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Biomarker compounds as source indicators for dissolved fulvic acids in a bog

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Abstract. In order to evaluate the sources of DOC in a subalpine bog, we compare the lignin and carbohydrate compositions of aquatic fulvic acid isolated from the bog (BogW-FA) with the fulvic acids isolated from water leachates of two local plants, sedge (sedgeL-FA) and willow leaves (willowL-FA), and of the peat in the bog (peatL-FA). The bogW-FA and peatL-FA have similar biomarker compositions and both differ significantly from either of the fulvic acids from the water leachate of the plants. Thus, the biomarker data strongly suggest that the peat, and not fresh leaffall, provides most of the aquatic fulvic acid to the bog at this time of year (Fall). However, additional sources of lignin- and carbohydrate-depleted fulvic acid to the bog are also needed to quantitatively match the relatively low levels of recognizable biochemicals in the aquatic fulvic acid from the bog. The unusual lignin compositions of both plant leachates suggest that nonstructural lignin phenol producing components, such as lignans, are part of the water-extractable component of plants. These extractable plant products could potentially contribute to aquatic fulvic acids in other environments or at different times of the year.

Introduction

Aquatic humic substances are colored organic material that comprise from 40 to 70% of the dissolved organic matter (DOM) in rivers and streams (Thurman 1985). Humic substances are a heterogeneous mixture of organic acids that can be isolated from water by adsorption onto non-ionic resins at low pH and elution at high pH. Traditionally, these organic acids are isolated using XAD-8 resins and then separated into the major component, acid-soluble fulvic acids, and a minor component, acid-insoluble humic acids (Thurman & Malcolm 1981).

Although humic substances represent an important riverine component with regard to water quality, stream geochemistry and overall energetics of the ecosystem, little conclusive data are available concerning specific biological sources of aquatic humic substances or the processes by which these compounds enter flowing water. In organic-rich river systems the water-soluble component of soil organic matter likely represents the major source of aquatic humic substances (Reuter & Perdue 1977; Thurman 1985; Ertel et al. 1986). However, in other aquatic systems contributions of dissolved humic substances from algal sources and leachate from plants and litter-fall can also be important and can be very seasonally dependent (Baron et al. 1991). In general, multiple primary sources, both autochthonous and allochthanous, exist for aquatic humic substances and the interplay of microbial and geochemical processes within the drainage basin dictate the relative contribution of these sources and thus the chemical composition of aquatic humic substances. Although the potential biological sources for humic substances differ significantly in their biochemical and chemical compositions (Tissot & Welte 1984), a problem for discerning the true origins and pathways for humic substances is that many analytical techniques commonly applied to aquatic humic substances lack the molecular specificity to unambiguously distinguish among these sources.

One geochemical approach that has been used to determine the primary sources for organic matter in soils and sediments is to compare the molecular composition of the organic matter to that of potential source plants (Tissot & Welte 1984). In particular, specific organic compounds, called biomarkers, are used as organic chemical probes to determine the relative contribution of different biological sources to the pool of organic matter found in a particular environment. Molecular biomarkers are organic compounds that have unique and identifiable molecular structures and whose biological distribution is limited to a specific plant or plant type. Although biomarker compounds usually represent trace components of the total organic mixture, their presence is unambiguous evidence that at least a portion of the organic matter is derived from that specific source material.

In the research presented here we use lignin-derived phenol and neutral sugar biomarkers as tracers for vascular plant material in aquatic humic substances. Lignins are phenolic polymers, found exclusively in vascular plants, (Sarkanen & Ludwig 1971) which can be converted to molecularly-specific phenols by alkaline CuO oxidation (Hedges & Ertel 1982). These lignin-derived phenols have been used as terrestrial biomarkers to characterize the vascular plant source material for organic matter in a variety of sedimentary (Ertel & Hedges 1984; 1985) and aquatic (Ertel et al. 1986; Alberts et al. 1992) environments. As determined by this approach, lignin residues appear to be a significant (up to 8% of the carbon) component of sedimentary, riverine and coastal humic substances (Ertel 1985; Moran et al. 1991). The suite of individual phenols released by CuO oxidation can provide information about the vascular plant and tissue types and also the diagenetic state of the lignin component (Hedges & Mann 1979; Ertel & Hedges 1984).

Neutral sugars released by hydrolysis of polysaccharides have also been used as biomarkers to distinguish between marine and terrestrial carbon sources and among different types of vascular plants in sediments and sediment trap materials (Cowie & Hedges 1984b; Hamilton & Hedges 1988). Polysaccharides are a significant residual component of aquatic (Sweet & Perdue 1982) and sedimentary (Ertel & Hedges 1985; Alberts et al. 1992) humic substances. In addition, polysaccharides, like lignins, have been found as a major component of the humic and fulvic acids of the base-extractable portion of fresh vascular plants (Ertel & Hedges 1985; Alberts et al. 1992).

We apply the lignin and carbohydrate biomarker technique here to investigate the sources of aquatic fulvic acids from a subalpine bog. The specific question addressed in this study is whether the colored organic matter in a stream draining the bog is leached from the peat in the bog or from relatively fresh litterfall in the drainage basin. Initial calculations (Caine 1982) suggest that if 10% of the seasonal plant material is converted to humic substances, there is sufficient productivity in the vicinity of the bog to account for the annual export of aquatic humic substances. However, the peat deposit, which has been accumulating over approximately 10,000 years, also has sufficient subaqueous mass that very low leaching rates could supply the humic substance flux. Since all the sources are derived from vascular plants, we hypothesize that lignin and carbohydrate analyses could yield unambiguous molecular-level evidence for the predominant sources of humic substances to the stream.

Site description

The bog under study here is a raised bog of approximately 3 m depth covering 13 hectares at an altitude of 3010 m within the Boulder Watershed in the Rocky Mountain Front Range (Caine 1982). Similar bogs in the region have been estimated to be derived from Upper Pleistocene and Holocene deposits (Gabole & Madole 1976). This bog is considered to be a transitional bog (Walmsley & Lavkulich 1975) due to the presence of the North Boulder Creek which flows through the northern portion of the bog, keeping it waterlogged throughout the year. Within the confines of the bog are a Sphagnum-Willow-Birch plant community. Estimated above ground net annual productivity is 40,000 kg, with perhaps 20% of the biomass remaining as peat (Caine 1982). The area surrounding the bog is composed mostly of sandy loams that support a sparse forest of Engelmann spruce with dense undergrowth of sedges and willows (Caine 1982).

Methods

Samples of sedge, willow leaves and peat were collected from the bog in October, air-dried and weighed. Aliquots of the peat and plant materials were immersed in distilled deionized water for 5 days at concentrations of approximately 10 g/l. The leachate solutions were stored at 2 °C to retard microbial activity. After 5 days the solutions were filtered through 0.45 μm silver filters, diluted with distilled water to approximately 10 mg C/l, and acidified to pH 2. Humic substances were isolated by passing the acidified solution through columns of pre-extracted Amberlite XAD-8 resin and back eluting with 0.1 N NaOH (Thurman & Malcolm 1981). Bog water (170 l), collected at the same time, was also processed similarly. Humic acid precipitates appeared upon acidification of the plant leachate eluant, but not the peat leachate or bog water sample, and were removed by filtration. The concentrated fulvic acid solutions were reabsorbed on XAD-8 resin, flushed with distilled water, eluted with base through a strong cation exchange resin (AgMP50) and freeze-dried (Caine 1982).

Approximately 10 mg of freeze-dried fulvic acids were reacted with CuO for 3 hours at 170 °C in 8% oxygen-free NaOH solution to release a series of lignin-derived phenols that were identified and quantified as trimethylsilyl (TMS) derivatives by capillary gas chromatography (Hedges & Ertel 1982). In addition, to look for free phenols 0.2 mg of the plant leachate fulvic acids was directly converted to TMS derivatives by the same procedure and analyzed by gas chromatography. The identify of all lignin phenols were confirmed by GC-MS.

To facilitate plant type identification, eight index phenols that are unambiguously derived from lignin are grouped into three families based upon the chemical substitution patterns. Vanillyl (V) phenols have monomethoxyl substitution and are present as an aldehyde, ketone and acid: vanillin (Vh), acetovanillon (Vo) and vanillic acid (Va). Syringyl (S) phenols have dimethoxyl substitution and similar oxidation states: syringaldehyde (Sh), acetosyringone (So) and syringic acid (Sa). Cinnamyl (C) phenols have propenoic acid substitution with one or no methoxyl group: p-coumaric (Ca) and ferulic (Fa) acids. Vanillyl phenols are produced from all vascular plants (gymnosperm and angiosperm) while syringyl phenols are only produced from angiosperms. Cinnamyl phenols are produced from the nonwoody tissues of both gymnosperm and angiosperm plants. In addition, a series of three phenols with no methoxyl substitution, p-hydroxybenzaldehyde (Ph), p-hydroxy-acetophenone (Po) and p-hydroxybenzoic acid (Pa) are also generally produced from lignin, but cannot be considered unambiguous lignin tracers since they can arise from other sources as well. The lignin-derived phenols are described by a series of intensive parameters which are weight ratios of individual phenols (e.g. Vo/Vh, Va/Vh) or sums of phenols in individual families (e.g. S/V). In addition, the total yield of lignin-derived phenols is represented by Λ, which is the sum of the weight in mg of all eight phenols normalized to 100 mg organic carbon. Lignin parameters are determined with a precision of 10%.

Neutral sugars were hydrolyzed from 5—10 mg samples by refluxing in 1.3 N H_2SO_4 for 3 hours and analyzed as equilibrated trimethylsilyl derivatives by capillary gas chromatography (Cowie & Hedges 1984a). Sugars are expressed as weight percents of the seven neutral sugars and TCH_2O, the weight of sugars in mg normalized to 100 mg of organic carbon. Elemental compositions were analyzed by Huffman Laboratories (Wheatridge, CO) and DOC was measured using a Beckman 915 Carbon Analyzer (Caine 1982).

Results

Leaching experiments

Initial short-term leaching experiments with the major vascular plants in the area — birch leaves, willow leaves, sedge and sphagnum moss — indicated that willow leaves and sedge had the highest yields of dissolved fulvic acids relative to plant material. Since these plants were major components of the plant community found in the bog, they were chosen for further study (Caine 1982). The yields and elemental compositions of the peat and vascular plant water leachate fulvic acids are presented in Table 1 along with the corresponding data for the aquatic fulvic acid from the bog. Approximately 25% of the plant particulate organic carbon was extracted with distilled water from the sedge and willow leaves compared to about 1% for the peat. Only 6 to 14% of the organic carbon leached from the plant and peat was isolated as fulvic acids. Water leachates from both the willow leaves and sedge yielded humic acids that were not quantified in this study.

All fulvic acids had elemental compositions within the range reported

Table 1. Yields and elemental compositions of the fulvic acids from the bog water and the water leachates of the sedge, willow leaves and peat.

	DOC/POC %	FA/DOC %	%OC	ATOMIC RATIOS N/C	H/C	S/C	O/C
BogW-FA	–	44	52.6	0.015	1.1	0.0027	0.70
PeatL-FA	1.2	6	52.1	0.026	1.1	0.012	0.71
SedgeL-FA	20	8	52.1	0.024	1.2	0.0070	0.71
WillowL-FA	28	14	52.4	0.0085	1.0	0.00064	0.78

Abbreviations: BogW-FA, fulvic acid isolated from the bog water; PeatL-FA, fulvic acid isolated from the water leachate of the peat; SedgeL-FA, fulvic acid isolated from the water leachate of sedge leaves; WillowL-FA, fulvic acid isolated from the water leachate of willow leaves; FA, fulvic acid; OC, organic carbon.

for aquatic fulvic acids (Thurman 1985). The peatL-FA was significantly enriched in sulfur and nitrogen relative to carbon as compared to the bogW-FA acid. A similar enrichment was seen for the sedgeL-FA, although to a lesser degree. The willowL-FA was enriched in oxygen but depleted in sulfur and nitrogen compared to the other fulvic acids.

Lignin and phenol analyses

All four fulvic acids contained significant concentrations of lignin-derived phenols (Table 2) as determined by CuO oxidation. These results clearly indicate that the bogW-FA has at least a partial vascular plant source and that the water leachate fulvic acids of peat and vascular plants contain an unambiguous lignin component. Highest yields of lignin phenols relative to fulvic acid carbon (Λ, Table 2) were from the plant leachate fulvic acids, particularly the sedgeL-FA, which had higher values than are usually seen in fresh grasses ($\Lambda = 4 - 10$) (Hedges and Mann 1979; Hedges et al. 1986; Alberts et al. 1992). Lowest levels of lignin were found in the bogW-FA ($\Lambda = 1$), which had lignin concentrations in the range of other riverine fulvic acids ($\Lambda = 0.7 - 1.2$) (Ertel et al. 1984; 1986). No free lignin-derived phenols were present in the fulvic acids as determined by derivatization and gas chromatographic analysis without prior CuO oxidation.

The entire suite of vanillyl, syringyl and cinnamyl phenols, plus the three p-hydroxyl phenols, were produced from all four fulvic acids in this study. All eight index phenols are only found in nonwoody angiosperm tissues (Hedges & Mann 1979), such as leaves and grasses. The fulvic acids from the plant leachates (willowL-FA and sedgeL-FA) contain the

Table 2. Lignin and carbohydrate data for the fulvic acids from the bog water and the water leachates of the sedge, willow leaves and peat.

PARAMETERS	BogW-FA	PeatL-FA	SedgeL-FA	WillowL-FA
S/V	0.804	1.04	1.06	0.240
C/V	0.184	0.244	0.611	0.593
P/V	1.44	1.79	1.05	0.450
Λ	1.03	1.70	8.49	2.94
Vo/Vh	0.51	0.53	0.94	2.4
Va/Vh	0.91	0.90	1.4	2.3
So/Sh	0.74	0.64	3.2	6.7
Sa/Sh	0.61	0.81	0.79	13.0
Po/Ph	0.83	0.74	4.6	12.0
Pa/Ph	1.9	1.8	4.9	19.0
Pc/Vc	1.5	0.72	0.60	6.8
TCH$_2$O	1.45	5.27	18.6	25.3
% Glucose	39.0	51.0	80.0	76.0
% Mannose	11.0	13.0	1.9	2.5
% Rhamnose	8.8	11.0	13.0	2.1
% Xylose	14.0	8.5	2.4	1.3
% Galactose	10.0	7.5	1.2	14.0
% Arabinose	8.5	4.9	1.4	3.9
% Fucose	7.6	3.0	0.5	0.

Abbreviations: S/V, weight ratio of Syringyl phenols to Vanillyl phenols; C/V, weight ratio of Cinnamyl phenols to Vanillyl phenols; P/V, weight ratio of p-hydroxyl phenols to vanillyl phenols; Λ, weight in mg of total vanillyl, syringyl and cinnamyl phenols normalized to 100 mg OC; Vo/Vh, acetovanillon to vanillin weight ratio; Va/Vh, vanillic acid to vanillin weight ratio; So/Sa, acetosyringone to syringaldehyde weight ratio; Sa/Sh, syringic acid to syringaldehyde weight ratio; Po/Ph, p-hydroxybenzophenone to p-hydroxybenzaldehyde weight ratio; Pa/Ph, p-hydroxybenzoic acid to p-hydroxybenzaldehyde weight ratio; Pc/Vc, p-coumaric acid to ferulic acid weight ratio; TCH$_2$O, weight in mg of the seven neutral sugars normalized to 100 mg OC.

same suite of lignin-derived phenols as would be expected from the plant material, suggesting that the water soluble lignin components found in the fulvic acids can also indicate the types of vascular plant source materials. In addition, the presence of all the index phenols in the peatL-FA and the bogW-FA suggests that these fulvic acids have at least a partial nonwoody angiosperm source as well. Significantly higher levels of p-hydroxyl phenols (P/V) and lower levels of cinnamyl phenols (C/V) were seen in the bogW-FA and peatL-FA than in the sedgeL-FA or willowL-FA (Table 2).

The relative distributions of individual phenols within the p-hydroxyl, vanillyl and syringyl families are presented in Fig. 1. The bogW-FA and peatL-FA have similar distributions of phenols and are in the range of the other aquatic fulvic acids (Fig. 2; Ertel et al. 1986). The sedgeL-FA and willowL-FA are relatively depleted in aldehydic phenols and enriched in ketonic compounds, especially in comparison to the average whole plant values (Fig. 2; Hedges & Mann 1979) and also base-extractable fulvic acids of woody and nonwoody plants (Fig. 2; Ertel & Hedges 1985). However, the dominant ketonic phenol is not the same between the willowL-FA (acetovanillon) and the sedgeL-FA (acetosyringone) fulvic acids, although all ketonic forms are relatively enriched.

Carbohydrate analyses

Both the willowL-FA and the sedgeL-FA have significantly higher concentrations of hydrolyzable neutral sugars relative to organic carbon (TCH_2O, Table 2) than the bogW-FA or the peatL-FA. The carbohydrate

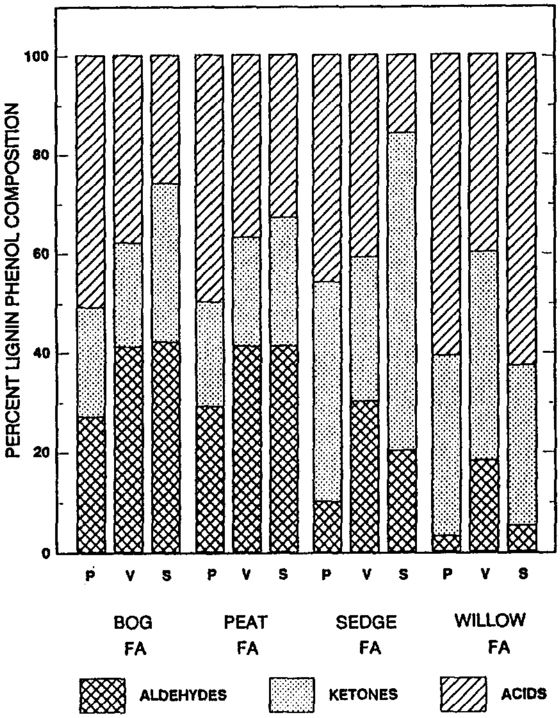

Fig. 1. The relative distributions of lignin-derived phenols according to families for the bog and water leachate fulvic acids. P, V and S are the p-hydroxyl, vanillyl and syringyl phenols, respectively, as defined in the text.

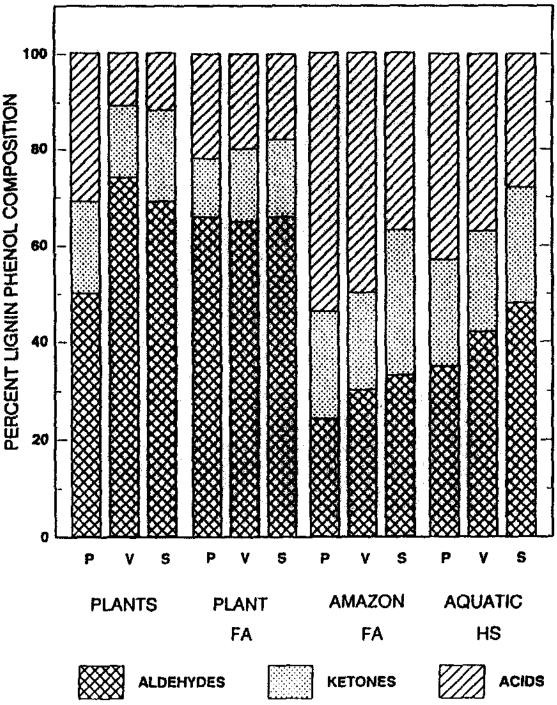

Fig. 2. The average distributions of lignin-derived phenols for a variety of sample types. The plants values are an average of 19 different vascular plants (Ertel & Hedges 1985). Plant FA were isolated from the basic extract of four vascular plants (Ertel & Hedges 1985). Amazon FA is the average of 16 aquatic fulvic acids isolated along the Amazon River (Ertel et al. 1986). Aquatic HS is the average of humic and fulvic acid fractions for two samples from the Pacific Northwest (Ertel et al. 1984).

compositions of both plant leachate fulvic acids are dominated by glucose (76—80%; Fig. 3) with rhamnose and galactose being the only other important sugars (13—14%) for the sedge and willow, respectively. The bogW-FA and the peatL-FA are also dominated by glucose (39—51%) although there are significant levels of the other six sugars.

The percentage of carbon contained as carbohydrate components in the fulvic acids are shown in Fig. 4, together with the organic carbon that can be accounted for by other analyzed biochemicals, lignins and amino acids (Caine 1982). The willowL-FA has 12% of its carbon present in hydrolyzable neutral sugars, 4% in lignin structural units and very little as amino acids (Caine 1982). The sedgeL-FA has the highest percentage of recognizable biochemical carbon (20%) while the bogW-FA has the least (3%). Surprisingly, the peatL-FA has the highest amino acid carbon levels, although low levels of carbohydrates and lignins. The levels of recogniz-

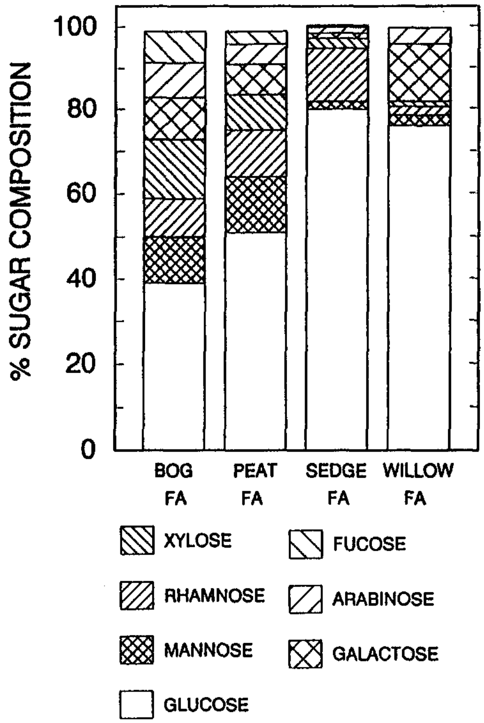

Fig. 3. The relative distributions of hydrolyzable sugars for the bog and water leachate fulvic acids.

able biochemicals are much lower in water leachate fulvic acids of plant material (sedgeL-FA and willowL-FA) than in fulvic acids obtained by leaching vascular plants with basic solutions (Ertel & Hedges 1985; Alberts et al. 1992).

Discussion

The approach for this research is to use specific lignin and carbohydrate biomarkers to compare the aquatic fulvic acid from the bog with similarly isolated fractions from the water leachates of possible vascular plant and peat sources. Since these biomarkers represent at best only a small portion of the total carbon in organic matter (Fig. 4) and since there are a variety of potential minor sources for aquatic humic substances that could effect the biomarker compositions, biomarker characteristics of the aquatic fulvic acids are not expected to exactly match those of the experimentally-derived leachate fulvic acids. This is particularly true with regard to the

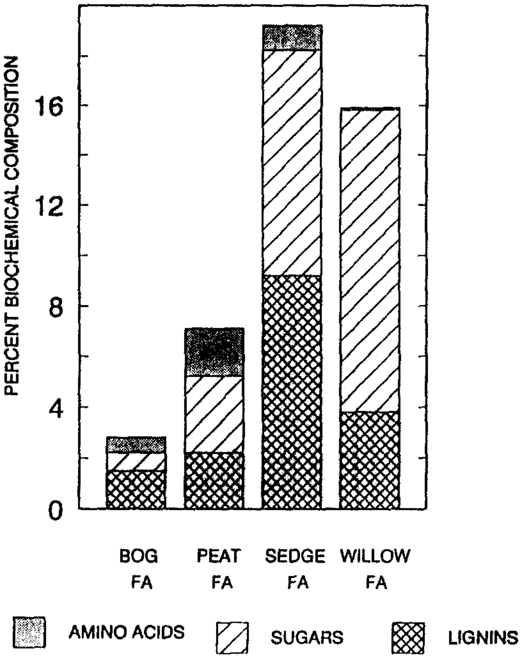

Fig. 4. The maximum amount of lignin, carbohydrate and amino acid components found in the bog and water leachate fulvic acids.

carbon-normalized concentrations of biomarkers, because these values can be increased by selective degradation (Hatcher et al. 1983) of other plant components (such as proteins or carbohydrates) or lowered by dilution with organic carbon from other sources. The discerning power of the biomarker approach lies in the ability to compare the molecular level compositional characteristics of these trace components like lignin-derived phenols to test the likelihood that these sources could contribute that biomarker suite and thus by analogy a significant portion of the carbon to aquatic humic substances. All four fulvic acids contained clearly recognizable lignin and carbohydrate components so that this evaluation can be made with the highly sensitive intensive parameters within each component.

Bog fulvic acid from leaf leachates

The lignin and carbohydrate compositional characteristics of the willowL-FA and sedgeL-FA are significantly different than the bogW-FA. For the lignin parameters, these differences are seen not only in the major compositional parameters — S/V, C/V and P/V, but also in the ratios of the individual aldehydic, ketonic and acidic phenols within the families, particularly Vo/Vh and So/Sh. These two plant leachate fulvic acids, alone, could not produce the lignin compositions seen in the bogW-FA.

The plant leachate fulvic acids examined here are both from nonwoody angiosperm tissues, which overall are expected to provide the greatest annual leaffall and thus sources of leachable material to the bog. The major differences in lignin compositions of the sedgeL-FA and willowL-FA indicate that, as with the nonwoody tissues themselves (Hedges and Mann 1979; Alberts et al. 1991), the water leachate fulvic acids of nonwoody angiosperms are chemically diverse. Thus, it is possible that contributions of leachable lignin residues in fulvic acids from other nonwoody angiosperm tissues could blend with those of the sedge and willow leaves to produce the lignin composition found in the bogW-FA. Due to the high concentrations of lignins in the sedgeL-FA and willowL-FA, any additional source plant must also yield high levels of lignin residues in the leachate fulvic acids to significantly alter the total compositional characteristics to match those of the bogW-FA. However, the existing data suggest that other potential source plants from the area yield significantly less leachable fulvic acids (Caine 1982).

Lignins are generally considered to be one of the more refractory components of vascular plants, particularly in comparison with carbohydrates. However, lignin degradation is known to proceed in aerobic soil environments (Kogel 1986) and in anaerobic aquatic environments (Benner et al. 1984; Colbert & Young 1985). Selective degradation of the lignin component in the plant water leachate fulvic acids could significantly alter the lignin compositional parameters and thus compromise our ability to discern potential sources. Fungal degradation of birch wood lowered S/V ratios and increased Va/Vh ratios (Hedges et al. 1988) and similar trends were found in wood isolated from sediments (Hedges et al. 1985). Likewise, the cinnamyl phenols are considered to be relatively labile in comparison to the vanillyl phenols (Hedges & Weilky 1988) and selective degradation could lower the high C/V ratios of the plant leachate fulvic acids to the range of the bog fulvic acid. However, the most outstanding differences in the lignin compositions between willowL-FA and sedgeL-FA and the bogW-FA are the unusual distributions seen within the individual phenolic families (Fig. 1). Lignin degradation can cause changes in the relative distributions of the individual vanillyl, syringyl and p-hydroxyl phenols such that the percentage of acidic phenols increases at the expense of aldehydic phenols while ketonic phenols remain relatively constant (Ertel & Hedges 1984; Hedges et al. 1988). The plant water leachate fulvic acids show generally lower % aldehydes than have been seen for other aquatic humic substances, for example from the Amazon

River (Fig. 2), although these low values could be reached by extensive degradation. However, the low % aldehydes are not counterbalanced by higher % acids as have been seen previously due to lignin diagenesis but by significantly higher % ketones than have been seen for any other lignin components (Fig. 2). High ketone levels have not been found in any samples that show other indications of extensive lignin degradation (Ertel et al. 1986; Hedges et al. 1985; 1988) and thus all evidence to date suggests that these distributions cannot be produced by diagenesis. Therefore, the lignin biomarker data strongly suggest that the plant water leachate fulvic acids cannot be contributing a significant portion of the fulvic acids to the bog.

Bog fulvic acid from peat leachates

In contrast to the situation seen for the plant water leachate fulvic acids, most of the lignin and carbohydrate compositional parameters for the peatL-FA are in the range of those of the bogW-FA. The peatL-FA is slightly enriched in syringyl and cinnamyl phenols (relative to vanillyl phenols) as compared to the bogW-FA, although the individual phenol ratios are generally within the 10% range of analytical variability. In fact, with the exception of a few parameters, the major differences between these fulvic acids are the quantitative yields of the recognizable biochemical components, Λ and TCH_2O, which are 2—4 times greater in the peatL-FA than in the bogW-FA (Fig. 4). The peatL-FA is also enriched in sulfur and nitrogen relative to carbon as compared to the bogW-FA. The carbon-normalized yields of biomarkers in the peatL-FA could be lowered to the levels seen in the bogW-FA either by selective degradation of the biomarker components (relative to the other DOC in the leachate solutions) or by dilution with fulvic acids that are carbon-rich and depleted in lignin and carbohydrate components. Since carbohydrates and proteins are among the more labile components of fulvic acids, the high TCH_2O and N/C levels of the peatL-FA could be reduced by selective degradation to levels in the bogW-FA. However, this is not the case for the refractory lignin component, which shows little differences in the diagenetic parameters (Va/Vh, etc.) between the peatL-FA and the bogW-FA. Thus, it seem likely that there are additional sources of fulvic acids to the bog. One potential source that has the required compositional characteristics is the fulvic acid fraction of soil organic matter (Ertel & Hedges 1985) which might be leached into the bog (Ertel et al. 1986). Thus, the good agreement in the intrinsic lignin biomarker parameters between the bogW-FA and the peatL-FA indicates that at least the lignin component in the bogW-FA is coming from leaching of the peat. By analogy, we propose that a significant portion of the fulvic acids in the bog water arise from continuous leaching of the peat.

If we assume that the peatL-FA is the major lignin source for the bogW-FA, then we can use the lignin composition of the peatL-FA to test for the minimum contributions of sedgeL-FA and willowL-FA that could be detected by this approach. For example, a blend of 95% peatL-FA with 5% willowL-FA would yield a Vo/Vh ratio of .616, which is more than 20% greater than the bogW-FA ratio. Also, addition of 5% sedgeL-FA to the peatL-FA would increase the So/Sa ratio to .90, which is also more than 20% greater than the bogW-FA. Since the analytical precision of the lignin technique is 10%, both of these changes would be clearly apparent. Thus, on a quantitative basis, less than 5% of the fulvic acid in the bog could be contributed by the water leachate fulvic acids from the plants.

The peatL-FA represents a much lower portion of the DOC in the water leachate of the peat than the bogW-FA is of the DOC in the bog water. If the peat is a major contributor of fulvic acids to the bog and if the leaching experiment at all resembles the natural mechanism of DOC formation, then the hydrophobic fulvic acid fraction is preferentially concentrated in the bog water compared to the more hydrophilic DOC fraction. Since the hydrophilic component is less likely to be physically or chemically removed from the water (McKnight et al. 1992), this observation suggests that the hydrophilic fraction of the water leachate from the peat is significantly more labile than the fulvic acid fraction and could be responsible for a portion of the DOC utilization found in streams (Kaplan & Bott 1983; Leff & Meyers 1991).

Plant leachate fulvic acids

Although it appears that in this environment the contribution of fulvic acids from plant water leachates is small in comparison to those from the peat, the relatively high yields of fulvic acids from the water leachate of plants (Table 1) and the high lignin concentrations in the plant leachate fulvic acids (Table 2) suggest that fulvic acids extracted from plants by water could be an important contributors to both the lignin and phenolic components in other, perhaps low color, streams (Larson 1978). The unusual lignin compositions of the plant water leachate fulvic acid suggests that lignin-derived phenols might be used as tracers to distinguish between peat or soil leachates and leachates of fresh plant (Ertel et al. 1984). Thus, further speculation on the sources of this lignin component is warranted.

As stated previously, the high relative concentrations of ketonic phenols in willowL-FA and sedgeL-FA are unique in comparison to the distributions seen in any other samples, particularly fresh whole plant material (Fig. 2). The basic hydrolysis and CuO oxidation during the lignin analysis is known to cleave the side-chain linkages of polymeric lignin molecules into a relatively constant distribution of aldehydic, ketonic and acidic phenols (Sarkanen & Ludwig 1971). This is true with all different types of lignin and is related to the statistical distribution of chemical linkages seen in lignins. Exactly what type of linkages result in ketonic phenols is unknown at the present time. However, from examining the distribution of p-hydroxyl phenols which can arise from other nonlignin sources, it appears that ketones are only formed from lignins, while the aldehydes and acids can be CuO oxidation products of other biochemicals (Hedges et al. 1988). Simple phenolic aldehydes and acids are found in aqueous (Zapata & McMillan 1979; Whitehead et al. 1981) and basic (Whitehead et al. 1983; Ertel & Hedges 1985) extracts of plant materials and soils, although, to the best of our knowledge, ketonic phenols have not been detected (Ertel & Hedges 1984; 1985). In addition, simple phenols would not be expected to be present in the fulvic acid fraction (Thurman 1985) and, in fact, were not detected by gas chromatographic analyses of the TMS-derivatives of the total (non CuO oxidized) plant water leachate fulvic acids.

Previous studies have shown that base-extraction of woody and non-woody gymnosperm and angiosperm tissues yielded fulvic acids whose ketonic distributions resembled those of the fresh plant material (Fig. 2; Ertel & Hedges 1985). Since the predominant form of lignin in fresh plants is structural lignins, these results indicate that strong base can solubilize a portion of structural lignins which then appear in the humic and fulvic acid fractions. That the lignin components of the plant water leachate fulvic acids produce the same suite of phenols as expected from the plants, but in different proportions, implies that the water-extractable components are related to but not identical with structural lignins. Due to the mild extraction technique that was used here, we propose that these lignin components might be related to plant extractives or natural products such as flavonoids, lignans or chalcones (Robinson 1980).

Lignans are dimers of the same phenylpropanoid structures found in lignins and are widely distributed in both gymnosperms and angiosperms (MacRae & Towers 1984). These compounds are in the molecular weight range of riverine fulvic acids (Thurman 1985) but generally too large to be detected as simple compounds dissolved in rivers (Larson 1978; Ekman 1976). Due to the presence of the p-hydroxyl groups, these compounds would generally undergo oxidative cleavage of the crosslinked propanoid sidechains during the CuO oxidation and, like lignins, could yield simple phenols. However, we suspect that lignans, upon CuO oxidation, would not yield the standard distribution of individual phenols as structural lignins, but would yield a suite of phenols dominated by compounds arising from the oxidation of the specific structures found in these natural products, thus yielding acetovanillon from the willow leachate and acetosyringone from the sedge. Although these interpretations are highly speculative, the ability to analyze lignans directly (without CuO oxidation) by gas chromatography (Ekman 1976) offers the possibility to test the hypothesis that individual vascular plant natural products like lignans are contributing to the phenolic component of the fulvic acid in streams.

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