ 3.0 or less) was associated with amino and sulfur-containing acid structures, oxalate half-ester structures, malonic acid structures, keto acid structures, and aromatic and olefinic carboxylic acid structures. The remaining 57% of strong acidity was attributed to aliphatic carboxylic acids in unusual and complex configurations for which limited model compound data are available. The objective of this paper is to account for the remaining strong-acid acidity in major structural arrangements of carboxylic acids in fulvic acid from the Suwannee River.

The approach of this study, isolation of strong-acid fractions of fulvic acid from the Suwannee River by normal-phase chromatography on silica gel (*2*) and characterization of these fractions by a combination of spectrometric, titrimetric, and colligative property measurements, was designed to obtain insight into possible configurations of carboxylic acids. A new approach to the analyses of titrimetric data was taken that assumed the heterogeneity in carboxyl-group $pK_a$ values in fulvic acid was primarily an intramolecular phenomenon in polyprotic acid structures rather than intermolecular variations in $pK_a$ values of simple carboxylic acids. Model compounds of proposed carboxylic acid configurations were purchased or synthesized and were characterized to correlate unique properties with acid groups in the fulvic acid fractions. Lastly, procedures that destroyed or modified specific functional groups were applied to the unfractionated fulvic acid to modify and quantify the carboxyl-group acidity associated with these functional groups.

## Experimental Section

**Reagents.** Polycarboxylic acids used in this study to model functional groups in fulvic acid were purchased from Aldrich Chemical Company (1,2,3,4-cyclobutanetetracarboxylic acid, isocitric acid lactone, tetrahydrofuran-2,3,4,5-tetracarboxylic acid) and American Tokyo Kasei, Inc. (1,2,3,4-cyclopentanetetracarboxylic acid). Phenoxysuccinic acid was synthesized (*3*). The Suwannee River fulvic acid used in this study was isolated as described by Leenheer (*2*).

**Silica Gel Fractionation of Fulvic Acid.** A total of 6 g of the tetrabutylammonium salt of fulvic acid from the Suwannee River was fractionated in two stages on a 2–L bed-volume column of activated silica (ICN Biochemicals) by normal-phase chromatography (*2*) with various organic solvents of increasing polarity. The first stage of the fractionation applied the tetrabutylammonium salt of fulvic acid dissolved in chloroform to the column, and the second stage used the acid form of the fulvic acid fractions from the first stage after conversion of the fractions to the acid form by a hydrogen-saturated cation-exchange resin. The acid fractions were dissolved in tetrahydrofuran for application to the column. The yields of the fractionation procedure are shown in Figure 1.

**Titrimetric Procedures.** Fulvic acid fractions (20 mg) from the silica gel fractionation were dissolved in 10 mL of

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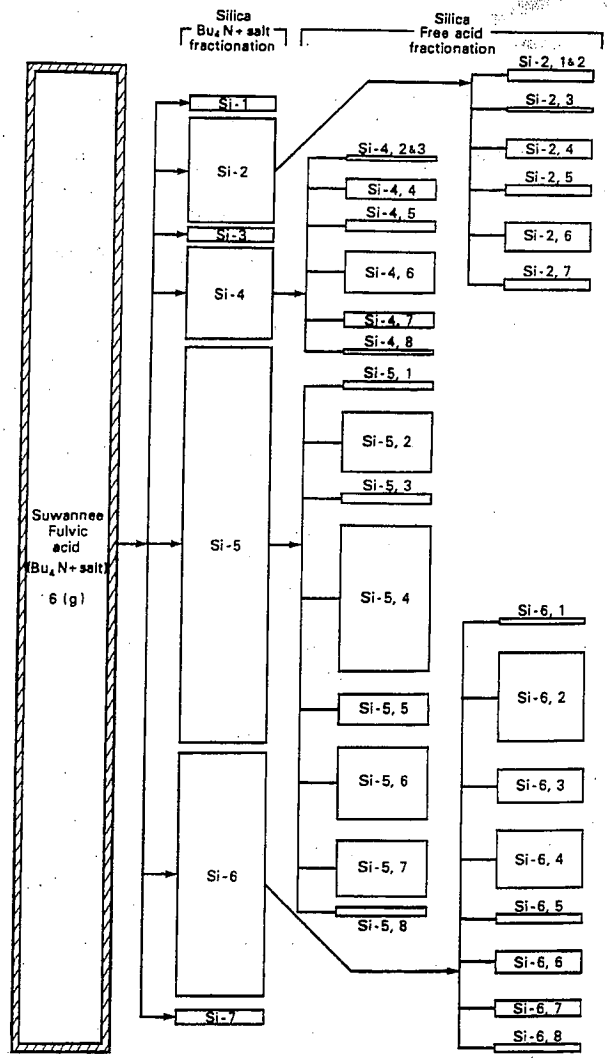



**FIGURE 1. Two-stage fractionation of Suwannee River fulvic acid on silica gel. Bar lengths are proportional to recoveries; the numbers correspond to the solvent elution sequence described in the text (2).**

water and were titrated with 0.1 M sodium hydroxide. Unfractionated fulvic acid samples were titrated at much greater concentrations (200–500 mg/5 mL of $H_2O$) to obtain more accurate determinations of the $pK_{a1}$ values of the strong-acid groups. For samples where sodium hydroxide and sodium chloride were used, an Orion Model 501 pH meter with an Orion 91-05 glass electrode was used. For samples where potassium hydroxide and potassium chloride were used, an automatic titrator (LIST Radiometer System) with a radiometer GK273920 combination pH glass was used. The radiometer pH meter was standardized using pH 4 and 7 buffer solutions verified against NIST certified buffers with an accuracy of ±0.01 pH at 25 °C. $pK_a$ values were determined as described in the previous paper (*1*).

**Cleavage of Esters and Ethers.** Esters in fulvic acid samples were hydrolyzed by dissolving 200–400 mg of sample in 10 mL of 0.5 N sodium hydroxide or potassium hydroxide, heating the sample in a boiling water bath for 2 h, and reacidifying the sample to pH 1.0–1.5 with 37% HCl. Carbon dioxide was removed from the reacidified samples prior to base titration with a 20-min nitrogen purge.

Esters, aliphatic alcohols, and ether linkages in fulvic acid were cleaved with hydriodic acid (*4*). Fulvic acid (500 mg) was dissolved in 5 mL of hydriodic acid plus 5 mL of glacial acetic acid. This 10-mL sample was sealed in a glass ampule and heated in a pressure vessel containing water at 130 °C for 15 h. Hydriodic acid, acetic acid, and iodine were removed from the fulvic acid by vacuum evaporation in a rotary evaporator. A mixture of 75% acetonitrile/25% water was repeatedly added to the reaction product and vacuum evaporated until there was no residual color of iodine in the distillate. Because certain carboxyl groups re-esterified with phenols in fulvic acid during the vacuum evaporation procedure, this sample was base hydrolyzed prior to titration.

**Spectrometric Measurements.** 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 HI-treated fulvic acid and fulvic acid fraction 6-3 were dissolved as the sodium salt form (pH 7) in $H_2O/D_2O$ (3:1); the acid form of the untreated fulvic acid samples was 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

**Titrimetry of Fulvic Acid Fractions.** Generally, the observed acid–base titration data of a given fulvic acid cannot be represented by a single ionization constant. Two different approaches have been used to rationalize the titration data.

In one approach, previous investigators have assumed that the necessity of using several different $pK_a$ values is needed to represent a single titration curve because of intermolecular or intramolecular heterogeneity of functional groups. For example, Perdue and others (*5*) have proposed that there is a normal distribution of individual ligand concentrations as a function of $pK_a$, the negative logarithm of the ionization constant. This distribution function may be represented by a mean $pK_a$ value and a variance parameter. Although a normal distribution of $pK_a$ values does generally fit fulvic acid titration data, comparison of literature $pK_a$ values of simple organic acid structures with operational $pK_a$ values for fulvic acid does not account for the majority of the strong-acid characteristics of fulvic acid from the Suwannee River (*1*)

A second approach is based upon a model in which the part of the variation in the observed $pK_a$ values for a single titration curve results from the electrostatic charge resulting from the ionization of more than one acidic group on a single molecule (*6–8*). Implicit in this treatment is the assumption that all of the ionizable functional groups are the same, and that they possess a single "intrinsic" dissociation that may be measured if the electrostatic effects are suppressed by high concentrations of background electrolyte. This treatment is correct when applied to certain carboxylated polymers (*9*); but it must be adjusted for several intrinsic (apparent) $pK_a$ values in fulvic acid (*7*). When electrostatic effects and carboxyl-group heterogeneity based on simple acid structures are both considered, the cumulative effect of both models does not completely explain the observed acidity characteristics of fulvic acid.

The new approach we have taken is to model fulvic acid as a polyprotic acid in which the clustering of acid groups in close proximity to each other or other electron-

withdrawing structures (ester, ketone, aromatic rings) gives rise to exceptionally strong inductive effects (*10*) and carboxylate stabilization via intramolecular hydrogen bonding (*11*) that lower $pK_a$ values. We shall demonstrate below that only a limited number of possible chemical structures exist, based upon model compound titrimetric data, to explain the titration data observed for fulvic acid. A tetraprotic acid molecule was assumed for unfractionated fulvic acid based upon the average number of carboxyl groups per molecule, and "operational" $pK_a$ values were determined as if each carboxyl group was titrated in a sequential manner as was discussed previously (*1*). The low value of $pK_{a1}$ (1.67) found previously (*1*) indicates strong electron-withdrawing functional groups in proximity to this carboxyl group with the low $pK_{a1}$ and possible intramolecular hydrogen bonding effects that also lower $pK_a$ values.

A plot of average $pK_a$ values versus the percentage of ionized carboxyl groups for the major fractions of the two-stage silica gel fractionation of fulvic acid from the Suwannee River (*2*) is shown in Figure 2. Number-average molecular weight values determined by equilibrium centrifugation were used for the data in Figure 2, and the number of $pK_a$ values for each fraction was calculated by multiplying the number-average molecular weight by equivalents per gram of acid-group content. The fractions contained two to five carboxyl groups per molecule.

Only two of the fractions (fractions 2-6 and 4-6) had significantly higher $pK_a$ values than the remainder of the fractions as plotted in Figure 2. This result indicates that the variation in $pK_a$ values is more of an intramolecular phenomenon than intermolecular because carboxyl groups of differing acidity that result from structural differences between fulvic acid molecules should have been separated by the silica gel fractionation. The slope of the curves in Figure 2 is caused by a combination of carboxyl-group heterogeneity and electrostatic effects. The number of carboxyl groups per molecule in the various fractions had little effect on the slope and y-intercept of the curves.

**Characterization of Fraction 6-3.** Fraction 6-3 isolated from fulvic acid from the Suwannee River (*2*) was characterized to determine the functional groups that cause the intramolecular heterogeneity and the low $pK_{a1}$ values. Fraction 6-3 was selected because: it is relatively monodisperse (degree of polydispersity = 1.14) in the molecular weight distribution as determined by equilibrium centrifugation. Its number-average molecular weight ($M_n$ = 640) is comparable to that of the unfractionated fulvic acid ($M_n$ = 780) (*12*). It has an average of four carboxyl groups per molecule as does the unfractionated fulvic acid. The $pK_{a1}$ of this fraction is low ($pK_{a1}$ = 1.8 in Figure 2). This

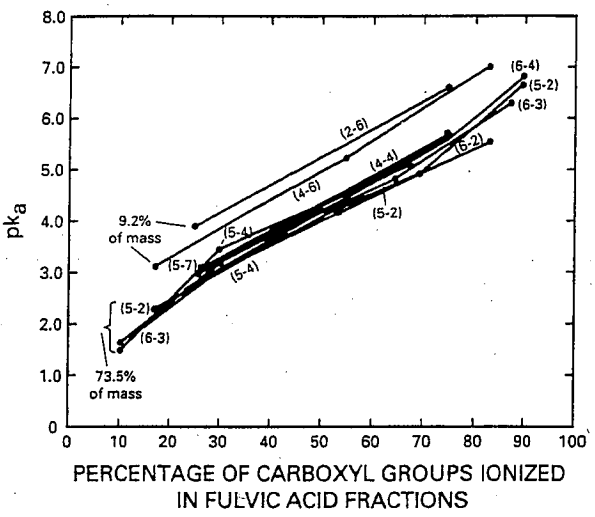



**FIGURE 2. Plots of $pK_a$ versus percentage of ionized carboxyl groups in fulvic acid fractions from the two-stage silica gel fractionation.**

fraction has an enhanced aliphatic carbon content (8 % greater than unfractionated sample) that is thought to be related to the strong-acid characteristics from the previous paper (*1*).

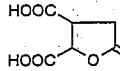
An average molecular model was derived for fraction 6-3 based upon molecular weight, elemental, titrimetric, and NMR spectrometric data. Specific methylations and acetylations coupled with NMR spectrometric determinations were used to assign oxygen distributions to carboxyl, ester, ether, hydroxyl, and ketone functional groups. The methods of data aquisition and model synthesis have been reported previously (*12, 13*). The average molecular model of fraction 6-3 indicated carboxyl groups that were predominantly aliphatic in nature and that ester and ether functional group contents were sufficiently large to possibly affect carboxyl-group acidity. Therefore, the focus of the investigation shifted to the effect of esters and ethers on acidity of the carboxylic groups in fulvic acid from the Suwannee River.

**Ester and Ether Structures.** Carbon–oxygen linkages located on the α-carbon to a carboxyl group result in acid strengthening that is dependent on the nature of the functional group as shown in Table 1.

Glycolic acid and methoxyacetic acid have nearly identical inductive effects caused by the alcohol and ether groups, but methoxyacetic acid is slightly stronger because of a solvation effect difference (the acid form of methoxyacetic acid is less soluble than the acid form of glycolic acid) (*16*). Acetoxyacetic acid (an α-ester acid) is significantly stronger than glycolic and methoxyacetic acids

**TABLE 1**

**Effects of Functional Groups on Acidity of α-Oxygen Substituted Carboxylic Acids[a]**

| compound | structure | ionic strength | $pK_{a1}$ | $pK_{a2}$ |
|---|---|---|---|---|
| glycolic acid[b] | $HOCH_2COOH$ | 0.5 | 3.57 | |
| methoxyacetic acid[b] | $CH_3OCH_2COOH$ | 0.1 | 3.31 | |
| acetoxyacetic acid[b] | $CH_3COCH_2COOH$ | 0.4 | 2.81 | |
| oxydiacetic acid[b] | $HOOCCH_2OCH_2COOH$ | 0.1 | 2.79 | 3.93 |
| *trans*-isocitric acid lactone[c] |  | ND | 2.13 | 3.95 |

[a] ND = not determined. [b] Data from Martell and Smith (*14*). [c] Data from Sergeant and Dempsey (*15*).

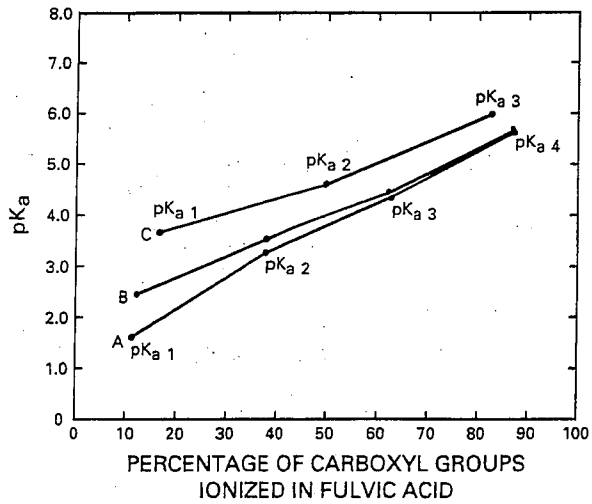
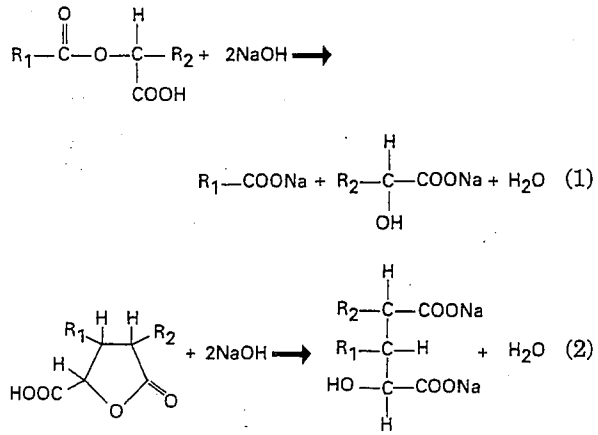



**FIGURE 3. Plots of p$K_a$ versus percentage of ionized carboxyl groups in fulvic acid from the Suwannee River (A), after alkaline hydrolysis (B), and after ether cleavage and reduction with hydriodic acid (C).**

because of the stronger inductive effect of the ester group compared to alcohol and ether groups. The combination of additional carboxyl groups with α-ether and α-ester structures as illustrated by oxydiacetic acid and isocitric acid lactone results in additional strengthening of the acidity of the first ionization constant because of additivity of inductive effects from carboxyl groups through the ether and ester linkages. The acid groups for α-hydroxy acids, even in combination with additional carboxyl groups such as in isocitric acid, are not as strong as the carboxyl groups for fulvic acid from the Suwannee River. However, polycarboxylic acids containing α-ether and α-ester groups may have sufficiently low p$K_{a1}$ values to account for the observed acidity of fulvic acid.

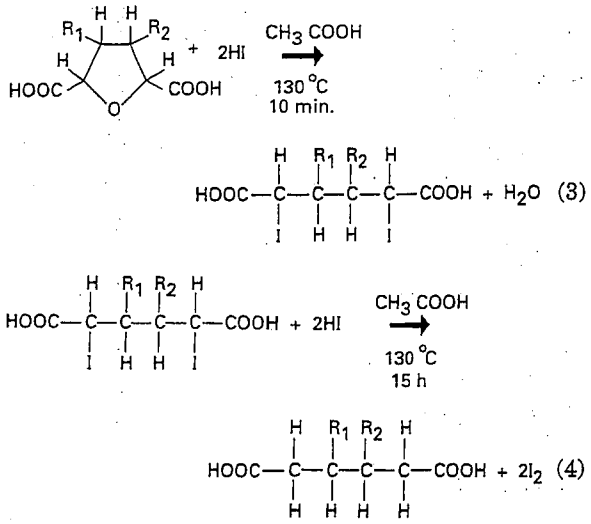
The effect of ester and ether linkages on carboxyl group acidity was assessed in fulvic acid from the Suwannee River by cleaving ester linkages by base hydrolysis and cleaving ester, ether, and alcohol linkages with hydriodic acid. After cleaving these linkages, the p$K_a$ distribution was redetermined by titration. The results are shown in Figure 3.

Ester hydrolysis can result in lower molecular weight products as shown in reaction 1 or in slightly greater molecular weight products with more carboxyl groups per molecule as shown in reaction 2.




A significant weakening of the carboxyl-group acidity was observed in Figure 3 as a result of base hydrolysis. The divergence of curve B from curve A with decreasing pH indicates the effect of neighboring ester-group structures is most pronounced on the ionization constants of the first two carboxyl groups.

The reaction of hydriodic acid with fulvic acid is a two-step reaction as shown in reactions 3 and 4.




Reaction 3 is reported to cleave ester, ether, and alcohol linkages and replace these linkages with iodine, except that phenols, phenolic ethers, and phenolic esters are converted to the free phenols (*4*). With extended heating in a sealed tube, some alkyl iodides produced in reaction 3 are reduced to hydrocarbon acids in reaction 4 (*17*).

The quantitative $^{13}$C-NMR spectrum of the products of HI treatment of fulvic acid is given in Figure 4. The aliphatic C–O linkages that occur in the region from 60 to 105 ppm in the spectrum of the untreated sample have largely disappeared, having been converted to a mixture of hydrocarbons and alkyl iodides that occur in the 10–50 ppm region of the spectra. There is only a 3 % loss of carboxyl plus ester carbon from decarboxylation reactions and production of volatile organic acids that are lost during the vacuum evaporation steps after HI treatment. Similarly, there is only a 4% increases in the aromatic/olefinic carbon that might be expected from dehydration reactions during the treatment of alcohols with strong acid. This selective degradation procedure at moderate temperature is the first application to humic substances; a previous study (*18*) of HI treatment at 250 °C resulted in severe alteration to humic substance structure.

The selective conversion of C–O linkages to hydrocarbons and alkyl iodides eliminated the strong-acid character of carboxyl groups in fulvic acid from the Suwannee River as shown by curve C in Figure 3. After HI treatment, the fulvic acid was no longer soluble in water and only went into solution at pH 3.2 during titration with base. This behavior is clear evidence that C–O linkages, specifically α-ether and α-ester linkages in association with additional carboxyl groups, produce the majority of strong-acid characteristics in fulvic acid from the Suwannee River.

**Model Compound Studies.** The p$K_a$ values of a number of model compounds with ether or ester linkages in proximity to a carboxyl group in its structure were measured. Table 2 lists those model compounds that were tested and the p$K_a$ values determined. The data in Table 2 indicate that only the aliphatic, alicyclic ether (tetrahydrofuran tetracarboxylic acid) and ester (isocitric acid lactone)

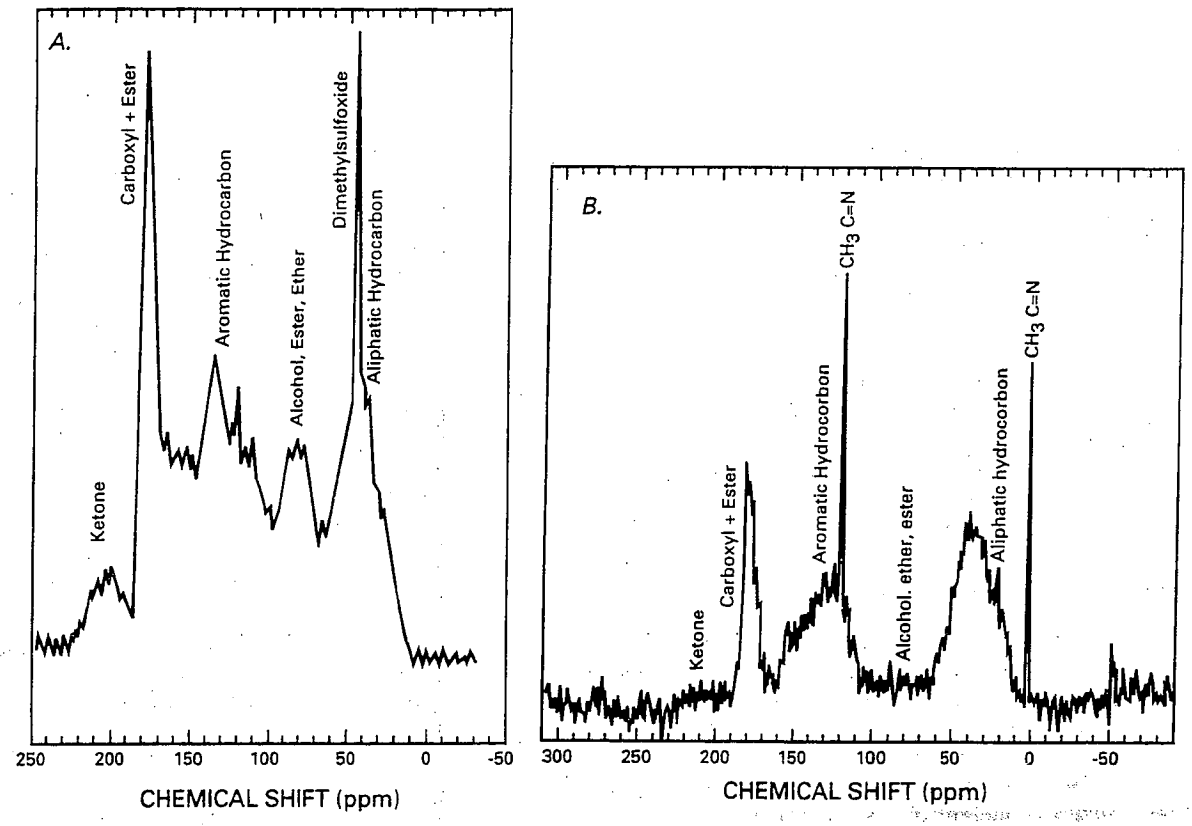



**FIGURE 4.** $^{13}$C-NMR spectra of fulvic acid from the Suwannee River before (A) and after (B) treatment with hydriodic acid.

**TABLE 2**

## $pK_a$ Values of Model Polycarboxylic Acids[a]

| compound | structure | ionic strength | $pK_{a1}$ | $pK_{a2}$ | $pK_{a3}$ | $pK_{a4}$ |
|---|---|---|---|---|---|---|
| succinic acid[b] | $HOOCCH_2CH_2COOH$ | 0.5 | 3.94 | 5.10 | | |
| phenoxysuccinic acid[c] | $HOOCCH_2CHCOOH$, O, benzene | 0.5 | 2.75 | 4.45 | | |
| carboxymethoxybutanedioic acid[b] | $OCH_2COOH$, $HOOCCH_2CHCOOH$ | 0.1 | 2.52 | 3.77 | 5.00 | |
| *trans*-cyclopentane dicarboxylic acid[d] | COOH, COOH | 0 | 3.96 | 5.85 | | |
| butane-1,2,3,4-tetracarboxylic acid[b] | COOH, $HOOCCH_2CHCHCH_2COOH$, COOH | ND | 3.43 | 4.58 | 5.85 | 7.16 |
| cyclobutane tetracarboxylic acid[c] | HOOC, COOH, HOOC, COOH | 0.5 | 2.52 | 3.68 | 4.80 | 5.97 |
| 1,2,3,4-cyclopentane tetracarboxylic acid[c] | HOOC, COOH, HOOC, COOH | 0.5 | 2.54 | 3.97 | 5.05 | 9.25 |
| tetrahydrofuran tetracarboxylic acid[c] | HOOC, COOH, HOOC, O, COOH | 0.5 | 0.95 | 3.40 | 5.55 | 6.42 |

[a] ND = not determined. [b] Data from Martell and Smith (*14*). [c] Data determined by titration. [d] Data from Dean (*19*).

substituted with three to four electron-withdrawing groups (carboxyl or ester) on the ring have sufficiently strong carboxyl groups to account for the acidity observed in fulvic acid. Acyclic α-ether structures (phenoxysuccinic and carboxymethoxybutanedioic acids) are not sufficiently acidic.

The importance of ring structures on the acidity of various polycarboxylic acids is also indicated by the data in Table 2. In dicarboxylic acids separated by two carbons, there is no significant difference in $pK_{a1}$ values between cyclic and acyclic structures. However, in tetracarboxylic acids in which each carboxyl group is separated by two

carbons, the $pK_{a1}$ values of the cyclobutane and cyclopentane structures is about 0.9 unit lower than the acyclic structure. Replacement of the carbon with an ether oxygen in the five-membered ring structure as is the case with tetrahydrofuran tetracarboxylic acid decreases the $pK_{a1}$ value an additional 1.6 units. We believe the lower values for $pK_{a1}$ in cyclic tetracarboxylic acids compared to linear tetracarboxylic acid analogs is related to the closer proximity of carboxyl groups in the cyclic structures that give rise to stronger inductive effects and also allow for intramolecular hydrogen bonding between adjacent carboxyl groups (*11*).

The exceptionally low $pK_{a1}$ values for the aliphatic, alicyclic α-ether and α-ester polycarboxylic acids are caused by a number of factors that include inductive effects, solvation effects, and steric effects of ring structures that allow intramolecular hydrogen bonding between carboxyl groups (*11*). In addition to a carboxylic acid being located α to an ester or ether linkage, two to three additional electron-withdrawing groups (carboxyl, ketone, ester, or aromatic groups) must be located on adjacent carbons on both sides of the ether or ester linkage. Model compounds incorporating aromatic structures such as 2,3-dicarboxy-2,3-dihydrobenzofuran, were considered for study, but they were not commercially available. Given the heterogeneity of fulvic acid, a variety of electronegative ring substituents and steric effects likely occur that contribute to the strong-acid characteristics of α-ether and α-ester acids.

**Structural Models of Carboxyl Groups in Fulvic Acid.** Three structural models of fulvic acid that illustrate possible carboxyl-group structures with $pK_a$ values less than 3.0 are shown in Figure 5. The structure in Figure 5A is based on the data obtained for fraction 6-3. A structure with greater aromatic character is given in Figure 5B, and an average structure that incorporates average structural data for unfractionated fulvic acid from the Suwannee River (*12*) is shown in Figure 5C. As seen from the variability between these three models, a wide variation in structural models is possible; the clustering of electronegative functional groups on ring structures with α-ether and α-ester linkages to carboxyl groups is reasonable given the structural characteristics and functional-group distribution in fulvic acid.

The acid structures shown in Figure 5 have additional implications for the structure–reactivity relations of fulvic acid. An estimate of the distribution of all acid structures in fulvic acid from the Suwannee River is listed in Table 3. The majority of carboxyl groups are accounted for by this study. From the low $pK_{a1}$ (1.67) of fulvic acid and from considerations of model compounds that give $pK_a$ values this low, the assumption of two additional carboxyl groups in cyclic succinic acid configurations associated with α-ether and α-ester structures is more likely than the one additional carboxyl-group assumption. Therefore, all carboxylic acids that comprise and are associated with α-ether and α-ester structures (the carboxylic acid in the α position to the oxygen plus two additional carboxyl groups) may be as great as 64%.

The clustering of carboxyl groups on aliphatic, alicyclic rings with α-ether and α-ester linkages should enhance the amphiphilic nature of fulvic acid structures compared to structures where carboxyl groups are evenly distributed. Polar and nonpolar regions are observable in the structures of Figure 5. Kile and others (*20*) have measured the water solubility enhancement factors for DDT and PCBs by fulvic acid from the Suwannee River, and they attribute this

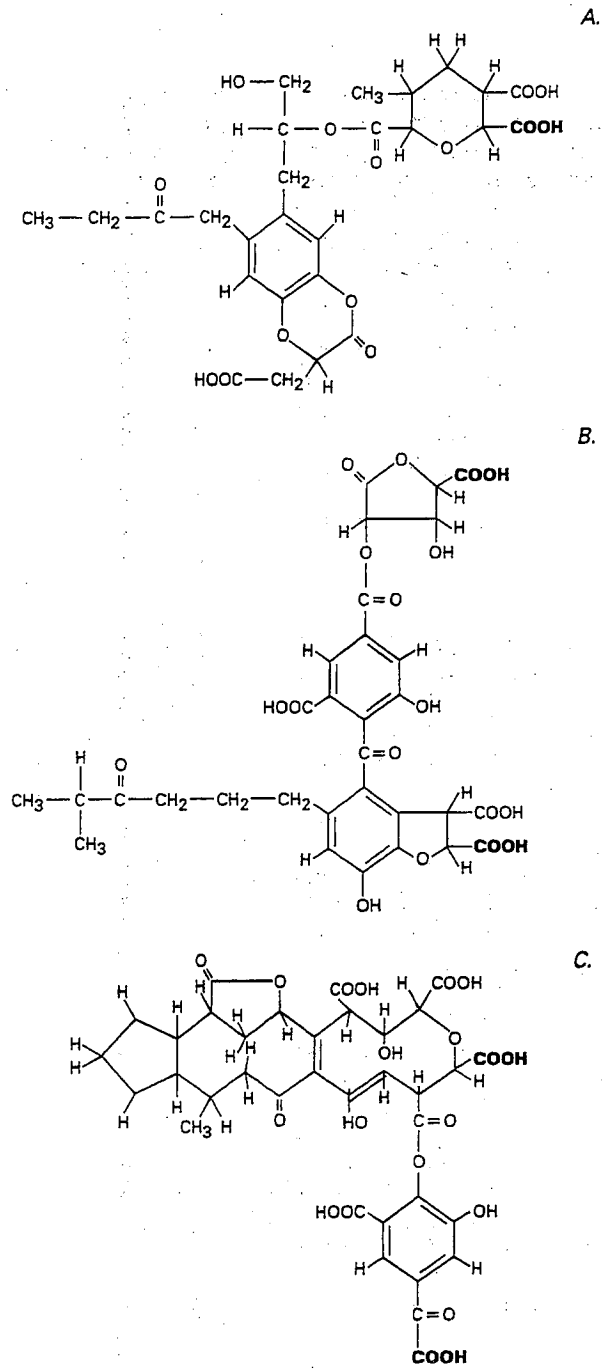



**FIGURE 5. Three structural models (A–C) of fulvic acid molecules from the Suwannee River. Carboxyl groups in bold print have $pK_a$ values less than 3.0.**

partition interaction to "molecular configuration and conformation that permits the formation of a sizable intramolecular nonpolar organic environment".

α-Ether carboxylic acids may have carboxyl groups on both sides of the ether linkage as in oxydiacetic acid. Polycarboxylic acids with oxydiacetate groups are excellent complexers of calcium, and a number of these structures have been proposed as builders in detergent formulations (*21*). There is a cyclic oxydiacetate structure in Figure 5C. The similarity of the calcium-binding constant of an aquatic fulvic acid to the calcium-binding constant of oxydiacetic acid was recently noted (*22*).

Finally, the α-ester and α-ether linkages in humic substances in general may result from either laboratory

**TABLE 3**

**Summation of Carboxyl-Group Structures Determined in Fulvic Acid from Suwannee River**

| acid-group structure | content (mmol/g) | % of total carboxyl groups |
|---|---|---|
| keto acids ($pK_a$ of 3.0 or less) | 0.20 | 3.0 |
| aromatic and olefinic acids ($pK_a$ of 3.0 or less) | 0.46 | 7.7 |
| aromatic and olefinic acids ($pK_a$ greater than 3.0) | 0.86 | 14.3 |
| α-ether and α-ester acids ($pK_a$ of 3.0 or less) | 1.28 | 21.3 |
| additional carboxylic acids associated α-ether and α-ester acids | | |
| (one additional acid assumption) | 1.28 | 21.3 |
| (two additional acids assumption) | 2.56 | 42.6 |
| total accounted acids | 4.1–5.4 | 68–90 |

processing or diagenetic processes. Laboratory acidification and drying of aliphatic $\tau$- and $\delta$-hydroxy acids will produce five- and six-membered ring lactones; five-membered ring lactones form so readily that acidification of aqueous solutions of $\tau$-hydroxy acids will cause lactone formation (*17*). Bowles and others (*23*) noted titrimetric characteristics of fulvic acid from the Suwannee River that were characteristic of lactone hydrolysis in alkali solution and re-esterification in acid solution. The α-ether linkages may result from selective preservation of ether linkages in lignin structure during microbial degradation (*24*). Additional ether linkages may be added to aromatic (*25*) and olefinic (*26*) structures through oxidative radical-coupling reactions initiated by enzymes or sunlight. Ester linkages also may result from radical-coupling processes.

## Summary

A major fraction of carboxyl groups in fulvic acid from the Suwannee River seem to be associated with cyclic aliphatic α-ester and α-ether structures. These structures give rise to exceptionally strong electron-withdrawing inductive effects and intramolecular hydrogen-bonding effects that strengthen the acidity of the first dissociation constant of these cyclic polyprotic acid structures. Destruction of these structures through alkaline ester hydrolysis and ether cleavage and reduction with hydriodic acid eliminates the strong-acid characteristics of the carboxyl group adjacent to the ether and ester structures.

## Acknowledgments

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## Literature Cited

(1) Leenheer, J. A.; Wershaw, R. L.; Reddy, M. M. *Environ. Sci. Technol.* **1995**, *29*, 393–398.
(2) Leenheer, J. A.; Brown, P. A.; Noyes, T. I. *Aquatic Humic Substances: Influence on the Fate and Treatment of Pollutants*; Advances in Chemistry Series 219; American Chemical Society: Washington, DC, 1989; pp 25–40.
(3) Julia, M.; Baillarge, M. M. *Bull. Soc. Chim. Fr.* **1954**, 470–473.
(4) Shriner, R. L.; Fuson, R. C., Curtin, D. Y. *The Systematic Identification of Organic Compounds;* Wiley: New York, 1964; pp 130–133.
(5) Perdue, E. M.; Reuter, J. M.; Ghosal, M. *Geochim. Cosmochim. Acta* **1984**, *48*, 1257–1263.
(6) Ephraim, J.; Alegret, S.; Mathuthu, A.; Bicking, M.; Malcolm, R. L.; Marinsky, J. A. *Environ. Sci. Technol.* **1986**, *20*, 354–366.
(7) Ephraim, J.; Reddy, M. M.; Marinsky, J. A. *Humic Substances in the Aquatic and Terrestrial Environment*; Springer-Verlag: New York, 1991; pp 263–276.
(8) Marinsky, J. A.; Ephraim, J. *Environ. Sci. Technol.* **1986**, *20*, 349–354.
(9) Marinsky, J. A. *J. Phys. Chem.* **1985**, *89*, 5294–5303.
(10) Perrin, D. D.; Dempsey, B.; Sergeant, E. P. *$pK_a$ Prediction for Organic Acids and Bases*; Chapman and Hall: New York, 1981.
(11) Brown, H. C.; McDaniel, D. H.; Hafliger, O. *Determination of Organic Structures by Physical Methods*; Academic Press: New York, 1955; pp 567–662.
(12) Averett, R. C.; Leenheer, J. A.; McKnight, D. M.; Thorn, K. A. *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*; U.S. Geological Survey Open-File Report 87-557; U.S. Geological Survey: Denver, CO, 1989.
(13) Leenheer, J. A. *Environmental Chemistry of Lakes and Reservoirs*; Advances in Chemistry Series 237; American Chemical Society: Washington DC, 1994; pp 195–221.
(14) Martell, A. E.; Smith, R. M. *Critical Stability Constants, Volume 3: Other Organic Ligands*; Plenum Press: New York, 1977.
(15) Sergeant, E. P.; Dempsey, B. *Ionization Constants of Organic Acids in Aqueous Solution;* IUPAC Chemical Data Series 23; Pergamon Press: New York, 1989.
(16) King, E. J. *Acid-Base Equilibria*; Macmillan: New York, 1965; pp 137–153.
(17) Fieser, L. F.; Fieser, M. *Advanced Organic Chemistry*; Reinhold: New York, 1961; p 114.
(18) Cheshire, M. V.; Cranwell, P. A.; Haworth, R. D. *Tetrahedron* **1968**, *24*, 5155–5167.
(19) Dean, J. A., Ed. *Lange's Handbook of Chemistry;* McGraw-Hill Book Co.: New York, 1985; pp 5–30.
(20) Kile, D. E.; Chiou, C. T.; Brinton, T. I. *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*; U.S. Geological Survey Open-File Report 87-557; U.S. Geological Survey: Denver, CO, 1989; pp 41–57.
(21) Nieuwenhuizen, M. S.; Kieboom, A. P. G.; Van Bekkum, H. *Tenside Deterg.* **1985**, *22*, 247–251.
(22) Paxeus, N.; Wedborg, M. *Humic Substances in the Aquatic and Terrestrial Environment*; Springer-Verlag: New York, 1991; pp 287–296.
(23) Bowles, E. C.; Antweiler, R. C.; MacCarthy, P. *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*; U.S. Geological Survey Open-File Report 87-557; U.S. Geological Survey: Denver, CO, 1989; pp 205–230.
(24) Crawford, R. L. *Lignin Biodegradation and Transformation*; Wiley: New York, 1981.
(25) Bollag, J. M. *Aquatic and Terrestrial Humic Materials*; Ann Arbor Science: Ann Arbor. MI, 1983; pp 127–142.
(26) Harvey, G. R.; Boran, D. A.; Chesal, L. A.; Tokar, J. M. *Mar. Chem.* **1983**, *12*, 119–132.

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