

# THE CARBOHYDRATE CONNECTION IN FULVIC ACID CHEMISTRY\*

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## SUMMARY

Carbohydrate, *Mother Nature's* foremost substance essential for life on earth, produced by plants by the well known process called photosynthesis, is always considered an *orphan*, if not unwanted *alien*, in humic chemistry. It is lignin and its weathering products that are considered the basis for formation of humic substances. When by the end of the twentieth century humic research turns to also examining dissolved organic matter, called DOM, and aquatic humic substances, the ever-presence of carbohydrates in aquatic as well as in terrestrial ecosystems seems to be more prevalent than that of lignin. Indigenous aquatic plants, especially, do not need lignin for growth and development, and release after cell death their photosynthates, mainly mono- and polymeric sugars, into the surrounding water. Leakage across cell membranes and active secretion are additional processes for enriching the aqueous medium with a variety of carbohydrates and other metabolically produced substances. The ubiquitous presence of carbohydrates in DOM and soil humus is obvious and tends to suggest that its contribution to formation of humic matter should be of more significance than has always been reported before. A futile effort in 1968 by the present author and coworkers at the Nitrogen laboratory, USDA-ARS, Fort Collins, Colorado, reporting a possible carbohydrate connection in fulvic acid formation and esterification of humatomelanic acids remained unnoticed. This is then the purpose of the present article to expose and highlight the importance of carbohydrates as components in humic substances. Fulvic acids extracted from lignite and a humic substance from the International Humic Substances Society (IHSS) were analyzed by CPMAS <sup>13</sup>C NMR (nuclear magnetic resonance) spectroscopy. Additional support was obtained from analyses by infrared absorption spectroscopy of fulvic acids extracted from Ultisols and humic water sampled in Georgia, USA. A literature reference of a plant polysaccharide, extracted from an edible fungus, was included as a standard comparison material. The NMR spectrum of lignite fulvic acid exhibits clearly the dominant features of carbohydrates with in some cases displaying strong signals for polysaccharides. In contrast, the NMR spectrum of humic acid is more aromatic in nature. Another difference of the NMR spectra of fulvic acids from that of humic acids is the very strong NMR signals for carboxyl (COOH) groups, indicating the larger contents of COOH groups in fulvic acids, as evident also in quantitative chemical analyses. These observations are supported by the NMR spectra of the IHSS humic samples. The infrared analyses show all fulvic acid samples to yield IR spectra resembling closely those of the polysaccharides. The spectral features also indicate that the polysaccharide is less weathered in aquatic fulvic acids than that in the soil fulvic acids.

## INTRODUCTION

Carbohydrate, nature's wonder product, seems to be considered till lately an *orphan* in humic matter chemistry. It was once believed in the old days that lignin and its weathering products were the basis

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for humic matter formation (Waksman, 1938; Russell and Russell, 1950; Bremner, 1954; Flaig, 1975). Carbohydrate was a contaminant and has to be removed during isolation of humic substances if true humic matter was to be obtained. An almost similar case was also noted with the nitrogenous part of humic matter, until Kononova showed in her book the necessity of protein substances hooking up to the molecule that could then justify the nitrogen content of the material called humic acid (Kononova 1966). The issue is that carbohydrate substances are perhaps the most abundant in the plant tissue. However, in soil humus it is allegedly second in amounts, with lignin always reported as the most abundant humus component (Stevenson, 1994). Considering the relative ease in decomposition of carbohydrates, this was of course to be expected. Carbohydrates are formed through absorption of the sun's energy by chlorophyll transforming gaseous CO<sub>2</sub> (carbon dioxide) and H<sub>2</sub>O (water) into carbohydrates, a process well known in crops and soil sciences by the name of *photosynthesis*. The substances—often also called *saccharides*—are usually divided into mono-, oligo-, and polysaccharides. The mono- and oligosaccharides are the common sugars, such as arabinose, fructose, glucose, xylose and others, which are soluble in water. On the other hand, the polysaccharides—the complex carbohydrates such as cellulose or starch—are not really soluble, but will form a colloidal suspension in water. In photosynthesis carbohydrates are always produced first, which is then followed by its transformation in the plant body into lignin, protein and other substances when conditions are favorable. In aquatic ecosystems, plants, such as algae and phytoplankton, do not even require lignification of their tissue, hence lignin is usually not formed after production of carbohydrates. These differences may create significant differences in chemistry and composition between terrestrial or soil humic matter and aquatic humic substances, with the former being more lignoid and the latter more carbohydrate in character (Tan, 2011). When at the turn of the century, biogeochemists and in particular hydrologists at the United States Geological Survey, Denver, Colorado, became interested in humic acid research, they turned their attention toward aquatic humic matter and especially the *dissolved organic matter* fraction that they called DOM (Leenheer and Croué, 2003; Wershaw, 2004). The material, currently recognized widely as DOM, is considered by limnologists a very important component for continuation of life, since it is providing the sources of food and energy for organisms living in aquatic systems. Hydrologists and geochemists, on the other hand, believe DOM to hold vital clues to solving the complex nature of humic matter chemistry. Though the bulk of DOM is cited to be composed of humic substances, this dissolved organic matter is reported to also contain an assortment of carbohydrates in the form of monomeric and polymeric saccharides. The indigenous aquatic biomass, in particular algae capable of photosynthesis, will release their photosynthates after cell death in the surrounding water (Bertilson, 2000; Tulong, 2004). Extracellular release of soluble compounds contributing to DOM has also been reported by the authors above to occur through leakage across cell membranes and by active secretion or excretion by the algae. In addition to the saccharides, the processes above have been noted to release some protein, amino acids, carboxylic acids, glycerols, mannitols and various organic substances of molecular weights smaller than 600 Da. Because of the ubiquitous presence of carbohydrates in DOM and soil humus, the present author tends to believe that its contribution to formation of humic matter should be of more significance than has always been reported before. In earlier investigations, the present author and co-workers at the Nitrogen laboratory, USDA-ARS, Fort Collins, Colorado, have discovered carbohydrate components in humatomelanic acids and especially in fulvic acids (Tan and Clark, 1968; Clark and Tan, 1969; Tan, 1975) Though in follow-

up investigations at later dates supporting evidence was obtained by carbon-13 nuclear magnetic resonance (NMR) analyses (Tan, 2011), the information remained unnoticed. Apparently, old ideas that carbohydrates were unwanted *aliens* in humic chemistry seem so entrenched in the beliefs of oldtimers and diehards that this outdated concept is difficult to change. The following report below is a new attempt to present evidence for a carbohydrate connection in fulvic acids with research data obtained by NMR and IR spectroscopic analyses. By analyzing and exposing the secrets hidden in the various NMR spectral signals and IR bands in a simple way the message these complex spectra want to convey can be made more obvious and easy to read. A more simple explanation in plain language will perhaps make the issue also more attractive and encourages more people to read it. This discovery does oppose neither Piccolo's supramolecular nor Wershaw's micellar concept, nor is it against the conventional polymer hypothesis or invalidate the nanotube concept as proposed lately by the author. The carbohydrate connection can be accommodated in either of the above concepts.

### **MATERIALS AND METHODS**

Humic and fulvic acids were extracted from lignite, Cecil soil (Hapludults) and Tifton soil (Kandiudults), major soils in Georgia, USA, with the NaOH method following procedures similar to those recommended by the International Humic Substances Society (IHSS) and the Soil Science Society of America (Tan, 1977; Tan et al., 1990). Humic and fulvic acids were in addition isolated from black water or humic water of the Little River watershed area located near Tifton in South Georgia, USA, by the IHSS XAD-8 resin method (Tan et al., 1990). Specimens of the extracted humic samples were then analyzed by cross polarization (CP) magic angle spinning (MAS)  $^{13}\text{C}$  NMR (nuclear magnetic resonance) spectroscopy, with a JEOL FX 90Q at the Department of Chemistry, University of Georgia, and/or with a Bruker MSL-300 NMR spectrometer at the United States Department of Agriculture, Agriculture Research Service (USDA, ARS), Athens, GA (Tan et al., 1991; Lobartini et al., 1992). For comparisons, pure carbohydrate in the form of corn starch and standard IHSS humic samples were also characterized by NMR analyses with the help of Dr. D. S. Himmelsbach at the USDA, ARS laboratory above. Infrared analyses were carried out at the author's laboratory at the Crops and Soils Department, University of Georgia, using a Beckman IR-18A spectrophotometer and the micro-KBr pellet technique (Tan, 2005).

### **RESULTS AND DISCUSSION**

***Carbon-13 NMR evidence.*** The NMR spectra, shown in Figure 1, are often considered as very confusing by the presence of so many peaks and this often discourages many people reading them. Therefore, a deliberate attempt is made here by the author how to read and interpret an NMR spectrum in a very simple way. The NMR spectrum of the lignite humic substance in Figure 1A can be divided into the three basic NMR regions, each exhibiting one or several peaks called signals, as follows:

- (1) an aliphatic region at 0 - 105 ppm chemical shifts. A signal at 65-105 ppm when present indicates the presence of polysaccharides. This region is identified by the bar on top of the spectrum labeled *aliphatic C* (see Figure 1A, top).
- (2) an aromatic region between 105 and 165 ppm signaling the presence of aromatic substances; See

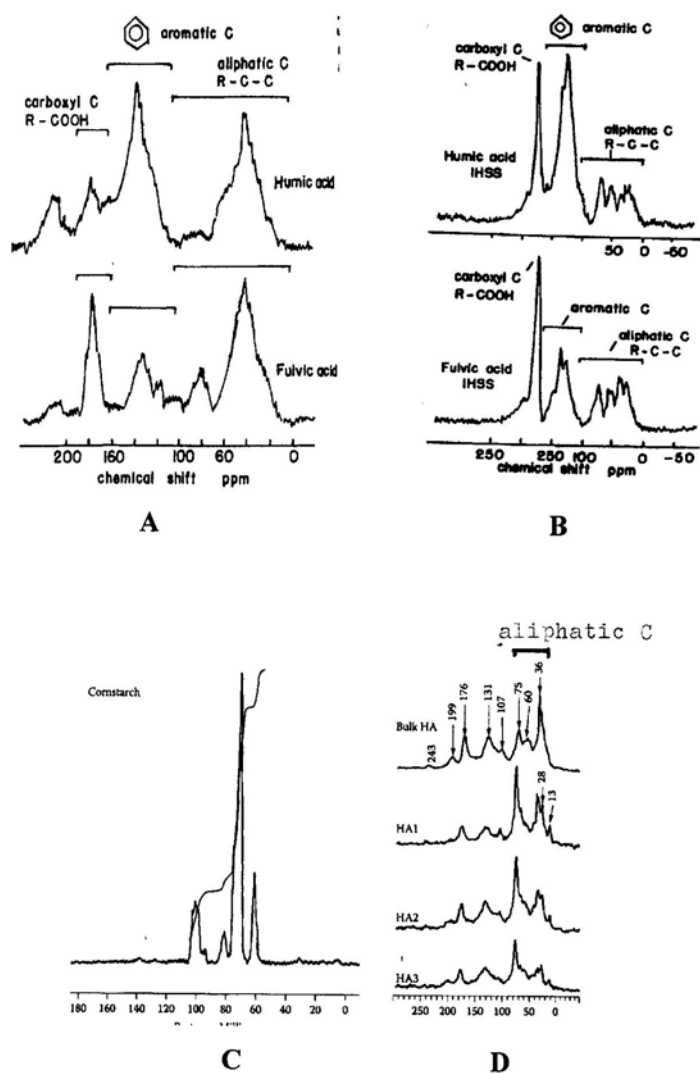
the bar labeled *aromatic C* in Figure 1A, top.

(3) a carboxyl region at 165 to 185 ppm for the presence of COOH (carboxyl) compounds. See the bar labeled *carboxyl C* in Figure 1A, top.

Invoking now the *fingerprinting concept* by using the three labeled regions above as *fingerprint markers*, it can be noticed that the lignite humic acid spectrum (top), though exhibiting clearly all the three basic regions, is dominated by only the first two of them, the aliphatic and aromatic regions. The third, the COOH, region is comparatively weak in resolution intensity. The spectrum of fulvic acid (bottom) is also displaying all the three regions, but at different resolution intensities. Compared to humic acid, the aliphatic region of the fulvic acid spectrum has increased visibly in intensity, and new signals have surfaced between 65 and 105 ppm for the presence of polysaccharides, which are nonexistent in the humic acid spectrum. The aromatic region of the fulvic acid spectrum is considerably weak in signals, but the carboxyl signal in the third region has gained substantially in intensity, which makes it stand out over the weak COOH signal in the humic acid spectrum. The message from the NMR spectra seems to convey that fulvic acid is more carbohydrate in nature than humic acid, whereas humic acid is more aromatic in composition than fulvic acid. The strong signals for COOH groups in fulvic acid are in support of the high contents of carboxylic groups detected in fulvic acids by quantitative chemical analyses (Tan, 2011).

Figure 1B shows the NMR spectra of humic substances isolated and processed by the International Humic Substances Society. They are adaptations from the United States Federal files, U. S. Geological Survey Open-file reports (Thorn, 1989; Thorn et al., 1989). The humic acid spectrum (top) seems to be dominated by strong signals in the aromatic and carboxyl regions, suggesting perhaps a type of humic acid somewhat different from that extracted from lignite (Figure 1A). It is more carboxylic in nature than the humic acid from lignite. Compared with the humic acid, the spectrum of the IHSS fulvic acid (bottom) shows an increase in signals in the aliphatic, sharp decreases in signals in the aromatic, accompanied by strong increased signals in the third, COOH, region. These differences are all indicative of fulvic acids being more aliphatic, less aromatic and substantially more carboxylic in nature than humic acids, as noted earlier for the lignite humic matter. Figure 1D lists NMR spectra of HPSEC (high performance size exclusion chromatography) elution fractions from Conte et al. (2006), identified as humic acids by the authors in question. However, all the NMR spectra only show strong signals in the aliphatic regions, which are NMR features normally exhibited by fulvic acids as explained above. The strong signals (peaks) between 60-100 ppm show close similarities, if not identical, with that in Figure 1C for cornstarch. Regardless of its possible mistaken identity — whether the material is humic or fulvic acid — the present author only wants to show here that carbohydrates in humic substances are not an isolated issue in his research only, but is apparently a worldwide though less noticed occurrence. Additional evidence of the carbohydrate nature of fulvic acids is provided by infrared analyses below.

***Infrared Absorption Spectroscopy.*** Figure 2 lists the infrared spectra of plant polysaccharides, fulvic acids extracted from two major soils in Georgia, USA, and from black water of the Little River watershed at the Tifton soil area. It is perhaps more justified to change the term *black water* into *humic water*, because of the predominance of humic matter that has imparted the dark colors on natural waters. This term is also used by European limnologists and hydrologists (Tulonen, 2004).



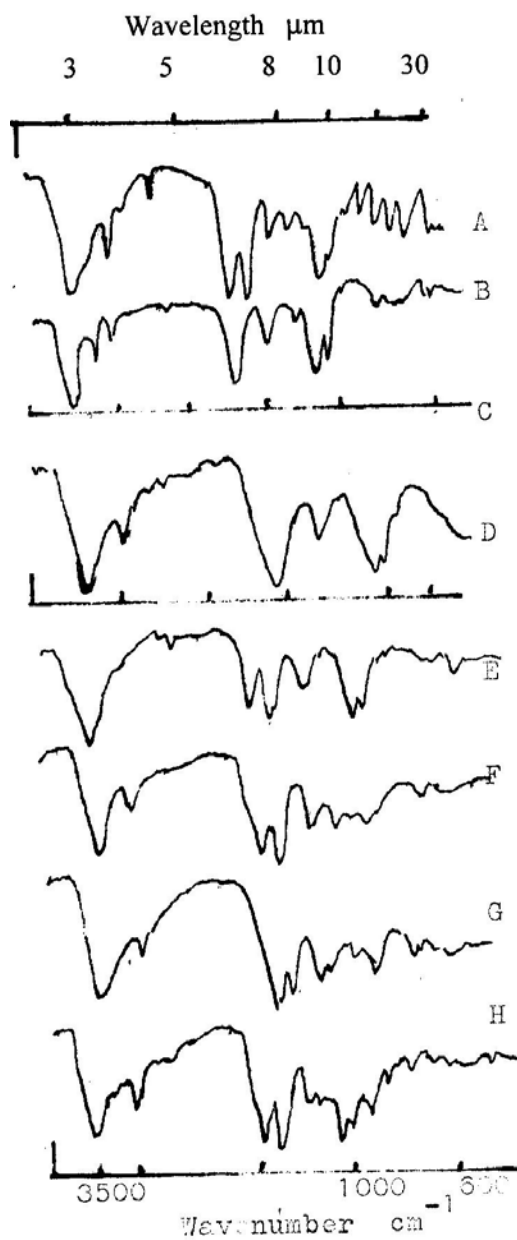
**Figure 1** Carbon-13 Nuclear Magnetic Resonance Spectra of:

(A) Humic acid and Fulvic acid specimens extracted by the NaOH method from lignite;

(B) Humic acid and fulvic acid from the IHSS (International Humic Substances Society);

(C) Carbohydrate, see Tan (2011; 2005),

(D) HPSEC (high-performance size-exclusion chromatography) elution fractions HA1, HA2 and HA3. The main NMR signal at 60 to 80 ppm—for polysaccharides or carbohydrates—was shown to increase in intensity in the HA fractions 1 to 11 which were the dominant signals of the spectra by P. Conte, R. Spaccini, and A. Piccolo (2006).



**Figure 2** Infrared Spectra of:

- (A) Plant polysaccharide (Thetsrimuang et al., 2011);
- (B) Soil polysaccharide (Tan and McCreery, 1970);
- (C) Blank;
- (D) Fulvic acid extracted from Cecil soil or Hapludults (Tan, 1977);
- (E) DOM from black water or humic water at Tifton, South Georgia, USA;
- (F) Fulvic acid isolated from DOM humic water (Tan et al., 1990);
- (G) Fulvic acid from Tifton soil (Kandiudults);
- (H) Humic acid from Tifton soil (Tan et al., 1990)

Figure 2A is the IR spectrum of a polysaccharide substance, extracted by Thetsrimuang et al. (2011) from an edible mushroom *Lentinus* sp. strain RJ-2, and Figure 2B is a spectrum of a polysaccharide isolated by the present author by cleavage from hymatomelanic acid (Tan and McCreery, 1970; Tan, 1975), which only for the sake of differentiating it from the plant polysaccharide is given the “ID” soil polysaccharide. Both spectra serve as standards or reference materials for comparison with the IR spectra of fulvic acids. As can be noticed, the spectrum of fulvic acid from Cecil soil (Figure 2D) exhibits IR features similar to those in Figure 2B. On the other hand, the spectra of DOM (Figure 2E) and fulvic acid isolated from humic water (Figure 2F) have IR features resembling more the spectrum of polysaccharide from the lentinus fungus (Figure 2A). Figure 2G shows the IR spectrum of the Tifton soil fulvic acid with IR features in between those of Figures 2A and 2B. The last one at the bottom (Figure 2H), a spectrum of humic acid, is added to show differences in degree of aromaticity as reflected in its IR spectrum from those of fulvic acids. Summarizing the above, it can be stated that the fulvic acid spectra are in general showing close resemblance to those of polysaccharide substances. These observations are in support with earlier reports that polysaccharides are major components of fulvic acids (Tan and Clark, 1968; Clark and Tan, 1969; Tan, 1975). Pending more developments of the issue from other researchers, the conclusion can be stated that the fulvic acids from soils—in the present research—are apparently more like the polysaccharide separated from hymatomelanic acid, whereas the fulvic acids isolated from DOM or humic water are more like the plant or fungus polysaccharide. The apparent difference is perhaps due to the possibility that the plant polysaccharide (Figure 2A) is less weathered in nature than its counterpart from hymatomelanic acid (Figure 2B)

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